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ScienceDirect

Environmental Pollution 155 (2008) 72–80

ENVIRONMENTAL POLLUTION

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Sodium and chloride accumulation in leaf, woody, and root tissue of *Populus* after irrigation with landfill leachate

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Received 24 May 2007; received in revised form 3 September 2007; accepted 28 October 2007

Sodium and chloride supplied via landfill leachate irrigation is accumulated at high concentrations in tissues of Populus.

Abstract

The response of *Populus* to irrigation sources containing elevated levels of sodium (Na^+) and chloride (Cl^-) is poorly understood. We irrigated eight *Populus* clones with fertilized well water (control) (N, P, K) or municipal solid waste landfill leachate weekly during 2005 and 2006 in Rhinelander, Wisconsin, USA (45.6°N, 89.4°W). During August 2006, we tested for differences in total Na^+ and Cl^- concentration in preplanting and harvest soils, and in leaf, woody (stems + branches), and root tissue. The leachate-irrigated soils at harvest had the greatest Na^+ and Cl^- levels. Genotypes exhibited elevated total tree Cl^- concentration and increased biomass (clones NC14104, NM2, NM6), elevated Cl^- and decreased biomass (NC14018, NC14106, DM115), or mid levels of Cl^- and biomass (NC13460, DN5). Leachate tissue concentrations were 17 (Na^+) and four (Cl^-) times greater than water. Sodium and Cl^- levels were greatest in roots and leaves, respectively.
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Keywords: Hybrid poplars; Short rotation woody crops; Phytoaccumulation; Salts; Waste management

1. Introduction

There is a need for environmental practices that merge intensive forestry with waste management (Mirck et al., 2005). For example, leachate remediation is accomplished in situ when used as an irrigation source for *Populus* trees (i.e. poplars) and other short rotation woody crops (SRWC) (Shrive et al., 1994). However, leachate application has been detrimental when applied to SRWC systems, with negative impacts on plant tissues including: leaf chlorosis and necrosis, decreased biomass accumulation, and increased mortality (Stephens et al., 2000). Such impacts are exacerbated when excessive salt levels in the leachate irrigation cause osmotic stress (Duggan, 2005), in addition to nutrient imbalance and toxic effects in the plant tissues (Lessani and Marschner, 1978). The chemical composition of most leachate is highly variable (Kjeldsen et al., 2002). Therefore, leachate chemistry needs to be evaluated to determine potential phytotoxic effects resulting from such elevated ionic concentrations, and to prevent reductions in photosynthesis, leaf area, height, and diameter of *Populus* genotypes (Fung et al., 1998; Stephens et al., 2000).

Poplars have shown potential for phytoremediation projects involving landfill leachate and high salinity environments (Shrive et al., 1994; Bañuelos et al., 1999; Shannon et al., 1999; Erdman and Christenson, 2000). Traits that make poplars suitable for such uses include: quick establishment, large biomass accumulation, extensive and deep root systems, high rates of transpiration, ease of asexual propagation, and

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0269-7491/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.envpol.2007.10.032

exceptional growth on marginal lands (Isebrands and Karnosky, 2001; Zalesny et al., 2005). However, there are few reports in the literature about the response of different genomic groups and clones of *Populus* to elevated levels of sodium (Na^+) and chloride (Cl^-), or the variation in salt tolerance and tissue composition of such genotypes over multiple growing seasons in field settings. Therefore, field trials of a mixture of genotypes representing numerous genomic groups offers an opportunity to identify and select clones that exhibit broad variation in tolerance to salt environments. Such information is important for making recommendations to resource managers that will help to increase the successful utilization of landfill leachate as a fertilization and irrigation source for species and interspecific hybrids of the genus *Populus* (Erdman and Christenson, 2000; Zalesny and Bauer, 2007). Additionally, such information is useful for establishing field-scale Na^+ and Cl^- thresholds for *Populus*.

The current study expands on our previous work evaluating ex situ genotype selection for phytoremediation projects (Zalesny et al., 2007a), along with testing the in situ growth and biomass accumulation of eight *Populus* clones when irrigated with landfill leachate for two growing seasons (Zalesny et al., 2007b). However, specific levels of Na^+ and Cl^- accumulation of the trees were not evaluated in either of these previous experiments. Therefore, the primary objective of the current study was to test the uptake and distribution of Na^+ and Cl^- into leaf, woody (stems + branches), and root tissue of eight *Populus* genotypes that were irrigated with fertilized well water (control) (N, P, K) or municipal solid waste landfill leachate for two growing seasons. Our hypotheses were that clones would respond differently to water and leachate irrigation, and that clones would vary for tissue concentration of Na^+ and Cl^- in leaf, woody, and root tissue. This information is useful to SRWC biomass production for environmental benefits, because there is a general lack of knowledge about the response of *Populus* genotypes to Na^+ and Cl^- concentrations in landfill leachate, especially when used as an irrigation source in field trials.

2. Materials and methods

2.1. Site description

The study was conducted at the Oneida County Landfill located 6 km west of Rhinelander, Wisconsin, USA (45.6°N, 89.4°W). Temperature, precipitation, and growing degree days across the experimental period were described previously (Zalesny et al., 2007b). The landfill soils are classified as mixed, frigid, coarse loamy Alfic Haplorthods (Padus Loam, PaB), with 0–6% slopes, and are considered well to moderately well drained with loamy deposits underlain by stratified sand and gravel glacial outwash.

2.2. Clone selection

Eight *Populus* clones were selected from 25 original genotypes, based on aboveground and belowground traits, after being irrigated with leachate in a series of greenhouse experiments that constituted three phyto-recurrent selection cycles (Zalesny et al., 2007a). The clones and their parentages (i.e. genomic groups) were: NC13460, NC14018 [(*P. trichocarpa* Torr. & Gray × *P. deltoides* Bartr. ex Marsh) × *P. deltoides* 'BC_1']; NC14104, NC14106, DM115 (*P. deltoides* × *P. maximowiczii* A. Henry 'DM'); DN5 (*P. deltoides* × *P. nigra* L. 'DN'); and NM2, NM6 (*P. nigra* × *P. maximowiczii* 'NM'). In this paper we use the *Populus* section names as specified by Eckenwalder (1996), but we have retained the species nomenclature for *P. maximowiczii* (Japanese poplar) now classified as a subspecies of *P. suaveolens* Fischer (Eckenwalder, 1996; Dickmann, 2001).

2.3. Tree establishment and experimental design

Shoots were collected during dormancy from stool beds established at Hugo Sauer Nursery in Rhinelander. Hardwood cuttings, 20 cm long, were prepared during January 2005, with cuts made to position at least one primary bud not more than 2.54 cm from the top of each cutting. Cuttings were stored at 5 °C and soaked in water to a height of 15 cm for 3 days before planting on 14 June 2005. Prior to planting, the soil was tilled to a depth of 30 cm. Cuttings were planted in a split plot design with eight blocks (i.e. replications), two irrigation treatments (whole plots), and eight clones (sub plots) at a spacing of 1.2 × 2.4 m (i.e. 3472 trees ha^{-1}). Clones were arranged in randomized complete blocks in order to minimize effects of any potential environmental gradients. Two border rows of clone NM2 were established on the perimeter of the planting and between treatment whole plots to reduce potential border effects (Hansen, 1981; Zavitkovski, 1981). Mechanical and hand weeding were performed weekly in 2005 and 2006 to ensure maximum tree survival. Electric fencing was used to prevent deer browse and injury to the trees. Polyvinylchloride (PVC) tubing, 15.24 cm in diameter, was installed after leaf senescence in November 2005 on each tree to protect the trunk from girdling by rodents during the winter.

2.4. Treatment application

Water (control) from a non-impacted well located 100 m from the study area was applied to all cuttings via hand irrigation for an establishment period of 14 days. Following establishment, trees were hand irrigated with either fertilized water or municipal solid waste landfill leachate that was collected weekly, using a low-flow distribution nozzle connected to a garden hose. Fertilizer (N, P, and K) was added to the control treatment during each irrigation application at a rate equal to that of the leachate to eliminate fertilization effects of these macronutrients. The 2005 weekly application rate was 3.8 L $tree^{-1}$ (23.1 mm ha^{-1} assuming an irrigated soil surface area of 0.16 m^2 per tree). Given eight applications, a total of 1.9 kL of each treatment was applied across the growing season. Drip irrigation was used to apply treatments during 2006. The treatment application rate for 2006 was increased to 22.7 L $tree^{-1}$ (34.6 mm ha^{-1} assuming an irrigated soil surface area of 0.66 m^2 per tree) because of root system development and increased water usage as the trees developed. Given 12 applications, a total of 17.4 kL of each treatment was applied across the growing season. To prevent substantial leaching from the experimental plot, application of treatments was adjusted based on precipitation events. Irrigation was postponed if greater than 0.5 cm of rainfall occurred within 2 days prior to watering or was expected to occur with a 40% chance or greater for 2 d following watering.

2.5. Sampling and measurements

2.5.1. Well water (control) and municipal solid waste landfill leachate

Water and leachate from the same source as the irrigation treatments were sampled from the Oneida County Landfill during April and October of 2005 and 2006. The water and leachate chemistry was analyzed (Northern Lake Service, Inc., Crandon, Wisconsin, USA) using approved United States Environmental Protection Agency methods. The leachate was brown in color and had a putrid odor. The composition of the water and leachate, including pH, electrical conductivity, biological oxygen demand, chemical oxygen demand, Na^+, Cl^-, nitrogen (N), phosphorus (P), and potassium (K) concentration, are given in Table 1. The rate per application of Na^+, Cl^-, N, P, and K, expressed on a kg ha^{-1} basis, is given in Table 2. Heavy metals and volatile organic compounds were not detectable in the leachate analysis, and therefore, not a concern with respect to plant establishment and development.

Table 1
Composition of well water (control) and leachate from the Oneida County Landfill (Rhinelander, Wisconsin, USA) during the 2005 and 2006 growing seasons

Component	2005		2006	
	Control	Leachate	Control	Leachate
pH	6.2 ± 0.1	8.8 ± 0.0	6.3 ± 0.2	8.4 ± 0.2
Electrical conductivity (mS cm^{-1})	0.2 ± 0.1	6.2 ± 0.5	0.1 ± 0.0	9.4 ± 0.2
Biological oxygen demand (mg L^{-1})	na[a]	21 ± 5	na	108 ± 83
Chemical oxygen demand (mg L^{-1})	nd[b]	660 ± 10	na	1050 ± 50
Na$^+$ (mg L^{-1})	na	690 ± 10	2.4[c]	1200 ± 0
Cl$^-$ (mg L^{-1})	nd	1093 ± 178	1.8 ± 1.8	1250 ± 50
N (mg L^{-1})	480	598 ± 86	660	685 ± 25
P (mg L^{-1})	1.5	1.9 ± 0.1	3.7	3.0 ± 0.7
K (mg L^{-1})	400	450 ± 24	420	450 ± 30

Data are means ± one standard error ($n = 2$), except N, P, and K for the control treatment in both years ($n = 1$).

[a] Not available.

[b] Not detectable.

[c] One sample collected at harvest.

2.5.2. Soil

Using a 5-cm diameter hand auger, nine soil samples at a depth of 0–30 cm were collected from each irrigation treatment plot 1 day before planting (13 June 2005) and harvesting (17 August 2006). For each date, soil from three sampling points was bulked, and three bulked samples were sent to the University of Wisconsin Soil & Plant Analysis Laboratory (Madison, Wisconsin, USA) for analysis of pH using a Fisher Scientific Accumet Model No. AR25 pH meter with combination reference-glass electrode (Orion, Ross® Sure-Flow™ combination, epoxy body Model No. 8165), electrical conductivity using a VWR Model No. 23226-523 digital conductivity meter with automatic temperature compensation, and Na$^+$, P, and K concentration using inductively coupled plasma optical emission spectrometry (ICP-OES). Nitrogen concentration of the samples was analyzed at the Institute for Applied Ecosystem Studies (Rhinelander, Wisconsin, USA) using a Flash EA1112 N-C analyzer (Thermo Electron, via CE Elantech, Inc., Lakewood, New Jersey, USA) with a model MAS 200 autosampler. Identical samples were sent to the Iowa State University Soil & Plant Analysis Laboratory (Ames, IA, USA) for analysis of Cl$^-$ concentration using a modified mercury thiocyanate method with a Lachat Flow Injection Analysis Auto-Analyzer. The composition of the soil, including pH, electrical conductivity, and Na$^+$, Cl$^-$, N, P, and K concentration, are given in Table 3.

Table 2
Rate per application of sodium (Na$^+$), chloride (Cl$^-$), nitrogen (N), phosphorus (P), and potassium (K) in well water (control) and leachate from the Oneida County Landfill (Rhinelander, Wisconsin, USA) during the 2005 and 2006 growing seasons

Element	Rate per application (kg ha^{-1})			
	2005[a]		2006[b]	
	Control	Leachate	Control	Leachate
Na$^+$	na[c]	163.88	0.83	412.73
Cl$^-$	0.00	259.59	0.62	429.92
N[d]	114.00	141.91	227.00	235.60
P	0.36	0.45	1.27	1.03
K	95.00	106.88	144.45	145.77

[a] Eight applications total. Rate based on an application volume of 3.8 L tree^{-1} and an irrigated soil surface area of 0.16 m^2 tree^{-1}.

[b] Twelve applications total. Rate based on an application volume of 22.7 L tree^{-1} and an irrigated soil surface area of 0.66 m^2 tree^{-1}.

[c] Not available.

[d] Nitrogen, P, and K fertilizer additions to the control treatment both years were based on April leachate analyses.

2.5.3. Plant tissues

All trees were destructively harvested in two stages on 18 August 2006. First, the aboveground portion of each tree was cut at 10 cm above the soil surface, and leaf and woody (stems + branches) components were separated and dried at 70 °C. Leaf and woody biomass was determined when dry mass values reached a constant mass. Second, root systems were excavated using a mechanized tree spade that removed a uniform, conical volume of soil (diameter × depth = 0.28 m^3) for each tree. Root systems were washed and dry mass was determined identically to shoot components. Leaf, woody, and root samples of three blocks (i.e. replications) for each irrigation treatment × clone interaction were sent to A & L Great Lakes Laboratories, Inc. (Fort Wayne, Indiana, USA) for analysis of Na$^+$ (ICP-OES) and Cl$^-$ (ion chromatography).

2.6. Data analysis

Soil Na$^+$ and Cl$^-$ data were analyzed using analyses of variance (PROC GLM; SAS Institute, Inc., 2004) assuming a completely random design with a fixed main effect for soil sample (preplanting, harvest control, and harvest leachate).

Tissue Na$^+$ and Cl$^-$ data were analyzed using analyses of variance (PROC GLM; SAS Institute, Inc., 2004) assuming a split split plot design with a random block (i.e. replication) effect and fixed main effects for irrigation treatment (whole plot), clone (sub plot), and plant tissue (sub sub plot). Where appropriate, non-significant ($P > 0.25$) interaction terms that included the block main effect were pooled into a common error term to increase precision of F-tests (Zalesny et al., 2005). Given the fixed main effects in both models, means were evaluated rather than variances. Fisher's protected least significant difference (LSD) was used to compare means of soil and tissue data. Principal component analyses (PROC PRINCOMP; SAS Institute, Inc., 2004) were used to assess irrigation × clone interactions for total tree Cl$^-$ concentration (Manly, 1986).

3. Results

The Na$^+$ and Cl$^-$ application rate in the leachate increased from 2005 to 2006, given the increased volume of leachate applied. The application rate of Na$^+$ in the leachate was 2.5 times greater in 2006 than 2005 (Table 2), while that during the 2006 irrigation season was 500 times greater than the fertilized well water (control). Likewise, the Cl$^-$ application rate was 1.7 times greater in 2006 than 2005, with the leachate treatment increasing the Cl$^-$ application 693 times over the water treatment in 2006. Similar results were observed for concentrations of Na$^+$ and Cl$^-$ in the soil when comparing preplanting levels with those at harvest for both irrigation treatments (Table 3). The soil Na$^+$ concentration of the leachate treatment was nearly three times greater than at preplanting and 24 times greater than the control. The leachate soil Cl$^-$ concentration was 4.7 times greater than at preplanting and three times greater than the control. Soil pH was significantly greater for the leachate treatment than the control, but neither irrigation pH differed from the preplanting level (Table 3). Electrical conductivity differed among all three soil groups, with preplanting levels being the greatest and control levels the least (Table 3).

Treatment effects of water and leachate irrigation differed for Na$^+$ and Cl$^-$ concentration across clones and tissues (Table 4). In contrast, clones and the irrigation × clone interaction

Table 3
Soil pH, electrical conductivity (EC), and concentration of sodium (Na^{+}), chloride (Cl^{-}), nitrogen (N), phosphorus (P), and potassium (K) (mean ± standard error, $n = 3$) before planting and at whole-tree harvest after irrigating for the 2005 and 2006 growing seasons with well water (control) and leachate from the Oneida County Landfill (Rhinelander, Wisconsin, USA)

Element	Preplanting		Harvest				LSD[a]
			Control		Leachate		
pH	5.9 ± 0.1	ab	5.7 ± 0.1	b	6.2 ± 0.1	a	0.4
EC (mS cm^{-1})	2.78 ± 0.17	a	0.28 ± 0.01	c	1.39 ± 0.18	b	0.49
Na^{+} (mg kg^{-1})	72.5 ± 0.9	b	8.5 ± 0.3	c	203.0 ± 21.9	a	43.9
Cl^{-} (mg kg^{-1})	19.5 ± 3.4	b	30.0 ± 3.9	b	90.8 ± 12.5	a	27.0
N (g kg^{-1})	1.44 ± 0.34	b	1.37 ± 0.59	b	3.45 ± 0.22	a	1.43
P (g kg^{-1})	3.55 ± 0.23	a	0.30 ± 0.01	b	0.35 ± 0.01	b	0.45
K (g kg^{-1})	0.83 ± 0.01	a	0.08 ± 0.00	b	0.10 ± 0.01	b	0.02

[a] Means for each row followed by different letters were different at $P < 0.05$, according to Fisher's least significant difference (LSD).

were not significant. However, the Na^{+} and Cl^{-} concentration differed among tissues and for the irrigation × tissue interaction and the clone × tissue interaction. Nevertheless, the irrigation × clone × tissue interaction was most important, influencing the distribution of Na^{+} and Cl^{-} in leaf, woody (stems + branches), and root tissue.

Sodium concentration was greatest in trees irrigated with leachate, along with being most concentrated in root tissue and least concentrated in woody tissue (Fig. 1). Leaf Na^{+} concentration was similar for genomic groups, except for NC14018 of the BC$_1$ genomic group [(*P. trichocarpa* × *P. deltoides*) × *P. deltoides*]. Clone NC14018 exhibited greater leaf Na^{+} concentration than all other genotypes. The Na^{+} concentration in woody tissue was not different among genomic groups and clones. In contrast, broad genotypic variation existed for Na^{+} concentration in the roots. The DM (*P. deltoides* × *P. maximowiczii*) and NM (*P. nigra* × *P. maximowiczii*) genomic groups performed similarly to one another and had greater root Na^{+} concentration than the BC$_1$ genotypes and clone DN5 (*P. deltoides* × *P. nigra*). Clones NM2 and NM6 of the NM genomic group differed, with NM6 having greater Na^{+} in the roots. Overall, clonal ranking for total tree Na^{+} concentration varied by treatment, with only one clone performing similarly regardless of being irrigated with water or leachate. Specifically, NM6 sequestered the greatest amount of Na^{+} across tissues in both the water (0.72 g Na^{+} kg^{-1}) and leachate (8.32 g Na^{+} kg^{-1}) treatments. The total tree Na^{+} concentration of the remaining clones irrigated with leachate was: NC14018 (8.23 g kg^{-1}), NC14104 (7.68 g kg^{-1}), NC14106 (7.17 g kg^{-1}), NM2 (7.05 g kg^{-1}), DM115 (6.57 g kg^{-1}), NC13460 (5.49 g kg^{-1}), and DN5 (3.87 g kg^{-1}).

Table 4
Probability values from analyses of variance comparing the concentration of sodium (Na^{+}) and chloride (Cl^{-}) across two irrigation treatments [well water (control) and landfill leachate], eight *Populus* clones (see Section 2) and three tissues (leaf, woody, and root)

Source of variation[a]	Element	
	Na^{+}	Cl^{-}
Irrigation	**0.0192**	**0.0035**
Clone	0.8190	0.2038
Irrigation × clone	0.8741	0.3245
Tissue	**<0.0001**	**<0.0001**
Irrigation × tissue	**<0.0001**	**<0.0001**
Clone × tissue	**0.0090**	**0.0007**
Irrigation × clone × tissue	**0.0240**	**0.0073**

Significant values are in bold.

[a] The experimental layout in the field included eight blocks (i.e. replications), of which three were randomly chosen for analysis in the current study. For simplicity, the block main effect and interaction terms including the block factor are not included in the table.

Chloride concentration was greatest in trees irrigated with leachate, along with being most concentrated in leaf tissue and least concentrated in woody tissue (Fig. 2). Broad variation existed among genomic groups and clones for leaf Cl^{-} concentration. The DM and NM genomic groups performed similarly to one another and had greater leaf Cl^{-} concentration than DN5. For the BC$_1$ clones, NC14018 exhibited greater leaf Cl^{-} concentration than NC13460, which was similar to the DM/NM clones (NC14018) and DN5 (NC13460). The Cl^{-} concentration in woody tissue was not different among genomic groups and clones. Similarly, there were no differences among genomic groups for Cl^{-} in the roots. However, for the BC$_1$ clones, NC14018 sequestered more Cl^{-} in the roots than NC13460. Overall, clonal ranking for total tree Cl^{-} concentration varied by irrigation treatment, with clones NC13460 (decreased Cl^{-}) and NM6 (increased Cl^{-}) ranking similarly regardless of water or leachate application. In contrast, clone NC14106 had the broadest Cl^{-} concentration range across tissues, with the greatest for leachate (19.07 g kg^{-1}) and the least for water treatment (3.17 g kg^{-1}). The total tree (leaf + woody + root) Cl^{-} concentration of the remaining clones irrigated with leachate was: NM6 (19.03 g kg^{-1}), DM115 (17.97 g kg^{-1}), NC14018 (17.33 g kg^{-1}), NC14104 (17.10 g kg^{-1}), NM2 (16.47 g kg^{-1}), DN5 (9.90 g kg^{-1}), and NC13460 (8.07 g kg^{-1}).

The distributional trends in the percent of total Cl^{-} allocated to leaf, woody, and root tissues was similar among genomic groups and clones (Fig. 3). With the exception of DN5, leachate-irrigated trees exhibited greater relative distribution of Cl^{-} into the leaves compared with those irrigated with water. The increased relative percent of Cl^{-} distributed to leaves was most apparent for clones NM2 and NM6. Although NM6 sequestered 16% more Cl^{-} into the combination of all plant tissues compared with NM2, the relative percent allocation to tissues was nearly identical.

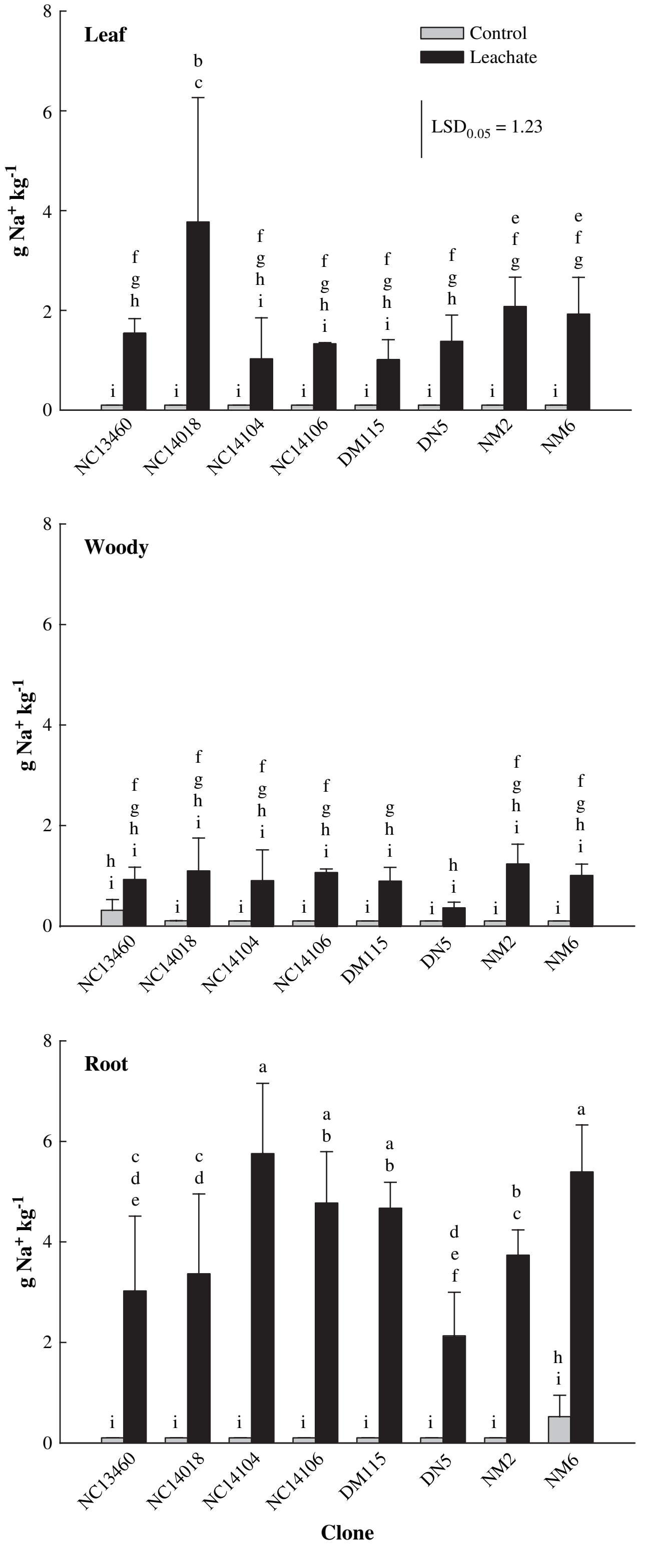

Fig. 1. Concentration of sodium for each combination of irrigation treatment [well water (control) and landfill leachate], *Populus* clone, and tree tissue (leaf, woody, and root). Error bars represent one standard error of the mean ($n = 3$). Bars labeled with the same letter were not different, according to Fisher's protected least significant difference (LSD).

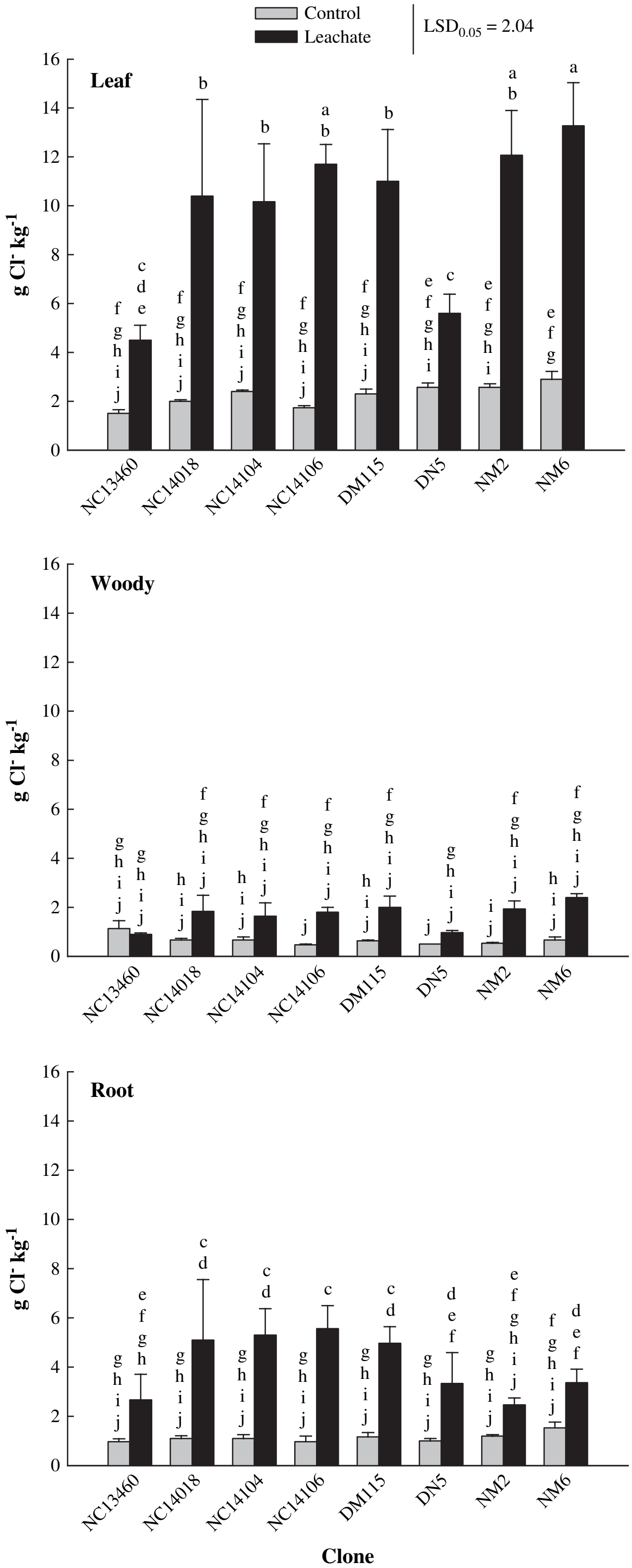

Fig. 2. Concentration of chloride for each combination of irrigation treatment [well water (control) and landfill leachate], *Populus* clone, and tree tissue (leaf, woody, and root). Error bars represent one standard error of the mean ($n = 3$). Bars labeled with the same letter were not different, according to Fisher's protected least significant difference (LSD).

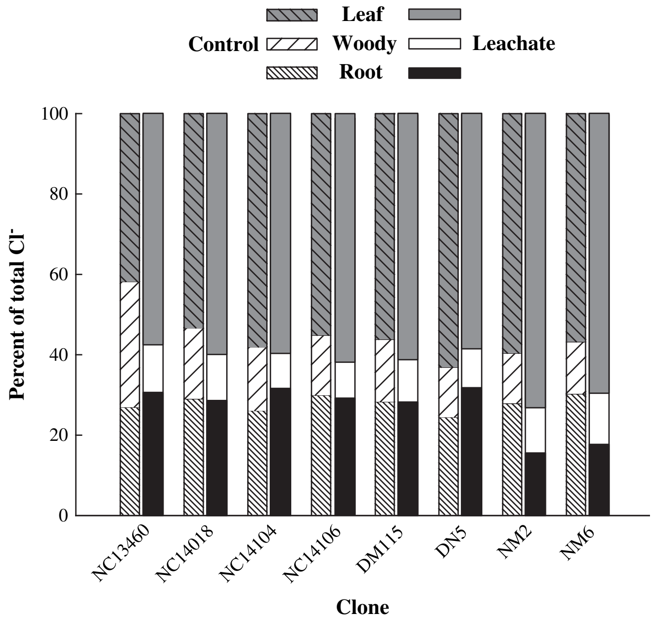

Fig. 3. Percent of total chloride allocated to leaf, woody, and root tissue of eight *Populus* clones irrigated with well water (control) or landfill leachate for two growing seasons.

There was broad clonal variation in the relationship between tissue Cl^- concentration and biomass production (Fig. 4). The range in total tree Cl^- concentration was narrow for clones irrigated with water, while total tree biomass was highly variable. Clones irrigated with leachate segregated into three response groups: (1) NC14104, NM2, and NM6 had elevated levels of total tree Cl^- concentration along with increased biomass; (2) NC14018, NC14106, and DM115 exhibited elevated levels of total tree Cl^- concentration along with decreased biomass; (3) NC13460 and DN5 exhibited mid levels of total tree Cl^- concentration and biomass. Principal component analyses corroborated these univariate results, with the first two principal components accounting for 100% of the variation in the irrigation × clone interaction data for total tree Cl^- concentration. A plot of the 16 irrigation × clone combinations substantiated the clustering of the three response groups described above (Fig. 5). Similar univariate and multivariate trends were exhibited for leaf, woody, and root tissue.

4. Discussion

The clone-specific uptake of Na^+ and Cl^- in leaf, woody, and root tissue of *Populus* was important, because there is a lack of information about the response of specific genotypes to elevated levels of Na^+ and Cl^- in irrigation sources such as landfill leachate. The 2006 leachate levels of 1200 ± 0 mg Na^+ L^{-1} and 1250 ± 50 mg Cl^- L^{-1} in the current study were six times (Na^+) and 1.9 times (Cl^-) greater than commonly accepted maximum concentration limits of 200 mg Na^+ L^{-1} and 650 mg Cl^- L^{-1} as constituents of irrigation water (Peavy et al., 1985). As expected, the higher concentrations of Na^+ and Cl^- in the leachate over the water irrigation treatment significantly increased the concentrations of these elements in leaf, woody, and root tissue. Across all genotypes, Na^+ levels were greatest in the roots, and Cl^- levels were greatest in the leaves.

The specific responses to and adaptations for salt stress of *Populus* species has not been well documented (Neuman et al., 1996). The visual damage to vegetative growth at the time of harvest in the current study ranged from no apparent salt stress to heavy defoliation, with an overall mortality rate of 22% that ranged from 6% (NM6) to 56% (NC13460). This variation in the response to salt stress is similar to that reported for other non-halophytes (Greenway and Munns, 1980; Munns and Termaat, 1986). In addition, horticultural and agricultural crop species used for the production of food and forage have been evaluated for salt tolerance more often

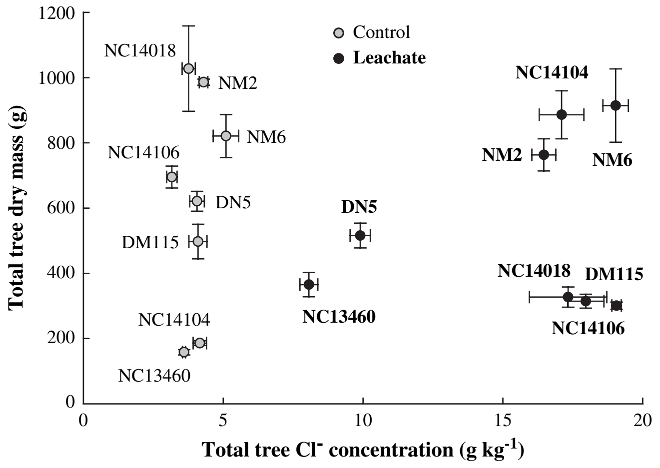

Fig. 4. Total tree chloride concentration versus biomass production of eight *Populus* clones irrigated with well water (control) or landfill leachate for two growing seasons. Error bars represent one standard error of the mean ($n = 9$).

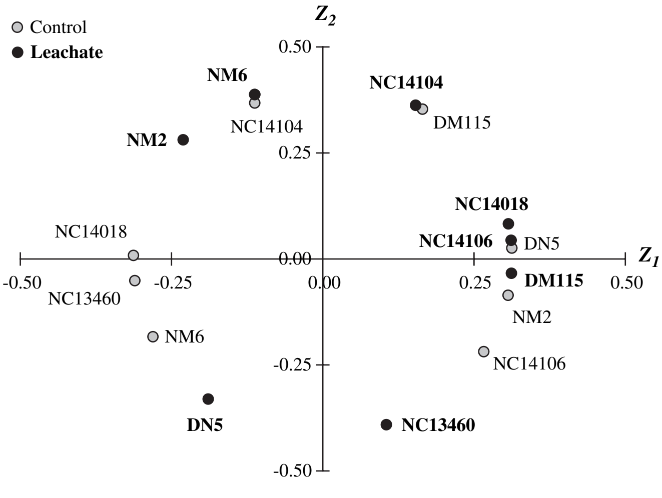

Fig. 5. Plot of first two principal components (Z_1, Z_2; 100% of variation) for total tree chloride concentration of eight *Populus* clones irrigated with well water (control) or landfill leachate for two growing seasons ($n = 9$).

than forest species, including SRWC, and have exhibited similar variability (Allen et al., 1994).

The genetic variation present in the genus *Populus* influences the ability of different genotypes to tolerate elevated levels of Na^+ and Cl^- in the rhizosphere. Variation in tissue Na^+ and Cl^- concentrations has resulted from the ability of the trees to exclude, compartmentalize, or translocate salts in an effort to reduce negative effects on growth (Neuman et al., 1996). In general, the initial tree responses to increased tissue Na^+ and Cl^- levels were decreased leaf growth and a corresponding increased root:shoot ratio (Munns and Termaat, 1986). Sodium levels were greatest in the root tissue of all but one clone tested in the current study, which had the greatest proportion of Na^+ in the leaves. Clone NC14018 [(*P. trichocarpa* × *P. deltoides*) × *P. deltoides*)] had the following tissue Na^+ allocations: leaf (46%), woody (13%), and root (41%). All other clones allocated the greatest percent of total tree Na^+ to root tissue, with clones in the DM genomic group (*P. deltoides* × *P. maximowiczii*) having the highest Na^+ percent in root tissue: NC14104 (75%), NC14106 (67%), and DM115 (71%). All eight clones allocated 18% or less of the total tree Na^+ into the woody tissue. Likewise, Stewart et al. (1990) reported that D (*P. deltoides*) and DN (*P. deltoides* × *P. nigra*) clones allocated the greatest percent of total Na^+ to root tissue when irrigated with municipal wastewater.

Similar variability in Na^+ distribution to that in the current study also has been reported. For example, after 30 days of irrigation with 300 mL NaCl solution (300 mM), *P. deltoides* × *P. nigra* cv. Italica had the highest concentration of Na^+ in leaf tissue, followed by root and stem tissue. *Populus popularis* (unknown authority) and *P. euphratica* Oliv., both species from the section *Turanga*, allocated the greatest amount of Na^+ to roots, followed by leaves and stems (Chen et al., 2002). Differences in salt tolerance of these three genotypes was likely the result of salt exclusion. *Populus euphratica* expressed the greatest ability to restrict Na^+ movement in the xylem, which is an important mechanism for the salt tolerance of woody species (Maas, 1993). Likewise, Chen et al. (2003) irrigated *P. euphratica* and *P. tomentosa* Carrière, section *Populus*, with NaCl for 20 days and reported the following total tree Na^+ allocation: *P. euphratica*, leaf (40%), stem (27%), and root (33%); *P. tomentosa*, leaf (36%), stem (30%), and root (34%).

A substantial long-term response to increased tissue Na^+ levels includes the abscission of older leaves that generally have higher salt concentrations than younger leaves, which was observed to some extent in the current study. Leaf abscission in response to long-term translocation of salts may be the cause of reduced biomass, which is often a yield-limiting factor due to decreased assimilation of carbon (Munns and Termaat, 1986). For this reason, an increase in salt tolerance is often related to decreased amounts of salts being translocated to leaves, whereby the plant is able to preserve more biomass in photosynthetic tissue. Thus, the whole plant response supports the assimilation of carbon for growth with the production of new leaves at a higher rate than the loss of old leaves (Munns and Termaat, 1986).

In general, several negative effects on plant growth occur due to increased Na^+ and Cl^-, including osmotic effects and water stress, nutrient and ion imbalance, and toxic effects on plant processes such as decreased photosynthesis and stomatal conductance (Lessani and Marschner, 1978; Neuman et al., 1996). One or more of these impacts may dominate and cause a physiological stress within the plant. However, some plants have adaptations to decrease the impact from the associated increase in ions. For example, a common stress response is the movement of salt ions into vacuoles in order to compartmentalize and translocate salts to aerial portions of the plant (Lessani and Marschner, 1978).

Of the movement of Na^+ and Cl^- in the plant, Na^+ translocation is generally under tighter root regulation than Cl^-. In contrast, Cl^- generally dominates in the shoot (Lessani and Marschner, 1978). In a study of seven crop species, applications of NaCl increased the foliar concentration of Cl^- over foliar Na^+ levels in all but one species [sugar beet (*Beta vulgaris* L.)], which had a 1:1 ratio of Na^+ to Cl^- (Lessani and Marschner, 1978). Likewise, all clones in the current study sequestered the majority of Cl^- in the leaf tissue. Leaves of clone NM2 had the highest percent of total tree Cl^- (73%) and NM6 had the second highest percent (70%), despite that the total amount of Cl^- in NM6 leaf tissue was 10% greater than that of NM2. Likewise, Zalesny and Bauer (2007) reported the greatest leaf Cl^- concentration for NM (*P. nigra* × *P. maximowiczii*) clones relative to DN genotypes. Similar trends in Cl^- distribution to that exhibited in the current study also have been reported. For example, after 30 days of irrigation with 300 mL of NaCl solution (300 mM), Italica had the highest concentration of Cl^- in leaf tissue, followed by root and stem tissue. *Populus popularis* and *P. euphratica* allocated the greatest amount of Cl^- to leaves, followed by roots and stems (Chen et al., 2002). Likewise, Chen et al. (2003) irrigated *P. euphratica* and *P. tomentosa* with NaCl for 20 days and reported the following total tree Cl^- allocation: *P. euphratica*, leaf (54%), stem (27%), and root (19%); *P. tomentosa*, leaf (55%), stem (29%), and root (16%).

Salt tolerance did not appear to be strictly related to salt uptake and distribution or biomass accumulation. There were three broad response categories of the eight clones for the relationship between total tree Cl^- concentration and total tree biomass. First, NC14104, NM2, and NM6 had elevated levels of total tree Cl^- concentration along with increased biomass. This relationship may have been the result of high ionic concentrations not reducing growth of these clones in relation to the other clones or in relation to the control, which might have occurred by compartmentalization and sufficient growth rates that replaced abscised foliage. Second, NC14018, NC14106, and DM115 exhibited elevated levels of total tree Cl^- concentration along with decreased biomass, suggesting that high concentrations of Cl^- did have a negative impact on growth compared to the other clones and the control. This impact most likely was due to osmotic effects and water stress, nutrient and ion imbalance, and/or toxic effects on plant processes. Additionally, premature leaf abscission may have reduced photosynthetic area and the ability of the plant to produce carbon compounds necessary for biomass accumulation. Third, NC13460 and DN5 exhibited mid levels of total tree Cl^- concentration and biomass, suggesting the ability of the plant to cope with the stress imposed by salts on plant processes at a cost of carbohydrates or growth inhibiting processes such as decreased photosynthesis and stomatal conductance.

The broad genetic variation that is the hallmark of the genus *Populus* may offer opportunities for introducing salt tolerance into breeding programs. However, despite variation among *Populus* genotypes in salt tolerance, the specific physiological response mechanisms are poorly understood. Therefore, there is an overwhelming need for genotypic screening among genetically distinct genomic groups and clones in order to determine levels of tolerance to salinity that are highly positively correlated with measurements of growth and yield, as well as, tissue concentrations of ions (Allen et al., 1994). Proper genotypic selection is necessary in order to select clones that perform well over a broad range of contaminants or that exhibit elevated phytoaccumulation potential for specific elements (Zalesny and Bauer, 2007). Selection of favorable clones is important for managed forests from economical and biological standpoints. A failed plantation depletes valuable resources associated with time, personnel, travel, and materials and supplies, while lengthening the time period to effective site remediation. Overall, differences that occur among clones are due to rate of uptake, salt retention in the roots, restricted translocation to the shoots (basipetally via xylem), and retranslocation back to the roots (acropetally via phloem) (Lessani and Marschner, 1978).

Biomass production is generally increased with irrigation. However, negative impacts to plant tissues and soil health need to be considered when utilizing a waste product such as high-salinity landfill leachate as the irrigation source (Neuman et al., 1996). The soil data collected before planting and at the time of harvest in the current study illustrated the importance of monitoring soil impacts from irrigation with leachate. Given that salt additions have the potential to alter the chemical, physical, and biological quality of soil after irrigation for lengthy periods (Bañuelos et al., 1999), it is especially meaningful in future studies to assess the amount of Na^+ and Cl^- that is lost through leaching, which may impact groundwater, to perform deeper soil sampling, and to test the release of salt into the soil from abscised leaves. Similar concerns with heavy metal concentrations in leaves also have been reported (Laureysens et al., 2004).

5. Conclusion

The impacts of soil salinity on ecosystem health are not as widespread in Wisconsin relative to other areas of North America. However, human activities have introduced increased salts into areas desired for plant growth, such as roadsides impacted from deicing salts (Sucoff et al., 1975), areas where Cl^- contributes to pollution due to agricultural irrigation (Stites and Kraft, 2001), and sites utilizing specialized irrigation regimes such as municipal solid waste landfills. Projects of this nature will benefit from *Populus* clones that are able to tolerate and sequester high amounts of Na^+ and Cl^- in leaf, woody, and root tissue. Clones NC14104, NM2, and NM6 exhibited high salt concentrations and biomass growth over two growing seasons, thereby expressing the necessary economical (woody biomass) and environmental (uptake) response for managed experimental plantations. Given the genetic variability among *Populus* clones, similar phytoaccumulation effectiveness is possible on other sites and with other high-salinity inputs.

Acknowledgements

This research was funded by Iowa State University, Department of Natural Resource Ecology and Management, US Forest Service Northern Research Station, Research Work Unit RWU-NRS-4158, and a Grant-in-Aid of Research from Sigma Xi, the Scientific Research Society. We are grateful to Neil Nelson for continual support throughout all stages of the project. In addition, we acknowledge the following people for assistance during field, greenhouse, and laboratory work: Edmund Bauer, Patricia Bauer, Daniel Baumann, Bruce Birr, David Coyle, Jennifer Crozier, Raymond Lange, JoAnne Lund, Paula Marquardt, and Nicanor Saliendra. Also, we appreciate review of earlier versions of the manuscript from: Gary Bañuelos, Edmund Bauer, Jud Isebrands, Neil Nelson, Thomas Schmidt, and Robert van den Driessche.

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