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Evaluation of **Populus** and **Salix** Continuously Irrigated with Landfill Leachate I. Genotype-Specific Elemental Phytoremediation

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EVALUATION OF *POPULUS* AND *SALIX* CONTINUOUSLY IRRIGATED WITH LANDFILL LEACHATE I. GENOTYPE-SPECIFIC ELEMENTAL PHYTOREMEDIATION

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There is a need for the identification and selection of specific tree genotypes that can sequester elements from contaminated soils, with elevated rates of uptake. We irrigated* Populus *(DN17, DN182, DN34, NM2, NM6) and* Salix *(94003, 94012, S287, S566, SX61) genotypes planted in large soil-filled containers with landfill leachate or municipal water and tested for differences in inorganic element concentrations (P, K, Ca, Mg, S, Zn, B, Mn, Fe, Cu, Al, Na, and Cl) in the leaves, stems, and roots. Trees were irrigated with leachate or water during the final 12 wk of the 18-wk study. Genotype-specific uptake existed. For genera, tissue concentrations exhibited four responses. First,* Populus *had the greatest uptake of P, K, S, Cu, and Cl. Second,* Salix *exhibited the greatest uptake of Zn, B, Fe, and Al. Third,* Salix *had greater concentrations of Ca and Mg in leaves, while* Populus *had greater concentrations in stems and roots. Fourth,* Populus *had greater concentrations of Mn and Na in leaves and stems, while* Salix *had greater concentrations in roots.* Populus deltoides × P. nigra *clones exhibited better overall phytoremediation than the* P. nigra × P. maximowiczii *genotypes tested. Phytoremediation for* S. purpurea *clones 94003 and 94012 was generally less than for other* Salix *genotypes. Overall, concentrations of elements in the leaves, stems, and roots corroborated those in the plant–sciences literature. Uptake was dependent upon the specific genotype for most elements. Our results corroborated the need for further testing and selecting of specific clones for various phytoremediation needs, while providing a baseline for future researchers developing additional studies and resource managers conducting on-site remediation.

KEY WORDS remediation, uptake, poplar, willow, heavy metals, salts, nutrients

INTRODUCTION

Phytoremediation is defined as the utilization of plants alone or the symbiotic relationship between plants and their rhizospheric microorganisms to destroy, remove, and stabilize contaminated soils *in situ* (Cunningham and Ow, 1996; Cunningham *et al.*, 1997; McIntyre and Lewis, 1997). Such plant-enhanced bioremediation is a potentially sustainable system for the remediation of landfills and similarly-contaminated sites (Gatliff, 1994). Species and hybrids belonging to the genera *Populus* (poplar) and *Salix* (willow)

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are currently used for phytoremediation of a variety of contaminants (Isebrands and Karnosky, 2001). Selected genotypes are ideal for phytoremediation due to their fast growth (Dickmann and Stuart, 1983; Zalesny *et al*., 2005c), elevated water usage (Vose *et al*., 2000; Zalesny *et al*., 2006), and extensive root systems (McLinn, Vondracek, and Aitchison, 2001; Zalesny, Riemenschneider, and Hall, 2005a). In addition, *Populus* and *Salix* genotypes exhibit broad genetic diversity and relatively successful interspecific hybridization (Aravanopoulos, Kim, and Zsuffa, 1999; Eckenwalder, 1984), which supports greater gains from selection than with other tree species (Dickmann, 2001; Orlovic *et al*., 1998). Vegetative propagation allows for the continuous availability of favorable genotypes once such genetic stock are identified and selected (Stanturf *et al*., 2001; Volk *et al*., 2004).

The design, implementation, and monitoring of vegetative caps for evapotranspiration and phytoremediation covers using *Populus* and *Salix* can be an effective technology from environmental and economical standpoints (Ensley, 2000; Glass, 1999; Isebrands and Karnosky, 2001). Likewise, these genera have been used for hydraulic control to mitigate the migration of contaminants (Ferro *et al*., 2001; Vose *et al*., 2000; Zalesny *et al*., 2006) and for riparian buffer strips because they are able to remediate surface and subsurface contaminants (McLinn *et al*., 2001; Perttu, 1993; Perttu and Kowalik, 1997). The efficacy of trees generally and *Populus*/*Salix* specifically for phytoremediation is verified because they extract and capture (phytoextraction, rhizofiltration), extract and release (phytovolatilization), degrade (phytodegradation, rhizodegradation), or confine (phytostabilization, phytoaccumulation) the contaminants (Bañuelos *et al*., 1999; Sander and Ericsson, 1998; Schnoor *et al*., 1995). Although organic compounds can be degraded/mineralized, inorganic contaminants must be removed or converted into inert forms (Cunningham and Ow, 1996). Thus, there is a need to identify trees that can sequester elements from contaminated soils, with elevated rates of uptake.

Information about specific phytoremediation capabilities of current *Populus* and *Salix* genotypes is important because there is a need for environmentally sound, sustainable, cost-effective strategies for the remediation of contaminated sites. Consequently, we seek knowledge about inorganic element concentrations in the leaves, stems, and roots of the trees. Such information will provide a baseline for future researchers in developing additional studies, along with helping resource managers to remediate their sites more cheaply than with traditional technologies. To address these issues, our overall objective was to identify specific genotypes that can be used for the phytoremediation of elements found in landfill leachate. The specific objective of the current study was to irrigate *Populus* and *Salix* genotypes with landfill leachate or municipal water and to test for differences in element concentrations within the leaves, stems, and roots. Our initial hypothesis was that elemental concentrations in the plant tissues would be genotype-specific, following treatment application (*i.e*., genera and clones-within-genera would respond differently to treatments). Given a lack of knowledge about such genotype-specificity, we believe that this information is necessary because proper genotype selection is required for success of every long-term phytoremediation system.

MATERIALS AND METHODS

Clone Selection

Five poplar (*Populus* spp.) clones and five willow (*Salix* spp.) clones were selected from two and four genomic groups, respectively, during spring of 1999. The genera, genomic

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groups, and clones were selected based on their current utilization and growth in the North Central United States, along with their phytoremediation potential. The *Populus* clones and their genomic groups were DN34, DN17, DN182 (*P. deltoides* Bartr. ex Marsh × *P. nigra* L.) and NM2, NM6 (*P. nigra* × *P. maximowiczii* A. Henry). The *Salix* clones and their genomic groups were 94003, 94012 (*S. purpurea* L.), S287 (*S. eriocephala* Michx.), S566 (*S. eriocephala* 28 × *S. eriocephala* 24), and SX61 (*S. sachalinensis* F. Schmidt). Dormant, unrooted cuttings of *Populus*, 25.4 cm long, were processed from whips grown for one growing season in stool beds established at Hugo Sauer Nursery in Rhinelander, WI, USA (45.6°N, 89.4°W). Dormant, unrooted cuttings of *Salix*, 25.4 cm long, were obtained from the State University of New York (Syracuse, NY, USA; 43.0°N, 76.2°W). For all planting stock, cuts were made to position at least one primary bud no more than 2.54 cm from the top of each cutting.

Tree Establishment

Trees were established outdoors and *ex situ* in Rhinelander in commercially-available cattle watering tanks that were constructed of black polyethylene. The 600-L tanks were tapped and fitted with a shutoff valve at the base, then placed on concrete blocks. A 5 × 5 cm piece of lumber measuring the length of the tank was placed on the blocks to provide a 4° slope toward the lower end to facilitate drainage of applied water into a collection system. The following three soil layers were used in the tanks: 1) 30 cm of topsoil obtained from the same source as the cover of the landfill; 2) middle layer composed of 30 cm of sand obtained from the same source as the sub-base of the landfill; and 3) 20 cm of washed 2- to 5-cm gravel as a bottom drainage layer. The tanks were fitted with a sheet of heavy-duty flat gray plastic to minimize introduction of precipitation. Twenty small incisions were made in the plastic to facilitate planting.

All cuttings were soaked, at a room temperature of 21°C, in water to a depth of 17.8 cm for 3 d before planting on June 14, 1999. The cuttings were planted to a depth that allowed only the uppermost bud to remain above the plastic cover. The cuttings were planted in paired rows according to genus, with the center line of the tank used as a separator between *Populus* and *Salix* clones. Spacing between paired cuttings and between rows was 30 × 30 cm for all clones.

Experimental Design

The treatments, of equal volume, consisted of leachate (L) from the landfill (pH 6.3) and a control with application of water (W) from the Rhinelander municipal water supply (pH 8.4). Also tested were the aforementioned genera and clones. Three tanks each were used for the combinations of leachate with trees and municipal water with trees. The cuttings were arranged in a nested split-plot design, with two treatments, two genera, five clones per genus, and three replications (tanks) per treatment. Treatments were arranged randomly per tank, while genera were considered whole plots because each genus was randomly isolated to a side of each tank. Clones-nested-within-genera were considered subplots and were randomly planted in two-tree plots. Treatments, genera, and clones were treated as fixed in the analysis and, therefore, we evaluated means rather than variances.

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Treatment Application

All tanks were irrigated with municipal water every other day for the initial 6 wk following planting to ensure the survival and health of the trees and to keep soil moisture as uniform as possible (*i.e.*, at field capacity) across all tanks. The volume of irrigation varied to simulate trends in natural precipitation events for northern Wisconsin (Zalesny and Bauer). The irrigation regime changed as the trees developed over the remaining 12 wk of the study. Tanks received two 75.7-L applications during the first treatment week, two 37.9-L applications per week during treatment weeks 2 to 6, and two 56.8-L applications per week during treatment weeks 7 to 9. One application per week of 56.8, 37.9, and 18.9 L was applied during treatment week 10, 11, and 12, respectively. Treatment application was accomplished using a dual trickle irrigation system, one for the leachate and the other for the municipal water. The irrigation system was plumbed using standard 1.6-cm polyvinylchoride (PVC) pipe at the top of each tank. The pipe was split using *t*-couplings to allow attachment of two distribution hoses that were placed on top of the soil between rows of trees for each genus.

Sampling and Measurements

Leachate and Municipal Water. Leachate for chemical analysis was collected, filtered, and preserved in laboratory bottles. Nine sample collections were prepared during the growing season for testing the concentration of 13 elements: phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), zinc (Zn), boron (B), manganese (Mn), iron (Fe), copper (Cu), aluminum (Al), sodium (Na), and chloride (Cl). In addition, the municipal water was tested four times during the growing season. All samples were sent to Northern Lake Service, Inc. (Crandon, WI, USA) for analysis using approved United States Environmental Protection Agency methods. Results from testing of elements in the leachate and municipal water are shown in Table 1, along with the sum total amount of each element applied.

Elements in Plant Tissues. Near the end of the growing season, leaves of all clones were collected by attaching nylon netting to each associated tree shortly before the leaves abscised from the tree. Once all leaves had abscised, they were placed in paper bags, dried at 70 °C for 72 h, and weighed. During excavation at the Forestry Sciences Laboratory in December 1999, stem and root components were processed for each clone × treatment interaction. The tissues were placed in paper bags, dried at 70 °C for 72 h, and weighed. Leaf, stem, and root subsamples of all clones were sent to the University of Wisconsin Soil & Plant Analysis Laboratory (Madison, WI, USA) for element analysis by inductively coupled plasma optical emission spectrometry (ICP-OES).

Data Analysis

Plant-tissue-related element data were subjected to analyses of variance according to SAS® (PROC GLM; SAS Institute, Inc., 2004) assuming a nested split-plot design with fixed main effects for treatment, genus, and clone-nested-within-genus (Table 2). There were three replications (tanks) for each treatment. The following linear additive model was used in the analysis:

$$Y_{ijklm} = \mu + T_i + G_j + TG_{ij} + E_{(ij)k} + C_{(j)l} + TC_{i(j)l} + E_{(ijkl)m}$$

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Table 1 Concentration of elements in leachate (n = 9) and municipal water (n = 4) applied to *Populus* and *Salix* in an experiment testing for clone-specific phytoremediation. Total = sum total amount of each element applied. All pairwise comparisons were different ($P = 0.0122$)

	Leachate				Municipal water			
	Concentration (mg L^{-1})				Concentration (mg L^{-1})			
Element[a]	Min	Mean ± SE	Max	Total (g)	Min	Mean ± SE	Max	Total (g)
K	67.60	73.20 ± 1.17	77.90	72.09	1.30	1.30 ± 0.00	1.30	1.28
Ca	133.00	200.00 ± 9.59	221.00	196.96	12.00	12.25 ± 0.25	12.50	12.06
Mg	36.90	52.60 ± 2.13	57.90	51.80	4.78	5.23 ± 0.17	5.48	5.15
S	15.40	22.20 ± 2.84	42.40	21.86	5.99	8.39 ± 0.72	9.25	8.26
B	1.40	1.63 ± 0.04	1.75	1.61	0.01	0.02 ± 0.01	0.06	0.02
Mn	0.25	0.73 ± 0.06	0.93	0.72	0.02	0.03 ± 0.01	0.07	0.03
Fe	0.76	3.32 ± 1.51	14.50	3.27	0.02	0.02 ± 0.00	0.02	0.02
Na	131.00	142.00 ± 1.69	146.00	139.84	2.70	2.90 ± 0.08	3.00	2.86
Cl	93.50	99.30 ± 2.34	117.00	97.79	6.29	6.50 ± 0.31	7.65	6.40

[a]Concentrations of P, Zn, Cu, and Al were not available. Note: Min = minimum, Max = maximum, SE = standard error.

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Table 2 Degrees of freedom and expected mean squares from analyses of variance (ANOVA) in an experiment testing for clone-specific phytoremediation of *Populus* and *Salix*. The main effects were: treatment (leachate, municipal water), genus (*Populus*, *Salix*), and clone (five per genus, see Materials and Methods)

Source	df	Expected mean squares
Treatment	1	$\sigma^2 + 5\sigma^2{}_{TGA} + 30\Phi_T$
Genus	1	$\sigma^2 + 5\sigma^2{}_{TGA} + 30\Phi_G$
Treatment × Genus	1	$\sigma^2 + 5\sigma^2{}_{TGA} + 15\Phi_{TG}$
Tank (Treatment × Genus)	8	$\sigma^2 + 5\sigma^2{}_{TGA}$
Clone (Genus)	8	$\sigma^2 + 6\Phi_{GC}$
Treatment × Clone (Genus)	8	$\sigma^2 + 3\Phi_{TGC}$
Error	32	σ^2
Pooled Error	59	

where Y_{ijklm} = response variable to be analyzed, μ = overall mean, T_i = main effect of the *i*th treatment, G_j = main effect of the *j*th genus, TG_{ij} = effect of interaction between the *i*th treatment and the *j*th genus, $E_{(ij)k}$ = whole-plot error, NID (o,σ^2), $C_{(j)l}$ = main effect of the *l*th clone nested within the *j*th genus, $TC_{i(j)l}$ = effect of interaction between the *i*th treatment and the *l*th clone nested within the *j*th genus, and $E_{(ijkl)m}$ = sub-plot error, NID (o,σ^2).

Analyses of covariance were conducted to test for the effect of cutting dry mass on all tissue-related variables, and cutting dry mass was not a significant covariate for any variable ($P > 0.05$). *Populus* genomic groups were differentiated according to single degree-of-freedom linear contrasts ($\alpha = 0.05$), while paired *t*-tests were used to compare element concentrations in the leachate with the municipal water ($\alpha = 0.05$). Fisher's protected least significant difference (LSD) was used to compare means of main effects and interactions (Chew, 1976). The variation associated with means is ± one standard error throughout this article.

RESULTS

Elements in Plant Tissues

Leaves. The treatment main effect was significant for B, Mn, and Na concentration of the leaves, while genera differed for P, Ca, Mg, S, B, Mn, Cu, Al, Na, and Cl (Table 3). The interaction between treatment and genus governed the concentration of Na. The concentration of B in the leaves was 99.63 ± 2.53 mg kg^{-1} (leachate) and 66.90 ± 2.61 mg kg^{-1} (municipal water); while that for Mn was 186.79 ± 5.43 mg kg^{-1} (leachate) and 159.46 ± 5.59 mg kg^{-1} (municipal water). The concentration of elements in the leaves varied greatly between *Populus* and *Salix* (Table 4). *Populus* exhibited greater leaf concentrations of P, S, Mn, Cu, Na, and Cl, while *Salix* exhibited greater leaf concentrations of Ca, Mg, B, and Al. The Na concentration for treatment × genus interactions was 80.25 ± 24.33 mg kg^{-1} (leachate × *Populus*), 25.03 ± 24.33 mg kg^{-1} (leachate × *Salix*), 262.43 ± 24.33 mg kg^{-1} (water × *Populus*), and 34.26 ± 25.81 mg kg^{-1} (water × *Salix*) ($LSD_{0.05,8}$ = 53.92 mg Na kg^{-1}, n = 15).

The main effect of clone was significant for the leaf concentration of Ca, Mg, S, Zn, Mn, Cu, Na, and Cl (Table 3). The concentration of significant elements in the leaves varied greatly among clones (Figure 1). In general, *Salix* clones had greater Ca in the leaves

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Table 3 Probability values from analyses of variance (ANOVA) testing the effects of treatment (leachate, municipal water), genus (*Populus*, *Salix*), and clone (five per genus, see Materials and Methods) on element concentration of leaves, stems, and roots in an experiment testing for clone-specific phytoremediation of *Populus* and *Salix*.

Source	P	K	Ca	Mg	S	Zn	B	Mn	Fe	Cu	Al	Na	Cl
							Leaves						
Treatment	ns[a]	ns	ns	ns	ns	ns	0.0028	0.0435	ns	ns	ns	0.0005	ns
Genus	0.0066	ns	<0.0001	0.0158	0.0012	ns	0.0227	0.0055	ns	0.0228	0.0014	<0.0001	0.0344
Treatment × Genus	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.0009	ns
Clone (Genus)	ns	ns	<0.0001	<0.0001	<0.0001	0.0045	ns	<0.0001	ns	0.0004	ns	0.0181	<0.0001
Treatment × Clone (Genus)	ns	ns	ns	ns	ns	ns	ns	0.0486	ns	ns	ns	ns	Ns
							Stems						
Treatment	ns	ns	ns	0.0319	ns	ns	ns	0.0080	ns	ns	ns	<0.0001	ns
Genus	0.0001	<0.0001	0.0103	<0.0001	0.0033	ns	ns	0.0050	ns	ns	ns	<0.0001	0.0022
Treatment × Genus	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.0011	ns
Clone (Genus)	<0.0001	0.0069	<0.0001	<0.0001	<0.0001	<0.0001	0.0037	0.0020	0.0355	<0.0001	ns	<0.0001	ns
Treatment × Clone (Genus)	0.0178	ns	0.0439	0.0042	ns	ns	ns	ns	ns	0.0457	ns	0.0039	Ns
							Roots						
Treatment	ns	ns	ns	0.0427	ns	ns	ns	0.0017	ns	ns	ns	<0.0001	ns
Genus	<0.0001	<0.0001	<0.0001	0.0002	ns	0.0001	ns	0.0367	0.0246	ns	0.0181	0.0032	ns
Treatment × Genus	ns	ns	0.0086	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Clone (Genus)	0.0010	<0.0001	<0.0001	0.0005	<0.0001	<0.0001	ns	ns	ns	ns	ns	ns	ns
Treatment × Clone (Genus)	ns	ns	ns	ns	0.0305	ns	ns	ns	ns	ns	ns	ns	Ns

[a]Not significant at $\alpha = 0.05$.

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Table 4 Mean concentration (mg kg^{-1} ± standard error, n = 30) of elements in leaves, stems, and roots of trees used in an experiment testing clone-specific phytoremediation capabilities of *Populus* and *Salix*.

	Leaves		Stems		Roots	
Element	*Populus*	*Salix*	*Populus*	*Salix*	*Populus*	*Salix*
P	0.24 ± 0.01	0.11 ± 0.01	0.151 ± 0.003	0.099 ± 0.003	0.26 ± 0.01	0.18 ± 0.01
K	ns	ns	0.66 ± 0.01	0.36 ± 0.01	0.89 ± 0.01	0.57 ± 0.01
Ca	1.40 ± 0.04	2.43 ± 0.04	0.47 ± 0.01	0.38 ± 0.01	0.82 ± 0.01	0.49 ± 0.01
Mg	0.26 ± 0.01	0.34 ± 0.01	0.158 ± 0.004	0.072 ± 0.004	0.140 ± 0.003	0.112 ± 0.003
S	0.42 ± 0.02	0.35 ± 0.02	0.073 ± 0.002	0.053 ± 0.002	ns	ns
Zn	ns	ns	ns	ns	50.06 ± 1.41	69.68 ± 1.42
B	72.44 ± 2.53	94.10 ± 2.61	ns	ns	ns	ns
Mn	194.58 ± 5.43	151.67 ± 5.59	58.40 ± 2.01	37.81 ± 2.03	73.66 ± 5.69	103.41 ± 5.75
Fe	ns	ns	ns	ns	521.60 ± 123.39	877.21 ± 124.82
Cu	6.49 ± 0.16	5.44 ± 0.17	ns	ns	ns	ns
Al	52.61 ± 2.66	81.40 ± 2.74	ns	ns	647.71 ± 148.45	1086.75 ± 150.16
Na	171.34 ± 17.21	29.64 ± 17.74	93.66 ± 2.42	58.64 ± 2.45	251.58 ± 17.11	349.20 ± 17.31
Cl	1496.70 ± 89.09	945.91 ± 91.83	95.53 ± 5.78	43.72 ± 5.85	ns	Ns

ns = pairwise comparisons not significantly different at $\alpha = 0.05$.

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than *Populus* clones, with clone SX61 exhibiting the greatest Ca concentration. Aside from clone NM2 having greater Ca in the leaves than NM6 within the NM genomic group, differences among *Populus* clones for Ca were negligible, while *Salix* genotypes were segregated based on parentage. Clone SX61 (*S. sachalinensis*) exhibited the greatest leaf concentration, while *S. eriocephala* clones (S287, S566) and *S. purpurea* clones (94003, 94012) ranked second and third, respectively. Overall, the concentration of Ca across clones ranged from 1.23 ± 0.10 to 3.57 ± 0.11 mg kg^{-1}, with a mean of 1.88 ± 0.09 mg kg^{-1}. For leaf Mg concentration, clone NM2 had greater levels than NM6 for *Populus* clones, while *Salix* genotypes were segregated based on parentage. Except for differences between S287 and S566 of the same genomic group, strategic level (*i.e.*, genomic group) differences existed for the leaf concentration of Mg. Specifically, clone S566 had the greatest Mg concentration, while S287, 94003, and 94012 were second best, and SX61 ranked third. Overall, the concentration of Mg across clones ranged from 0.21 ± 0.04 to 0.50 ± 0.03 mg kg^{-1}, with a mean of 0.30 ± 0.01 mg kg^{-1}. There was broad clonal variation for S concentration of the leaves, with selected *Populus* and *Salix* genotypes exhibiting similar levels. Overall, the concentration of S across clones ranged from 0.24 ± 0.04 to 0.53 ± 0.04 mg kg^{-1}, with a mean of 0.38 ± 0.02 mg kg^{-1}. Zinc concentration was similar across genera and clones. *Salix* clone SX61 and *Populus* clone NM6 exhibited the greatest and least Zn concentration, respectively. Overall, the concentration of Zn across clones ranged from 171.30 ± 26.41 to 343.09 ± 30.26 mg kg^{-1}, with a mean of 233.82 ± 9.94 mg kg^{-1}. The leaf concentration of Cu was greatest for *Populus* clones, where differences within genomic groups were prevalent. Clone DN17 had greater levels of Cu than DN182 and DN34, while leaf Cu concentration for NM2 was greater than NM6. Across clones, NM2 exhibited the greatest concentration, while that for DN17 was similar to NM2 and *Salix* clone 94012. Overall, the concentration of Cu across clones ranged from 4.50 ± 0.42 to 7.74 ± 0.36 mg kg^{-1}, with a mean of 6.00 ± 0.17 mg kg^{-1}. The greatest clonal variation was exhibited by the leaf concentration of Na. In general, *Populus* clones exhibited greater leaf Na concentrations than *Salix* clones, with clones DN17, DN34, and DN182 exhibiting the greatest Na levels. Overall, the concentration of Na across clones ranged from 17.36 ± 38.47 to 263.68 ± 38.47 mg kg^{-1}, with a mean of 101.57 ± 18.15 mg kg^{-1}. There were genomic group differences for leaf Cl concentration, with NM clones exhibiting greater levels than their DN counterparts. Clone 94003 had greater leaf Cl concentration than all other *Salix* genotypes (including 94012 in the *S. purpurea* genomic group). Despite genus, Cl concentration varied greatly across clones, with *Populus* clones NM2 and NM6, along with *Salix* clone 94003, exhibiting the greatest Cl concentration in the leaves. Overall, the concentration of Cl across clones ranged from 431.21 ± 228.23 to 2205.08 ± 199.22 mg kg^{-1}, with a mean of 1231.20 ± 98.79 mg kg^{-1}.

The interaction between treatment and clone governed the leaf concentration of Mn (Table 3). In general, an advantage of using clones belonging to either genus was non-existent. However, broad genotypic variation existed, with clone-specific responses to leachate and water treatments influencing the concentration of Mn in the leaves (Figure 2). Overall, the concentration of Mn across treatments and clones ranged from 77.49 ± 17.16 to 272.81 ± 21.88 mg kg^{-1}, with a mean of 171.66 ± 7.79 mg kg^{-1}.

Stems. The treatment main effect was significant for Mg, Mn, and Na concentration of the stems, while genera differed for P, K, Ca, Mg, S, Mn, Na, and Cl (Table 3). The interaction between treatment and genus governed the concentration of Na. The concentration of Mg in the stems was 0.125 ± 0.004 mg kg^{-1} (leachate) and 0.105 ± 0.004 mg kg^{-1} (municipal water), while that for Mn was 57.54 ± 2.01 mg kg^{-1} (leachate)

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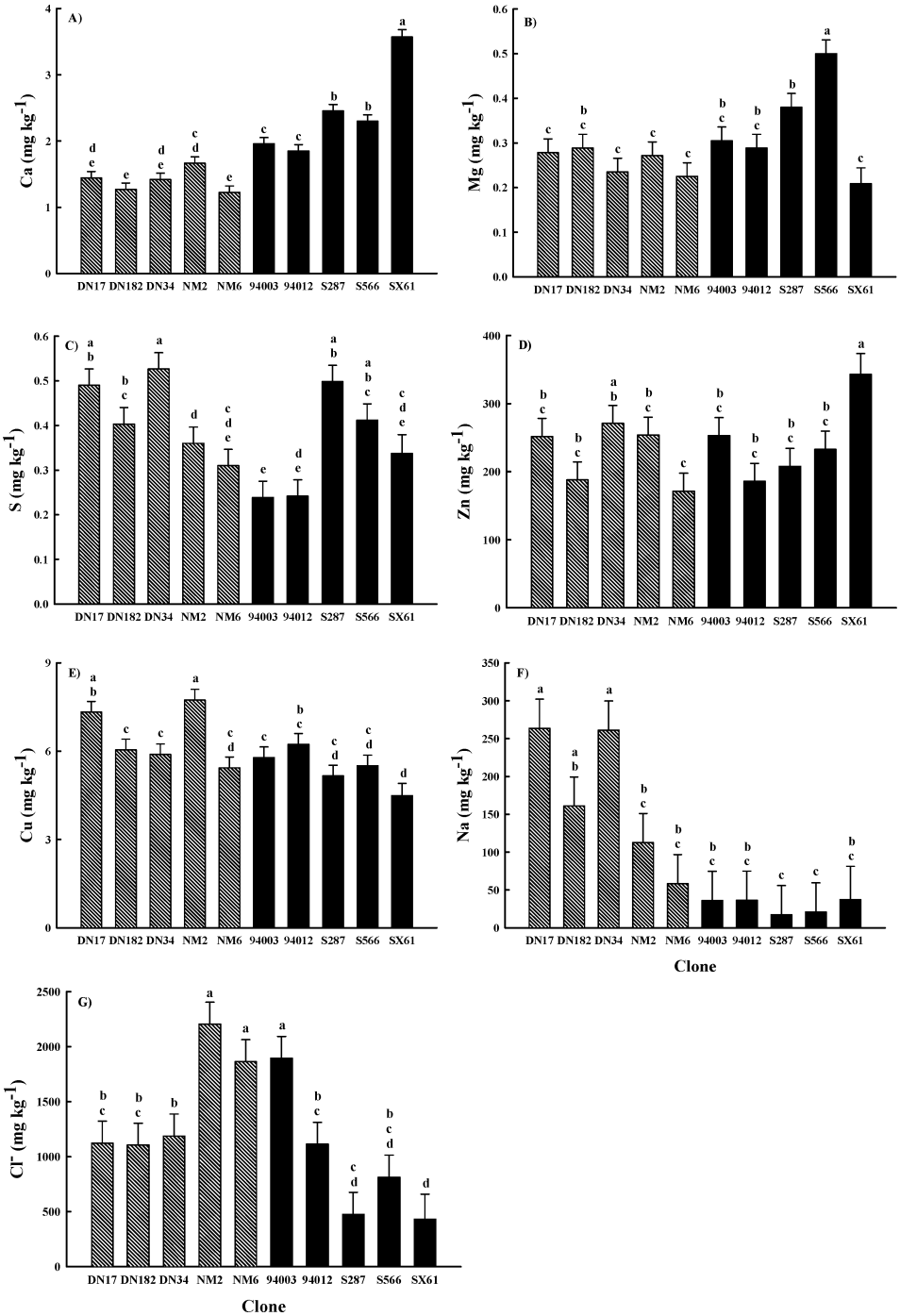

Figure 1 Leaf concentration of Ca, Mg, S, Zn, Cu, Na, and Cl pooled across treatments for *Populus* (dashed bars) and *Salix* (black shaded bars) clones in an experiment testing genotype-specific phytoremediation capabilities. Standard error bars represent one standard error of the mean, n = 6 trees for each clone. Concentrations with different letters above the bars are different according to Fisher's protected least significant difference (LSD) ($\alpha = 0.05$, LSD for Ca, Mg, S, Zn, Cu, Na and Cl is 0.31, 0.10, 0.12, 86.13, 1.18, 124.47, and 649.68 mg kg^{-1}, respectively).

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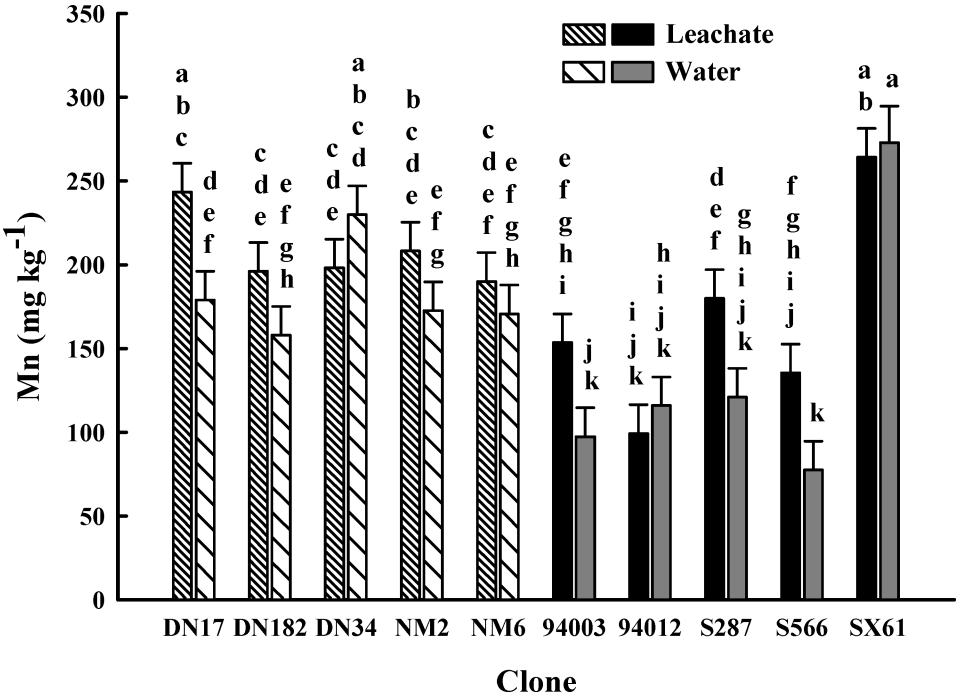

Figure 2 Leaf concentration of Mn for each combination of treatment (leachate, municipal water) and clone in an experiment testing genotype-specific phytoremediation capabilities of *Populus* (dashed bars) and *Salix* (shaded bars). Standard error bars represent one standard error of the mean, n = 3 trees for each interaction. Concentrations with different letters above the bars are different according to Fisher's protected least significant difference (LSD) ($\alpha = 0.05$, LSD = 55.97 mg kg^{-1}).

and 38.67 ± 2.03 mg kg^{-1} (municipal water). The concentration of elements in the stems varied greatly between *Populus* and *Salix*, with *Populus* exhibiting the greatest stem concentrations for all significant elements (Table 4). The Na concentration for treatment × genus interactions was 26.29 ± 3.38 mg kg^{-1} (leachate × *Populus*), 14.00 ± 3.46 mg kg^{-1} (leachate × *Salix*), 161.02 ± 3.46 mg kg^{-1} (water × *Populus*), and 103.27 ± 3.46 mg kg^{-1} (water × *Salix*) ($LSD_{0.05,8}$ = 14.97 mg Na kg^{-1}, n = 15).

The main effect of clone was significant for the stem concentration of P, K, Ca, Mg, S, Zn, B, Mn, Fe, Cu, and Na (Table 3). The concentration of significant elements in the stems varied greatly among clones (Figure 3). *Populus* clones had greater K in the stems than *Salix* clones. Overall, the concentration of K across clones ranged from 0.31 ± 0.03 to 0.73 ± 0.03 mg kg^{-1}, with a mean of 0.51 ± 0.02 mg kg^{-1}. Similarly, S levels were greater for *Populus* than *Salix*, with *Populus* clones DN34 and DN17 exhibiting the greatest S concentration. Overall, the concentration of S across clones ranged from 0.042 ± 0.004 to 0.090 ± 0.004 mg kg^{-1}, with a mean of 0.060 ± 0.002 mg kg^{-1}. Genomic group differences for stem Zn concentrations were negligible for *Populus*, while clones of *S. eriocephala* and *S. sachalinensis* parentage had greater stem Zn concentration than *S. purpurea* clones. *Salix* clone 94012 exhibited the least concentration, while the other *Populus* and *Salix* clones exhibited similar concentrations of Zn in the stems. Overall, the concentration of Zn across clones ranged from 41.05 ± 4.30 to 88.78 ± 4.30 mg kg^{-1}, with a mean of 70.96 ± 2.31 mg kg^{-1}. Boron concentration was similar across genera and

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clones. *Populus* clone DN34 and *Salix* clones 94012 and SX61 exhibited the greatest and least B concentrations, respectively. Overall, the concentration of B across clones ranged from 26.23 ± 1.12 to 35.74 ± 1.12 mg kg^{-1}, with a mean of 31.46 ± 0.61 mg kg^{-1}. The stem concentration of Mn was greatest for *Populus* clones, with DN34 exhibiting the greatest concentration and DN17 performing equally well, yet similar to other *Populus* and *Salix* clones. Overall, the concentration of Mn across clones ranged from 22.16 ± 4.54 to 70.08 ± 4.54 mg kg^{-1}, with a mean of 48.18 ± 2.62 mg kg^{-1}. The least amount of clonal variation was exhibited by the stem concentration of Fe. *Salix* clone S287 had the greatest Fe concentration, with *Populus* clone DN17 and *Salix* clone S566 having similar concentration to S287 and the remaining clones. Overall, the concentration of Fe across clones ranged from 21.59 ± 3.90 to 40.43 ± 3.90 mg kg^{-1}, with a mean of 26.20 ± 1.39 mg kg^{-1}.

The interaction between treatment and clone governed the stem concentration of P, Ca, Mg, Cu, and Na (Table 3). In general, an advantage of using clones belonging to either genus was non-existent. However, broad genotypic variation existed, with clone-specific responses to leachate and water treatments influencing the concentration of all significant elements in the stems (Figure 4). Overall, the concentration of P across treatments and clones ranged from 0.067 ± 0.009 to 0.193 ± 0.009 mg kg^{-1}, with a mean of 0.130 ± 0.004 mg kg^{-1}, while the concentration of Ca across treatments and clones ranged from 0.20 ± 0.04 to 0.68 ± 0.04 mg kg^{-1}, with a mean of 0.42 ± 0.02 mg kg^{-1}. In addition, the concentration of Mg across treatments and clones ranged from 0.04 ± 0.01 to 0.26 ± 0.01 mg kg^{-1}, with a mean of 0.12 ± 0.01 mg kg^{-1}, while the concentration of Cu across treatments and clones ranged from 3.95 ± 0.35 to 8.81 ± 0.35 mg kg^{-1}, with a mean of 6.15 ± 0.19 mg kg^{-1}. The concentration of Na across treatments and clones ranged from 10.95 ± 7.74 to 204.72 ± 7.74 mg kg^{-1}, with a mean of 75.27 ± 8.12 mg kg^{-1}.

Roots. The treatment main effect was significant for Mg, Mn, and Na concentration of the roots, while genera differed for P, K, Ca, Mg, Zn, Mn, Fe, Al, and Na (Table 3). The interaction between treatment and genus governed the concentration of Ca. The concentration of Mg in the roots was 0.131 ± 0.003 mg kg^{-1} (leachate) and 0.121 ± 0.003 mg kg^{-1} (municipal water), while that for Mn was 116.01 ± 5.69 mg kg^{-1} (leachate) and 61.06 ± 5.75 mg kg^{-1} (municipal water). The concentration of Na in the roots of the leachate and municipal water treatment was 206.18 ± 17.11 mg kg^{-1} (leachate) and 394.60 ± 17.31 mg kg^{-1} (municipal water). The concentration of elements in the roots varied greatly between *Populus* and *Salix* (Table 4). *Populus* exhibited greater root concentrations of P, K, Ca, and Mg, while *Salix* had greater concentrations of Zn, Mn, Fe, Al, and Na. The Ca concentration for treatment × genus interactions was 0.87 ± 0.01 mg kg^{-1} (leachate × *Populus*), 0.48 ± 0.01 mg kg^{-1} (leachate × *Salix*), 0.77 ± 0.01 mg kg^{-1} (water × *Populus*), and 0.51 ± 0.01 mg kg^{-1} (water × *Salix*) ($LSD_{0.05,8}$ = 0.06 mg Ca kg^{-1}, n = 15).

The main effect of clone was significant for the root concentration of P, K, Ca, Mg, S, and Zn (Table 3). The concentration of significant elements in the roots varied greatly among clones (Figure 5). *Populus* clones had greater levels of P in the roots than *Salix* clones, with clone DN34 exhibiting the greatest P concentration. Overall, the concentration of P across clones ranged from 0.16 ± 0.01 to 0.30 ± 0.01 mg kg^{-1}, with a mean of 0.22 ± 0.01 mg kg^{-1}. Root K concentration was greater for *Populus* clones than *Salix* clones. There was a difference in root K concentration within DN and NM genotypes. Clones DN17 and DN34 had greater levels of K than DN182, while clone NM6 exhibited greater K concentration than NM2. Clones DN34 and DN17 exhibited the greatest K concentration. Overall, the concentration of K across clones ranged from 0.51 ± 0.03 to

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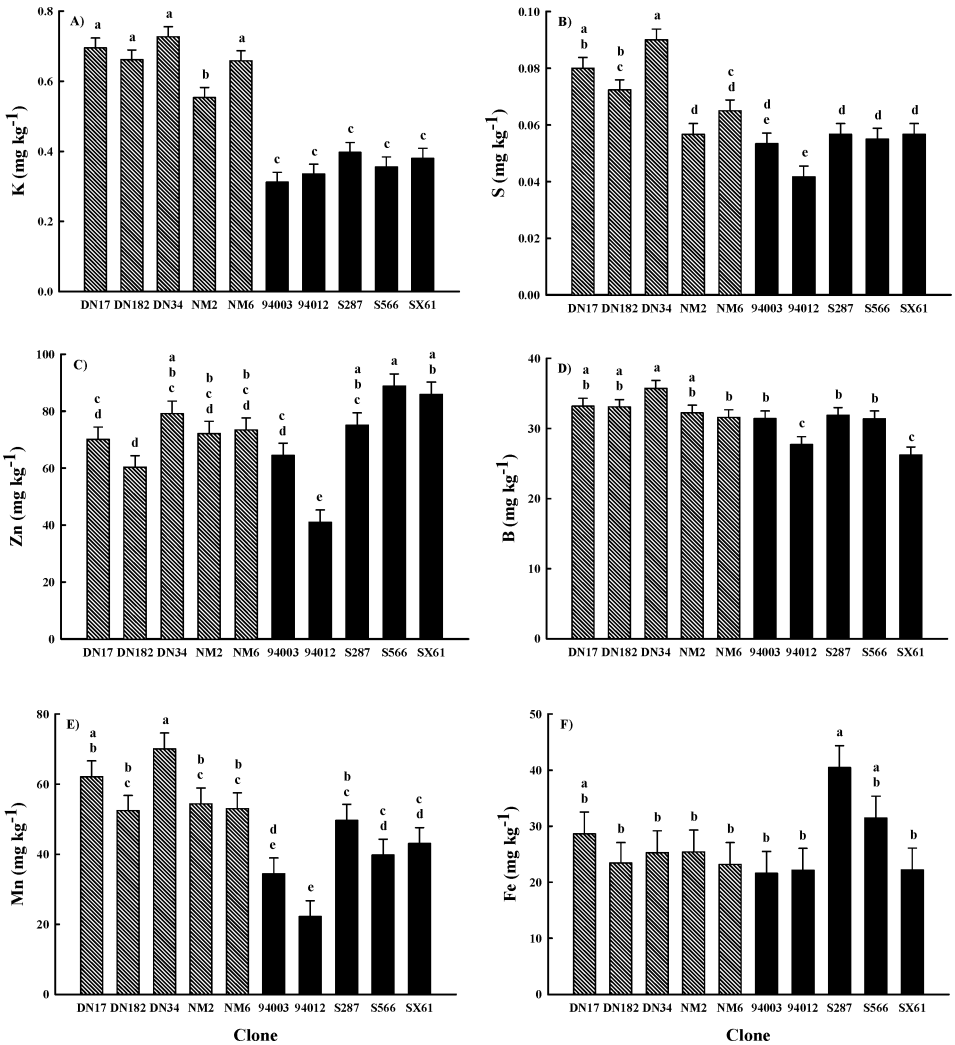

Figure 3 Stem concentration of K, S, Zn, B, Mn, and Fe pooled across treatments for *Populus* (dashed bars) and *Salix* (black shaded bars) clones in an experiment testing genotype-specific phytoremediation capabilities. Standard error bars represent one standard error of the mean, n = 6 trees for each clone. Concentrations with different letters above the bars are different according to Fisher's protected least significant difference (LSD) ($\alpha = 0.05$, LSD for K, S, Zn, B, Mn, and Fe is 0.09, 0.01, 14.02, 3.64, 14.82, and 12.72 mg kg^{-1}, respectively).

1.02 ± 0.03 mg kg^{-1}, with a mean of 0.74 ± 0.03 mg kg^{-1}. The root concentration of Ca was greater for *Populus* than *Salix*. The DN clones exhibited similar performance as for root K concentration, with DN17 and DN34 having greater Ca than DN182. There was a reversal for the NM clones, with NM2 having greater root Ca concentration than NM6. Overall, the concentration of Ca across clones ranged from 0.43 ± 0.02 to 0.90 ± 0.02 mg kg^{-1}, with a mean of 0.66 ± 0.02 mg kg^{-1}. The root concentration of Mg was greatest for *Populus* clones, with DN34, DN17, and DN182 having the greatest levels. Overall, the concentration of Mg across clones ranged from 0.098 ± 0.007 to 0.157 ± 0.007 mg kg^{-1}, with a mean of 0.130 ± 0.003 mg kg^{-1}. In contrast, *Salix* clones had greater Zn in the roots

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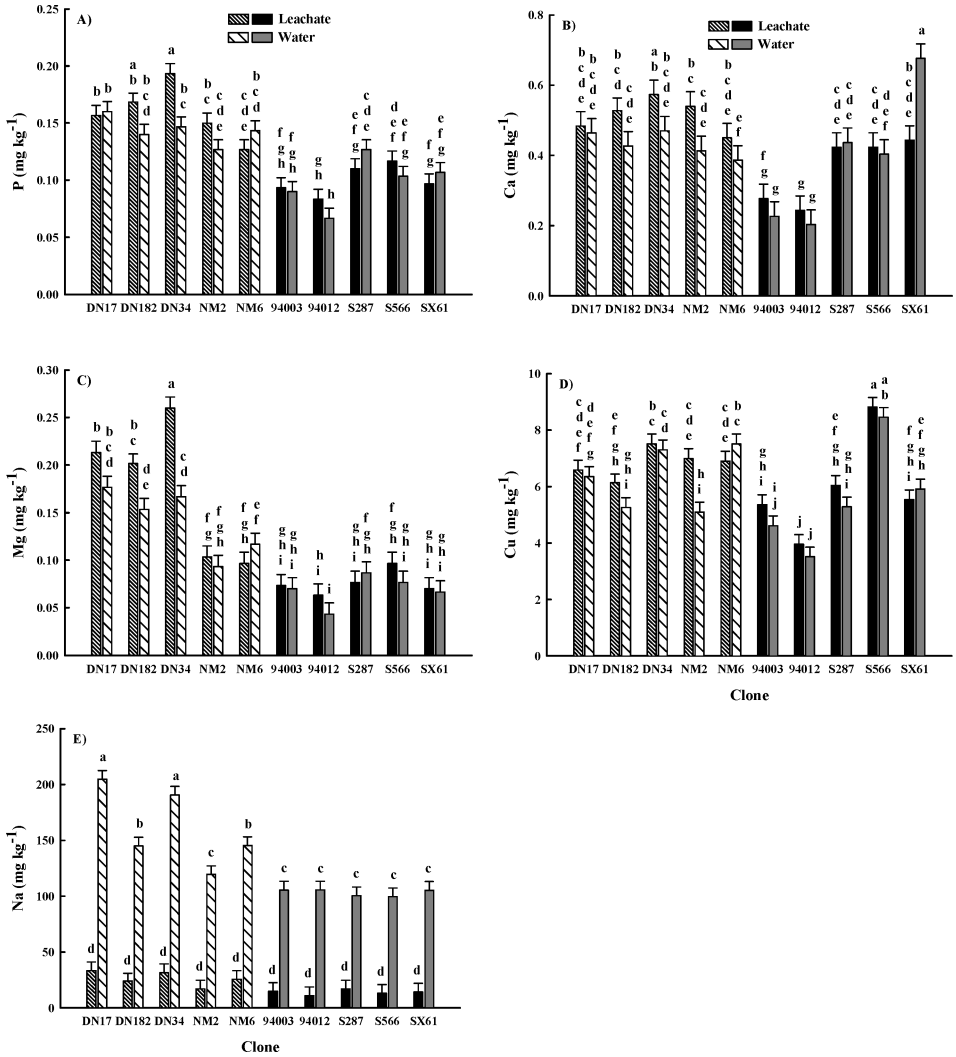

Figure 4 Stem concentration of P, Ca, Mg, Cu, and Na for each combination of treatment (leachate, municipal water) and clone in an experiment testing genotype-specific phytoremediation capabilities of *Populus* (dashed bars) and *Salix* (shaded bars). Standard error bars represent one standard error of the mean, n = 3 trees for each interaction. Concentrations with different letters above bars are different according to Fisher's protected least significant difference (LSD) ($\alpha = 0.05$, LSD for P, Ca, Mg, Cu, and Na is 0.03, 0.13, 0.04, 1.13, and 25.25 mg kg^{-1}, respectively).

than *Populus* genotypes, with clone SX61 exhibiting the greatest concentration. Overall, the concentration of Zn across clones ranged from 45.04 ± 2.99 to 87.98 ± 3.18 mg kg^{-1}, with a mean of 59.63 ± 2.03 mg kg^{-1}.

The interaction between treatment and clone governed the root concentration of S (Table 3). Once again, an advantage of using clones belonging to either genus was non-existent. However, broad genotypic variation existed, with clone-specific responses to leachate and water treatments influencing the concentration of S in the roots (Figure 6).

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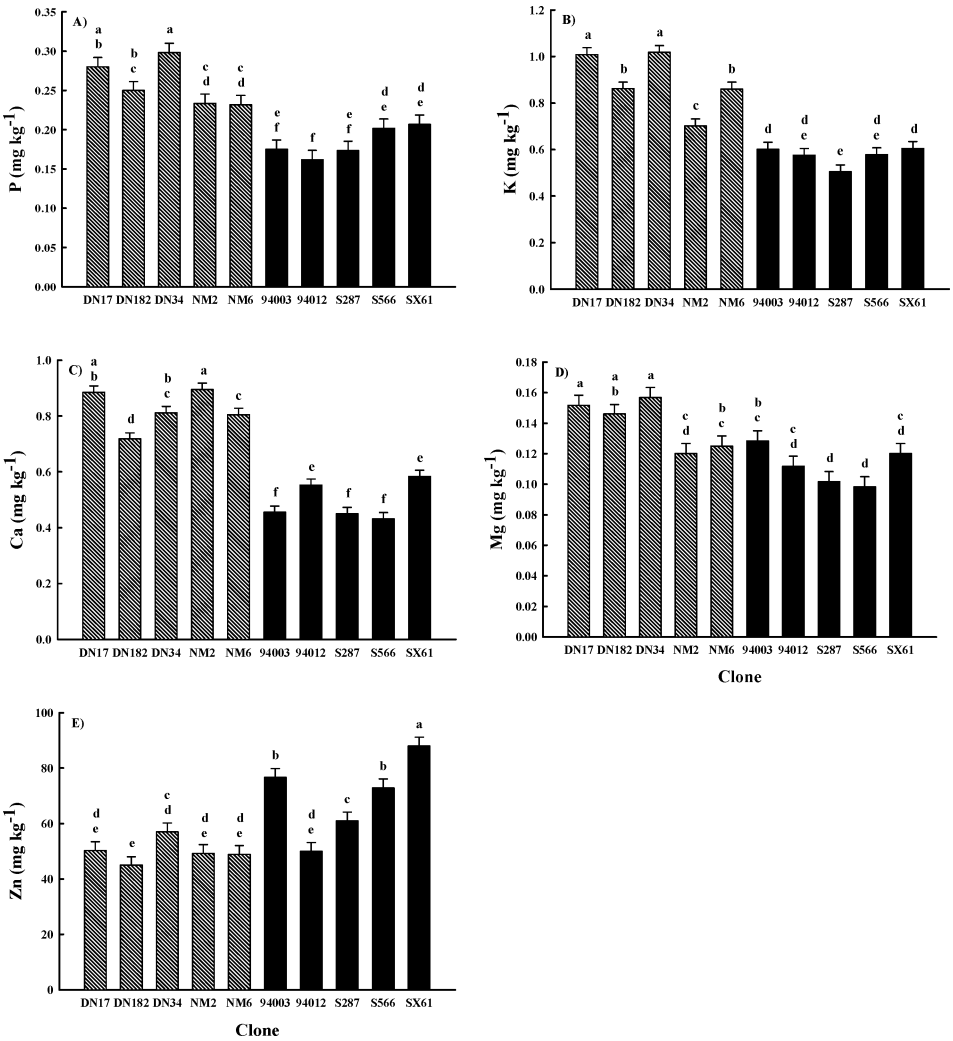

Figure 5 Root concentration of P, K, Ca, Mg, and Zn pooled across treatments for *Populus* (dashed bars) and *Salix* (black shaded bars) clones in an experiment testing genotype-specific phytoremediation capabilities. Standard error bars represent one standard error of the mean, n = 6 trees for each clone. Concentrations with different letters above the bars are different according to Fisher's protected least significant difference (LSD) ($\alpha = 0.05$, LSD for P, K, Ca, Mg, and Zn is 0.04, 0.10, 0.07, 0.02, and 10.37 mg kg^{-1}, respectively).

Overall, the concentration of S across treatments and clones ranged from 0.083 ± 0.010 to 0.140 ± 0.010 mg kg^{-1}, with a mean of 0.100 ± 0.003 mg kg^{-1}.

DISCUSSION

The goal of this study was to irrigate *Populus* and *Salix* genotypes with landfill leachate or municipal water and to test for element concentration differences in the leaves, stems, and roots. Although levels for some elements were greater than those recommended for various plant species, the overall concentration of elements in *Populus* and *Salix* plant

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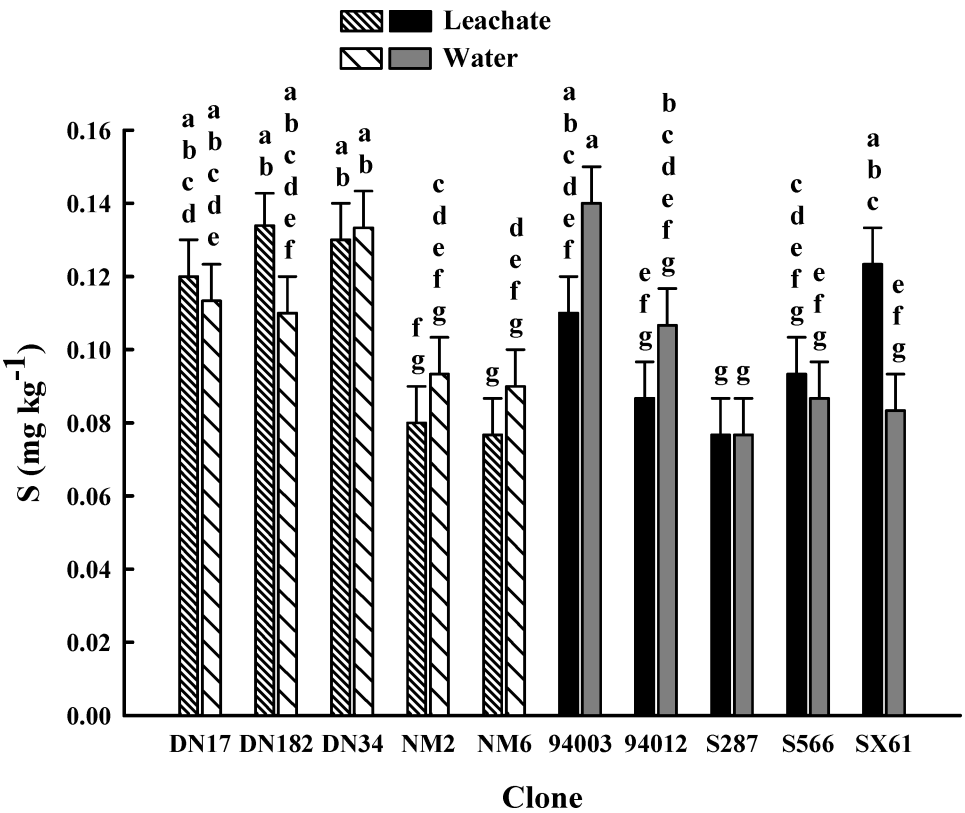

Figure 6 Root concentration of S for each combination of treatment (leachate, municipal water) and clone in an experiment testing genotype-specific phytoremediation capabilities of *Populus* (dashed bars) and *Salix* (shaded bars). Standard error bars represent one standard error of the mean, n = 3 trees for each interaction. Concentrations with different letters above the bars are different according to Fisher's protected least significant difference (LSD) ($\alpha = 0.05$, LSD for S is 0.03 mg kg^{-1}).

tissues across treatments corroborated those necessary for plant growth and development (Marschner, 1995). Genotype-specific phytoremediation of the elements existed for our genera and clones. Thus, our information is useful for researchers and site managers who need to make decisions about what genotypes to deploy, based on the element in need of remediation. We created Table 5 to be used as a tool for such decisions. Moreover, the results should be interpreted taking into consideration that the treatments were applied over one growing season. Changes in the specific differences and/or the magnitude of differences most likely would exist in a longer-term study. In addition, the lack of nitrogen data should be considered. Nitrogen is a key element for tree growth and development, along with its importance for determining application rates for landfill leachate used as irrigation.

At the genus level, the element concentrations in the leaves, stems, and roots exhibited four responses (Table 5). First, across all plant components, *Populus* had the greatest capability for phytoremediation of P, K, S, Cu, and Cl. Second, *Salix* exhibited the greatest phytoremediation capability for Zn, B, Fe, and Al. Third, *Salix* had greater concentrations of Ca and Mg in the leaves, while *Populus* exhibited greater levels of these elements in the stems and roots. Fourth, *Populus* had greater concentrations of Mn and Na in the leaves

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Table 5 Landfill leachate phytoremediation summary, according to element concentrations in the leaves, stems, and roots of *Populus* and *Salix* clones

	Leaves		Stems		Roots	
Element	*Populus*	*Salix*	*Populus*	*Salix*	*Populus*	*Salix*
P	All clones	—	All clones	—	DN17, DN34	—
K	—	—	All clones	—	All clones	—
Ca	—	S287, S566, SX61	*, All clones	SX61	All clones	—
Mg	—	S287, S566	DN17, DN182, DN34	—	DN17, DN182, DN34	—
S	*, DN17, DN34	S287	DN17, DN182, DN34	—	DN17, DN182, DN34	SX61
Zn	DN34	SX61	All clones	94003, S287, S566, SX61	—	94003, S287, S566, SX61
B	—	All clones	All clones	94003, S287, S566	—	—
Mn	*, DN17[a]	SX61	DN17, DN34	—	—	All clones
Fe	—	—	—	S287	—	All clones
Cu	DN17, NM2	—	DN34	S566	—	—
Al	—	All clones	—	—	—	All clones
Na	DN17, DN182, DN34	—	DN17, DN182, DN34, NM6	—	—	All clones
Cl	*, DN34, NM2, NM6	94003	All clones	—	—	—

[a]Interpretation: use *Populus* clone DN17 or *Salix* clone SX61 for uptake and translocation of Mn into the leaves (the *Populus* genus is significantly better). Asterisks (∗) indicate the significantly greater genus at $\alpha = 0.05$, while dashed lines (—) indicate a lack of an advantage for either genus.

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and stems, while *Salix* had greater levels of these elements in the roots. At the clone level, *P. deltoides* × *P. nigra* "DN" clones exhibited better overall phytoremediation capabilities; however, *P. nigra* × *P. maximowiczii* "NM" genotypes performed well for some plant components and elements. For example, the concentration of Cl in the leaves was greater for NM clones than DN genotypes ($P = 0.0029$). Likewise, the stem K concentration was similar among DN genotypes and clone NM6. The phytoremediation capabilities for *S. purpurea* clones 94003 and 94012 generally were less than those of the other *Salix* genotypes. However, clone 94003 did perform well for some elements (Table 5), which corroborates our assertion of clone-specific phytoremediation and the need for proper genotype selection.

The elevated concentration of elements in the plant tissues illustrated the effectiveness of the phytoremediation system (Bañuelos *et al.*, 1999; Greger and Landberg, 1999; Sander and Ericsson, 1998). However, in some cases, the relative uptake of elements was similar regardless of the irrigation treatment. Nevertheless, our hypothesis was upheld for element concentrations in the leaves, stems, and roots. Genera and clones-within-genera responded differently to leachate and water treatment. This result is an important step toward developing environmentally-sound, cost-effective strategies for remediation of leachate-contaminated sites. Our results were equally-important for the presence of elements in the leaves, stems, and roots, because this information provides a finer scale with which to select genotypes for specific applications. Other researchers have reported similar tissue-specific concentrations of elements (Black, Fuchigami, and Coleman, 2002; Klang-Westin and Eriksson, 2003; Moffat, Armstrong, and Ockleston, 2001).

Researchers and resource managers must be cautious when choosing genotypes for phytoremediation (Zalesny *et al.*, in press), because those that have broad utilization potential may exhibit inadequate performance for specific elements (Symeonidis, McNeilly, and Bradshaw, 1985). Thus, there is a need for careful selection of clones within genomic groups, given the genetic variability within the genera *Populus* and *Salix* (Stettler, Bradshaw, and Zsuffa, 1992; Zalesny *et al.*, 2005a; 2005b). Matching genotypes to specific elements may contribute to enhanced efficiency and productivity of the system (Bañuelos *et al.*, 1999; Perttu and Kowalik, 1997). For example, Greger and Landberg (2001) and Landberg and Greger (1994) selected specific *Salix* genotypes for the phytoextraction and accumulation of Cd (cadmium) in the shoots that were more effective than *S. viminalis* L. "78183" (osier willow) tested by Klang-Westin and Eriksson (2003) and Sander and Ericsson (1998). Similar methodologies have been developed and refined in the plant sciences, especially in tree breeding programs, where phenotypic plasticity (*i.e.*, genotype × environment interaction) caused a range of responses across varying environments (Stearns, 1989; Stettler *et al.*, 1992). Thus, there was a need to select either generalist genotypes that performed well over broad geographic areas (low plasticity) or specialist genotypes that performed well in designated breeding/production zones (high plasticity), depending on the traits of interest (Dickmann and Keathley, 1996; Orlovic *et al.*, 1998; Zalesny *et al.*, 2005a).

We recognize that there are numerous possibilities for classifying the aforementioned 13 elements into groups that support logical interpretation and discussion of the results. Also, we acknowledge that the interactions of elements in the soils and tissues contributed to the variable responses of *Populus* and *Salix* genotypes (Kahle, 1993; van den Driessche, 2000). Therefore, given the complexity of the data, we have separated the elements into three groups: heavy metals, soluble salts, and additional plant nutrients. Our classification of heavy metals is based on those elements (Zn, Mn, Fe, and Cu) that have a density greater than 5 g cm^{-3} (Morris, 1992), whereas elements (Ca, Na, and Cl, hereafter referred to as

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salts) comprising and disassociating from soluble salts are those that commonly have been studied and reported in the phytoremediation literature (Glenn, Brown, and Blumwald, 1999; Wang *et al*., 2002). The additional plant nutrients category was defined previously as macronutrients (Mg, K, P, and S), micronutrients (B), and beneficial nutrients (Al) (Marschner, 1995).

Variability in heavy metal phytoextraction corroborated previous results for short rotation woody crops (Greger and Landberg, 1999). There were broad differences among the genotypes studied for leaf, stem, and root element concentrations of heavy metals (Zn, Mn, Fe, and Cu). At the genus level, *Populu*s exhibited the greatest concentration of Mn (leaf and stem) and Cu (leaf), while *Salix* had greater concentrations of Zn (root), Mn (root), and Fe (root). Clone main effects governed the concentration of Zn (leaf, stem, and root), Mn (stem), Fe (stem), and Cu (leaf). *Salix* genotypes exhibited the greatest Zn in the roots, especially clones 94003, S566, and SX61. This *Salix* advantage is not surprising, given that root concentrations of 161 ± 5 mg Zn kg^{-1} have been reported for *S. viminalis* clone 78183 (Boye, 2002), which was more than 2.5 times greater than the concentration across clones in the current study (59.63 ± 2.03 mg kg^{-1}). The Zn concentrations are notable given similar clonal performance across genomic groups, rather than the best clones having the same parentage. In this regard, there were differences within genomic groups. Specifically, the root Zn concentration was greater for clone 94003 than 94012 and greater for clone S566 than S287.

The capability of our genotypes for the phytoextraction and translocation of Zn to stems and leaves was greater than those reported previously for *Populus* and *Salix*. For example, van den Driessche (1999) reported leaf Zn concentrations ranging from 26 to 191 mg kg^{-1} with a mean of 74 mg kg^{-1} for a *P. trichocarpa* Torr. & Gray (western black poplar) $\times$ *P. deltoides* clone, while Moffat et al. (2001) provided leaf Zn concentrations of 32.7 mg kg^{-1} (*P. trichocarpa* $\times$ *P. deltoides* "Beaupré") and 51.6 mg kg^{-1} (*P. trichocarpa* "Trichobel"). Landberg and Greger (1996) reported $\sim$ 14 to 314 mg kg^{-1} (median $\approx$ 57 mg kg^{-1}) Zn in the shoots across 34 *Salix* clones belonging to five species, while Boye (2002) provided shoot Zn concentrations of 160 ± 7 and 290 ± 20 mg kg^{-1} for *S. viminalis* clone 78183. The collective mean values of Zn concentration in the leaves and stems of our clones was $\sim$ 300 mg kg^{-1} ($\sim$ 400 mg kg^{-1} when adding the root concentration) after 84 d of leachate treatment (126 d of growth), which did not reach a threshold for phytotoxicity (500 to 1500 mg Zn kg^{-1}) reported for agronomic species (Madejon *et al*., 2002, cited by Angle and Linacre, 2005).

The greater Zn concentrations in the aboveground tissues, relative to the roots, was a reversal from a common trend for northern hardwood species with similar levels of tolerance/uptake (Kahle, 1993). Furthermore, there were reports of greater Zn phytoextraction than with our genotypes. Zalesny *et al*. (2005c) reported a mean leaf concentration of 455.63 mg Zn kg^{-1} across 20 *Populus* clones and two *Salix* clones, while Erdman and Christenson (2000) reported leaf Zn concentrations ranging from 1200 to 5400 mg kg^{-1} for *P. deltoides* grown in soils contaminated with landfill leachate. Greger and Landberg (1999) provided a range of 14 to 1813 mg Zn kg^{-1} (shoots) and 72 to 2140 mg Zn kg^{-1} (roots) across nearly 150 *Salix* clones (mostly *S. viminalis*). Overall, despite extensive variation among the species reported, our results demonstrated the potential of *Populus* and *Salix* for Zn phytoextraction, especially when comparing our mean leaf Zn concentration (233.82 ± 9.94 mg kg^{-1}) with the range of reported normal concentrations among different plant species (6 to 126 mg kg^{-1}) (Kabata-Pendias and Pendias, 1984, cited by Boye 2002; Marschner, 1995).

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Our hypothesis was upheld for variation in the concentration of Mn (leaves) and Cu (stems). Genera and clones-within-genera responded differently to leachate and water treatment. Despite extensive variation in leaf Mn concentration across genotypes, differences in treatment responses within clones were negligible except for *Populus* clone DN17 and *Salix* clones 94003, S287, and S566 that exhibited greater concentrations with leachate treatment than water. We assert that these genotypes benefited from Mn levels in the leachate (0.73 ± 0.06 mg L^{-1}) that were consistent with commonly-accepted maximum concentration limits ranging from 0.10 to 10 mg L^{-1} for constituents of irrigation water (Rowe and Abdel-Magid, 1995). In addition, the leachate-advantage supported soil chemistry theory, wherein Mn and Cu availability should be greater for acidic conditions such as with the leachate irrigation (pH 6.3) compared with the alkaline water treatment (pH 8.4) (Foth, 1990). In contrast, the lack of a treatment $\times$ clone interaction for Zn and Fe was surprising, given that availability of these heavy metals also should have been greater under more acidic conditions, and that Fe levels in the leachate (3.32 ± 1.51 mg L^{-1}) were consistent with limits ranging from 1 to 20 mg L^{-1} (Rowe and Abdel-Magid, 1995). Nevertheless, stem Fe concentration was approximately one third (26.20 ± 1.39 mg kg^{-1}) of that reported for the poplar clones Beaupré (83.4 mg kg^{-1}) and Trichobel (93.3 mg kg^{-1}) (Moffat *et al.*, 2001). In contrast, our genotype-specificity in the aboveground concentration of Mn that ranged from $\sim$ 100 to 350 mg kg^{-1}, with a mean of $\sim$ 220 mg kg^{-1}, was greater than 10 times that for Beaupré and Trichobel (19.4 mg kg^{-1} each) (Moffat *et al.*, 2001).

In addition, differences in treatment responses within clones for stem Cu concentration were negligible except for the *Populus* clone NM2, which exhibited greater concentrations with leachate treatment than water, and the *Salix* clone SX61, which had greater levels with water treatment than leachate. Overall, the leaf Cu concentration in the current study (6.00 ± 0.17 mg kg^{-1}) agreed with reported normal values for various plant species (1 to 29 mg kg^{-1}) (Marschner, 1995) and was greater than for a *P. trichocarpa* $\times$ *P. deltoides* clone that ranged from 1.8 to 3.6 mg Cu kg^{-1} with a mean of 2.4 mg Cu kg^{-1} (van den Driessche, 1999). Similarly, the aboveground Cu concentration ($\sim$ 12 mg kg^{-1}) was greater than those reported previously that ranged from 0.4 to 9.0 mg kg^{-1} (Greger and Landberg, 1999; Landberg and Greger, 1996; Moffat *et al.*, 2001). The shoot Cu concentrations were less than those reported for the *S. viminalis* clone 78183 (26 ± 3 and 25 ± 5 mg kg^{-1}) (Boye, 2002). Nevertheless, the current *Populus* and *Salix* genotypes exhibited potential for Mn and Cu phytoextraction.

There were broad genotypic differences for leaf, stem, and root concentrations of soluble salts (Ca, Na, and Cl), which corroborated descriptions of plants either excluding or accumulating salts in their tissues (Glenn *et al.*, 1999; Shannon, 1997; Wang *et al.*, 2002). The response of specific *Populus* and *Salix* genotypes to elevated salt concentrations in soil generally is unknown. Although the level of Na (142.00 ± 1.69 mg L^{-1}) and Cl (99.30 ± 2.34 mg L^{-1}) in the leachate of the current study was below commonly-accepted maximum concentration limits of 200 mg Na L^{-1} and 650 mg Cl L^{-1} as constituents of irrigation water (Peavy, Rowe, and Tchobanoglous, 1985), the broad amount of variation within these genera supports the potential for selection of promising genotypes (Neuman *et al.*, 1996). For example, van den Burg (1984) reported a broad range in salt sensitivity for *P. deltoides* "D", *P. nigra* "N", *P. maximowiczii* "M", *P. trichocarpa* "T", and *P. tremuloides* L. "Q" (quaking aspen) comprising seven genomic groups (D, N, Q, T, DN, MN, and MT).

The sensitivity of our genotypes varied at strategic and operational scales. At the genus level, *Populus* exhibited the greatest concentration of Ca (stem and root), Na (leaf and stem), and Cl (leaf and stem), while *Salix* had the greatest concentrations of Ca (leaf)

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and Na (root). Operationally, clone main effects governed the concentration of Ca (leaf, root), Na (leaf), and Cl (leaf). Our leaf Na concentrations with a mean of 101.57 ± 18.15 mg kg^{-1} were much less than those ranging from 2280 to 4680 mg kg^{-1} for *P. deltoides* (Erdman and Christenson, 2000).

Our hypothesis was upheld for variation in the stem concentrations of Ca and Na, where genera and clones-within-genera responded differently to leachate and water treatment. However, despite extensive variation across genotypes, differences in treatment responses within clones were negligible except for the *Salix* clone SX61, which exhibited greater concentrations with water treatment than leachate. This may have been due to a sensitivity of this clone to salinity stress in the stems (Wang *et al*., 2002). In addition to being important for cellular division, nitrate assimilation, and being a cofactor for some enzymes (Vaillant *et al*., 2004), Ca contributes to membrane integrity and, thus, is a structural component for plants (Redfield *et al*., 2003). These results corroborated those of Zalesny and Bauer (), where root dry mass of SX61 was significantly less with leachate irrigation than water. Similar salinity stress was observed for stem Na concentration, where all clones, regardless of genus, responded better to water than leachate. These results are intuitive, given that the Na concentration in our leachate (142 mg L^{-1}) was 31 times that of a standard nutrient solution (4.6 mg L^{-1}) (Vaillant *et al*., 2004) and that Ca availability should be greater for alkaline conditions such as with the water treatment (pH 8.4) compared with the acidic leachate irrigation (pH 6.3) (Foth, 1990). However, *Populus* clones Beaupré (63.4 mg Na kg^{-1}) and Trichobel (70.7 mg Na kg^{-1}) did not exhibit similar salinity stress (Moffat *et al*., 2001), despite nearly-identical stem Na levels to the mean of the current study (75.27 ± 8.12 mg kg^{-1}).

The Cl concentrations in the leaves of our genotypes indicated that some clones were salt excluders (S287 and SX61) while others were includers (DN17, DN182, DN34, NM2, NM6, 94003, 94012, and S566). Overall, the leaf Cl concentrations ranged from 431.21 ± 228.23 to 2205.08 ± 199.22 mg kg^{-1}, with a mean of 1231.20 ± 98.79 mg kg^{-1}, and were consistent with those reported from similar genetic trials evaluating variation among a variety of genotypes. For example, Yang and Blanchar (1993) tested 60 *Glycine max* (L.) Merr. (soybean) cultivars and, for plants at the end of the growing season, reported leaf Cl concentrations of 300 mg kg^{-1} (excluders) and 1800 mg kg^{-1} (includers). Regardless, despite a Cl concentration in our leachate (99.3 mg L^{-1}) that was 14 times greater than a standard nutrient solution (7.1 mg L^{-1}) (Vaillant *et al*., 2004), none of our genotypes exhibited symptoms of Cl toxicity (*i.e*., leaf chlorosis/abscission, growth inhibition). The lack of phytotoxicity for *Salix* was not surprising, however, given reports of successful establishment of clone 78183 when irrigated with leachate containing nearly 800 mg Cl L^{-1} (Boye, 2002). From a practical standpoint, these results are important in areas such as the North Central United States, where Cl contributes substantially to pollution due to agricultural irrigation (Stites and Kraft, 2001; Wang *et al*., 2002). Thus, utilization of *Populus* and *Salix* clones that are Cl includers may help to reduce the impact of such pollution. However, detailed physiological studies across entire rotations of *Populus* and *Salix* are needed to test whether the trees can withstand long-term salt accumulation, along with addressing the issue of what to do with salts stored in the leaves.

There were broad differences among the genotypes studied for leaf, stem, and root concentrations of additional plant nutrients (P, K, Mg, S, B, and Al). At the genus level, *Populu*s exhibited the greatest concentration of P (leaf, stem, and root), K (stem and root), Mg (stem and root), and S (leaf and stem), while *Salix* exhibited the greatest levels of Mg (leaf), B (leaf), and Al (leaf and root). Clone main effects governed the concentration of P

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(root), K (stem and root), Mg (leaf and root), S (leaf and stem), and B (stem). In general, *Populus* clones exhibited similar stem K concentrations that were greater than levels that were similar for *Salix* clones. There were differences within the NM genomic group, with NM6 having greater K concentration than NM2. Overall, despite the lower K concentrations in the leachate ($\sim$ 73 mg L^{-1}) and water ($\sim$ 1 mg L^{-1}) relative to some nutrient solutions ($\sim$ 203 mg L^{-1}), the trees did not show signs of K deficiency (brown scorching and curling of leaf tips, yellowing of leaf veins), which is important because K is the second-most required element for plants (by quantity) and is important for photosynthesis (Vaillant *et al*., 2004). The lack of a treatment $\times$ clone interaction corroborated the expected lack of a chemical relationship between K availability and pH, wherein the treatments of leachate (pH 6.3) and water (pH 8.4) should have provided similar K availability (Foth, 1990). Furthermore, DN *Populus* clones exhibited greater leaf S concentrations than NM genotypes ($P = 0.0495$). In general, *Salix* clones segregated such that *S. eriocephala* genotypes had the greatest leaf S levels, while those of *S. sachalinensis* and *S. purpurea* ranked second and third, respectively. DN *Populus* clones exhibited greater stem S concentration than NM clones ($P < 0.0001$) and all *Salix* genotypes.

Populus F_1 hybrids from three genomic groups (*P. deltoides* $\times$ *P. nigra*, *P. deltoides* $\times$ *P. maximowiczii*, and *P. nigra* $\times$ *P. maximowiczii*) have exhibited better height, diameter, and biomass when irrigated with landfill leachate containing 12 mg B L^{-1} compared with negligible concentrations in municipal water (Zalesny *et al*., submitted). The B concentration in the leachate of the current study was 1.63 ± 0.04 mg L^{-1}, which did not reach a similar threshold of testing for elevated levels of tolerance and/or phytoremediation. However, these levels were in the range of commonly-accepted maximum concentration limits for constituents of irrigation water, which have ranged from 0.5 to 2.0 mg B L^{-1} (Rowe and Abdel-Magid, 1995).

The plant requirement for B on a molar basis is greater than any other micronutrient (Marschner, 1995). However, despite a report that *P. deltoides* extracted and accumulated B in its leaves more readily than other elements (Erdman and Christenson, 2000), B concentrations were significant only in the stems of our genotypes. Additionally, the lack of a treatment $\times$ clone interaction for B was surprising, because the availability of B under the acidic leachate treatment (pH 6.3) should have been greater than for the alkaline water treatment (pH 8.4) (Foth, 1990). Nevertheless, there were no genomic group differences for B concentrations in the stems, wherein clone DN34 exhibited the greatest concentration. Once again, there was variation within the *S. purpurea* genomic group, with clone 94003 exhibiting greater levels than 94012. The DN *Populus* clones exhibited a trend similar to that for the Zn and Mn in the stems. Overall, the clonal performance in stem B concentration across our clones (31.46 ± 0.61 mg kg^{-1}) was double that of Moffat *et al*. (2001), who reported stem B concentrations of 11.2 mg kg^{-1} (*P. trichocarpa* $\times$ *P. deltoides*) and 14.6 mg kg^{-1} (*P. trichocarpa*). Our results corroborated that of Bañuelos *et al*. (1999) (14 to 40 mg kg^{-1} for lower and upper stems, respectively, at moderate salinity levels), who tested the effects of B and Se (selenium), along with various salinity levels, on the phytoextraction capability of nine *Populus* clones belonging to three genomic groups: *P. trichocarpa* $\times$ *P. deltoides*, *P. deltoides* $\times$ *P. nigra*, and *P. trichocarpa* $\times$ *P. nigra*. The similar outcomes between the two studies were intuitive, given that the B concentration in the leachate of the current study was in the range (1 to 5 mg L^{-1}) of Bañuelos *et al*., (1999) and that *P. deltoides* and *P. nigra* were used as parents in the F_1 crosses of both studies. From a genetics standpoint, it is important that *P. maximowiczii* and *P. trichocarpa* belong to the *Tachamahaca* section of *Populus*, which infers a greater relative level of genotypic

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similarity than if parental species from different sections were utilized and/or compared (Zalesny and Wiese, 2006).

Our hypothesis was upheld for variation in the stem concentrations of P and Mg and the root S concentration, where genera and clones-within-genera responded differently to leachate and water treatment. However, despite extensive variation across genotypes, differences in treatment responses within clones were negligible except for *Populus* clones DN34 (P; Mg) and DN182 (Mg), which exhibited greater concentrations with leachate treatment than water, and *Salix* clone SX61 that had greater levels with water treatment than leachate. The contrasting treatment effects between these clones were not surprising, given that P and Mg availability are similar for slightly-acidic and medium-alkaline soils (Foth, 1990). From a genetics standpoint, clones DN34 and DN182 were promising for Mg phytoremediation, despite the fact that the Mg level (52.60 ± 2.13 mg L^{-1}) in the leachate was below a commonly-accepted maximum concentration limit of 150 mg L^{-1} (Peavy *et al*., 1985). From a practical standpoint, the results for P are important in areas such as the Eastern United States, where P is detrimental to freshwater and coastal systems (Jordan *et al*., 2003; Kleinman and Sharpley, 2003), as well as the North Central United States, where P contributes substantially to pollution due to agricultural fertilization (Daverede *et al*., 2003; Zhao *et al*., 2001). Thus, utilization of *Populus* and *Salix* clones that are able to extract P may help reduce the impact of such pollution. In addition, despite extensive variation in root S concentration across genotypes, differences in treatment responses within clones were negligible except for the *Salix* clone SX61 that exhibited greater concentrations with leachate treatment than water. Once again, these results are intuitive, given that the availability of S is similar for the soil conditions of both treatments (Foth, 1990).

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