Phytoremediation of Hydrocarbon-Contaminated Soil Using Native Plants

D.B. Robson[1], J.J. Germida[1], R.E. Farrell[1], and J. D. Knight[1]

[1]Department of Soil Science, University of Saskatchewan, Saskatoon, SK, S7N 5A8

Abstract

Phytoremediation of hydrocarbon-contaminated soil involves plants and their associated microorganisms. However, few cold-tolerant plants have been identified for reclamation in the native grasslands and woodlands of Canada. We assessed 35 native grasses, legumes and forbs, and seven exotic grasses and legumes for their ability to germinate and survive in crude oil contaminated soil. Based on germination, survival, growth rate, and above and below ground biomass five native (*Artemisia frigida, Bromus ciliatus, Glycyrrhiza lepidota, Potentilla pensylvanica,* and *Psoralea esculenta*) and three exotic (*Medicago sativa, Melilotus officinalis* and *Trifolium repens*) plants exhibited phytoremediation potential. The ability of these species to degrade specific hydrocarbons and mixtures of hydrocarbons is currently being assessed.

Introduction

Phytoremediation is the use of plants and their associated microorganisms to degrade or contain contaminants in soil (Cunningham and Ow, 1996). Phytoremediation is a promising biotechnology for the treatment of hydrocarbon-contaminated soil as it causes minimal disturbance to the ecosystem and is more cost-effective than engineering techniques (Frick et al., 1999). An additional benefit of phytoremediation in areas of native grassland is that it can fulfill two reclamation goals of the oil and gas industry: soil remediation and revegetation.

Although research on phytoremediation with cultivars and wild plants native to other parts of the world has been conducted, there has been little research on selecting plants native or naturalized to the prairie and boreal plain ecozones—the major oil and gas producing areas in western Canada (Frick et al., 1999). We screened 35 native grasses, legumes and non-leguminous forbs, and seven exotic forage plants for their ability to germinate and survive in contaminated soil. The above- and below-ground biomass was measured to help assess the phytoremediation potential.

Materials and Methods

Five commercially available topsoils, were used for the plant screenings (Table 1). For the preliminary germination screening, soils 1, 2 and 3 were artificially contaminated with crude oil in amounts equaling 0.1%, 0.5%, 1% and 5% (wt/wt). For the biomass testing, soils 4 and 5 were artificially contaminated with crude oil in amounts equaling 0.5%, 1% and 5% (wt/wt). Two crude oil-contaminated field soils from Alberta were also obtained for use in the screenings, one having 2% hydrocarbons and the other having 10% (wt/wt).

Table 1. Selected Characteristics of Soils Used in this Study

Soil	Soil Texture	Organic Carbon %	pH	EC mS/cm	$N0_3$-N µg/g	P µg/g	K µg/g
Soil 1	CL	78	5.9	0.47	230	8.8	67
Soil 2	C	47	7.6	0.52	73	26.5	280
Soil 3	SCL	16	7.4	1.0	36	190	720
Soil 4	SC	31	7.4	0.54	90	21	180
Soil 5	SCL	18	6.9	0.33	23	5.5	65
Field 1	SL	2.3	9.0	0.45	3.2	2.0	87
Field 2	SL	22	8.0	1.8	2.5	2.5	230

Seven exotic forage plants (Table 2) and thirty-five native plants (Table 3) including 13 grasses, seven legumes and 15 forbs were screened in the germination testing. Prior to planting, 28 of the native species were subjected to either scarification or stratification or both to increase their germination.

Table 2. Exotic Species Screened.

Scientific Name	Common Name	Plant Category
Agropyron cristatum	Crested wheat grass	Grass
Bromus inermis	Smooth brome	Grass
Medicago sativa	Alfalfa	Legume
Melilotus officinalis	Yellow sweet-clover	Legume
Phleum pratense	Timothy	Grass
Poa pratensis	Kentucky blue grass	Grass
Trifolium repens	Clover	Legume

* Seed obtained from Early's Farm and Garden

For the germination screening, the soils were poured into 25 cm x 50 cm x 5.5 cm trays. After moistening the soil, 10 seeds of each species were placed in furrows running along the width of the trays. The trays were covered with plastic domes and placed in a growth chamber (16 hours light/25ºC and 8 hours dark/15ºC) for 35 days. The soil in the trays was kept evenly moist by misting daily with distilled water.

For the biomass screening, the soil was poured into 4 cm x 20 cm cone-tainers (Stuewe & Sons, Corvallis, OR). Seeds of the eight species that performed the best in the germination screening were planted in individual cone-tainers (n = 7) for each soil treatment. The cone-tainers were placed in a growth chamber receiving 16 hours light/25ºC and 8 hours dark/15ºC for 28 days. Soil was kept at 60% of field capacity by daily watering. After 28 days of growth, above- and below-ground biomass was harvested, oven dried at 60°C for at least 24 hours, and weighed.

Table 3. Seed Treatment Applied to Native Species Screened in this Study.*

Scientific Name	Common Name	Plant Category	Seed Treatment
Achillea millefolium	Common yarrow	Forb	Stratification
Agropyron dasystachyum	Northern wheatgrass	Grass	Stratification
Agropyron smithii	Western wheatgrass	Grass	Stratification
Agropyron trachycaulum	Slender wheatgrass	Grass	Stratification
Artemisia frigida	Pasture sage	Forb	Stratification
Aster ciliolatus	Lindley's aster	Forb	Stratification
Aster ericoides	Tufted white prairie aster	Forb	Stratification
Astragalus crassicarpus	Ground plum	Legume	Scarification
Astragalus striatus	Ascending purple milk-vetch	Legume	Scarification
Bouteloua gracilis	Blue grama	Grass	Stratification
Bromus ciliatus	Fringed brome	Grass	Stratification
Calamagrostis canadensis	Marsh reed grass	Grass	None
Elymus canadensis	Canada wild rye	Grass	Stratification
Epilobium angustifolium	Fireweed	Forb	Stratification
Festuca hallii	Plains rough fescue	Grass	Stratification
Gaillardia aristata	Gaillardia	Forb	None
Galium boreale	Northern bedstraw	Forb	None
Geum triflorum	Three-flowered avens	Forb	Stratification
Glycyrrhiza lepidota	Wild licorice	Legume	None
Hedysarum alpinum	Hedysarum	Legume	Scarification
Helianthus subrhomboideus	Rhombic-leaved sunflower	Forb	Stratification
Heterotheca villosa	Hairy golden aster	Forb	Stratification
Koeleria macrantha	June grass	Grass	Stratification
Liatris punctata	Dotted blazingstar	Forb	Stratification
Linum lewisii	Wild blue flax	Forb	None
Oxytropis monticola	Late yellow locoweed	Legume	Scarification
Potentilla pensylvanica	Prairie cinquefoil	Forb	None
Psoralea esculenta	Indian breadroot	Legume	Scarification
Ratibida columnifera	Prairie coneflower	Forb	None
Solidago rigida	Stiff goldenrod	Forb	Stratification
Sporobolus cryptandrus	Sand dropseed	Grass	Stratification
Stipa comata	Needle-and-thread	Grass	Scarification & stratification
Stipa spartea var. *curtiseta*	Porcupine grass	Grass	Stratification
Stipa viridula	Green needle grass	Grass	Scarification & stratification
Thermopsis rhombifolia	Goldenbean	Legume	Scarification & stratification

* Seed obtained from Last Mountain Lake Prairie Habitat Restoration Project, Prairie Mountain and Blazing Star

Results & Discussion

Germination screening revealed that 80% of the species tested had poor germination (<40%) in two of the three soils artificially contaminated with 5% crude oil. Three species had less than

10% germination in all soil treatments, including the uncontaminated control soils: *Elymus canadensis*, *Epilobium angustifolium* and *Sporobolus cryptandrus*. Poor seed viability was the likely cause of the low germination of these species. Eight species had germination greater than or equal to 40% in two of the three artificially-contaminated soils: *Artemisia frigida, Bromus ciliatus, Glycyrrhiza lepidota, Medicago sativa, Melilotus officinalis, Potentilla pensylvanica, Psoralea esculenta* and *Trifolium repens*. These eight species also had germination equal to or greater than 40% in field soil 1. Germination of all 42 species was less than 20% in field soil 2. Poor seed to soil contact due to the formation of hydrophobic aggregates and potential toxic effects are possible causes of poor germination in soils with 5% or more hydrocarbons (Baker, 1970).

Germination during the biomass study was similar to that noted in the germination screening except for *Glycyrrhiza lepidota* (Figure 1). The cause of the poor germination of this species was likely a fungal infection since fungal hyphae were observed on some of the ungerminated seeds. Seeds of this species will be sterilized and tested again. Germination was similar in both soils for most species. However, *Bromus ciliatus* and *Psoralea esculenta* germination in the 5% crude oil treatment was lower in soil 2 than in soil 1. The texture difference between the two soils may have affected germination of these species.

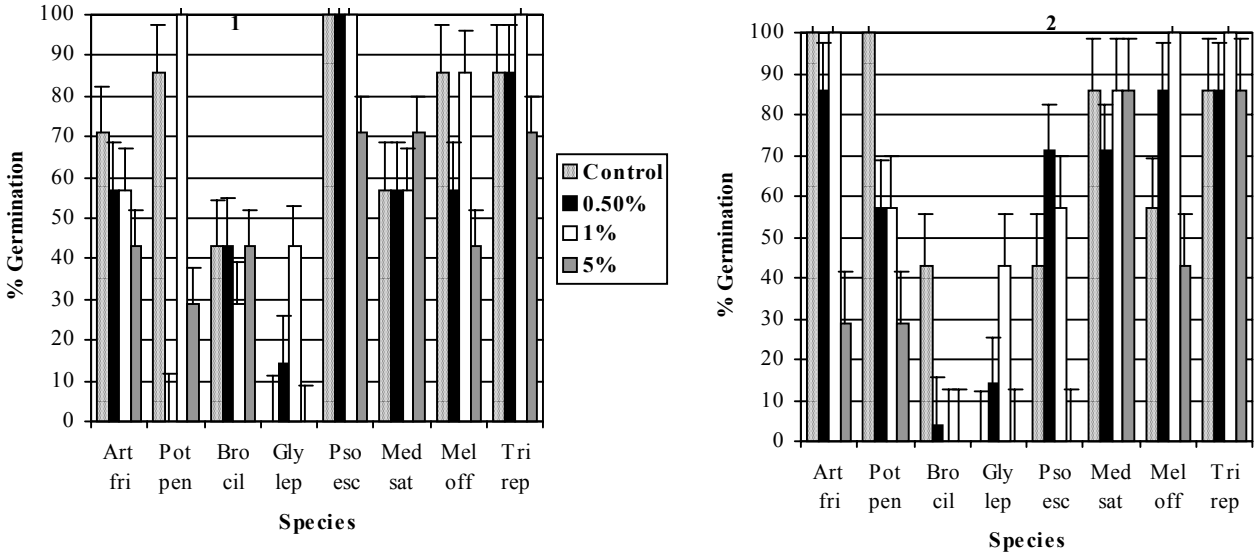

Figure 1. Germination of *Artemisia frigida* (Art fri), *Bromus ciliatus* (Bro cil), *Glycyrrhiza lepidota* (Gly lep), *Medicago sativa* (Med sat), *Melilotus officinalis* (Mel off), *Potentilla pensylvanica* (Pot pen), *Psoralea esculenta* (Pso esc) and *Trifolium repens* (Tri rep) in soils 1 and 2 (Table 1).

In both soils, above-ground biomass of all species decreased when crude oil was added, although the affect was greater in some species (Figure 2). *Psoralea esculenta* performed the best, exhibiting an average biomass decline of less than a third at the 0.5% and 1% levels in both soils. Average above-ground biomass of *Melilotus officinalis* and *Medicago sativa* declined gradually in both soils as more crude oil was added. In all other species the addition of even 0.5% crude oil cause a drastic decline (80-90%) in above-ground biomass.

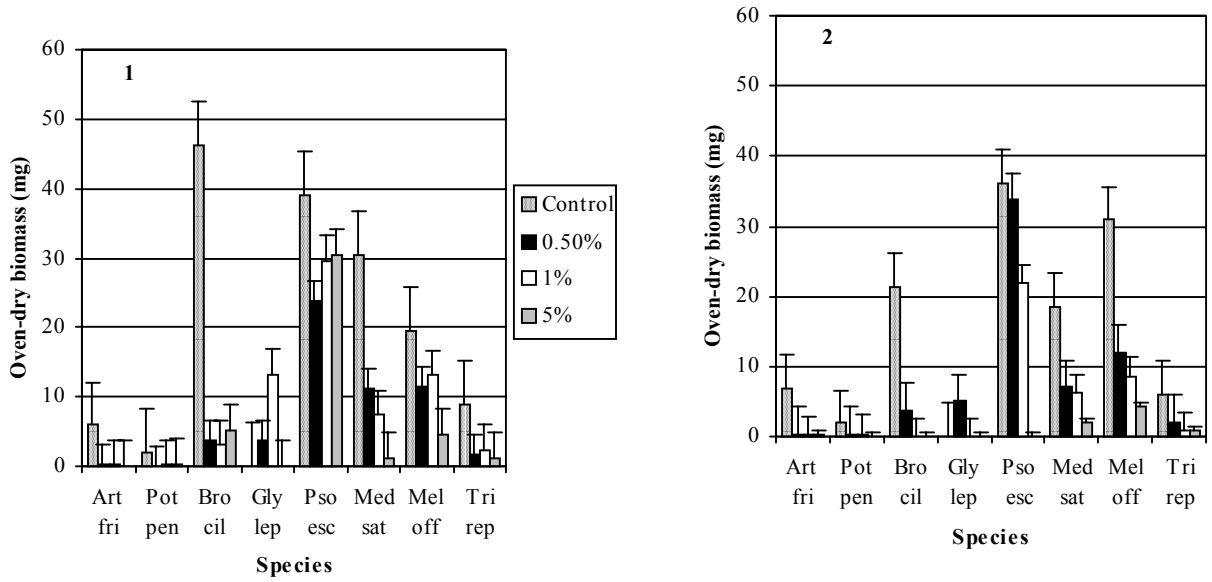

Figure 2. Above ground biomass of *Artemisia frigida* (Art fri), *Bromus ciliatus* (Bro cil), *Glycyrrhiza lepidota* (Gly lep), *Medicago sativa* (Med sat), *Melilotus officinalis* (Mel off), *Potentilla pensylvanica* (Pot pen), *Psoralea esculenta* (Pso esc) and *Trifolium repens* (Tri rep) in soils 1 and 2 (Table 1).

The pattern for below-ground biomass was similar to that of above-ground biomass (Figure 3). All species, except *Psoralea esculenta* and *Melilotus officinalis*, saw a decrease in average below-ground biomass by at least 50% with the addition of 0.5% crude oil to both soils.

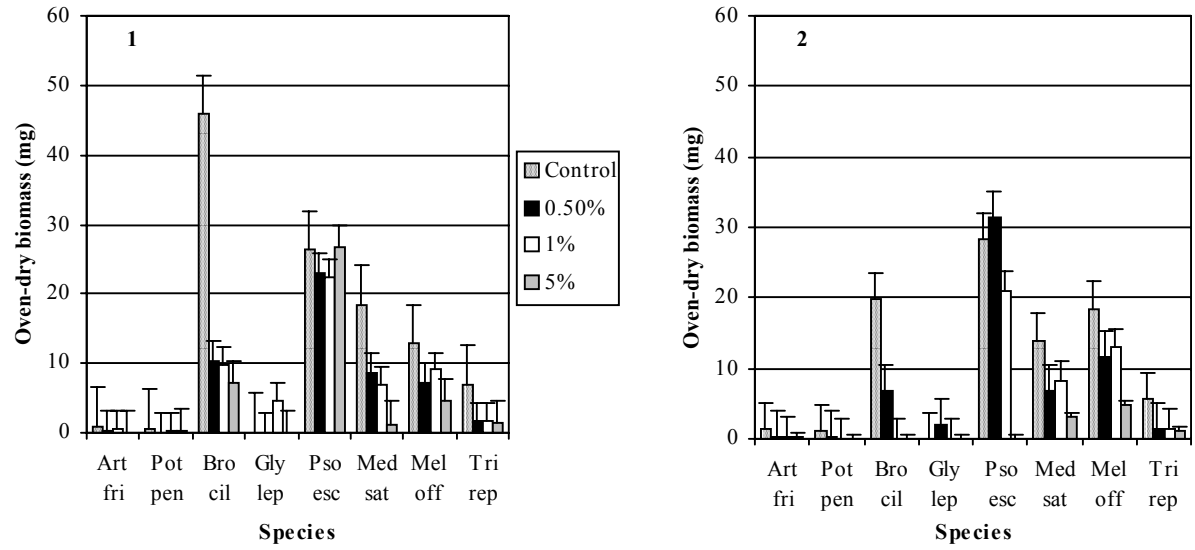

Figure 3. Below ground biomass of *Artemisia frigida* (Art fri), *Bromus ciliatus* (Bro cil), *Glycyrrhiza lepidota* (Gly lep), *Medicago sativa* (Med sat), *Melilotus officinalis* (Mel off), *Potentilla pensylvanica* (Pot pen), *Psoralea esculenta* (Pso esc) and *Trifolium repens* (Tri rep) in soils 1 and 2 (Table 1).

Conclusions

Germination of most species tested was significantly affected by crude oil addition. Above- and below-ground biomass of all species except *Psoralea esculenta* and *Melilotus officinalis* was greatly affected by increasing concentrations of crude oil.

Of the species examined, *Psoralea esculenta* and *Melilotus officinalis* show the most promising phytoremediation potential. They both germinated well in contaminated soil and their biomass was not as negatively affected by the contamination as the other species examined. Because these two plants are both legumes, they are capable of fixing nitrogen; this is an important trait since hydrocarbon-contaminated soils are often low in this nutrient. Further testing will determine if they are capable of degrading hydrocarbons in addition to being able to tolerate their presence.

References

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