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Natural and induced cadmium-accumulation in poplar and willow: Implications for phytoremediation

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Abstract

Potentially poplars and willows may be used for the *in situ* decontamination of soils polluted with Cd, such as pasturelands fertilised with Cd-rich superphosphate fertiliser. Poplar (Kawa and Argyle) and willow (Tangoio) clones were grown in soils containing a range (0.6–60.6 μg g^{-1} dry soil) of Cd concentrations. The willow clone accumulated significantly more Cd (9–167 μg g^{-1} dry matter) than the two poplar clones (6–75 μg g^{-1}), which themselves were not significantly different. Poplar trees (Beaupré) sampled *in situ* from a contaminated site near the town of Auby, Northern France, were also found to accumulate significant quantities (up to 209 μg g^{-1}) of Cd. The addition of chelating agents (0.5 and 2 g kg^{-1} EDTA, 0.5 g kg^{-1} DTPA and 0.5 g kg^{-1}NTA) to poplar (Kawa) clones caused a temporary increase in uptake of Cd. However, two of the chelating agents (2 g kg^{-1} EDTA and 0.5 g kg^{-1} NTA) also resulted in a significant reduction in growth, as well as abscission of leaves. If the results obtained in these pot experiments can be realised in the field, then a single crop of willows could remove over 100 years worth of fertiliser-induced Cd contamination from pasturelands.

Introduction

Elevated Cd concentrations in soils are of concern because of their risks to human health and the productivity of plants and animals (Underwood and Suttle, 1999). They could even be used as a non-tariff trade barrier to exports. Many years of fertilisation with Cd-rich superphosphate has increased concentrations of Cd in New Zealand soils, in some cases to levels above a self-imposed limit of 3 μg g^{-1} in dry soil (Zanders, 1998).

Conventional treatments for Cd-contaminated soils fall into three general categories: Isolation, cleansing and 'inerting'. Isolation may involve removal of the topsoil, covering with concrete or non-contaminated soil, or hydraulic isolation from surrounding areas. Cleansing involves the leaching of pollutants with acids. 'Inerting' is the addition of other chemicals to the soil that render the pollutants into a non-toxic form. Conventional procedures cost between US$ 100 000 and 1 000 000 per hectare (Russel et al., 1991). Furthermore, these methods may leave the soil infertile, cause further pollution by leaching or only be a temporary solution.

Chaney (1983) suggested that some heavy-metal contaminated soils may be cleaned up by growing a crop of plants which accumulate the pollutants, then harvesting the plants and disposing of them in a 'safe area'. This process was termed *phytoremediation.* The first feasibility experiments using 'hyperaccumulator' plants (see below) were conducted by McGrath et al. (1993), although Huiyi et al. (1991) had already shown that some forest species, including poplar, could be used to remove Cd from polluted soils. The cost of these operations is estimated to be US$ 60 000 to 100 000 per hectare (Salt et al., 1995) considerably

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less than the cost of conventional operations. Unlike some conventional operations the solution is permanent, there is a low risk of pollution due to leaching, and the soil is left fertile.

Plants used for phytoremediation should be fast-growing, deep-rooted (in the case of immobile contaminants at depth), easily propagated and accumulate the target metal. Accumulation of the target metal is of paramount importance, ideally the species used should have a high *bioaccumulation coefficient* which is defined as the plant/soil metal concentration quotient. Poplars and willows exhibit the first three of the aforementioned properties making them good candidates for phytoremediation, providing they have a high Cd bioaccumulation coefficient. Plant-metal uptake is usually a function of the 'bioavailability' of the metal in the soil, the transpiration rate of the plant and the selective uptake of that metal by the plant (Chardonnens et al., 1998). Certain plants naturally accumulate inordinately high concentrations of heavy metals in their aerial portions. Such plants, termed 'hyperaccumulators' (see Brooks, 1998) are able to sequester heavy metals in the rhizosphere, then transport them and store them in the above ground portions. Poplars and willows have not been reported to exhibit this phenomenon, however, their water use is high relative to other plants so their relative uptake of Cd may also be high (Hinchman et al., 1996). Göransson and Philippot (1994) and Landberg and Greger (1994) reported high Cd accumulation by willow and suggested they could be used for Cd phytoextraction. Greger et al. (1997) discussed the potential of willows for the decontamination of Cd-contaminated soils, reporting that 12 years of growth would be required to remove the Cd accumulated in Swedish soils during the last century. Felix (1997) calculated that a hybrid willow could remove 0.222 kg of Cd per hectare per annum from a soil containing 6.6 μg g^{-1} Cd. It would take 77 years to reduce the soil Cd burden to 0.8 μg g^{-1}.

Huang and Cunningham (1996) reported that the addition of chelates such as EDTA to Pb-contaminated soils greatly increased plant-uptake. In this way, high-biomass non-hyperaccumulator plants such as *Zea mays* could be used to remove Pb from contaminated soils. The effect of chelating agents on plant-Cd uptake is unknown. Any chelating agent used in a phytoremediation operation should be cheap and, most importantly, have a low environmental impact. It is imperative that the chelating treatments do not lead to leaching of the mobilised heavy metal. Means et al. (1980) reported that the degradation rates of the chelating agents used in this study are 10–40 days, however, more recent studies (Hong et al., 1999) indicate that EDTA can persist for extended periods in soils. The addition of EDTA may also lead to the loss (either by leaching or plant accumulation) of essential plant micro-nutrients such as Cu and Zn.

The aim of this study was to determine the tolerance and accumulation of Cd by poplar and willow clones grown in soil containing a range of Cd concentrations and investigate the effect of chelating agent addition on Cd uptake. We also sought to investigate the accumulation of Cd by poplars growing *in situ* in contaminated soil. These results would thus establish the potential of poplar and willow for the phytoremediation of Cd-contaminated soils.

Materials and methods

Shade house experiments using potted trees

Shade house experiments were performed at Hort-Research, Palmerston North, New Zealand (latitude 40.2 ° S, longitude 175.4 ° E). Average monthly vapour pressure deficits (in Pascals) were: Oct. 408, Nov. 503, Dec. 638, Jan. 857, Feb. 907. Poplar and willow clones were grown in 20 l buckets with varying Cd additions as described in Table 1. The soil used was Manawatu silt loam which has a Kd value for Cd of 640 (soil/solution cadmium concentration quotient) over the concentration ranges used. The pH of the soil was 5.7, total organic carbon 6.3% and an exchange capacity of 13.4 cmol(+) kg^{-1} Soils were prepared by adding varying amounts of a 1% Cd solution to 20kg of soil and mixed using a concrete mixer. The Cd was added as the nitrate.

The varieties of poplar used were Kawa (*Populus deltoides* × *P. yunnanensis* NZ 5006) and Argyle (*P. deltoides* × *P. nigra* NZ 5015). The willow variety was Tangoio (*Salix matsudana* × *S. alba* NZ 1040). Kawa was chosen for the soil-amendment experiment because this variety has one of the highest rates of water use amongst poplars. Soils were fertilised with an Osmocote^{TM} slow release fertiliser at a rate recommended by the manufacturer. Planting occurred at the end of September, 1998, some 2 months after preparation of the pots. This delay was to allow time for the added Cd to come to equilibrium with the soil. Trees were grown from c.a. 0.2 m cuttings. Buckets were arranged in a randomised block design within the shade house. The chelating agents NTA (nitrilotriacetic acid)

Table 1. Treatments used in experiments

Number of buckets	Soil Cd concentration (μg g^{-1}) dry weight
12 (4 replicates of Kawa, Argyle, and Tangoio)	0.6
8 (4 replicates of Kawa and Tangoio)	1.6
12 (4 replicates of Kawa, Argyle, and Tangoio)	5.6
4 Kawa +0.5 g kg^{-1} NTA (added 19/1/1999)	5.6
4 Kawa +0.5 g kg^{-1} DTPA (added 19/1/1999)	5.6
4 Kawa +0.5 g kg^{-1} EDTA (added 19/1/1999)	5.6
4 Kawa +2 g kg^{-1} EDTA (added 19/1/1999)	5.6
12 (4 replicates of Kawa, Argyle, and Tangoio)	20.6
8 (4 replicates of Kawa and Tangoio)	60.6

EDTA (ethylenediaminetetraacetic acid), and DTPA (diethylenetriaminepentaacetic acid) were added on the 19/1/1999. Leaf samples (first mature leaf from apical bud) were taken on the 12/12/98, 26/1/99 and 3/2/99. Periodic measurements of transpiration rates were made using porometery (LiCor LI1600 steady state porometer) to indicate the water use of the trees. Plants were harvested on 5/3/99.

Collection of poplar samples from Auby, Northern France

The accumulation of Cd *in situ* was investigated by taking leaf, stem and root samples as well as root zone soil samples from poplar trees (Beaupré: *P. tricocapa* × *P. deltoides*) growing near the town of Auby, Northern France. The site was chosen because the levels of Cd pollution vary from relatively unpolluted ($<$1 μg g^{-1}) to highly polluted ($>$300 μg g^{-1}). This is due to the presence of a nearby base metal smelter. Beaupré has been used in many revegetation and beautification projects in the area.

Sample preparation and Cd determination

Leaves, stems and roots were separated and placed in a drying cabinet at 80 °C until a constant weight was reached. Samples were weighed, and ground. Approximately 0.2 g of material from each sample was accurately weighed into 50 mL Erlenmeyer flasks. Concentrated nitric acid (10 mL) was added to each tube and the mixtures heated on a heating block until a final volume of ca. 3 mL was reached. The samples were then diluted to 10 mL using deionized water and stored in polythene containers.

Table 2. Dry biomass (g) per plant (average of 4 replicates) grown in soils with varying Cd concentrations

Soil Cd concentration (μg g^{-1} dry weight)	Kawa	Argyle	Tangoio
0.6	205	179	478
1.6	228		n.d.
5.6	207	165	379
20.6	158*	161	n.d.
60.6	111*		315

*Significantly different (p=0.05) to 0.6 μg g^{-1} plant. n.d. = not determined.

Soil samples were dried at 80 °C and sieved to $<$1 mm size using a nylon sieve. About 0.2 g quantities of sieved soil were accurately weighed into boiling tubes. Ten mL of concentrated nitric acid was then added and the mixtures boiled until a final volume of 3 mL was reached. A further 10 mL of concentrated hydrochloric acid was then added and the mixtures again evaporated to 3 mL. After filtration, the solutions were diluted to 10 mL with deionized water.

Chemical analyses on the plant and soil solutions were performed using a GBC 904 atomic absorption spectrometer. Differences between means were determined using ANOVA on Microsoft Excel.

Results and discussion

Effect of Cd concentration on plant growth

Table 2 shows the biomass production of the clones for each Cd concentration. Growth of Kawa was significantly reduced in the two highest Cd concentrations. Argyle and Tangoio also showed growth reduction at 20.6 μg g^{-1} and above, but these decreases were not significant. The visible effects of high soil Cd concentrations were shortened shoots, increased branching, and chlorosis of the leaves. Growth was unaffected at levels below 5.6 μg g^{-1}, the range where the plants may be used for phytoremediation.

The accumulation of Cd by poplar and willow

Shade house experiments

Figure 1 shows the Cd accumulation of all trees tested. The level of Cd in the plants always exceeded the respective soil levels. There was a general decrease in the bioaccumulation coefficient with increasing soil

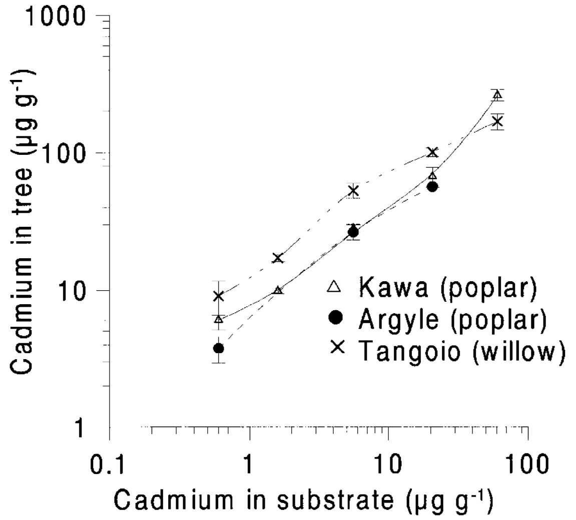

Figure 1. Above-ground Cd concentrations (dry stems and leaves) of poplar and willow clones as a function of the Cd concentration in the substrate. Vertical bars indicate standard error of the mean.

Cd concentrations. There was no significant difference between the varieties of poplar (at the p=0.05 level), but the willow clone accumulated significantly more (p<0.01) Cd than poplars in all, but the highest soil Cd-concentration. The anomalous high accumulation of Cd by Kawa clones grown in soil containing 60.6 μg g^{-1} Cd may be explained by a lower 'dilution by growth' due to the significantly reduced growth at this soil concentration (Table 2). The higher bioaccumulation factor of willows make them more suitable than poplars for Cd phytoremediation in high-rainfall areas.

Leaves contained on average 1.6 times the Cd concentration of the stems. Leaves were typically 49% of the total above-ground biomass of the plant. Root Cd concentrations were highly variable and their measurement was unreliable due to soil contamination. On average they were 0.9 times the concentration of the shoots, in contrast to the findings of Dickinson et al. (1994) who reported that most Cd accumulated by willows grown in solution culture was in the roots. The biomass of the roots was not determined.

Leaf Cd-concentrations increased over the course of the experiment. Table 3 shows the concentration of Cd in Kawa leaves on four different occasions. If Cd uptake is a function of plant water use, then the leaf-Cd increase over time may be explained by cumulative water use.

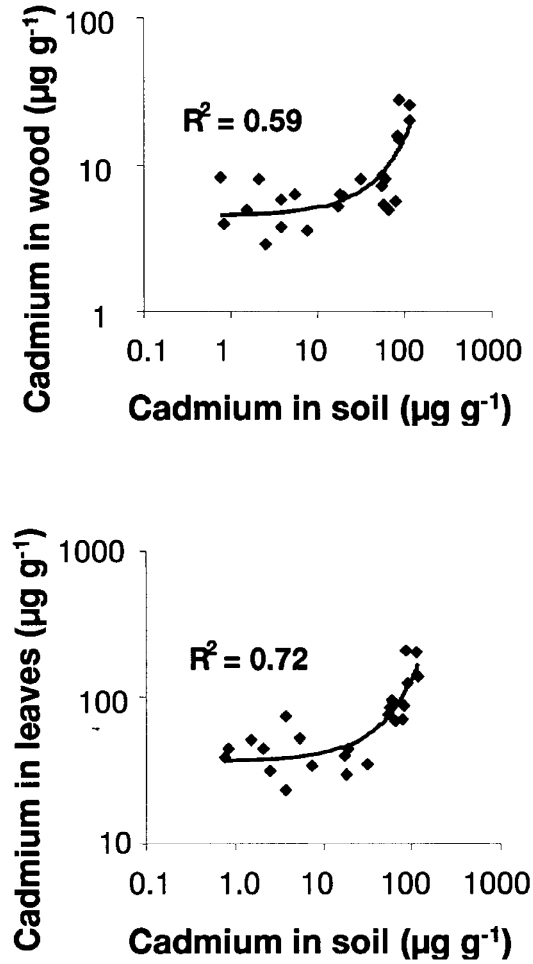

Figure 2. Cadmium accumulation by poplar (var. Beaupré) from Auby, Northern France, as a function of the soil Cd concentration.

Poplars growing in situ on contaminated soils

Figure 2 (A, B) show the Cd concentrations in Beaupré clones growing around the town of Auby, Northern France. In soils that were heavily polluted with Cd, leaf Cd concentrations reached values in excess of 200 μg g^{-1} (Figure 2B). These results indicate that poplar growing *in situ* will accumulate Cd to a similar degree to plants grown in our controlled studies. It should be noted, however, that within the window of 0.6–60.6 μg g^{-1} Cd in the soil (the range used in the pot trials), there was no significant increase in plant Cd. Smelter emissions were stopped in 1997, so it is unlikely that the high Cd values reported here are the result of air-borne contamination. Most soil Cd was deposited as the insoluble oxide in the top ten centimetres of soil (Deram, 1999). The high accumulation of Cd by poplars growing in this soil may be the result

Table 3. Cd concentrations in dry poplar (Kawa) leaves growing in soil containing 5.6 μg g^{-1} Cd. The treatments were added on the 19th of January 1999

	20th Dec 1998	26th Jan 1999	3rd Feb 1999	5th Mar 1999
No treatment	12	31	34	39
0.5 g kg^{-1} EDTA	–	34	61*	41
2 g kg^{-1} EDTA	–	52*	62*	39
0.5 g kg^{-1} DTPA	–	46	59*	31
0.5 g kg^{-1} NTA	–	54*	62*	28

*Significantly different (p=0.05) to controls.

of the CdO reacting with the soil to render the Cd in a more plant-available form.

The effect of chelating agents on Cd accumulation

Table 3 shows leaf concentrations of Cd before and 1, 2 and 6 weeks after the addition of chelating agents to the soil. Two weeks after the addition of the chelating agents, all treatments had significantly higher leaf Cd concentrations than the control. At the end of the experiment, however, there was no significant difference between the controls and any of the treatments, both for leaves and stems. Treatments with 2 g kg^{-1} EDTA and 0.5 g kg^{-1} NTA caused necrosis and abscission of most of the leaves by 1 week after treatment. The plants slowly recovered and had significantly lower biomass production at the end of the experiment relative to the controls. Plants treated with 0.5 g kg^{-1} EDTA showed reduced growth and leaf discoloration. There was no reduction in growth for the 0.5 g kg^{-1} DTPA treatment. Porometory measurements showed a reduction in the stomatal conductance of the DTPA and 0.5 g kg^{-1} EDTA treatments, indicating reduced plant water-use due to a loss of leaf functioning. This may have adversely affected metal uptake over time.

These results indicate that if chelating agents are to be used to enhance plant Cd uptake in a phytoremediation operation, then the plants should be harvested shortly after their addition to take immediate advantage of the surge in uptake. The 0.5 g kg^{-1} NTA and 2 g kg^{-1} EDTA treatments should not be used as they caused leaf abscission.

The potential of poplar and willow for phytoremediation

Results indicate that poplar and willows have excellent potential for Cd phytoremediation because of their high biomass production and high Cd bioaccumulation coefficient. If the plant-uptake in field conditions is similar to that which has been achieved in these experiments then poplars could be used to decontaminate soils weakly contaminated with Cd. The use of poplar or willow for phytoremediation would require annual harvesting to avoid the recycling of leaf-bound Cd in autumn. Both poplars and willows coppice readily.

An annual application of superphosphate fertiliser at a rate of 300 kg ha^{-1} adds about 10g of Cd per hectare (Loganathan and Hedley, 1997). On a soil containing 5 μg g^{-1} Cd, poplars harvested shortly after a pulse of EDTA, or willows, would have a Cd concentration in the dry above-ground biomass of 53 μg g^{-1}. The biomass production of poplars and willows under optimal conditions is 30 tonnes per hectare per annum. If we adopt a conservative approach and use a figure of 20 t ha^{-1} per year, a single crop would remove 1.06 kg of Cd per hectare. This equates to 106 years of fertiliser addition. A soil containing 5 μg g^{-1} Cd in the top 100 mm of soil contains 6 kg of Cd per hectare, assuming a bulk density of 1.2. Four crops of willows or treated poplars would reduce the soil Cd burden to 2.35 μg g^{-1}, well below the threshold of 3 μg g^{-1}. These calculations make the assumption that the results from the pot trials can be realised under field conditions.

Conclusions

Cadmium levels in poplars growing *in situ* on contaminated soils indicate that bioaccumulation factors under field conditions are the same, or better, than those obtained in this shade-house study. To verify the link between pots and the field, future work will involve small-scale plot trials and different regimes of chelating agent addition to maximise Cd uptake. Poplars and willows have the potential to provide a cheap method of cleaning up Cd-contaminated soils in New Zealand.

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