Interactive effects of salinity and lime on the phytoextraction of cadmium by S*alix* species in biosolids

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Abstract
A glasshouse experiment was set to examine the effect of lime amendments and increasing salinity on the phytoextraction of Cd by three *Salix* species grown in biosolids. Liming greatly enhanced the Cd phytoextraction by *Salix* crop by up to 12 times, via increasing shoot biomass, Cd uptake and translocation. Increases in salinity (NaCl) further enhanced Cd uptake, however salt stress dramatically reduced plant growth. In biosolids of low salinity *S. reichardtii* had the highest rate of Cd extraction, whereas in moderately saline biosolids *S. viminalis* had the highest biomass and Cd accumulation. This study revealed that lime addition to saline biosolids is an effective method of increasing Cd phytoextraction and reducing salt stress, thus allowing irrigation of phytoextraction crops with saline water to be a feasible option.

Key Words
Phytoremediation, salty water irrigation, species variation, willow.

Introduction
Fresh water is limited in many areas of Australia and much of the water available for the irrigation of phytoremediation crops is burdened with dissolved salts. Hence, to phytoextract metals from many areas, and from substrates that are naturally saline, such as biosolids, plants must not only accumulate high concentrations of metals but also be able to tolerate increasingly saline conditions. Currently, the potential of short rotation *Salix* stands for the phytoextraction of Cd is being investigated at Werribee Treatment Plant (Laidlaw *et al.* 2007). It is suggested that saline lagoon water is used for the irrigation of *Salix* plantations. However, the effects of introducing additional salts to the already moderately saline biosolids (EC 2.6 dS/m) on: (i) Cd mobility; (ii) plant growth and (iii) the overall phytoextraction of Cd are unknown. As biosolids at the Western Treatment Plant are acidic (pH =4.6, 1:5 H_2O) liming may heighten the growth of *Salix* crops and thus enhance phytoremediation practices. The combined effects of salinity and lime on the phytoremediation of Cd from biosolids requires further research and hold potential for enhanced phytoextraction efficiency. The aim of this study was to determine the effects of increasing concentrations of salts (NaCl) and lime on Cd bioavailability and phytoextraction by three *Salix* species (*Salix viminalis, Salix reichardtii* and *Salix purpurea).*

Materials and methods
A glasshouse experiment was set up which consisted of two levels of lime (0 and 8 g $CaCO_3$/kg dry biosolids) applications, three S*alix* species and five salinity levels with three replicates. The salt application rates were 0, 1.5, 4, 8 and 11 g NaCl/kg biosolids. The three species of *Salix* used were: 1) *Salix purpurea*; 2) *Salix reichardtii*, which is a hybrid taxon of *Salix caprea* × S*alix cinera* and has been found to be most successful at extracting Cd from biosolids (Laidlaw *et al.* 2007); and 3) *Salix viminalis*, which is well known for its high biomass production, but is less tolerant to heavy metals than other *Salix* species (Punshon and Dickinson 1997). Stem cuttings (10 cm length) of *Salix* species were collected from 3-year plantations. Prior to planting, the cuttings were chilled (4^{o}C) for three weeks to stimulate growth, as winter approached. All cuttings were dipped in rooting hormone and grown in a heated sandbed with a mist watering system in a glasshouse (25^{o}C). After 30 days, uniform cuttings were transferred to lined 3-L pots containing 1.8 kg of biosolids. Lime treatments were added in powder form to the biosolids, well mixed and then incubated for 14 days prior to planting. Basal nutrients were added at the time of planting (5 g Grocote fertilizer/kg biosolid). After allowing the cuttings to stabilize in the biosolids for 16 days, salt treatments were added in 4 parts over 14 days. Pots were watered with tap water to field capacity every second day.

Plant shoots and roots were harvested 70 days after transplanting into the biosolids. Roots were rinsed four times in distilled water and then soaked in ice cold 5 mM $PbNO_3$ (1h) to desorb metals adhering to the root apoplasts (Assunçao *et al.* 2001). The dry weights of shoots and roots were recorded before grinding the samples. Cadmium concentrations in plant tissues were determined by atomic absorption spectroscopy after plant material (0.1 g) was digested in a 1:4 mixture of nitric: perchloric acids using Tecator DS40 Digestion systems. The translocation percentage was used to indicate the ability of *Salix* plants to transport Cd from the roots to the shoots and was calculated by dividing the total metal content in shoots by the total metal content in roots (Ghnaya *et al.* 2007).

Results

Plant growth

The growth rate of all *Salix* species was low. By Day 10 after salt addition, new growth of all plants grown with high salt levels (8 to 11 g NaCl/kg) showed signs of chlorosis, particularly *S. viminalis.* By Day 21 after salt addition, the leaves of many of the plants, particularly those grown in non-limed biosolids, began to senesce. Liming increased the shoot and root dry mass of *Salix* species by up to 4 fold (Figure 1). Conversely, increasing salinity significantly decreased plant growth, most markedly when salt additions exceeded 1.5 g NaCl/kg. Shoot and root dry weights differed significantly between *Salix* species, in varying salt and lime environments. *Salix purpurea* had the greatest shoot biomass in biosolids with low salt additions (compared other *Salix* species), whilst *S. viminalis* had the greatest biomass in the most saline biosolids.

The pH and EC of bulk and rhizosphere biosolids

Salt treatments increased the bulk EC from 1.45 to 3.58 dS/m in the non-limed biosolids and from 1.55-4.72 dS/m in the limed biosolids. The EC was highly variable between replicates, particularly in the rhizosphere, and did not significantly differ between species. The addition of lime to the biosolids increased the pH from 4.5 to 6.2 in the bulk and rhizosphere biosolids and did not significantly differ between *Salix* species. Salt treatments did not significantly influence the bulk soil or rhizosphere pH.

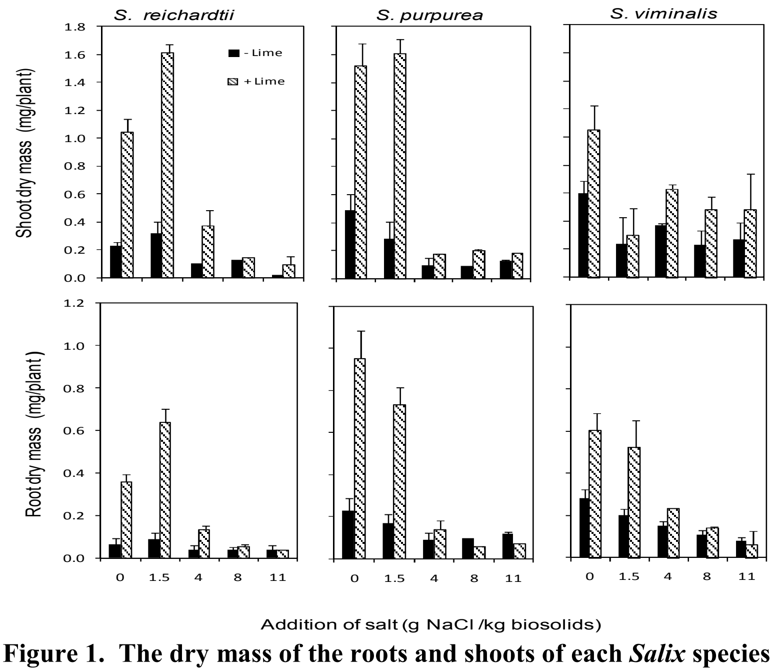

Figure 1. The dry mass of the roots and shoots of each *Salix* species after 70 days growth in biosolids with various salt treatments with (+ lime, 8 g/kg) and without lime (-lime, 0 g/kg). The bars indicate the standard error of means of 3 replicates.

Cadmium concentration and content in Salix shoots and roots

Liming significantly elevated Cd concentrations in the shoots of all *Salix* species (Figure 2). In particular, liming increased Cd uptake in *S. reichardtii* by 12 times at 1.5 g NaCl/kg. However, liming did not consistently increase Cd concentrations in the plant roots. The highest concentrations of Cd in shoots were found in *Salix* grown on limed biosolids with 4 g/kg salt treatments. However, total Cd uptake in the plant shoots was greatest for biosolids with 1.5 g/kg salt addition, due to greater plant growth. Overall Cd extraction in biosolids with low salt additions was revealed with the following decreasing order: *S. reichardtii* > *S. purpurea* > *S. viminalis*. However, *S. viminalis* had the greatest total Cd uptake in the moderate saline environments.

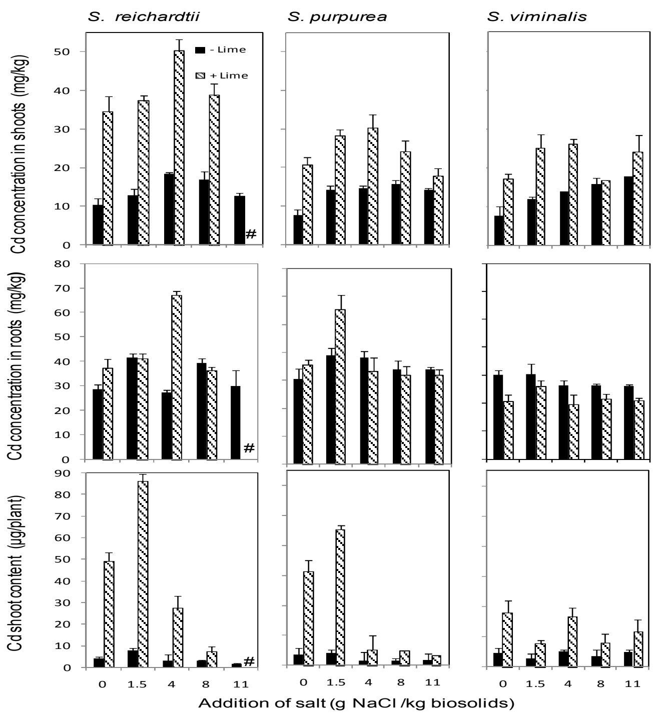

Figure 2. Concentration of Cd in the shoots and roots and total Cd content in the shoots of each *Salix* species after 70 days growth in biosolids with various salt treatments with (+ lime 8 g/kg) and without lime (-lime 0 g/ kg). The bars indicate the standard error of means of 3 replicates. Missing data are indicated by "#".

Cadmium translocation

Salix grown on limed biosolids had significantly greater translocation of Cd from the roots to the shoots, as shown in Figure 3. The translocation rates of Cd ranged from 29 to 135%. *Salix viminalis* had significantly greater translocation of Cd compared to the other *Salix* species, particularly in limed biosolids with high salt levels. Both salt and lime additions were shown to increase Cd translocation.

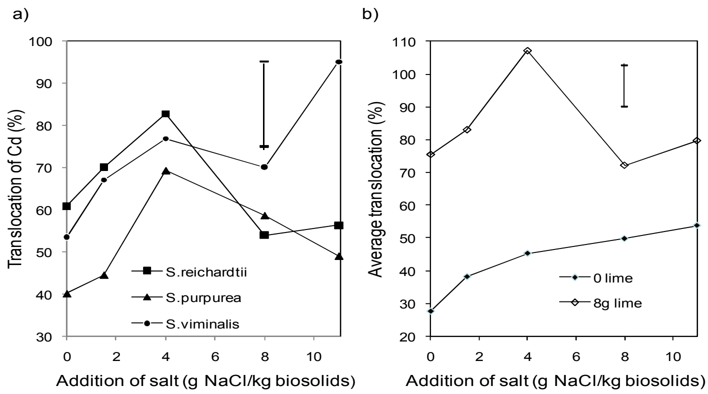

Figure 3. a) Average translocation of Cd in *S. reichardtti*, *S. purpurea* and *S. viminalis* with increasing salt additions. b) Average translocation of Cd from the roots to the shoots of *Salix* plants when grown with lime (lime 8 g lime) and without lime (0 lime), with increasing salt additions. The bars represent the L.S.D of the means.

Discussion

Salix species varied in their capacity to tolerate increasing salinity and accumulate Cd. *Salix viminalis* had the greatest shoot biomass at high salt levels. Correspondingly, *S .viminalis* had the greatest Cd accumulation in the most saline biosolids. *Salix reichardtii* had the highest concentrations of Cd when grown in biosolids with low salt additions. *Salix viminalis* showed greater selectivity for K accumulation than other *Salix* species and consequently had the lowest Na/K concentration ratios in its shoots (Dannatt, unpublished), indicating its tolerance to salinity (Schachtman and Liu 1999).

Liming significantly reduced salt stress in all *Salix* species. Lime reduced Na concentrations in plant shoots by more than 50 % (unpublished). This was most likely due to increased Ca availability in the root zone of limed plants. In addition, lime may have alleviated salt stress indirectly by amelioration of biosolid acidity and increased nutrient availability. The alleviation of simultaneous environmental stresses, such as acidity, metal toxicity and salinity by lime remain largely unknown.

Liming enhanced Cd uptake in *Salix* but did not significantly decrease the DTPA-extractable Cd in the biosolids (Dannatt unpublished). These results are contradictory to the results of most studies, which suggest that liming decreases Cd bioavailability and thus plant uptake (Singh *et al.* 1995). There are several mechanisms that may have caused the observed increases in Cd concentrations found in limed *Salix*: (i) liming ameliorated biosolid acidity and hence increased shoot biomass and root function; (ii) liming reduced competition of other metal elements for plant uptake; (iii) addition of Ca increased Cd bioavailability and/or (iv) liming increased translocation of Cd due to increased transpiration and improved root function.

Increasing salinity increased Cd accumulation in *Salix* shoots. It is likely that increases in salinity elevated Cd translocation due to the formation of chloro-complexes with Cd, resulting in heightened Cd solubility, increased uptake of Cd in the form of chloro-complexes and/or increased transport of Cd within the root apoplasts, in the presence of Cl (Helal *et al.* 1999). In addition, lime increased plant vitality and transpiration, augmenting Cd translocation. Further research on the influence of Cl on Cd translocation is required, and holds potential for increasing the efficiency of Cd phytoremediation methods.

Conclusion

Liming has great potential at increasing the efficiency of Cd phytoextraction by *Salix* crops. Amendment of biosolids with lime, at a rate of 8 g/kg, increased *Salix* growth by 4 fold and increased total Cd content by up to 12 times. Liming also increased Cd translocation from plant roots to shoots, probably due to increased transpiration. The use of saline water to irrigate phytoextraction crops can further increase Cd phytoextraction as a result of increased Cd bioavailability and transport within plant tissues. However, *Salix* species have limited tolerance to salinity. Further research is required to elucidate the influence of lime on *Salix* growth in field conditions and to determine if phytoextraction of other contaminants such as Zn and Cu is also enhanced in these environments.

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