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Short Communication

Cadmium accumulation by willow clones used for soil conservation, stock fodder, and phytoremediation

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Abstract

Elevated levels of cadmium are often found in the soil of New Zealand pasturelands due to the long-term use of Cd-contaminated fertilisers. The accumulation of Cd in willow biomass used as stock fodder could therefore adversely affect agricultural productivity and human health. Alternatively, willows may be used for phytoremediation of Cd-contaminated soil at polluted sites. An investigation was carried out to determine the variation in Cd as well as Zn, Mn, and Fe accumulation in 15 willow clones that had been bred for soil conservation purposes. These clones were grown under controlled conditions in 20-L pots of soil containing Cd, Zn, Mn, and Fe at concentrations of 0.3, 64, 597, and 56000 mg/kg, respectively. Daily water use was measured over the final 2 weeks of the experiment and biomass accumulation was determined at the end of the experiment. We found that shrub willows had significantly higher leaf and stem Cd, Mn, and Zn concentrations than tree willows. Average leaf Cd concentrations varied widely between clones from 1.5 to 10 mg/kg. Clones with a high Cd accumulation capacity may be selected to improve the efficacy of Cd-phytoremediation, whereas clones that accumulated lower Cd concentrations may be used for stock fodder. Metal concentrations were not significantly correlated with plant water-use, or biomass production.

Additional keywords: bioaccumulation, heavy metal, pastureland, water use.

Introduction

Willows are used extensively in New Zealand for soil conservation and supplementary stock fodder during times of drought (Wilkinson *et al.* 1999). Both foliage and small twigs can be browsed by sheep and cattle (Hathaway 1986; Douglas *et al.* 1996). The majority of New Zealand pasturelands have elevated cadmium (Cd) concentrations due to repeated applications of Cd-rich superphosphate fertiliser (Bramley 1990). The average concentration of Cd in dry topsoil of New Zealand pasturelands is 0.44 mg/kg (Roberts *et al.* 1994). Robinson *et al.* (2000) showed that a commonly used willow clone, Tangoio (*Salix matsudana* × *S. alba*), accumulated Cd at levels of up to 14 mg/kg in the dry leaves when grown in a soil containing just 0.6 mg/kg of this element. This concentration is above levels (1–5 mg/kg) shown to adversely affect livestock (Underwood and Suttle 1999). Thus Cd accumulation by willows that might be used for fodder is of concern. Willows may also facilitate the entry of Cd into the food chain via insect browsing. The recently introduced willow saw fly (*Nematus oligospilus*) can completely defoliate willows, going through several generations in 1 year (Charles and Allan 2000). This could introduce another exposure pathway for Cd.

The ability of willows to accumulate Cd has been exploited to remove Cd from contaminated soils (Östman 1994; Greger and Landberg 1997, 1999; Robinson *et al.* 2000). The phytoremediation of Cd-contaminated soil would involve short rotation coppicing of willows. Harvested material could then be burnt in an incinerator equipped with a Cottrell

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precipitator (Graham and Cragg 1959) that prevents the metal being lost in the smoke. The remaining ash product could be stored in an appropriately designed landfill. The energy produced by the incineration of the crop might even be utilised for generating electricity (Nixon *et al.* 2001).

The efficacy of willows to extract soil-metals is due to a number of attributes including high biomass production, and water-use (Chmelar 1973). Mills *et al.* (2000) demonstrated that within a single clone, Cd uptake was influenced primarily by tree water-use. It has been demonstrated that there is a wide variation in the ability of different willow clones to accumulate heavy metals in the bark and wood (Riddell-Black *et al.* 1997). Using hydroponic experiments, Punshon and Dickinson (1999) showed interclonal variation in tolerance to Cu, Cd, Ni, and Zn, as well as in the uptake of Cu.

Given the potential economic importance to New Zealand of Cd accumulation by willows when used as stock fodder or in phytoremediation, an investigation was warranted into Cd accumulation in the leaves and wood of New Zealand bred willow clones. This study aimed to determine variation in Cd, Zn, Mn, and Fe accumulation, as well as water use, in 15 willow clones that have been bred or selected for soil conservation purposes in New Zealand. It was anticipated that suitable clones could be found for both stock fodder (low Cd accumulation), and phytoremediation (high Cd accumulation). Thus clones could be matched for soil conditions, whether that soil was pastureland or a contaminated site.

Materials and methods

Experimental design

The experiment was conducted at the Horticultural and Food Research Institute of New Zealand Ltd (HortResearch), Palmerston North, New Zealand (40.2°S, 175.4°E). Fifteen willow clones were selected for the experiment from the HortResearch poplar and willow nursery at Aokautere. Eight were shrub willows (sub-genus *Caprisalix*) and 7 were tree willows (sub-genus *Salix*). Information on the clones is shown in Table 1. Plant material and soils were taken from a stool bed containing 9-year-old willows growing in a fine sandy loam (Manawatu series) with pH 5.7, CEC (cation exchange capacity) 13.4 $cmol_c$/kg, organic carbon content of 63 g/kg, and a resident Cd concentration of 0.3 mg/kg. No further Cd was added to the soil. The soil was homogenised using a soil mixer, and Osmocote fertiliser added at rates recommended by the manufacturer.

Table 1. Willow clones used in the experiments

Clone number	Cross	Tree/shrub	Sex
1	*S. viminalis* (L.)	Shrub	M
2	*S. opaca* (Anderss. ex. Seemen)	Shrub	M
3	*S. caprea* (L.) × *S. viminalis* (L.)	Shrub	F
4	*S. viminalis* (L.)	Shrub	F
5	*S. schwerinii* (E. Wolf)	Shrub	M
6	*S. disperma*	Shrub	F
7	*S. dasyclados* (Wimm.)	Shrub	F
8	*S. triandra* (L.)	Shrub	M
9	*S. matsudana* (Koidz) × *S. alba* (L.) clone 1	Tree	F
10	*S. matsudana* (Koidz) × *S. alba* (L.) clone 2	Tree	F
11	*S. matsudana* (Koidz) × *S. alba* (L.) clone 3	Tree	M
12	*S. matsudana* (Koidz) × *S. alba* (L.) clone 4	Tree	F
13	*S. babylonica* (L.) × *S.alba* (L.) clone 1	Tree	F
14	*S. babylonica* (L.) × *S.alba* (L.) clone 2	Tree	F
15	*S. fragilis* (L.)	Tree	F

Three cuttings of each willow clone were grown separately in 20-L plastic buckets from 5 January 2000 to 8 April 2000. Each planted pole was 200–300 mm in length and 20 mm in diameter. Plants were grown in a shade-house in a randomised block design and watered twice daily using an automatic watering system, dispensing *c.*1 L of water per application. The first leaves appeared after 8 days, and at the end of the experiment, the average dry weight of the new growth was 61.6 g/plant.

Water-use

From 23 March 2000 to 3 April 2000, the daily water-use of individual plants was measured. Automatic watering was discontinued and the buckets were weighed daily using a balance accurate to 0.001 kg. The pots were watered manually on alternate days according to each plant's water use. A control pot containing bare soil was used to calculate soil evaporation.

Sample preparation and metal determination

On 4 April 2000, each tree was defoliated, and all current season's wood was removed. Total leaf area per tree was evaluated using a leaf-area meter (LICOR 3000). Leaves and stems of each cutting were placed in a drying cabinet at 80°C until a constant weight was reached. These samples were then weighed separately and ground. A 0.15-g subsample was placed into a 50-mL Erlenmeyer flask and digested with 10 mL of concentrated nitric acid. These flasks were placed on a heating block until a final volume of 3 mL was reached. It was then diluted to 10 mL in a measuring cylinder using deionised water and stored in polyethylene containers. Cadmium determinations were performed using a graphite furnace atomic absorption spectrometer (GBC 909 AA). Zinc, Fe, and Mn were determined using flame atomic absorption spectroscopy (GBC Avanta Σ).

Data analysis

Data from the metal concentration measurements were analysed using MINITAB. Fisher's least significant difference method was used to indicate significant difference between the metal concentrations of different clones. The Spearman Rank Correlation test was then used to test rankings of clones given water-use, and Cd, Zn, Mn, and Fe concentration data.

Results and discussions

Cd accumulation

Table 2 summarises the average metal concentrations from all 15 willow clones. Clearly the concentration of Cd is higher in the leaves of the willows than in the stems. There was, however, a significant positive correlation ($r = 0.79$, $P < 0.001$) between leaf and stem Cd concentrations. The higher Cd concentrations in the leaves compared with the stems is consistent with the findings of Greger and Landberg (1997) and Robinson *et al.* (2000). The data also clearly illustrate the ability of some willows to take up significant amounts of Cd from soil, with a mean bioaccumulation coefficient of 13 (Table 2). The bioaccumulation coefficient, as used here, is defined as the leaf/soil metal concentration quotient on a dry weight basis. Given that the Cd concentration in the soils of this experiment (0.3 mg/kg) is

Table 2. Metal concentrations (mg/kg dry matter) in leaves, stems, and associated soils of the willow clones

		Cd	Mn	Zn	Fe
Leaf	Median	3.9	236	137	136
	s.e.	0.5	32	8	70
Stem	Median	2.7	65	54	20
	s.e.	0.2	10	4	4
Soil		0.3	597	64	5.6%
Bioaccumulation coefficient[A]		13.0	0.4	2.1	0.002

[A]The leaf/soil metal concentration quotient on a dry weight basis.

lower than the national average (0.44 mg/kg), the Cd concentrations in willow trees used for soil conservation and fodder purposes may well exceed our experimental values.

All the clones tested had Cd concentrations above levels (1–5 mg/kg) shown to adversely affect livestock (Underwood and Suttle 1999). It is unclear, however, what adverse effect (if any) willow leaves may have if they only form a small part of the animal's diet. Not all the Cd from the ingested leaves will be adsorbed by the animal's gut. The nutritional implications of using willow for stock fodder warrants further research.

Figure 1*a* illustrates the mean leaf Cd concentration for each clone. Corresponding bioaccumulation coefficients ranged from 6 to 33. Shrub willows (sub-genus *Caprisalix*) had significantly ($P < 0.01$) higher leaf Cd concentrations than tree willows (sub-genus *Salix*) with mean values of 5.81 and 3.05 mg/kg, respectively.

Accumulation of Zn, Mn, and Fe

Evaluation of the leaf concentrations of Zn, Mn, and Fe was sought to provide additional information about the potential fodder value of different willow clones, and provide clues as to how and why there are differences in Cd accumulation between clonal types. Significant clonal variation was found for Mn and Zn (Fig. 1*b*, *c*) but not for Fe (Fig. 1*d*). These 3 elements were concentrated mostly in the leaves (Table 2). With the exception of Clone 2, the Zn bioaccumulation coefficient in the leaves was >1, whereas the Mn and Fe bioaccumulation coefficients were <1. As with Cd, shrub willows showed a higher level of Zn and Mn accumulation than tree willows. Average leaf metal concentrations for shrub and tree willows were, respectively, 391 and 159 mg/kg for Mn, 153 and 141 mg/kg for Zn, and 291 and 236 mg/kg for Fe.

Experiments investigating Cd toxicity revealed direct effects on both Mn and Zn metabolism in calves and sheep and showed that Zn consumption may annul toxic Cd effects (Van Bruwaene *et al.* 1986). Willows with a high Zn concentration may therefore be selected for fodder use so as to reduce Cd toxicity to stock. In addition, supplemental feeding of willow leaves to stock may alleviate Zn deficiency in areas with a low soil Zn concentration. Clark and Millar (1983) reported a Zn concentration of <5 mg/kg in New Zealand pasture species, compared with the mean Zn concentration in willow leaves of 148 mg/kg found here.

Metal accumulation in relation to water use and biomass production

Figure 1*e* illustrates plant water use per unit of leaf area. The Spearman Rank Correlation test of data presented in Fig. 1*a–d* (leaf metal concentrations) and Fig. 1*e* (water-use per unit leaf area) showed no significant relationships. This finding indicates that differences in metal accumulation between clones are controlled mostly by factor(s) other than water use. Our findings differ from those of Mills *et al.* (2000) because we investigated the relationship between Cd concentration and water-use over several different clones whereas Mills *et al.* (2000) investigated one single clone. There was no significant relationship between plant biomass production (Fig. 1*f*) and either leaf or stem metal concentration.

Caution should be applied when extending our results to the field. The clonal variations with prospects for fodder or phytoremediation are nonetheless exciting and merit further exploration. Our experiments, however, conducted over one growing season, may not represent the long-term metal accumulation behaviour of the clones. The root morphology and soil-Cd distribution in the field need not bear any resemblance to our buckets filled with homogenised soil. The accumulation of metals could be affected by different soil types and the concentrations of other elements (Punshon and Dickinson 1997). The soil used in

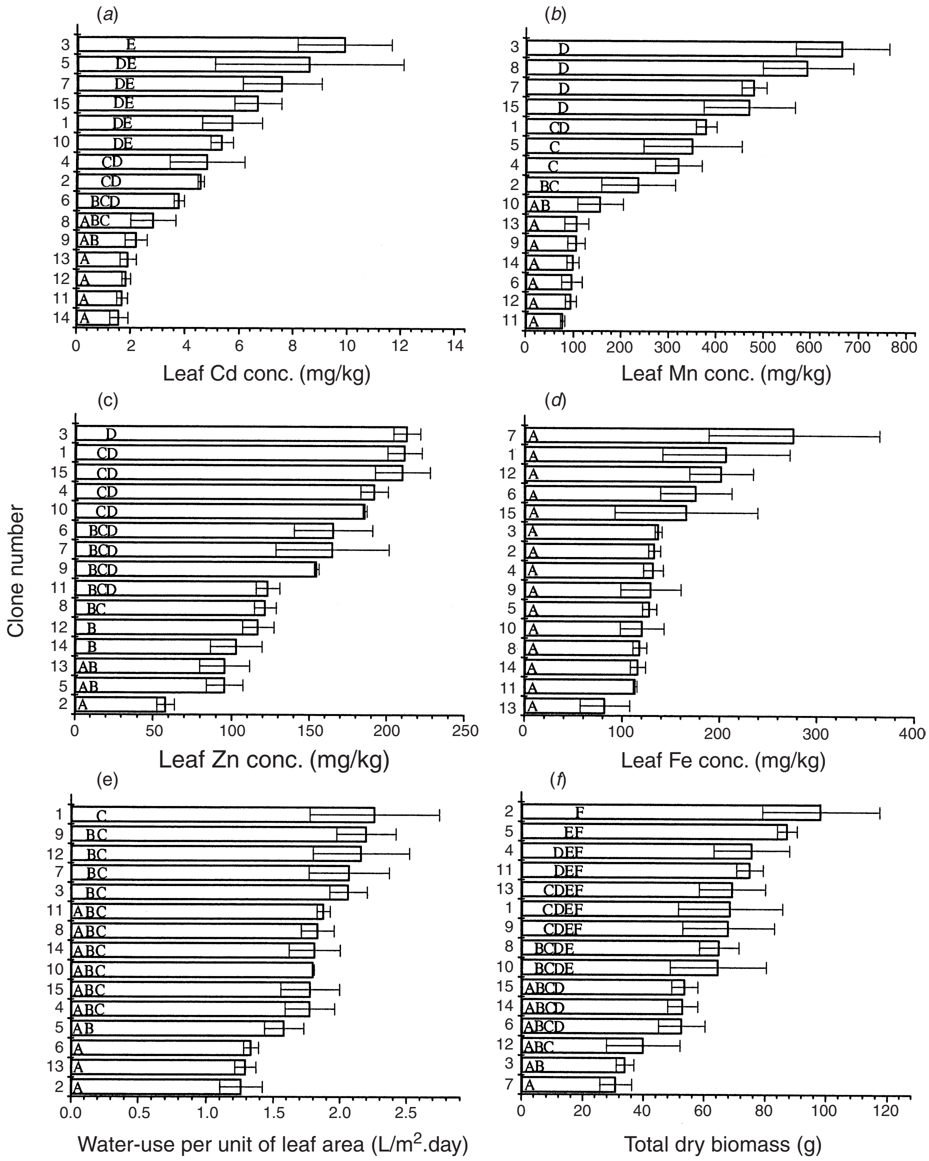

Fig. 1. Variations in the willow clones of (*a*) leaf Cd concentrations, (*b*) leaf Zn concentrations, (*c*) leaf Mn concentrations, (*d*) leaf Fe concentrations, (*e*) water use per unit leaf area, and (*f*) dry biomass production. All concentrations are expressed on a dry weight basis. Capped lines represent the standard error of the mean. Clones with a common letter are not significantly as determined by Fisher's least significant difference test.

our experiments has a relatively low clay-fraction and organic matter content. This may have resulted in increased metal bioavailability and plant uptake, relative to other soils. The presence or absence of arbuscular mycorrhizal fungi may also play a role in the metal accumulation and tolerance of *Salix* (Harris and Jurgensen 1977).

Conclusions

Willow clones have been found to display a large interclonal variation with respect to Cd accumulation from soil. The most efficient clones for Cd-extraction have leaf Cd concentrations 6 times higher than the lowest one and they can concentrate Cd within leaves at levels 33 times that of the soil. These clonal differences cannot be attributed to either biomass or water-use. They may be due to some genetic expression and could be related to differing root microflorae. Shrub willows generally showed greater concentrations of elements within their leaves when compared with tree willow.

Willows with both high-biomass production and low leaf Cd concentrations, such as clones 11 and 13, can be selected for stock fodder on Cd-contaminated soils. These characteristics are typically found in tree willows. Shrub willows, such as clones 1 and 5, showed good biomass production, and their Cd concentration in their leaves tended to be higher indicating their suitability as phytoremediation plants for Cd-contaminated soil. Our results illustrate the potential for manipulating the characteristics of specific willow types to improve the appropriateness and efficacy for particular land management strategies.

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