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Cadmium and Copper Uptake and Translocation in Five Willow (*Salix* L.) Species

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ABSTRACT

The efficacy for phytoremediation of five willow species was tested by experimental copper and cadmium uptake in a greenhouse hydroponic system. Five treatments included two concentrations (5 and 25 μM for each metal) and a control. Metal concentrations in solution as well as solution uptake were monitored. Metal resistance was assessed through effects on the dry weight of roots and shoots. The willow species tested were generally resistant of increased Cu and Cd content. Metal accumulation was found in all plant organs of all species. Growth and transpiration were not decreased by 5 μM of copper and 25 μM of cadmium in the solution for most species. 25 μM copper caused injury and reduced the dry weight for all species after 21 d. *Salix nigra* was highly resistant of both Cu and Cd and accumulated more metals than other species. Future field study should be conducted to confirm the findings and feasibility of the phytoremediation technology using those species.

KEY WORDS: *Salix*, willow, heavy metals (cadmium and copper), metal resistance, hydroponic culture.

INTRODUCTION

The accumulation of heavy metals in the upper soil layers due to mining, smelting activities, atmospheric deposition, and the disposal of sewage sludge onto agricultural land represents a serious environmental problem. Phytoremediation is an area of applied research, using green plants to remove, contain, or render harmless environmental contaminants (Cunningham and Berti, 1993). Phytoremediation is developing in the United States and other countries as a potential cost-effective solution for cleaning a variety of contaminated sites (Salt, Smith *et al.*, 1998). The search for hyperaccumulators has led to intensive screening of many plant species. Metal resistance in plants varies among genera, species, and clones; some herbaceous species, such as grasses and mustards, have been found to be capable of accumulating significant amounts of metal in their tissues.

The search for fast-growing woody species that are able to remove metals from contaminated sites has been a focus of recent research in both North America and Europe. Some trees such as *Acer pseudoplatanus* (Turner and Dickinson, 1993), *Salix caprea* (Eltrop *et al.*, 1991), and *Betula pubescens* (Kozlov *et al.*, 1995) are known to be able to colonize metalliferous soil. The idea of using *Salix* for purification of metal-contaminated ecosystems is emerging (Greger and Landberg, 1999; Ostman, 1994). The genus *Salix* comprises about 450 species, which all are easily propagated, fast-growing, and tolerant of diverse soil conditions. The ability of *Salix* to resprout after harvesting of aboveground biomass, along with significant transpiration rates and potential production of energy biomass, makes it an attractive group of plants for phytoremediation purposes. Different species of willow, as well as some clones, vary considerably in their metal translocation patterns and their ultimate resistance of heavy metals (Dickinson *et al.*, 1994; Riddell-Black, 1994). Resistance to some metals, such as Cd, Cu, and Zn, has been documented for a few European *Salix* species (Punshon and Dickinson, 1997; Watson *et al.*, 1999). Some temperate Asian species are able to accumulate significant amounts of Fe, Zn, and Pb (Ali *et al.*, 1999).

This research extends the study of willows' response to heavy metals to North American species. We hypothesized that New World species of *Salix* would show metal resistance through continued growth and health in the presence of cadmium and copper. These two metals are of great importance as environmental pollutants because they are very commonly found in industrially contaminated soils and in wastewater and sewage sludge (Salt *et al.*, 1998). Cadmium is not an essential element for plant metabolism and can be strongly phytotoxic, causing rapid death (Baker, 1993). Copper is an essential micronutrient for plants, but it can become toxic at high concentrations. It has been proposed that the metabolic mechanism for cadmium and copper detoxification involves two classes of phytochelating peptides in plant tissue: metallothioneins and phytochelatins (Salt *et al.*, 1998). Metallothioneins are induced by Cu, to which they have an affinity, while phytochelatins bind Cd and Cu. Internal detoxification of metal ions takes place by chelation with organic acid residues and sequestration in the vacuole, as well as in leaf trichomes (Salt *et al.*, 1995). The cell wall of the root can also serve as a storage site and can be very efficient in immobilizing metal ions (Kahle, 1993).

The first objective of this study was to determine the metal resistance of five North American willow species. The second objective was to document the uptake and translocation patterns of the metals. Knowledge of the patterns of metal uptake and sequestration is important for environmental projects, where metal translocation into aboveground shoots or foliage, as opposed to roots, is essential for harvesting.

MATERIALS AND METHODS

The greenhouse experiment was replicated twice: in April–May and August–September of 2000 (here we present set of data collected in April–May; we have found the same trends for the repeated experiment).

Ambient temperature fluctuated between 20 and 27°C and average relative humidity was 80% with a natural photoperiod. Plants for the experiment were set out in a randomized complete block design with four replicates. Each block consisted of 25 hydroponic units, or treatments, with two factors: five species and five solutions (control, two concentrations of Cu or Cd).

The willow species used in the study were S. *discolor* Muhl., *S. eriocephala* Michx., *S. exigua* Nutt., *S. nigra* Marsh., and *S. lucida* Muhl. Cuttings of these species were obtained from native habitats in rural Ohio (*S. discolor*, *S. eriocephala*, and *S. lucida*, Lucas County; *S. exigua* and *S. nigra*, Franklin County). Each species was represented by one clone. Hardwood cuttings of uniform 20-cm length had been rooted hydroponically in half-strength Hoagland's nutrient solution for 5 wk prior to the beginning of the experiment. Each cutting was mounted into a plastic pot cover to prevent algal growth and set into a pot containing 900 ml of constantly aerated solution. After 5 wk, when the root systems were well developed, the hydroponic solution was replaced with half-strength Hoagland's nutrient solution containing either 5 or 25 μM additional Cu or Cd (added as $CuSO_4$ or $CdSO_4$). A fifth control treatment maintained the Hoagland's solution. All solution loss was made up twice a week. Solution pH was maintained with the range of 5.5–6.0. The experiment continued for 28 d after the addition of metals.

Solution uptake by plants was recorded twice a week by weighing the pots. Measurements of free metal ions present in solution were conducted each 3–4 d using cupric (Model 9629, ORION, USA) and cadmium (Model 9648, ORION, USA) selective electrodes. The measurements of Cd and Cu concentration were made just before the addition of new metals every 3–4 d. At the end of the experiment, all plants were harvested and washed; plant material was separated into roots, wood (original cutting), and shoots (leaves and secondary stems), dried in the oven for 48 h at 70°C, and dry weights were recorded. Metal content was determined in dry plant tissues using an Inductively Coupled Plasma Mass Spectrometer (ELAN 6000 ICP-MS, Perkin-Elmer Corporation). Roots, wood, and shoots of each plant were analyzed separately. After recording the dry weight, all samples ($N = 300$) were ground, digested with HNO_3 and HF; 0.02 g of the tissue were dissolved in 5 ml of HNO_3 and 1 ml 48% HF in a Teflon beaker. Samples were heated on a hot plate for 5–10 min until all organic matter had decomposed; digests were poured into 50-ml plastic bottles and then brought up to volume with deionized water prior to analysis.

A standard protocol to calculate indices of plant resistance to metals involves measurement of the length of the main root in control and metal treatments (Baker, 1993). The cuttings used in this experiment lacked a main root, but developed dense adventitious root systems. Since it was impossible to measure elongation of any single root, the metal resistance was evaluated based on dry root weight values compared to control.

Statistical Procedure

Data were subjected to analysis of variance (ANOVA) (SAS release 6.11, SAS Institute, Cary, NC) and the treatment effects reported were significant according to an F-test at the $\alpha = 0.05$ level. Data for metal concentration in solution was analyzed using the SAS general linear model (GLM) procedure.

RESULTS

Overall Vitality

Throughout the experiment, all cuttings continued active growth and there was 100% survival in all treatments. Wilting and curling of leaf tips were recorded at 25 μM Cu for *S. exigua* and *S. eriocephala*, but the plants continued to grow.

Plant Growth

Plant growth in all treatments was estimated through effects on root and shoot dry weight (Figures 1 and 2). The addition of metals to the solution caused significant changes of root and shoot weight. All treatment, species, and species x treatment interactions effects were significant (Table 1).

Five μM Cd had little inhibitory effects on root and shoot biomass of any species and *S. eriocephala*, *S. exigua*, and *S. nigra* actually had higher shoot biomass than in the control plants. Twenty-five μM Cd was inhibitory, especially for *S. lucida*, but had no negative effect for *S. exigua* and *S. nigra*: 5 μM Cu increased root and shoot growth of *S. exigua* and *S. nigra*, but 25 μM was strongly inhibitory. Root biomass of *S. discolors* and *S. nigra* decreased less dramatically in 25 μM Cu compared with other species, indicating their higher resistance to the metal. Decrease of shoot growth in the 25-μM treatment for most species was not as great as for roots. Roots were the organs most affected by metals; there was a 34–84% reduction of root weight depending on species in 25 μM of copper compared to a 25–58% reduction in shoot growth.

Solution Uptake

The addition of both metals to the solution caused significant changes in solution uptake ($F_{2,45} = 4.02$, $P < 0.0247$ for Cd and $F_{2,45} = 35.19$, $P < 0.0001$ for Cu treatments). Depressed solution uptake was observed for *S. eriocephala* in most metal treatments with the lowest at 25 μM of Cu where it dropped from 23.0 g/d in control to 16.3 in g/d (Table 2). In *S. discolor* and *S. nigra*, some increase of solution uptake was observed in Cd treatments (from 27.6 in control to 34.9 and 31.4 g/d in 5 and 25 μM Cd, respectively, for *S. discolor*; from 21.4 in control to 22.3 and 24.2 g/d in 5 and 25 μM Cd, respectively, for *S. nigra*), but solution uptake decreased at 25 μM Cu for *S. discolor* to 24.4 g/d and at 5 and 25 μM Cu for *S. nigra* to 16.4 and 16.7 g/d. For *S. lucida*, the lowest solution uptake rate was recorded in 25 μM of Cd (16.3 g/d compared to 23.0 g/d in control). For the remaining species it fluctuated less.

Metal Concentration in Solution

Average Cd concentration in solutions throughout the experiment (Table 3) did not change significantly among all species, suggesting an inability of plants to avoid metal uptake; it is probable that Cd ion uptake by the roots of trees is directly proportional to concentrations in the solution. Species responded differently to the Cu treatments, where a significant change in metal concentration ($F_{4,42} = 3.06$, $P = 0.03$)

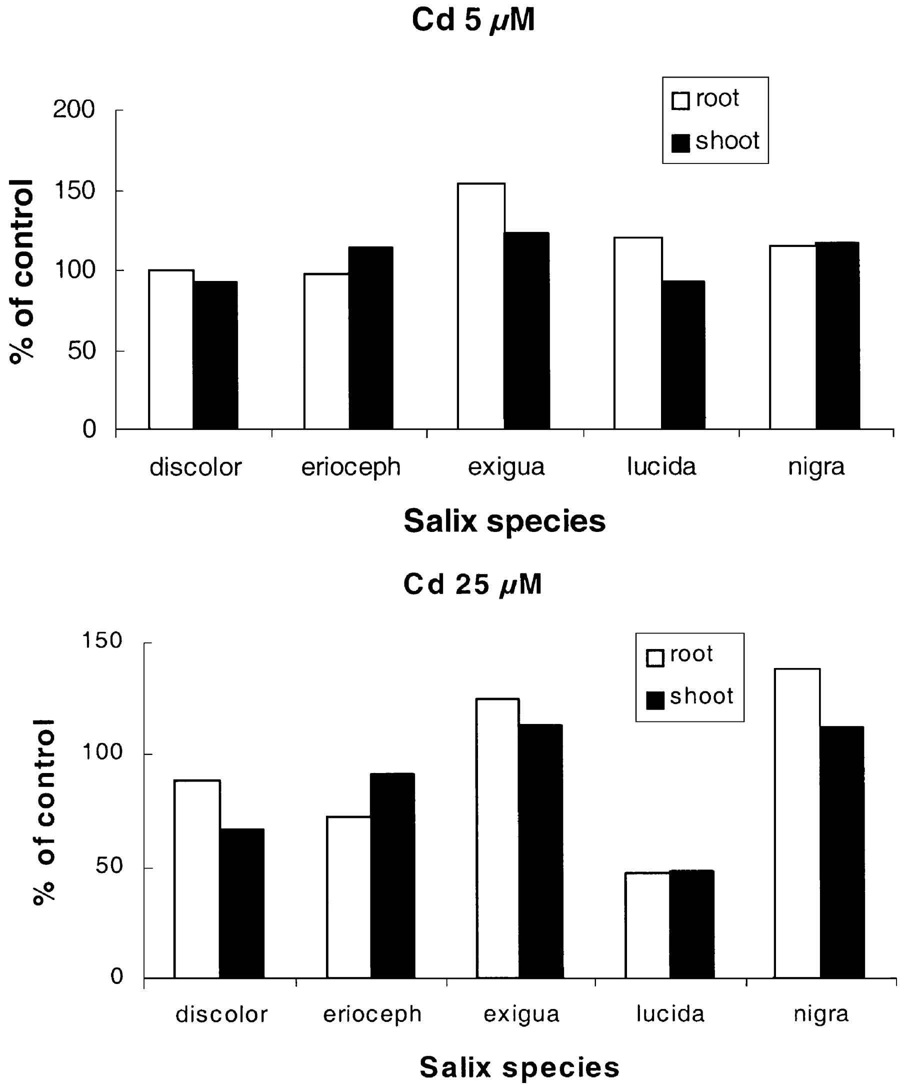


FIGURE 1. Effect of two cadmium concentrations on root and shoot dry weight relative to control for 5 *Salix* species. Plants were treated with Cd for 21 d ($n = 4$).

was recorded for different species. Two species (*S. exigua* and *S. eriocephala*) had noticeably lower average concentrations of Cu in solutions throughout the experiment (8.4 and 10.6 µM, respectively).

Metal Concentration in Plant Tissues

We found that metal concentration in plant tissues differed significantly among species, treatments, and plant organs (Table 4). The higher concentration of metal in solution corresponded with the higher concentration of metal in the plant.

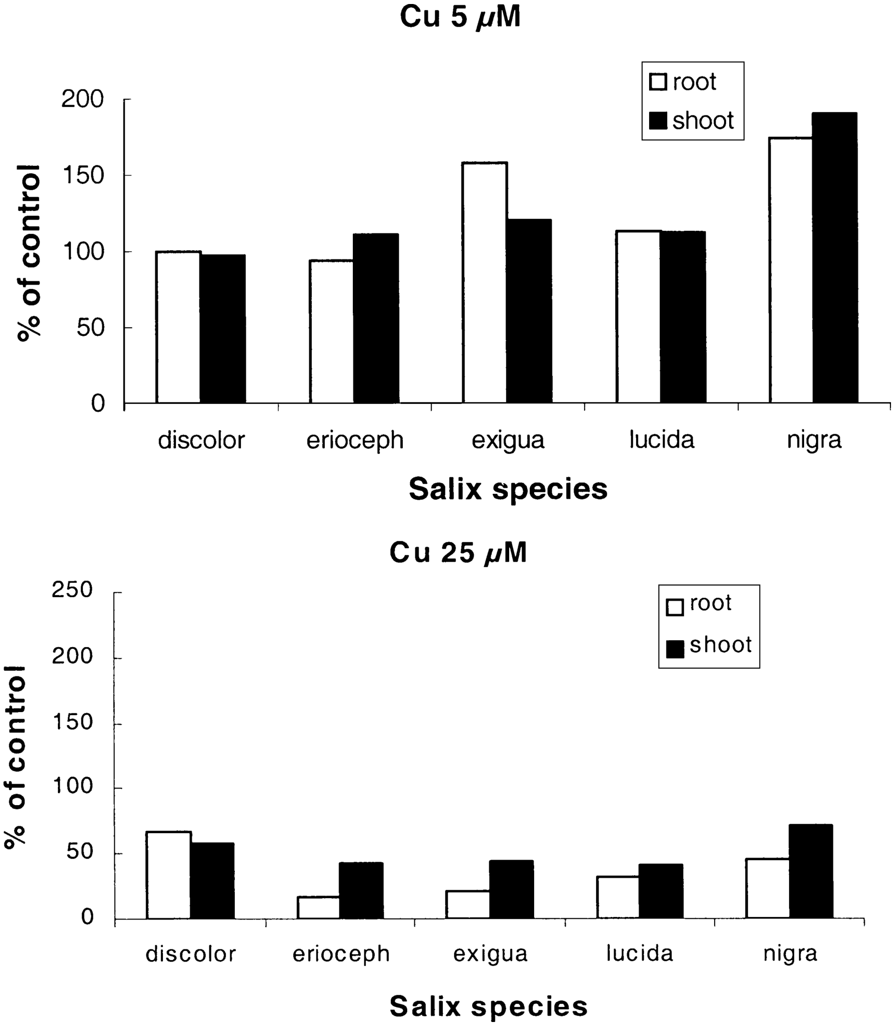


FIGURE 2. Effect of two copper concentrations on root and shoot dry weight relative to control for five *Salix* species. Plants were treated with Cu for 21 d ($n = 4$).

All species accumulated the highest concentration of Cd and Cu in their roots (Figures 3 and 4). Cadmium concentration in roots ranged from 75.9 to 577.3 $\mu g\ g^{-1}$ in different treatments, with the most found in *S. lucida* at 25-μM treatments. The concentration of Cd in wood ranged from 18.3 to 181.0 $\mu g\ g^{-1}$ for all species and treatments with the highest value recorded for *S. nigra* (100 and 181.08 $\mu g\ g^{-1}$ in 5- and 25-μM treatments, respectively) and *S. exigua* (49 and 90 $\mu g\ g^{-1}$). Foliar concentrations of Cd were the lowest for *S. discolor* (6.45 and 27.44 $\mu g\ g^{-1}$) and for the rest of the species it stayed more or less in the same range (30–40 and 60–80 $\mu g\ g^{-1}$ in 5- and 25-μM treatments, respectively) with the highest values for *S. eriocephala* and *S. nigra* at 25 μM.

TABLE 1. Probabilities of null effect on root and shoot growth for all species in Cd and Cu treatments. Bold type indicates significance at the $\alpha = 0.05$ level.

Treatment variable	Cd				Cu			
	Root weight		Shoot weight		Root weight		Shoot weight	
	$\boldsymbol{F}_{\mathbf{d,f}}$	***P***	$\boldsymbol{F}_{\mathbf{d,f}}$	***P***	$\boldsymbol{F}_{\mathbf{d,f}}$	***P***	$\boldsymbol{F}_{\mathbf{d,f}}$	***P***
Species	$F_{4,45} = 10.24$	<**0.0001**	$F_{4,45} = 84.37$	<**0.0001**	$F_{4,45} = 11.87$	<**0.0001**	$F_{4,45} = 85.06$	<**0.0001**
Treatment	$F_{2,45} = 32.08$	<**0.0001**	$F_{2,45} = 28.08$	<**0.0001**	$F_{2,45} = 40.02$	<**0.0001**	$F_{2,45} = 334.2$	<**0.0001**
Species*treatment	$F_{8,45} = 6.19$	**0.001**	$F_{8,45} = 11.17$	<**0.0001**	$F_{8,45} = 4.10$	**0.001**	$F_{8,45} = 11.07$	<**0.0001**

TABLE 2. Effect of Cu and Cd on average transpiration rate of 5 *Salix* species (g/day) ($n = 4$) (±SE).

	Treatment				
Species	Control	Cd 5 μM	Cd 25 μM	Cu 5 μM	Cu 25 μM
S. discolor	27.6 ± 0.77	34.9 ± 1.06	31.4 ± 0.88	28.1 ± 0.66	24.4 ± 0.81
S. eriocephala	23.0 ± 0.91	17.6 ± 0.66	17.8 ± 1.08	16.4 ± 1.33	16.3 ± 0.54
S. exigua	28.0 ± 0.71	24.5 ± 1.72	24.1 ± 1.61	27.9 ± 0.55	22.9 ± 0.68
S. lucida	23.0 ± 1.01	22.4 ± 1.07	16.3 ± 0.99	18.6 ± 0.74	18.8 ± 0.89
S. nigra	21.4 ± 0.58	22.3 ± 0.82	24.2 ± 0.55	16.4 ± 0.68	16.7 ± 1.02

The average copper concentrations in the control plants were 12.4 μg g^{-1} for roots, 4.5 μg g^{-1} for stems, and 5.1 μg g^{-1} for shoots. Copper is an essential element for plants and normal copper concentration in plant material is 2.5–25 μg g^{-1} (Grimshaw *et al.*, 1989); 8 μg g^{-1} is the normal foliar value in *Salix* species growing in unpolluted conditions (Allen, 1989).

The copper concentration in plant organs showed a trend similar to that of cadmium for all species, with maximum concentrations found in roots and less in aboveground organs. Copper accumulation in roots was in the range of 150–1372.7 μg g^{-1}, with the highest concentration in *S. exigua* at 25-μM treatments. For most species, the Cu concentration in the wood averaged from 8 to 15 and 27 to 36 μg g^{-1} in 5- and 25-μM treatments and in the shoots from 13–21 to 14–19 μg g^{-1} in 5- and 25-μM treatments. *S. nigra* accumulated the highest Cu concentration in the wood (18.3 and 61.6 μg g^{-1} in 5- and 25-μM treatments) and shoots (16.4 and 30.3 μg/g in 5- and 25-μM treatments).

Metal Content in Plant Tissues

The metal content in plant parts was calculated by multiplying the dry weight of part by the metal concentration in the respective treatment and the significant differences were found between species, treatments, and species x treatments interaction for each part (Table 5). Metal content varied significantly different among tissues ($F_{2.114} = 28.54$, $P < 0.0001$ for Cd and $F_{2.174} = 30.43$, $P < 0.0001$ for Cu for all species) (Table 6). Total metal content in whole plant varied among species and between treatments (Table 7) and ranged from 72.7 to 697.6 μg for Cd treatments and from 70.7 to 294.1 μg for Cu treatments (Table 8).

TABLE 3. Average concentration of Cd and Cu in 5- and 25-μM solution throughout the experiment for each species ($n = 8$) (± SE).

Species	Cd treatments (μM)	Cu treatments (μM)
S. discolor	12.13 ± 2.25	12.17 ± 0.95
S. eriocephala	13.07 ± 2.49	10.63 ± 1.01
S. exigua	13.43 ± 2.51	8.43 ± 1.32
S. lucida	12.37 ± 2.32	13.09 ± 0.71
S. nigra	12.59 ± 2.38	13.81 ± 1.14

TABLE 4. Probabilities of null effects on tissue metal concentration in Cd and Cu treatments for all *Salix* species. Bold type indicates significance at the $\alpha = 0.05$ level.

	Root		Wood		Shoot	
Variable	F_{df}	P	F_{df}	P	F_{df}	P
Cd						
Species	$F_{4,45} = 19.47$	**<0.0001**	$F_{4,45} = 74.03$	**<0.0001**	$F_{4,45} = 30.79$	**<0.0001**
Treatment	$F_{2,45} = 825.46$	**<0.0001**	$F_{2,45} = 379.3$	**<0.0001**	$F_{2,45} = 423.1$	**<0.0001**
Species* treatment	$F_{8,45} = 12.97$	**<0.0001**	$F_{8,45} = 21.19$	**<0.0001**	$F_{8,45} = 12.5$	**<0.0001**
Cu						
Species	$F_{4,45} = 11.47$	**<0.0001**	$F_{4,45} = 39.24$	**<0.0001**	$F_{4,45} = 7.65$	**<0.0001**
Treatment	$F_{2,45} = 1080.8$	**<0.0001**	$F_{2,45} = 487.1$	**<0.0001**	$F_{2,45} = 145.61$	**<0.0001**
Species* treatment	$F_{8,45} = 15.94$	**<0.0001**	$F_{8,45} = 20.12$	**<0.0001**	$F_{8,45} = 7.36$	**<0.0001**

The extent of metal translocation to the aboveground organs was estimated based on the percentage of distribution between plant parts (Figures 5 and 6). For most species the highest Cd content was found in wood; it was intermediate in roots and lowest in shoots. An exception was *S. exigua*, which accumulated the highest amount of cadmium in roots in the 5 μM of Cd treatments. For Cu treatments the trend was different and the highest amount of metal was found in roots, intermediate in wood, and least in shoots. The amount of copper in new growth in 25-μM treatments was lower than that in 5-μM solution.

Relationship Between Metal Uptake from Solution and Metal Concentration in Plants

The amount of metal taken up by the whole plant from the solution calculated through solution uptake over 21 d. An increase of Cd content in plant tissue was correlated to metal uptake through solution uptake ($R^2 = 0.5$) (Figure 7). A different pattern of Cu uptake and accumulation shown in Figure 8, where the Cu content of plant tissues did not increase commensurately to its calculated uptake from solution. As the experiment proceeded, copper was being excluded from plants in 25-μM treatments.

DISCUSSION

Plant Growth and Tissue Metal Content

The observed ability of *Salix* species to continue growth in the presence of Cd and Cu and to accumulate metals in their tissues demonstrated their resistance of moderate-to-high levels of metals. Metal uptake, translocation, and growth response varied with metal, application levels, and species. The difference in sensitivity between species to high metal content ranged from the stimulation of root and shoot growth to their severe inhibition.

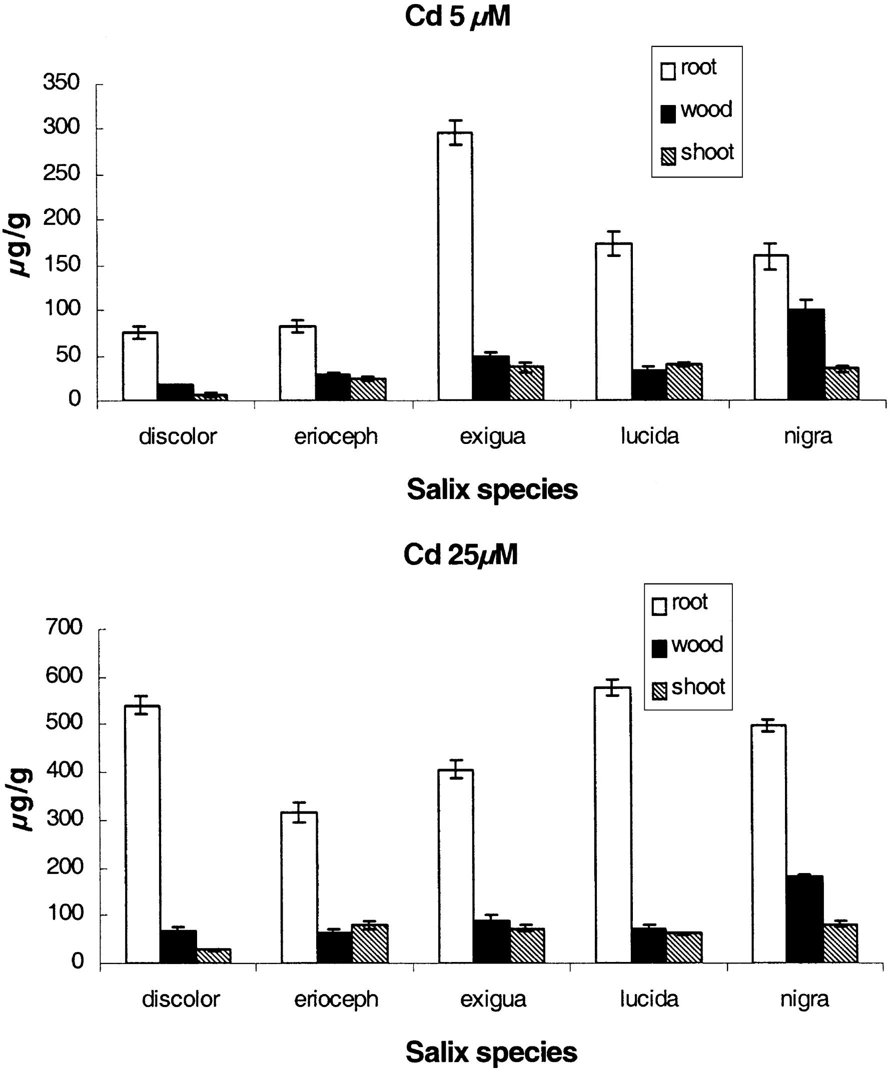


FIGURE 3. Average Cd concentration in dry plant tissues (μg/g) of five *Salix* species after 21 d of growth in metal contained solution ($n = 4$).

The general trend for copper was the stimulation of growth at 5 μM and considerable depression of growth at 25 μM. Solution uptake was also inhibited by 25 μM Cu for all species. *S. exigua* and *S. eriocephala* had noticeably lower average concentration of Cu in solution and higher concentration of metals in roots than other species. The same species exhibited foliar damage at 25 μM of copper, but they did not have high leaf Cu, indicating reduced resistance for this metal.

Willows were less sensitive to Cd than to Cu and plant growth, for most species were not inhibited even at high concentrations. Inhibition of growth was evident only

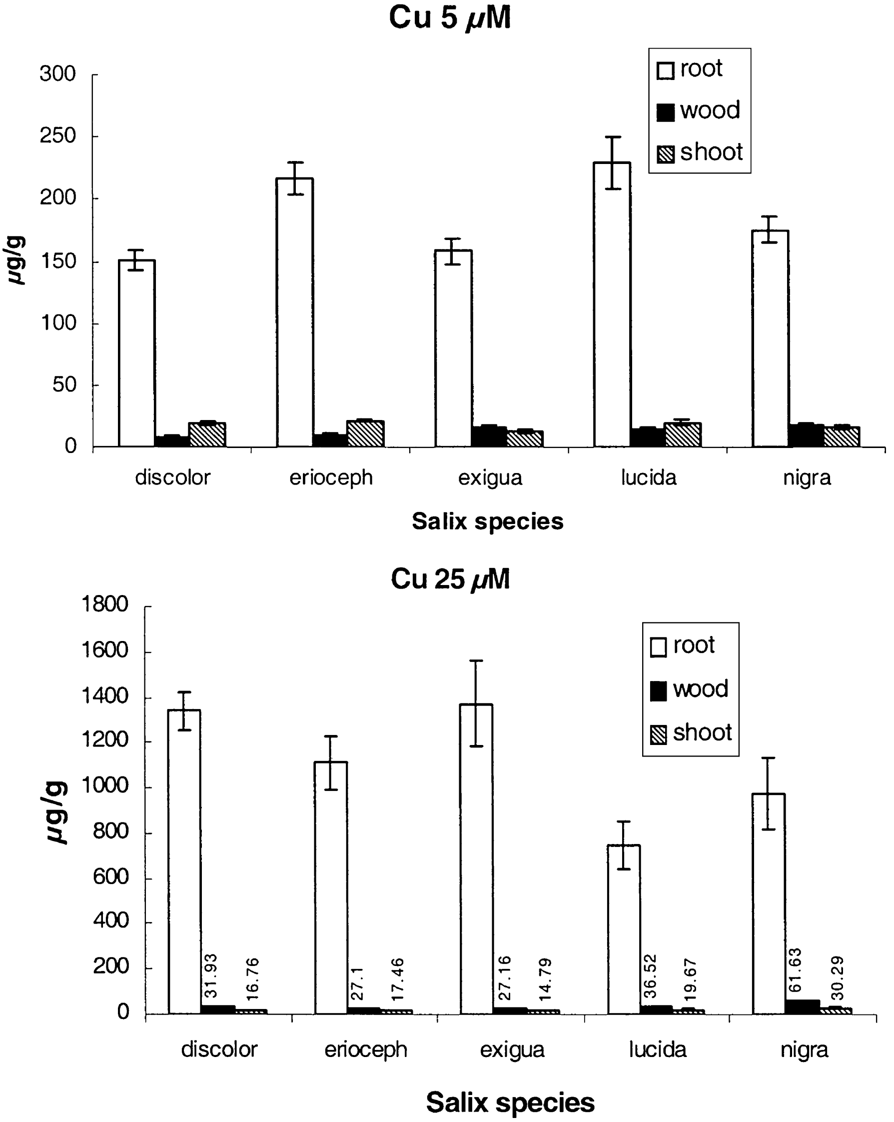


FIGURE 4. Average Cu concentration in dry plant tissues (μg/g) of five *Salix* species after 21 d of growth in metal contained solution ($n = 4$).

for *S. lucida*, for which the highest concentration of Cd was found in roots of the 25-μM treatment. This coincided with the lowest solution uptake and low values for root and shoot biomass. High Cd concentrations were toxic for this species. In contrast, growth of *S. nigra* and *S. exigua* was stimulated even at high Cd concentration. Cadmium (<50 μM) has been shown to stimulate root growth in sugar beets (Greger and Lindberg, 1986). There is no evidence that cadmium is essential for growth of any organism and further research is necessary to clarify the effect of Cd.

TABLE 5. Probabilities of null effects on root, wood, shoot metal content in Cd and Cu treatments. Bold type indicates significance at the $\alpha = 0.05$ level.

	Root		Wood		Shoot	
Variable	F_{df}	P	F_{df}	P	F_{df}	P
Cd						
Species	$F_{4,30} = 17.18$	$<$**0.0001**	$F_{4,30} = 10.64$	$<$**0.0001**	$F_{4,30} = 30.04$	$<$**0.0001**
Treatment	$F_{1,30} = 25.90$	$<$**0.0001**	$F_{1,30} = 134.9$	$<$**0.0001**	$F_{1,30} = 48.75$	$<$**0.0001**
Species* treatment	$F_{4,30} = 2.85$	**0.0407**	$F_{4,30} = 0.64$	$<$0.6384	$F_{4,30} = 10.50$	$<$**0.0001**
Cu						
Species	$F_{4,45} = 2.01$	0.1091	$F_{4,45} = 20.71$	$<$**0.0001**	$F_{4,45} = 34.25$	$<$**0.0001**
Treatment	$F_{2,45} = 53.18$	$<$**0.0001**	$F_{2,45} = 197$	$<$**0.0001**	$F_{2,45} = 196.9$	$<$**0.0001**
Species* treatment	$F_{8,45} = 1.98$	0.0707	$F_{8,45} = 4.40$	**0.0006**	$F_{8,45} = 9.55$	$<$**0.0001**

The analysis of total metal content in plant tissues revealed that the metals taken up by the plant go to the aerial tissues. The shoot and wood metal values are probably affected by leaf area and transpiration. The copper content of aerial tissues was relatively lower than that of cadmium. Cadmium appears to be more mobile within the plant, so that the highest accumulation was in wood tissues, while new growth also contained appreciable quantities.

Most of the Cu was immobilized in the roots of the willows tested. The metal could be bound to the root surface or adsorbed in the apoplast, where its effects are less detrimental. However, elevated levels of metal in aerial tissues indicated that excess Cu may have passed through the root endodermis to enter the transpiration stream or that the direct access of solution Cu to woody tissue via diffusion through the epidermis/bark region and through the cut end of the cutting took place. An increase from 5 to 25 μM Cu in solution did not lead to an increase in shoot accumulation. This is evidence of exclusion. Low mobility of Cu in *Salix* has also been observed in previous research (Nissen and Lepp, 1997; Punshon and Dickinson, 1997).

TABLE 6. Probabilities of null effects on metal tissue content (root, wood or shoot) for each *Salix* species in Cd and Cu treatments. Significance at the $\alpha = 0.05$ level is indicated by bold type.

	Cd treatments		Cu treatments	
Species	F_{df}	*P*	F_{df}	*P*
S. discolor	$F_{2,18} = 7.8$	**0.0036**	$F_{2,30} = 3.91$	**0.0311**
S. eriocephala	$F_{2,18} = 3.41$	0.0554	$F_{2,30} = 9.39$	**0.0007**
S. exigua	$F_{2,18} = 19.35$	$<$**0.0001**	$F_{2,30} = 9.86$	**0.0005**
S. lucida	$F_{2,18} = 16.37$	$<$**0.0001**	$F_{2,30} = 5.02$	**0.0132**
S. nigra	$F_{2,18} = 5.48$	$<$**0.0138**	$F_{2,30} = 4.21$	**0.0245**
For all species	$F_{2,114} = 28.54$	$<$**0.0001**	$F_{2,174} = 30.43$	$<$**0.0001**

TABLE 7. Probabilities of null effects on total metal content in plant in Cd and Cu treatments. Bold type indicates significance at the $\alpha = 0.05$ level.

	Cd		Cu	
Variable	F_{df}	P	F_{df}	P
Species	$F_{4,45} = 4.52$	**0.0037**	$F_{4,30} = 19.89$	<**0.0001**
Treatment	$F_{2,45} = 115$	<**0.0001**	$F_{1,30} = 99.88$	<**0.0001**
Species* treatment	$F_{8,45} = 2.21$	**0.0446**	$F_{4,30} = 1.41$	0.2545

Two basic strategies of plant response to heavy metal were proposed by Baker (1981). Plants are able to detoxify metal ions at different locations. “Excluders” detoxify metals in the roots, whereas “accumulators” transport metal ions to the shoot where they can be stored in the vacuoles of leaf cells. Accumulators are able to dispose of metals through seasonal leaf drop. Our results indicate that *Salix* functions as an accumulator for cadmium and an excluder for copper. But this categorization is not absolute, since some copper translocation to the aboveground tissues was observed. Copper appears to be more toxic for plants and less mobile than cadmium.

Willows vary in their resistance to Cu and Cd in terms of growth rate, metal concentration, and total metal content. *S. nigra* was resistant to both metals, indicating that this species possesses *general* resistance to heavy metals. *S. exigua* exhibited resistance to Cd, but not to Cu. The experimental plant material was obtained from uncontaminated soils, so our results suggest an innate resistance of wild plants to abnormal metal contamination in soil water.

Prospects for Phytoremediation

Phytoremediation requires the accumulation of metal primarily in aboveground organs, to facilitate harvesting. A maximum of 0.01–0.02% of Cd of dry matter of aboveground tissues was found in the wood of *S. nigra*, but the considerable biomass production of willows may increase the efficiency of soil decontamination. The economic viability of metal extraction from *Salix* leaves and stems should be evaluated. Metal recovery from the roots is also possible, but only after final harvest of the plants. In a biofuel production cycle, recovery from root biomass would occur only every 25–30 yr (Landberg and Greger, 1994).

TABLE 8. Total metal content in plant (μg) after treatment with metals for 21 days ($n = 4$).

	Cd		Cu		
Species	5 μM	25 μM	0.25 μM (control)	5 μM	25 μM
S. discolor	72.7	309.5	16.3	70.7	248.8
S. eriocephala	218.8	572.8	24.6	249.2	256.9
S. exigua	467.5	697.6	23.8	202	246.7
S. lucida	334.6	558.5	47.9	236.3	294.1
S. nigra	261.5	641.5	15.4	173.4	256.4

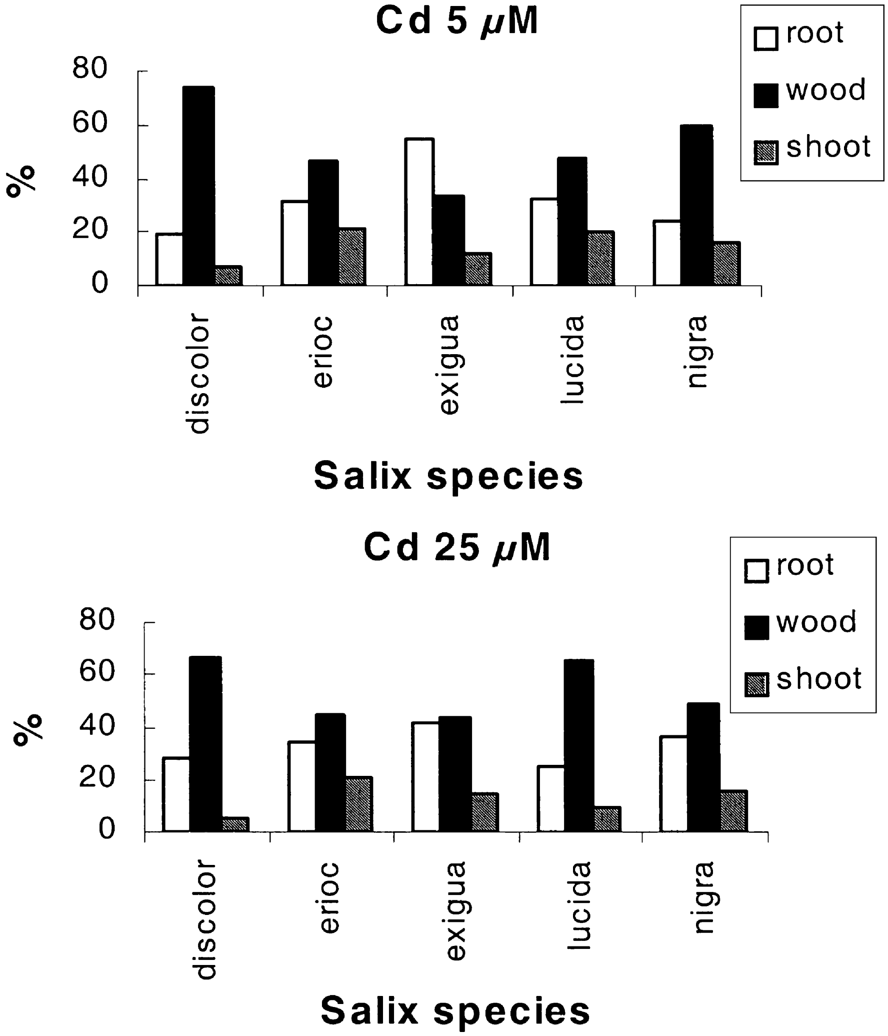
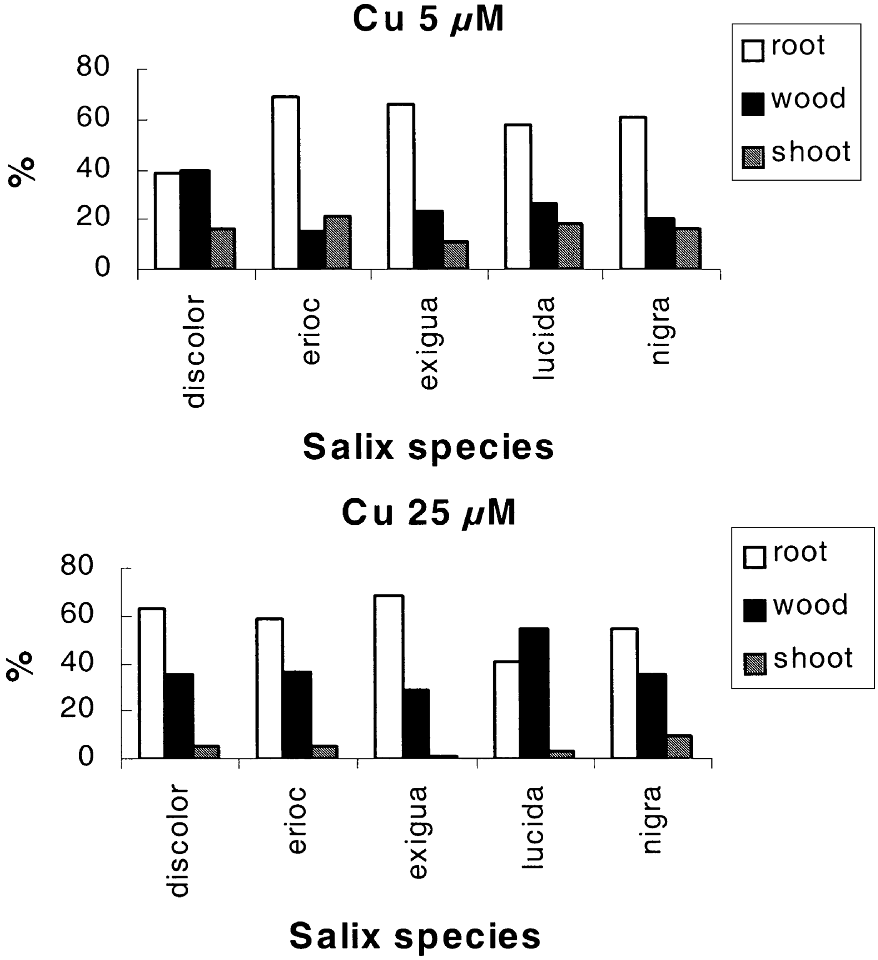


FIGURE 5. Tissue distribution (%) of cadmium in *Salix* species calculated as total Cd content in plant tissue compare with total Cd content in whole plant. Plants ($n = 4$) were treated with Cd for 21 d.

Salix nigra is the most promising North American species for phytoremediation research because of its high total metal content, its ability to translocate Cu and Cd into wood and leaves, and its capacity to maintain high biomass during the experiment, especially in Cd treatments. Our results indicate that *S. exigua* was able to maintain high biomass throughout the experiment with high internal concentration of cadmium in the aboveground tissues as well. The ability of S. *exigua* to sucker into dense stands with vast root systems in short periods could also be useful in the phytoremediation of Cd contaminated soils. Future field study should be conducted to confirm the productivity of these species under field conditions.



Cu 5 μM

Cu 25 μM

FIGURE 6. Tissue distribution (%) of copper in *Salix* species calculated as total Cu content in plant tissue compare with total Cu content in whole plant. Plants were treated with Cu for 21 d ($n = 4$).

Future Field Experiments

Plant behavior in metal-contaminated soils could differ from that in solution, so species performance in the field should be evaluated. Other factors affect the growth of field plants, including:

1. Spatial contamination of soils is heterogeneous, but roots will tend to proliferate in less contaminated regions. This decreases the metal uptake and translocation into the plant. In this research, plant roots were in contact with a homogeneous hydroponic medium.

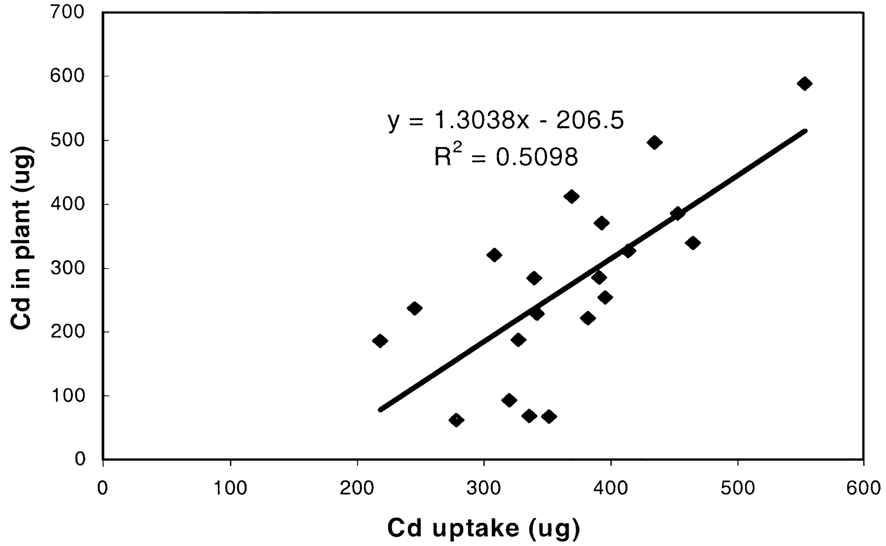


FIGURE 7. Relationship between apparent Cd uptake (water uptake *x* metal concentration) and metal accumulation in plant tissues for five *Salix* species.

2. Mycorrhizae can protect plant roots in heavy-metal-polluted soils and probably decrease the translocation of metal. It is known that willows benefit from vesicular–arbuscular endomycorrhizal as well as ectomycorrhizal associations (Lodge, 1989). There is evidence that *Betula* tolerance to Zn may depend on

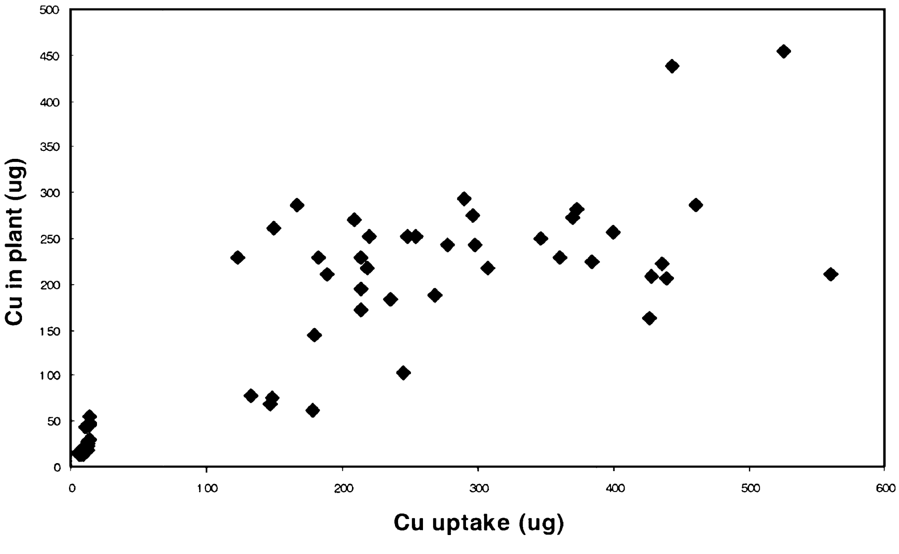


FIGURE 8. Relationship between apparent Cu uptake (water uptake *x* metal concentration) and metal accumulation in plant tissues for five *Salix* species. No trend is revealed.

an ectomycorrhizal association that limits plant tissue concentration of this metal because of metal adsorbtion to the surface of hyphae (Denny and Wilkins, 1987). In this instance, a cell wall, either of the root or of the associated mycorrhizal fungus, can serve as a storage site, thus efficiently immobilizing metal ions belowground (Kahle, 1993).

3. Heavy-metal field contamination usually comprises multiple elements. The effect of the interaction between heavy metals on plant growth, whether independent, antagonistic, additive, or synergistic, should be studied (Kahle, 1993).

Environmental Impact

Due to growing interest of using willow as an alternative biofuel, various agricultural techniques have been proposed for cultivation. These include fertilization with sewage sludge, which usually is contaminated with metals and the use of initially contaminated soils for biofuel plantation sites (Greger and Landberg, 1999; Hansson *et al.*, 1999; Perttu, 1993; Riddell-Black *et al.*, 1997).

From an ecological perspective, metal contamination of vegetation could injure herbivorous organisms that consume willows. There is some evidence that passerine bird species from subalpine areas of central Norway have high accumulations of Cd in their livers, probably as a result of high consumption of *Salix* seeds (Hogstad, 1996). High concentrations of Cd (9 ppm in twigs during winter) in naturally grown *S. aurita* in Norway resulted in toxic metal intake by moose (Ohlson and Staaland, 2001). From this perspective, only species with low shoot uptake but with metal accumulation in roots should be used for nonurban revegetation purposes.

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