

CHLORIDE AND SODIUM UPTAKE POTENTIAL OVER AN ENTIRE ROTATION OF *POPULUS* IRRIGATED WITH LANDFILL LEACHATE

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There is a need for information about the response of Populus genotypes to repeated application of high-salinity water and nutrient sources throughout an entire rotation. We have combined establishment biomass and uptake data with mid- and full-rotation growth data to project potential chloride (Cl^-) and sodium (Na^+) uptake for 2- to 11-year-old Populus in the north central United States. Our objectives were to identify potential levels of uptake as the trees developed and stages of plantation development that are conducive to variable application rates of high-salinity irrigation. The projected cumulative uptake of Cl^- and Na^+ during mid-rotation plantation development was stable 2 to 3 years after planting but increased steadily from year 3 to 6. Year six cumulative uptake ranged from 22 to 175 $\text{kg Cl}^- \text{ ha}^{-1}$ and 8 to 74 $\text{kg Na}^+ \text{ ha}^{-1}$, while annual uptake ranged from 8 to 54 $\text{kg Cl}^- \text{ ha}^{-1} \text{ yr}^{-1}$ and 3 to 23 $\text{kg Na}^+ \text{ ha}^{-1} \text{ yr}^{-1}$. Full-rotation uptake was greatest from 4 to 9 years (Cl^-) and 4 to 8 years (Na^+), with maximum levels of Cl^- (32 $\text{kg ha}^{-1} \text{ yr}^{-1}$) and Na^+ (13 $\text{kg ha}^{-1} \text{ yr}^{-1}$) occurring in year six. The relative uptake potential of Cl^- and Na^+ at peak accumulation (year six) was 2.7 times greater than at the end of the rotation.

KEY WORDS salt accumulation, hybrid poplar, wastewater recycling, sustainable forestry, short rotation woody crops

INTRODUCTION

There is a growing opportunity to incorporate sustainable recycling of wastewaters as irrigation and fertilization for alternative biomass-feedstock production systems (Shrive, McBride, and Gordon, 1994; Erdman and Christenson, 2000; Zalesny and Bauer, 2007b). However, the success of such systems, including short rotation woody crops (e.g., *Populus* and *Salix*), is largely based upon the selection of genotypes that are productive on the sites where they are deployed (Riemenschneider *et al.*, 2001), as well as the response of such genotypes to contaminants present in the wastewaters (Zalesny and Bauer, 2007a). Although chloride (Cl^-) and sodium (Na^+) are constituents of wastewaters often requiring regulation, there is limited information about the long-term response of *Populus* to salinity (Neuman *et al.*, 1996). The north central United States does not have inherent soil salinity problems

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relative to regions such as the western United States (Bañuelos *et al.*, 1999; Shannon *et al.*, 1999). Yet wastewaters, including landfill leachate (Stephens, Tyrrel, and Tiberghien, 2000; Kjeldsen *et al.*, 2002), whey (a major liquid byproduct of the dairy industry) (Marwaha and Kennedy, 1988; Gonzalez-Siso, 1996), and runoff from road deicing salts (Sucoff, Feller, and Kanten, 1975; Godwin, Hafner, and Buff, 2003), pose substantial environmental and economic problems in many areas, including the north central region. Therefore, it is pertinent for long-term regional sustainability to assess phytotechnologies that reduce the ecological impact of high-salinity wastewaters, along with decreasing the costs (*e.g.*, money and carbon emissions) associated with proper treatment and disposal in rural and urban areas (Wong and Leung, 1989; Duggan, 2005).

To address this concern, we irrigated and fertilized *Populus* trees with municipal solid waste landfill leachate during plantation establishment (two years) to assess the potential phytoaccumulation capability of differing clones (Zalesny *et al.*, 2007a; 2007b; 2008). Unfortunately, data are completely lacking about the cumulative response of these and similar genotypes to long-term (*i.e.*, rotation age) exposure to high-salinity water and nutrient sources. There is a growing need for such information for regulators and resource managers so that decisions can be made about acceptable Cl^- and Na^+ thresholds. Therefore, in the current study, we combined our establishment biomass and accumulation data with growth data from mid-rotation (Riemenschneider *et al.*, 2001) and full-rotation (Netzer *et al.*, 2002) regional field testing programs to project the potential Cl^- and Na^+ uptake throughout an entire rotation (*i.e.*, 2 to 11 years) in the north central United States. Our objectives were to identify potential levels of uptake as the trees developed and stages of plantation development that are conducive to variable application rates. This information is vital for wastewater resource planning in order to adjust the cumulative quantities of contaminants recycled, thereby maintaining environmental quality, and local and regional agriculture, forestry, and recreation resources.

MATERIALS AND METHODS

Establishment Data

Phyto-recurrent selection (Zalesny and Bauer, 2007b) consisting of four cycles of greenhouse and field studies was conducted in 2005 and 2006 to determine favorable *Populus* genotypes that combined elevated biomass accumulation with chloride (Cl^-) / sodium (Na^+) uptake into leaf, woody (stems + branches), and root tissues. Trees were irrigated with fertilized well water (control) or municipal solid waste landfill leachate. Nitrogen, phosphorus, and potassium were added to the water at a rate equal to the leachate. Eight of the original 25 clones (Table 1) were selected for field-testing after the first three cycles in the greenhouse (Zalesny *et al.*, 2007a). Cycle 4 consisted of two growing seasons at the Oneida County Landfill located 6 km from Rhinelander, Wisconsin, USA (45.6°N, 89.4°W). The trees were harvested on 18 August 2006 to assess growth and biomass (Zalesny *et al.*, 2007b), as well as uptake of Cl^- and Na^+ into leaf, woody, and root tissue (Zalesny *et al.*, 2008). These biomass and accumulation data were the basis for the projections of cumulative and annual uptake described below.

In cycle 4, the trees were grown in a split plot design at a spacing of 1.2×2.4 m (*i.e.*, 3472 trees ha^{-1}), with eight trees (*i.e.*, replications) for each combination of two irrigation treatments (whole plots; water or leachate) and eight clones (sub plots). The three tissues (sub sub plots) were added as a factor in the split split plot model used for analysis. Only

Table 1 Genomic groups and clones of *Populus* compared in the current study.

Genomic group ^a	(Establishment, 2 yrs) Zalesny <i>et al.</i> (2008)	(Mid-rotation, 2 to 6 yrs) Riemenschneider <i>et al.</i> (2001)	(Full-rotation, 3 to 11 yrs) Netzer <i>et al.</i> (2002)
(<i>P. trichocarpa</i> × <i>P. deltoides</i>) × <i>P. deltoides</i>	NC13460, NC14018	—	—
<i>P. deltoides</i> × <i>P. maximowiczii</i>	DM115, NC14104, NC14106	Belgian 25	—
<i>P. deltoides</i> × <i>P. nigra</i>	DN5 ^b	DN34	DN17, DN34, DN182
<i>P. nigra</i> × <i>P. maximowiczii</i>	NM2, NM6	NM6	—

Uptake and biomass data are from plantation establishment during landfill leachate irrigation, while tree diameter and estimated biomass data are from identical genomic groups utilized in regional field testing programs in the north central United States through mid- and full-rotation.

^aAuthorities for the species are: *P. deltoides* Bartr. ex Marsh; *P. trichocarpa* Torr. & Gray; *P. nigra* L., *P. maximowiczii* A. Henry.

^bCommercial check clone.

results from the leachate irrigation treatment are presented in the current study, because the concentrations of Cl[−] and Na⁺ were negligible in the water treatment. In addition, we are interested in practical implications of irrigating the trees with high-salinity wastewaters rather than traditional water and nutrient regimes. Overall, 1.9 kL of leachate was applied in 2005, with the volume increasing to 17.4 kL in 2006. At the end of the experiment, the leachate had an electrical conductivity of 9.4 ± 0.2 mS cm^{−1}, a pH of 8.4 ± 0.2, and elemental concentrations of 1250 mg Cl[−] L^{−1} and 1200 mg Na⁺ L^{−1}.

The aforementioned biomass and accumulation data were input into the following model to estimate Cl[−] and Na⁺ uptake during establishment (for simplicity, only Cl[−] is listed here):

Cl[−] Uptake = Tissue Biomass × Concentration (Sample) × Conversion Factor × Stocking

$$\left(\frac{\text{kg Cl}^-}{\text{ha}}\right) = \left[\left(\frac{\text{kg biomass}}{\text{tree}}\right) \times \left(\frac{\text{g Cl}^-}{\text{kg}}\right) \times \left(\frac{\text{kg}}{1000 \text{ g}}\right) \times \left(\frac{3472 \text{ trees}}{\text{ha}}\right)\right], \tag{1}$$

where Cl[−] uptake = total mass of Cl[−] in tissue per unit land area; tissue biomass = total tree (leaf + woody + root) biomass of establishment data or woody (stem + branch) biomass of mid- and full-rotation data; concentration (sample) = Cl[−] concentration in tissue sample; conversion factor = number of grams per kilogram; and stocking = number of trees per unit land area.

Mid-Rotation Data

Riemenschneider *et al.* (2001) described mid-rotation results at year six only of a *Populus* regional field-testing program in the north central United States. Fifty-nine clones belonging to six unique genomic groups were planted in 1995 at Ames, Iowa (42.0 °N, 93.6 °W), Arlington, Wisconsin (43.3 °N, 89.4 °W), and Westport, Minnesota (45.7 °N, 95.2 °W). Only clone NM6 was common to Zalesny *et al.* (2008) and Riemenschneider *et al.* (2001). Therefore, we used biomass data from their clone Belgian 25 to estimate uptake of

our NC14104, because both belong to the *P. deltoides* × *P. maximowiczii* genomic group (Table 1). Likewise, clone DN34 was used for our DN5 (*P. deltoides* × *P. nigra*; commercial check clone). Riemenschneider *et al.* (2001) used diameter at breast height (dbh) to estimate woody (stem + branch) biomass in a model developed following destructive aboveground harvests of multiple genotypes across sites ($r^2 = 0.98$, $P < 0.0001$, $n = 152$) (Hansen, 1992):

$$\text{Woody Biomass} = [6.16 - (2.23 \times \text{dbh}) + (0.353 \times \text{dbh}^2)]. \quad (2)$$

Additional data from years 2 to 5 (R.S. Zalesny Jr., U.S. Forest Service; unpublished data) were added to the dataset to get a complete analysis for years 2 to 6 of plantation development. We estimated cumulative mid-rotation Cl^- and Na^+ uptake according to equation (1) using the woody (stem + branch) biomass estimated from equation (2), establishment woody concentration data described above, and a reported stocking of 1075 trees ha^{-1} (3.05×3.05 m spacing) (Riemenschneider *et al.*, 2001). We used subtraction to estimate annual mid-rotation Cl^- and Na^+ uptake (*i.e.*, Year 3 Current Uptake = Year 3 Uptake–Year 2 Uptake, etc.).

Full-Rotation Data

Netzer *et al.* (2002) described the results of a *Populus* regional field-testing program in the north central United States. One hundred forty clones belonging to 30 unique genomic groups were tested across 15 locations. For the current study, we used their full-rotation results (years 3 to 11) from the mean of three *P. deltoides* × *P. nigra* clones (DN17, DN34, DN182) (Table 1) that were planted in 1987 at Ashland, Wisconsin (46.6 °N, 90.9 °W), Mondovi, Wisconsin (44.6 °N, 91.7 °W), Milaca, Minnesota (45.8 °N, 93.7 °W), Granite Falls, Minnesota (44.8 °N, 95.5 °W), Fargo, North Dakota (46.9 °N, 96.8 °W), and Sioux Falls, South Dakota (43.6 °N, 96.7 °W). We used the mean across sites of their data for clones DN17, DN34, and DN182 to estimate uptake of our clones NM6, NC14104, and DN5. Netzer *et al.* (2002) used dbh to estimate woody (stem + branch) biomass using the model presented in equation (2). Overall, we estimated cumulative full-rotation Cl^- and Na^+ uptake according to equation (1) using the mean woody biomass across the six sites, establishment woody concentration data described above, and a reported stocking of 1680 trees ha^{-1} (2.44×2.44 m spacing) (Netzer *et al.*, 2002). As with our mid-rotation analysis, we used subtraction to estimate annual full-rotation Cl^- and Na^+ uptake.

RESULTS

Chloride (Cl^-)

Establishment. The percent of Cl^- from municipal solid waste landfill leachate that accumulated in the total tree biomass (leaf + woody + root) following two years of establishment ranged from 3% (NC13460) to 21% (NM6), with a mean of 10% (Table 2). Only clones NM6 (218%), NC14104 (192%), and NM2 (151%) had above average Cl^- uptake. The remaining genotypes segregated into two response groups, with DM115, NC14018, and NC14106 accumulating nearly twice as much Cl^- as DN5 and NC13460.

Table 2 Percent of chloride (Cl^-) and sodium (Na^+) from municipal solid waste landfill leachate in the total tree biomass (leaf + woody + root) of eight *Populus* clones irrigated for two growing seasons in northern Wisconsin

Clone	Percent Cl^- removed ^a		Clone	Percent Na^+ removed ^a	
	Absolute	Relative to mean		Absolute	Relative to mean
NM6	21	218	NM6	6	202
NC14104	18	192	NC14104	5	164
NM2	14	151	NM2	5	146
DM115	7	77	NC14018	3	96
NC14018	7	70	DM115	2	56
NC14106	6	67	NC14106	2	49
DN5	4	45	NC13460	2	48
NC13460	3	28	DN5	1	47
Mean	10		Mean	3	

^aA total of 96 kg $\text{Cl}^- \text{ ha}^{-1}$ and 92 kg $\text{Na}^+ \text{ ha}^{-1}$ was applied.

Mid-Rotation. The projected cumulative uptake of Cl^- during mid-rotation plantation development was stable from 2 to 3 years after planting but increased steadily from year 3 to 6 (Figure 1). Cumulative chloride uptake was greatest at Arlington and least at Westport, with clone NM6 consistently accumulating the most Cl^- and DN5 the least. Across sites, uptake levels at year six were 32 (DN5), 60 (NC14104), and 117 kg $\text{Cl}^- \text{ ha}^{-1}$ (NM6), while the overall range for specific combinations of sites and clones was 22 (DN5, Westport) to 175 kg $\text{Cl}^- \text{ ha}^{-1}$ (NM6, Arlington) (Figure 1). The projected annual uptake of Cl^- was highly variable, with optimal mid-rotation levels dependent upon specific clone $\times$ site interactions from year 3 to 6 (Table 3). Annual chloride uptake was greatest at Westport for three-year-old trees, while trees at Arlington accumulated the most Cl^- during the fourth and fifth growing seasons. Ames had the most Cl^- uptake in year six. Clone NM6 generally accumulated the most Cl^- and DN5 the least, with NM6 being the only genotype to have increasing levels as the plantations developed (Table 3). Across sites, annual uptake levels at year six were 8.6 (DN5), 13.0 (NC14104), and 39.6 kg $\text{Cl}^- \text{ ha}^{-1} \text{ yr}^{-1}$ (NM6), while the overall range for specific combinations of sites and clones was 7.5 (DN5, Westport) to 53.7 kg $\text{Cl}^- \text{ ha}^{-1} \text{ yr}^{-1}$ (NM6, Arlington).

Full-Rotation. The projected cumulative uptake of Cl^- during full-rotation plantation development followed a sigmoidal curve that mirrors mean annual growth increment in forest trees. The greatest accumulation of Cl^- occurred during the period from year 4 to 9 (Figure 2). At the end of the rotation (year 11), Cl^- uptake was greatest for clone NM6 (200 kg $\text{Cl}^- \text{ ha}^{-1}$) and least for DN5 (81 kg $\text{Cl}^- \text{ ha}^{-1}$), with NC14104 (136 kg $\text{Cl}^- \text{ ha}^{-1}$) exhibiting a level similar to the mean. Likewise, the projected annual uptake of Cl^- followed a bell-shaped curve that typically represents current annual growth increment in forest trees (Figure 3). Maximum levels of Cl^- uptake were exhibited in year six: 13 (DN5), 22 (NC14104), and 32 kg $\text{Cl}^- \text{ ha}^{-1} \text{ yr}^{-1}$ (NM6). The relative uptake potential at peak accumulation (year six) for all clones was 2.7 times greater than at the end of the rotation.

Sodium (Na^+)

Establishment. The percent of leachate Na^+ accumulated in the total tree biomass (leaf + woody + root) following two years of establishment ranged from 1% (DN5) to

Table 3 Projected annual uptake of chloride (Cl^-) and sodium (Na^+) into the woody biomass of three *Populus* clones

Clone	$\text{kg Cl}^- \text{ ha}^{-1} \text{ yr}^{-1}$				$\text{kg Na}^+ \text{ ha}^{-1} \text{ yr}^{-1}$			
	Tree age (yrs)							
	3	4	5	6	3	4	5	6
Ames, Iowa								
NM6	3.4	25.7	26.0	34.9	1.4	10.8	10.9	14.6
NC14104	3.7	19.7	22.9	14.6	2.1	10.9	12.7	8.1
DN5	1.5	9.9	7.2	9.4	0.6	3.7	2.7	3.5
Mean ^a	2.9	18.3	17.9	18.4	1.3	8.3	8.1	8.4
Arlington, Wisconsin								
NM6	3.7	48.6	54.6	53.7	1.6	20.4	22.9	22.5
NC14104	2.7	28.9	12.2	10.5	1.5	16.0	6.7	5.8
DN5	0.4	11.7	14.3	8.9	0.2	4.4	5.3	3.3
Mean	2.0	27.8	21.4	7.2	0.9	12.6	9.7	3.3
Westport, Minnesota								
NM6	3.5	18.3	18.8	30.2	1.5	7.7	7.9	12.7
NC14104	4.3	11.9	10.3	13.9	2.4	6.6	5.7	7.7
DN5	1.5	5.7	4.8	7.5	0.5	2.1	1.8	2.8
Mean	3.1	11.5	10.6	16.1	1.4	5.2	4.8	7.3
Across sites								
NM6	3.6	30.9	33.2	39.6	1.5	13.0	13.9	16.6
NC14104	3.6	20.2	15.1	13.0	2.0	11.1	8.4	7.2
DN5	1.1	9.1	8.7	8.6	0.4	3.4	3.3	3.2
Mean	2.7	19.2	17.6	17.4	1.2	8.7	8.0	7.9

Projected uptake is based on mid-rotation diameter and estimated biomass at three sites in the north central United States.

^aMean projected annual uptake based on mean Cl^- and Na^+ uptake during establishment (equation 1) and mean woody biomass (equation 2), not the mean of projected annual uptake across clones.

6% (NM6), with a mean of 3% (Table 2). Only clones NM6 (202%), NC14104 (164%), and NM2 (146%) surpassed the mean, while NC14018 was nearly identical (96%). The remaining genotypes removed similar percentages, ranging from 47% (DN5) to 56% (DM115) of the mean.

Mid-Rotation. The projected cumulative uptake of Na^+ during mid-rotation plantation development was stable from 2 to 3 years after planting, but increased steadily from year 3 to 6 (Figure 4). Cumulative sodium uptake was greatest at Arlington and least at Westport, with clone NM6 consistently accumulating the most Na^+ and DN5 the least. Across sites, uptake levels at year six were 12 (DN5), 33 (NC14104), and 49 $\text{kg Na}^+ \text{ ha}^{-1}$ (NM6), while the overall range for specific combinations of sites and clones was 8 (DN5, Westport) to 74 $\text{kg Na}^+ \text{ ha}^{-1}$ (NM6, Arlington) (Figure 4). The projected annual uptake of Na^+ was highly variable, with optimal mid-rotation levels dependent upon specific clone $\times$ site interactions from year 3 to 6 (Table 3). Annual sodium uptake was greatest at Westport for 3-year-old trees, while trees at Arlington accumulated the most Na^+ during the fourth and fifth growing seasons. Ames had the most Na^+ uptake in year six. Clone NM6 generally accumulated the most Na^+ and DN5 the least, with NM6 being the only genotype to have steadily increasing levels as the plantations developed (Table 3). Across sites, annual uptake levels at year six were 3.2 (DN5), 7.2 (NC14104), and 16.6 $\text{kg Na}^+ \text{ ha}^{-1} \text{ yr}^{-1}$ (NM6), while

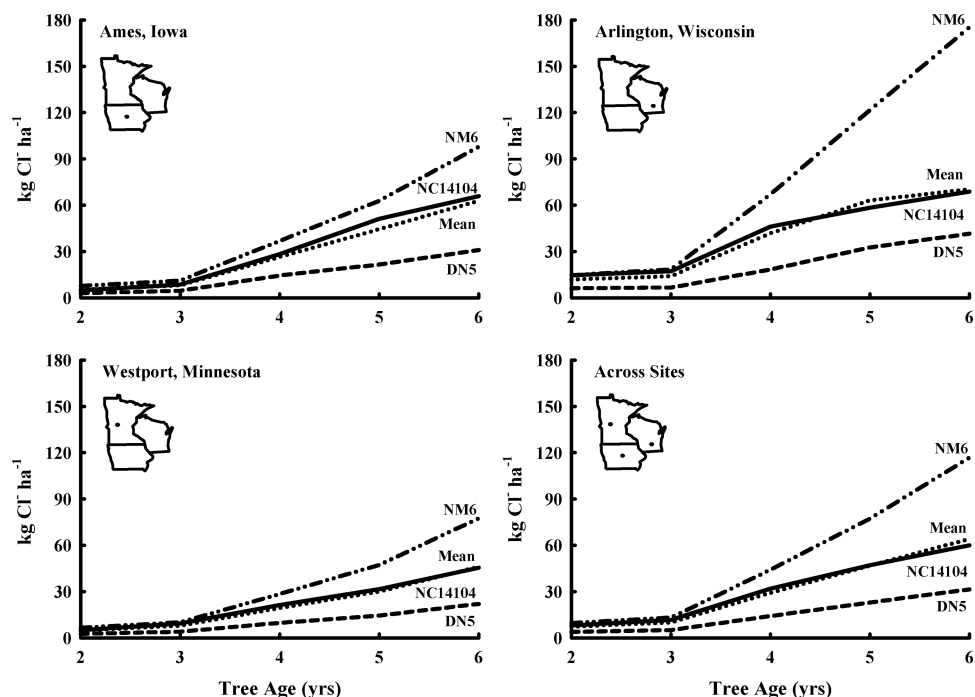


Figure 1 Projected cumulative uptake of chloride (Cl^-) into woody biomass of three *Populus* clones, based on mid-rotation diameter and estimated biomass at three sites in the north central United States.

the overall range for specific combinations of sites and clones was 2.8 (DN5, Westport) to 22.5 $\text{kg Na}^+ \text{ha}^{-1} \text{yr}^{-1}$ (NM6, Arlington).

Full-Rotation. The projected cumulative uptake of Na^+ during full-rotation plantation development followed a sigmoidal curve that mirrors mean annual growth increment in forest trees. The greatest accumulation of Na^+ occurred during the period from year 4 to 8 (Figure 2). At the end of the rotation (year 11), Na^+ uptake was greatest for clones NM6 (84 $\text{kg Na}^+ \text{ha}^{-1}$) and NC14104 (75 $\text{kg Na}^+ \text{ha}^{-1}$), with DN5 (30 $\text{kg Na}^+ \text{ha}^{-1}$) exhibiting below-average accumulation potential. Likewise, the projected annual uptake of Na^+ followed a bell-shaped curve that typically represents current annual growth increment in forest trees (Figure 3). Maximum levels of Na^+ uptake were exhibited in year six: 5 (DN5), 12 (NC14104), and 13 $\text{kg Na}^+ \text{ha}^{-1} \text{yr}^{-1}$ (NM6). The relative uptake potential at peak accumulation (year six) for all clones was 2.7 times greater than at the end of the rotation.

DISCUSSION

Phytotechnologies that merge intensive forestry strategies with waste management practices are suitable to many regions of the world, including the north central United States (Mirck *et al.*, 2005). In addition to phytoremediation of contaminants in wastewaters such as landfill leachate, secondary benefits from these systems include carbon sequestration, erosion control, aesthetic improvement, wildlife habitat, and woody biomass production for fiber and energy (Duggan, 2005). Results of the current study can be used to estimate

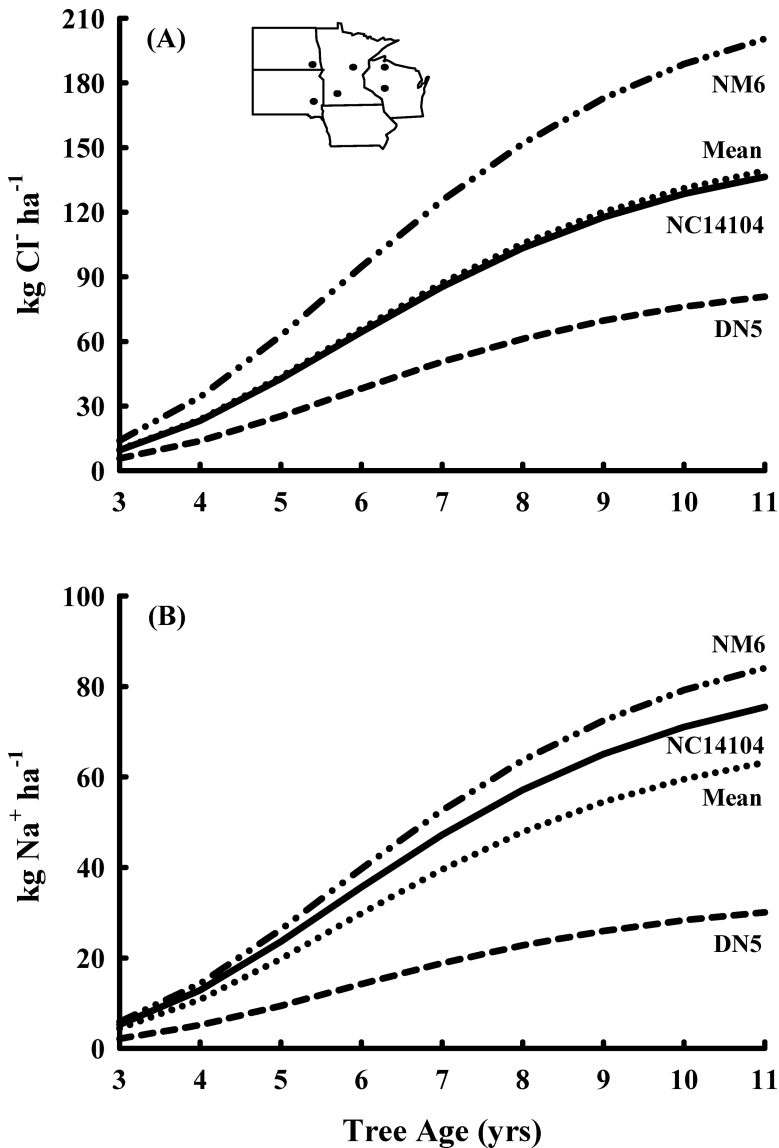


Figure 2 Projected cumulative uptake of chloride (Cl^-) (A) and sodium (Na^+) (B) into woody biomass of three *Populus* clones, based on full-rotation diameter and estimated biomass across six sites in the north central United States. Note different scales of the y-axes.

field-scale thresholds for chloride (Cl^-) and sodium (Na^+) uptake that are needed by resource managers and regulators to fulfill compliance requirements, and by researchers interested in testing long-term responses of *Populus* to salts.

In the north central United States, the response of *Populus* to high-salinity irrigation has not been well studied over an entire rotation, and growth suppression due to high ion concentration may introduce limitations to the projected field-scale uptake of Cl^- and Na^+ into tree tissues. In general, *Populus* genotypes are variably affected by salt accumulation,

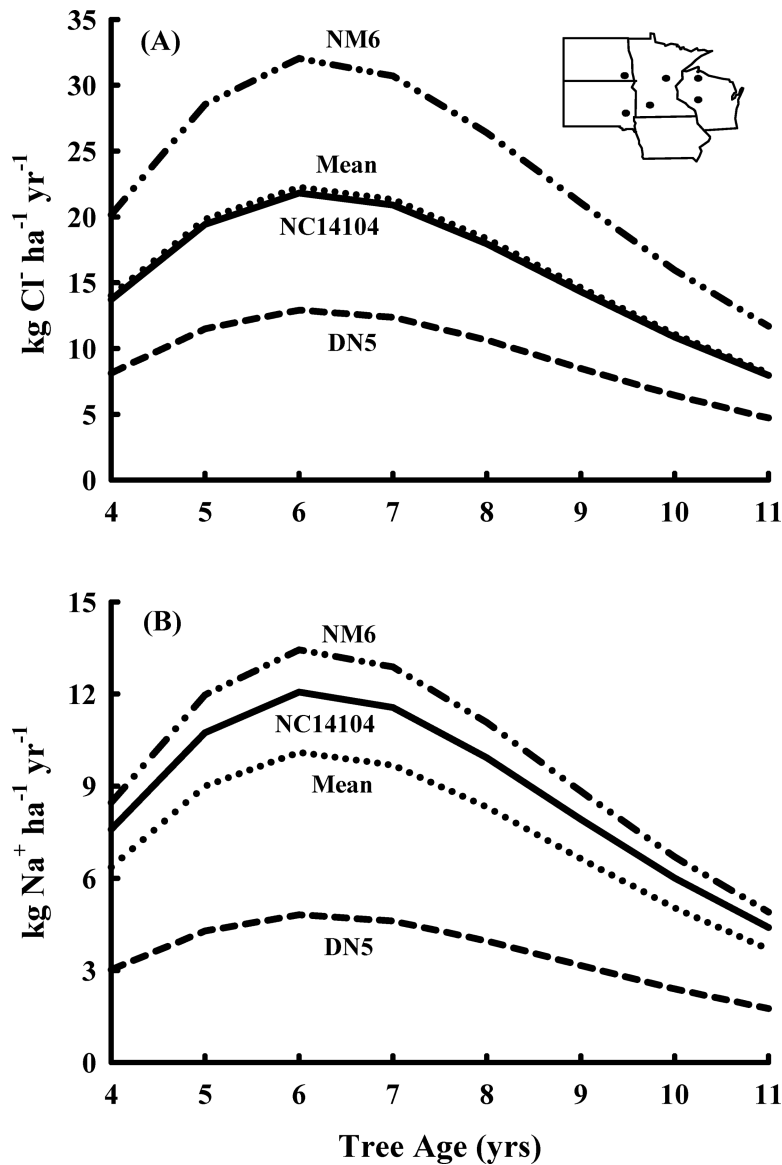


Figure 3 Projected annual uptake of chloride (Cl⁻) (A) and sodium (Na⁺) (B) into woody biomass of three *Populus* clones, based on full-rotation diameter and estimated biomass across six sites in the north central United States. Note different scales of the y-axes.

and such clones have produced phenotypic responses ranging from little observed stress to complete mortality (Zalesny *et al.*, 2008). Salt tolerance of adapted genotypes has resulted from salt exclusion, translocation, or compartmentalization within cell vacuoles (Lessani and Marschner, 1978; Neuman *et al.*, 1996). In contrast, increased Cl⁻ and Na⁺ concentration creates osmotic imbalance, water stress, and toxic effects that are disruptive to physiological life-supporting processes (Stephens *et al.*, 2000). For example, elevated Na⁺ concentration in the leaves produces symptoms, including tissue chlorosis and leaf

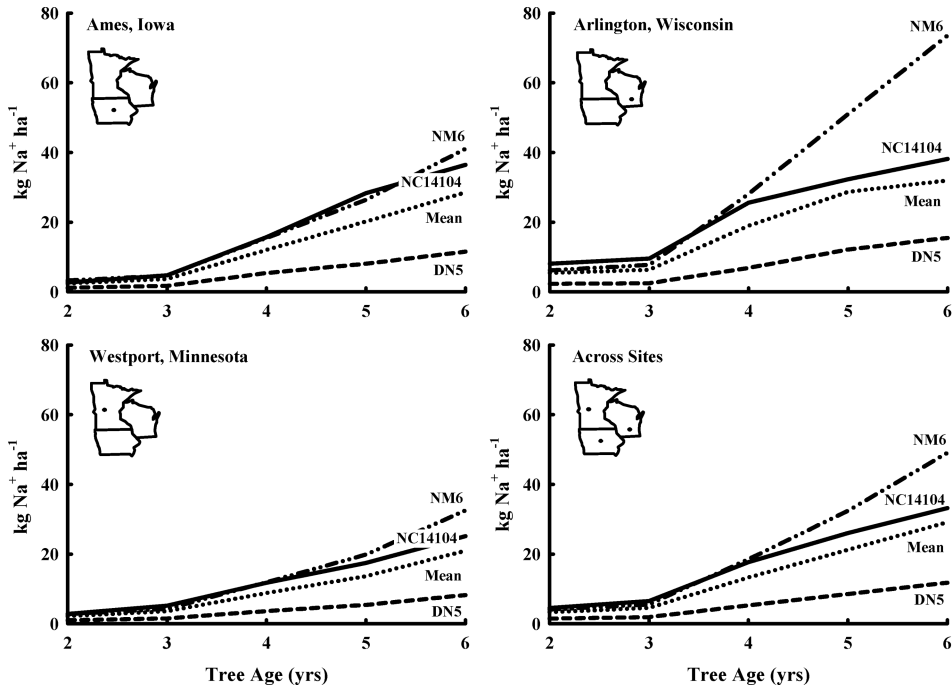


Figure 4 Projected cumulative uptake of sodium (Na^+) into woody biomass of three *Populus* clones, based on mid-rotation diameter and estimated biomass at three sites in the north central United States.

abscission, that lead to loss of photosynthetic area, decreased carbon assimilation, and reduced biomass accumulation (Fung *et al.*, 1998). *Populus* clones may overcome some of these negative impacts, with fast growth rates that exceed the loss of abscised foliage.

In the current study, the identified uptake of Cl^- and Na^+ into woody tissue followed a bell-shaped curve (*i.e.*, current annual increment) that was greatest during year six (Figure 3). Thus, the projected results may have underestimated uptake over the entire rotation. For example, increased biomass accumulation and greater potential shoot:root ratios, especially during years 4 to 9 (Cl^-) and 4 to 8 (Na^+), would likely lead to sequestration of additional amounts of salt ions within woody tissue than was predicted from the two-year field establishment data. Moreover, drought-induced biomass reductions during both the 2005 and 2006 growing seasons may have decreased the uptake of salts into tissues due to physiological stress and reduced transpiration (Neuman *et al.*, 1996). Inadequate natural precipitation, as well as regulatory short duration discharge permits with limits on total Cl^- that could be applied, constrained the establishment irrigation regime. Furthermore, Zalesny *et al.* (2008) reported 15% of total Cl^- and 26% of total Na^+ was sequestered in the woody tissue across clones after two seasons of leachate irrigation. Ten percent and 75% of total Cl^- was in root and leaf tissue, respectively, while these values for Na^+ were 32% (root) and 42% (leaf). The concentration of Cl^- and Na^+ is typically least in the woody tissue (harvestable biomass) of young *Populus* trees. Specifically, Cl^- is generally transported out of the root and into the shoot, where it has the greatest concentration in the leaves and least in the wood. In contrast, tighter root regulation of Na^+ controls its transport from roots into leaves and woody tissue, typically restricting most Na^+ to the roots and

least to the wood (Lessani and Marschner, 1978). Therefore, relative distributional trends may also have led to conservative projections in the current study.

The genotypes evaluated (*i.e.*, NM6, NC14104, DN5) have exhibited broad variation when irrigated with well water (control) or leachate from the Oneida County Landfill (Zalesny *et al.*, 2007a; 2007b; 2008). However, variation due to simple clone effects is not surprising, given the inherent variability among species and hybrids (intra- and inter-specific) of the genus *Populus* (Rajora and Zsuffa, 1990). Therefore, it is important to assess genotype $\times$ environment interactions to understand clonal stability for the traits of interest. In the current study, such interactions included those between irrigation treatment and clone, as well as between site and clone. The irrigation treatment $\times$ clone interaction that dominated the variability associated with uptake of Cl^- and Na^+ during establishment was extensively described in Zalesny *et al.* (2008). Riemenschneider *et al.* (2001) (mid-rotation) and Netzer *et al.* (2002) (full-rotation) detailed the site $\times$ clone interactions that controlled the growth of the trees used as the source of the growth data in the current study. The practical long-term implications of the combination of these genotype $\times$ environment interactions to site managers is that it is necessary to select clones that can tolerate elevated levels of Cl^- and Na^+ , along with being well-adapted to sites of deployment.

Therefore, from a genetics standpoint, phyto-recurrent selection cycles must include multiple-trait selection strategies that involve complex phenotypic expressions associated with quantitative inheritance. Overall, the outcome of such selection should be to either match clones with specific contaminants and sites (specialists) or to deploy genotypes that are adapted to a wide range of contaminants and growing conditions (generalists) (Zalesny and Bauer, 2007a). For example, although changes in clonal rank did not exist for cumulative Na^+ uptake (Figure 4), the magnitude of uptake potential was much greater for NM6 at Arlington than either of the other sites. Therefore, of the three clones tested, NM6 was better adapted in Wisconsin, while NC14104 exhibited similar uptake potential to NM6 in Iowa and Minnesota. Furthermore, volume of irrigation/precipitation and particular soil type have a direct influence on genotype $\times$ environment interactions associated with Cl^- and Na^+ uptake potential. For example, coarse-textured soils and elevated quantities of irrigation/precipitation combined with mobility of the salt ions may cause leaching from the soil and reduced availability for uptake, especially in the early stages of plantation development when root systems are less developed (B.T. Sexton, Oneida County Solid Waste Department; personal communication). Reduced Cl^- and Na^+ availability was likely minimal for the trees of our establishment data (Zalesny *et al.*, 2007c), however, given a drought during both growing seasons. Nevertheless, it may be beneficial to only irrigate with water during the first growing season in order to avoid the potential for such leaching. Given the stunted size of the trees after the first growing season (Zalesny *et al.*, 2007b), irrigating with water only may also help to avoid potential above- and below-ground biomass reductions associated with exposure to salts in the wastewater.

Negative impacts on soil and water resources must be carefully evaluated when land-applying wastewater irrigation containing elevated levels of Cl^- and Na^+ . Routine field monitoring and leachate analyses should be conducted throughout the rotation to detect negative impacts on ecosystem health. The irrigation regime may require modifications as the chemical constituents of the leachate change over time (Kjeldsen *et al.*, 2002), along with increased plant water and nutritional needs. For example, dilution may be necessary early in the rotation when root systems are smallest and transpiration rates are lowest. However, as the trees approach the maximum annual growth rate, the irrigation volume could potentially be increased to accommodate the water and nutritional needs of the

plantation. Until now, estimates of long-term, rotation-age data from *Populus* plantations irrigated with high-salinity inputs such as municipal solid waste landfill leachate were unavailable to resource managers, regulators, and researchers throughout the north central United States. Thus, development of appropriate strategies utilizing such waste products has been complicated, especially given necessary environmental protection. The results of the current study highlight projected levels of Cl^- and Na^+ uptake, along with stages of plantation development that are conducive to variable application rates. This information will greatly support critical decision making for projects requiring compliance with regulatory standards. In addition, researchers will benefit from the field-scale, rotation-age uptake thresholds.

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