# Phytoremediation of Brine-Affected Soil with Salt-Tolerant Plants: a Screening Study

S. Gerrard<sup>1</sup>, R.E. Farrell<sup>1</sup>, J.D. Knight<sup>1</sup>

<sup>1</sup>Department of Soil Science, University of Saskatchewan, Saskatoon, SK S7N 5A8

Keywords: phytoremediation, saline soil, salt tolerance, sodium

### Abstract

Phytoremediation is an attractive alternative to traditional soil remediation for brine-affected areas. Potential phytoremediation plants must possess several key characteristics including adaptation to semi-arid climate, moderate to high salt tolerance, accumulation of Na in above ground tissues and an extensive root system to obtain Na and improve soil structure. Eighteen salt-tolerant species were identified from literature and screened for sodium uptake ability in a sand culture experiment. Plants were treated with 0, 200, 400 and 600 mM NaCl and grown for 50 d. Plants were harvested and biomass analyzed for Na content. Sea blite (*Suaeda calceoliformis*) and salt grass (*Distichlis stricta*) showed promise for phytoremediation by surviving high levels of Na while developing extensive root systems. These species did not accumulate as much Na in their aboveground tissue as less tolerant plants. However, during this short screening study, cell wall integrity was maintained and a longer study would allow plants to accumulate more biomass and Na. These two species will be tested on contaminated saline-sodic field soil to provide an understanding of sodium removal over the length of a Saskatchewan growing season.

#### Introduction

Saline and saline-sodic soils may result from brine (NaCl) spills associated with oil and gas extraction in Western Canada. High levels of soluble sodium in soil are detrimental to plant growth and survival by causing physiological drought, ion toxicity and nutrient competition (Flowers 1986). As well, poor soil structure and water infiltration, soil crusting, high electrical conductivity (EC) and sodium absorption ratio (SAR) are common symptoms of brine-contamination (De Jong 1982). These conditions can be difficult and costly to treat using conventional remediation techniques.

Phytoremediation presents an economical long-term solution with minimal inputs and site disturbance. Salt tolerant (halophytic) species that accumulate sodium in aboveground biomass have the potential to reduce soil sodium (Flowers 1986; Zhou 1991). In addition species with extensive root systems can improve soil structure and water infiltration. Nitrogen availability improves plant salt tolerance (Donovan et al. 1997); therefore, N<sub>2</sub>-fixing species which are typically non-halophytic are still worth investigating to enhance plant community health. In order to develop this system for Western Canada, potential species must be identified.

This study was designed to identify plants with the ability to remediate brine-contaminated soil. Species in four categories were identified through a literature search:  $N_2$ -fixers that are moderately salt tolerant, ubiquitous species with moderate salt tolerance, salt-excreting and salt-accumulating halophytes. Plants were treated with four levels of NaCl in a sand culture system to determine which species had the greatest phytoremediation potential.

# Methods

Seeds of 18 potential species (Table 1) were germinated in Petri dishes at (16 hours light/25° C and 8 hours dark/15° C) and transferred to 10 cm pots filled with silica sand after 2-7 d (n=4). Pots were sub-irrigated with \_ strength Hoagland's nutrient solution (Hoagland and Arnon 1950). Solution was maintained at 1.5 cm depth with distilled-deionized water. Seven d following transplanting, solution was supplemented with NaCl to increase salinity in four treatments. NaCl concentration was increased incrementally by changing solutions on alternating days (over a 24 d period) to final concentrations of 0, 200, 400 and 600 mM NaCl (corresponding to EC values of 0.25, 18, 36 and 54 dS m<sup>-1</sup>). Solutions were then changed weekly until harvest (50 d after transplant). Pots were randomized within each treatment with each solution change.

| Scientific Name         | Common Name                | Characteristic          |
|-------------------------|----------------------------|-------------------------|
| Atriplex gardneri       | Nuttall's saltbush         | Na accumulation         |
| Chenopodium salinum     | rocky mountain goosefoot   | Na accumulation         |
| Salicornia rubra        | red samphire               | Na accumulation         |
| Suaeda calceoliformis   | sea blite                  | Na accumulation         |
| Distichlis stricta      | salt grass                 | Na excretion            |
| Glaux maritima          | sea milkwort               | Na excretion            |
| Puccinellia distans     | Fult's weeping alkaligrass | Na excretion            |
| Puccinellia nuttalliana | Nuttall's alkali grass     | Na excretion            |
| Spartina gracilis       | alkali chordgrass          | Na excretion            |
| Elymus angustus         | Altai wild rye             | Na tolerant             |
| Festuca arundinaceae    | tall fescue                | Na tolerant             |
| Festuca rubra           | red fescue                 | Na tolerant             |
| Kochia scoparia         | kochia                     | Na tolerant             |
| Psathyrostachys juncea  | Russian wildrye            | Na tolerant             |
| Medicago lupina         | black medic                | N <sub>2</sub> fixation |
| Medicago sativa         | alfalfa                    | N <sub>2</sub> fixation |
| Melilotus albus         | white dutch clover         | N <sub>2</sub> fixation |
| Melilotis officinalis   | yellow sweetclover         | N <sub>2</sub> fixation |

Table 1. Species Screened for Phytoremediation Ability and Characteristic of Interest.

Plant survival was recorded at 5 d intervals. At harvest, biomass was separated into above and below ground portions and fresh weight, dry weight height and length of longest root were measured. Biomass was digested in sulphuric acid (modified from Thomas et al. 1967) and Na<sup>+</sup> content was determined by atomic absorption according to Jones and Case (1990).

### **Results and Discussion**

## Survival

Plant survival decreased with increasing NaCl except for kochia, Fult's weeping alkali grass (FWAG) and sea blite, which tolerated all NaCl treatments (data not shown). Red samphire and salt grass survived 400 and 600 mM NaCl treatments better than 200 mM NaCl, reflecting their halophytic nature. Nitrogen-fixing species, except yellow sweet clover, died around the midpoint of both the 400 and 600 mM NaCl treatment and were completely desiccated at time of harvest. These species appear poorly suited for brine phytoremediation.

# Growth

Increased NaCl decreased biomass for all species (Figure 1). At 600 mM NaCl, of the N<sub>2</sub>-fixing species dry weight decreased in this order: yellow sweet clover > alfalfa > white dutch clover > black medic. Tolerant species ranked: kochia > tall fescue > Altai wild rye > Russian wild rye > red fescue. Na excreting species ranked: salt grass > FWAG > Nuttall's alkali grass > alkali chord grass = sea milkwort. Na accumulating species ranked: sea blite > rocky mountain goosefoot > Nuttall's saltbush = red samphire. All species except sea blite produced little biomass at the 400 and 600 mM NaCl levels. Salt grass biomass production was stimulated at the 200 mM NaCl level. The response of these two species indicates they are strongly halophytic and though they are surpassed by other species in non-saline soil, perform well at high salt levels.



Figure 1. Effect of NaCl on above ground biomass (g) of eight species  $\pm$  S.E.

Figure 2 shows a trend of decreased length of the longest root with increased NaCl for most species. Salt grass was an exception; root length remained constant through treatment and was stimulated by 200 mM NaCl. Sea blite root length was surpassed only by salt grass at the 600 mM level. At the 600 mM NaCl treatment N<sub>2</sub>-fixing species longest root length ranked in this order: yellow sweet clover > alfalfa = white dutch clover > black medic. Tolerant species ranked: kochia = Russian wild rye = Altai wild rye > tall fescue > red fescue. Na Excreting species ranked: salt grass > alkali chord grass > FWAG > Nuttall's alkali grass > sea milkwort. Na accumulating species ranked: sea blite > red samphire > rocky mountain goosefoot > Nuttall's saltbush.



Figure 2. Effect of NaCl on length of longest root (mm) of eight species  $\pm$  S.E.

#### Na Removal per Plant

The most salt tolerant species accumulated the least Na from solution (Figure 3). Total Na accumulated per plant of N<sub>2</sub>-fixing species ranked in this order: yellow sweet clover > white dutch clover > black medic > alfalfa. Tolerant species ranked: Altai wild rye > red fescue > Russian wild rye = tall fescue = kochia. Na excreting species ranked: FWAG > salt grass= alkali chord grass > Nuttall's alkali grass > sea milkwort. Na accumulating species ranked: rocky mountain goosefoot > sea blite > Nuttall's saltbush = red samphire. Alfalfa, Altai wild rye and FWAG removing 10 fold the Na of sea blite and salt grass from the 600 mM NaCl treatment. This is misleading, however, as alfalfa and Altai wild rye plants had poor survival. Most plants died near the midpoint of the experiment and were completely desiccated by harvest. The solution provided constant moisture to dead plants, which then acted as wicks, and water evaporated, leaving Na behind in the tissues. This resulted in erroneously high Na contents. Harvest date was kept constant for all plants to allow for a realistic comparison of performance over time.



Figure 3. Effect of NaCl on total Na accumulated per plant (ppm) of 8 species  $\pm$  S.E.

The efficiency of brine phytoremediating plants is related to biomass production and Na accumulation (Keiffer and Ungar (1997). When examining the overall biomass and Na accumulation performance of the 18 species studied in the four categories, the ranking was as follows: N<sub>2</sub>-fixers: yellow sweet clover > alfalfa > white dutch clover > black medic; tolerant plants: kochia > Russian wild rye > tall fescue > Altai wild rye > red fescue; Na excreters: salt grass > FWAG> alkali chord grass> Nuttall's alkali grass > sea milkwort; Na accumulators: sea blite > rocky mountain goosefoot > red samphire > Nuttall's saltbush. A community of species with key traits for brine phytoremediation should contain plants from several of these categories. The results of this screening study indicate yellow sweet clover, kochia, salt grass and sea blite perform best under highly saline conditions. Low survival of yellow sweet clover reduces it's value for phytoremediation and kochia is a challenging agricultural weed that will also be excluded from further considerations. FWAG's performance rivaled salt grass, however, low germination limited it's usefulness and was therefore not chosen for further study at this time.

Halophytic species grow slowly (Yeo 1983) and a longer study to simulate a growing season allowing plants to accumulate more biomass would be beneficial. Plant establishment is also important to consider as low germination and establishment rates are problematic in brine-affected soil (Keiffer and Ungar 2002). Osmotic shock resulting in low survival restricts the suitability of transplanting seedlings to strongly halophytic species. Thus, despite low short term biomass production and Na accumulation in aboveground tissues, sea blite and salt grass show potential for phytoremediation of brine-contaminated soil.

# Conclusion

Sea blite (*Suaeda calceoliformis*) and salt grass (*Distichlis stricta*) show promise for phytoremediation by surviving high levels of Na<sup>+</sup> in their environment while developing extensive root systems. Sea blite stores Na in aboveground tissues, while salt grass excretes Na. These species did not accumulate as much sodium in their aboveground tissue as less tolerant plants, however, during this short study cell wall integrity was maintained in these plants and over a longer period plants may accumulate more Na. These two species will be tested on contaminated saline-sodic field soil with chemical amendments to improve plant vigour in repacked PVC columns. The experiment will run for 90 d and will provide an understanding of sodium movement and removal over the length of a Saskatchewan growing season.

# References

- De Jong, E. 1982. Reclamation of soils contaminated by sodium chloride. Can. J. Soil Sci. 62: 351-364.
- Donovan, L.A., J.H. Richards and E.J. Schaber. 1997. Nutrient relations of the halophytic shrub, *Sarcobatus vermiculatus*, along a salinity gradient. Plant and Soil. 190: 105-117.

Flowers, T.J. 1986. Halophytes. Q. Rev. Biol. 61: 313-337.

- Hoagland, D.R. and D.I. Arnon. 1950. The water-culture method for growing plants without soil. Calif. Agr. Exp. Sta. Berkley, CA. Cir. 347. 32 pp.
- Jones , J.B. and V.W. Case. 1990. Sampling, handling, and analyzing plant tissue samples. p. 389-428. *In* R.L. Westerman (ed) Soil Testing and Plant Analysis. SSSA. Madison, USA.

- Keiffer, C.H. and I.A. Ungar. 1997. The effects of density and salinity on shoot biomass and ion accumulation in five inland halophytic species. Can. J. Bot. 75: 96-107.
- Keiffer, C.H. and I.A. Ungar. 2002. Germination and establishment of halophytes on brine effected soils. J. Appl. Ecol. 39: 402-415.
- Thomas, R.L., R.W. Sheard and J.R. Moyer. 1967. Comparison of conventional and automated procedures for N, P and K analysis of plant material using a single digestion. Agron. J. 59: 240-243.
- Zhou, K.F. 1991. Desalinization of saline soils by Suaeda salsa. Plant Soil. 135: 303-305.