Field-scale assessments of phytotechnologies applied to sites impacted with weathered hydrocarbons

Co-Principle Investigators: J.J. Germida and R.E. Farrell
Collaborating Scientists: J.D. Knight and K.C.J. Van Rees
Research Personnel: Nancy Evenson (Research Technician), Mike Solohub (Research Officer), Nyla Dubiel (Undergraduate Research Assistant), Jennifer Fernet (Undergraduate Research Assistant)

What is the purpose of the project?

Put simply, phytotechnologies involve the plant–assisted bioremediation of organic and inorganic contaminants and are essentially a form of ecological engineering that depends on natural, synergistic relationships among plants, microorganisms and the environment. However, the various plant-associated remediation pathways—and the interactions between these pathways and the biochemical and ecological interactions between plants, microbes, and the environment—give rise to a high level of complexity surrounding phytoremediation. Likewise, the presence of mixed contaminants in the soil adds an even greater level of complexity to the development of practical phytoremediation strategies. Understanding this complexity is crucial to the success of any phytoremediation effort.

Since 1998, our research has focused on assessing the effectiveness of phytotechnologies as a means of reducing petroleum hydrocarbon (BTEX, TPH & PAH) concentrations in soils contaminated with weathered oil product. This focus reflects the fact that (i) many types of PHCs are amenable to microbial degradation; (ii) the phytoremediation of organic contaminants often involves enhanced microbial degradation in the rhizosphere; and (iii) there are an estimated 200,000 PHC-contaminated sites in the Prairie provinces alone. During the past three years, the Phytoremediation team at the University of Saskatchewan has formed effective collaborations with numerous scientists, regulators, and project managers in both government and industry which has culminated in the successful establishment of a strong, interdisciplinary research effort on phytoremediation based in the Department of Soil Science.

The Environmental Biotechnology Applications Division (EBAD) of Environment Canada has been working, since 1997, in partnership with other federal departments, provincial governments, universities and the private sector to assess the utility of plants in a remediation capacity under prevailing Canadian environmental conditions and associated regulatory oversight. The results of this work indicate that to fully exploit and use phytoremediation we need to gain a better understanding of: (i) the pool of phytoremediation species found in Canada; (ii) how phytoremediation operates under unique Canadian climatic conditions; (iii) the mechanisms employed by phytoremediator plants to restore contaminated sites; and (iv) the agronomic requirements needed to maximize phytoremediation as an efficient and cost-effective cleanup technology. So, while there is clear recognition that phytotechnologies have the potential to play an important role in future remediation strategies in Canada, there remains a ***critical need*** for 'field performance data' to verify this potential, as well as to assess its limitations and determine appropriate uses of the newly emerging phytotechnologies. To address this need, we have been working with our industry and government collaborators to establish two new field sites for inclusion in the USEPA Remediation Technologies Development Forum (RTDF) 'Phytoremediation Action Team–TPH Subgroup Cooperative Field Trials' program. The purpose of the program is to "bring together technological, environmental, and regulatory interests to

develop and demonstrate phytoremediation technologies that can clean up soils and ground water contaminated with organics, and to achieve regulatory and public acceptance of these technologies". The site is especially unique in that it involves a flare pit soil in a modified land-farming operation.

The long-term objective of this study is to establish field research sites in Saskatchewan and Alberta to assess and demonstrate the utility of phytoremediation as a means of reducing petroleum hydrocarbon levels in oil-contaminated soils to environmentally acceptable endpoints.

How is the project being conducted?

Protocols used to evaluate the effectiveness of phytoremediation at both sites were adapted from the *Phytoremediation of Petroleum Hydrocarbons in Soil Field Study Protocol* developed by the RTDF-Phytoremediation of Organics Action Team. The complete field study protocol can be viewed at http://www.rtdf.org/public/phyto/protocol/protocol99.htm.

What are the results?

The phytoremediation field trials are being conducted at sites in SK (in co-operation with Talisman Energy) and AB (in co-operation with Husky Energy). Both sites involve excavated flare pit soils and were specifically selected with the USEPA-RTDF protocol in mind. General climate and site conditions for the sites are summarized in Table 1. Experimental details and anticipated timelines are summarized in Table 2.

Table 1. Summary of the climate and site conditions.

	Site L (SK)	Site M (AB)
Ecozone	Mixed grassland/parkland	Boreal fringe
Soil type	Dark Brown/Black Chernozem	Black Chernozem/Gray Luvisol
Soil texture	Clay	Sandy clay loam
Analytical: TPH (ppm)	5,440	3,000
Mean annual precipitation (mm)	422	411
Growing season length (days)	125	113
Average last frost	May 19th	May 24th
Average first frost	September 21st	September 15th
Depth to groundwater (m)	5–6	unknown
Contaminant source	Crude oil & brine from flare pit	Crude oil from flare pit
Depth of contamination (cm)	45	45

Site L, SK. Whereas site selection and construction, and the initial site characterization were conducted during the summer and fall of 2001, the research plots were not established until late spring 2002. Prior to this, however, plant selection studies in soil from the site were conducted in early spring 2002, with 21 plant species (Table 3) evaluated for their ability to germinate and grow in the contaminated soil. The soil used in the growth chamber study received the same amendments (gypsum, straw, compost & fertilizer) that were to be applied to the field plots. These amendments were required to establish a suitable plant growth environment, and were intended to (i) alleviate salt effects (EC = 5.8; SAR = 25), (ii) improve soil structure and water holding capacity (the structure of the soil was 'massive', resulting in extremely low infiltration rates and water storage), (iii) increase the organic matter content of the soil (the flare pit 'soil' consisted primarily of B and C horizon material, with very little native organic matter), and (iv)

improve the fertility status of the soil (esp. N & P). Based on the results of the plant screening, and the requirements of the RTDF protocol, seven plant species were selected for use in the field.

Table 2. Summary of experimental details for the Canadian RTDF sites.

Site		Site L (SK)	Site M (AB)
Treatments:	1	Unvegetated (Unfertilized)	Unvegetated (unfertilized)
	2	Unvegetated (fertilized)	Unvegetated (fertilized)
	3	Vegetated (fertilized)	Vegetated (fertilized)
	4	Vegetated (fertilized)	Vegetated (fertilized)
Experimental Design		RCBD	RCBD
Replicates		4	4
Experimental Plot Size		6.5m x 6.5m	6.5m x 6.5m
Planting Date		June 2002	May/June 2003
Sampling Dates:	Time = i	November 2001	May 2003
	Time = 0	June 2002	May/June 2003
	Time = 1	October 2002	October 2003
	Time = 2	October 2003	October 2004
	Time = 3	October 2004	October 2005
Cores per plot		8	8
Sampling Depth (cm)			
Shallow		0-15	0-15
Deep		15-45	15-45
Fertilization:			
Vegetated plots		Treatments 3 and 4	Treatments 3 and 4
Unvegetated plots		Treatment 2 only	Treatment 2 only

The experimental design at the Saskatchewan site consists of a randomized complete block design (RCBD) with four treatments, replicated four times. The treatments are: (1) a control with no amendments and no plants; (2) a control with amendments but no plants; (3) a localized version of the standard RTDF grass mix; and (4) the *U of S* mixture.

As indicated above, soil tests revealed severe nitrogen and phosphorus deficiencies–requiring the addition of 264 kg ha^{-1} of 34-0-0 (urea) and 96 kg ha^{-1} of 12-51-0 (mono-ammonium phosphate) to each plot. Moreover, because the soil is a heavy clay–and because PHC-contaminated soils are often hydrophobic–wheat straw and manure also were added to the plots to improve soil structure and increase water infiltration. Four bales of wheat straw, weighing approximately 17 kg each were spread over each plot. This resulted in approximately 68 kg of wheat straw per plot (equivalent to 16.2 t ha^{-1}). The plots were then rototilled to incorporate the fertilizer and straw amendments into the soil to a depth of 10- to 15-cm. Aged manure was then applied to each plot at a rate of 3.1 m^3 $plot^{-1}$ (equivalent to 225 t ha^{-1}) and the plots rototilled a third time to incorporate the manure. The T = 0 soil sampling for hydrocarbons (after seedbed preparation; before seeding) was performed at this time. A total of 8 sub-samples (0-15 cm) were taken from each plot, combined to form a composite, placed in glass jars with Teflon lined lids and stored on ice. Finally, approximately 2.3 m^3 (396 t ha^{-1}) of composted manure was applied to each plot, spread evenly over the entire area, and then packed to a depth of ca. 3-cm with a drum roller. This provided a sodium- and hydrocarbon-free seed bed in which seedlings could become

established before encountering the contaminant. In addition, the entire site received an application of gypsum (50 t $CaSO_4 \cdot 2H_2O$ ha^{-1}; rototilled in to a depth of ca 15-cm.) prior to seed bed preparation.

Table 3. Plant species screened in soil from the Saskatchewan phytoremediation site.

Common Name	Latin Name	Origin	Germ (%)*
Perennial ryegrass	*Lolium perenne*	Introduced	94
Altai wildrye	*Elymus angustus*	Introduced	89
Russian wildrye	*Elymus junceas*	Introduced	91
Creeping red fescue (var. Laurel)	*Festuca ruba*	Native	90
Hard fescue	- - -	Unknown	77
Sheep's fescue	*Festuca ovina*	Native	83
Crested wheatgrass (var. A.C. Parkland)	*Agropyron cristatum*	Introduced	78
Western wheatgrass (var. Rosanna)	*Agropyron cristatum*	Native	42
Slender wheatgrass (var. Revenue)	*Agropyron trachycaulum*	Native	74
Tall wheatgrass	*Agropyron elongatum*	Introduced	72
Northern wheatgrass (var. unknown)	*Agropyron dasystachyum*	Native	50
Nuttall's salt-meadow grass	*Puccinella nuttalliana*	Native	26
Orchard grass	*Dactylis glomerata*	Introduced	53
Indian rice grass	*Oryzopsis hymenoides*	Native	0
Fult's weeping alkai grass	*Puccinella distans*	Introduced	20
Alfalfa (A.C. Longview)	*Medicago sativa*	Introduced	89
Alfalfa (var. Hornet)	*Medicago sativa*	Introduced	88
Alfalfa (var. Rambler)	*Medicago sativa*	Introduced	92
Red clover	*Trifolium pratense*	Introduced	96
Alsike clover	*Trifolium hybridum*	Introduced	90
White Dutch clover	*Trifolium repens*	Introduced	77
Cicer milk vetch (var. Oxley II)	*Astragalus cicer*	Introduced	61
Purple prairie clover	*Petalostemon purpureum*	Native	48
Narrow leaved sunflower	*Helianthus maximilianii*	Native	27

* Percent germination in a standard petri dish assay.

Upon completion of the seed bed preparation, the site was left unplanted for two weeks so that any weed seeds introduced with the straw or manure applications would have time to germinate. The plots were then sprayed with glyphosate (2.0 L ha^{-1}) to kill the weeds and left unplanted for an additional week. In addition, the plots were hand weeded immediately prior to seeding. The plots were seeded on June 5th, using a hand applicator to broadcast the appropriate seed mixture across each plot in Treatments 3 & 4. The seeds were then lightly raked into the surface, which was then packed using the roller. Seeding rates for each treatment were calculated based on the percent germination for each seed type and were then quadrupled to compensate for potential salt-induced mortality. Weed control during the growing season, consisted of hand weeding the plots and spraying the beams and alleyways.

Seed germination and plant emergence were generally good and, by July, the stands were beginning to establish. By August, plant stands had become well-established in Treatments 3 & 4. However, plants in some areas of the plots were showing a slight discoloration or yellowing of

the leaves–suggesting a slight nutrient deficiency (most likely, N). The plots were hand weeded every 3–4 weeks, though at no time during the 2002 growing season were any plants observed to be growing on the unplanted/unlamented control plots (Treatment 1).

Plant assessments were carried out in September 2002, at which time visual observations were made of percent cover and percent species composition in each of the vegetated plots (Figure 1). As well, the above-ground biomass was sampled from three quadrats within each vegetated plot and combined to form a composite sample. Upon their return to Saskatoon, these samples were dried and weighed to obtain shoot biomass (Figure 2). It is important to note, however, that there was a severe infestation of grasshoppers during the latter part of the summer which, based on visual observations, resulted in significant losses of above-ground plant material. Below-ground biomass was determined by collecting triplicate soil cores (0-15 cm & 15-45 cm depths; collected using a bucket auger) from each of the sampling quadrats in the vegetated plots. These samples were then combined to form a composite sample for each plot and stored on ice for transport back to the lab before being processed to characterize root parameters (distribution & density).

Soil samples for hydrocarbon analyses (T=1) were collected from all 16 plots in late October. A total of 8 sub-samples (0- 15 cm) were collected from each plot using a stainless steel, split core sampler; combined to form a composite sample and transferred to glass jars with Teflon-lined lids. A single 15-45 cm core also was collected from each plot using the split core sampler, but with a butyrate plastic sleeve. Immediately upon removal, the sleeves were sealed by placing a polyethylene cap on each end. Soil samples (0-15 cm & 15-45 cm) for microbial analysis also were obtained from all treatments. All soil samples were immediately packed on ice for shipment back to Saskatoon. Also in October, additional seed from the appropriate mix was broadcast onto each plot in Treatments 3 & 4 and lightly raked into the soil.

Site M, AB. Work conducted at the Alberta site during the 2002-03 reporting period consisted of (i) consultations with our industrial partner (Husky Energy) to choose a suitable site, (ii) an on-site assessment, and (iii) obtaining the necessary approval from the Alberta Energy & Utilities Board (AEUB) for the project.

The material to be remediated is crude oil-contaminated soil (containing weathered PHCs) from a tank farm. The soil was excavated and stockpiled on-site in November 2002, at which time samples were collected and preliminary hydrocarbon analyses performed. These included: total petroleum hydrocarbons (TPH) by GC-FID (EPA 8015) and TPH fractions by GC-FID (CCME protocol). The average TPH concentration of the soil was 3,647 ppm. Mean values for the various CCME fractions were: F1(C6-C10) = 24 ppm; F1(BETX) = 23 ppm; F2(C10-C16) = 635 ppm; F3(C16-C34) = 2,100 ppm; and F4(C34-C50) = 1,070 ppm. A more refined assessment will be conducted once the experimental pots are established (May/June 2003). Plant screenings are currently underway using soil from the Alberta site.

What happens next?

Site L, SK. Work will include completing the hydrocarbon analyses for the T=0 and T=1 sampling times and a preliminary site assessment in May 2003 to assess the status of the plant stands and collect soil samples for fertility analysis. This will be followed by the addition of fertilizer and, if necessary, a reseeding of the vegetated plots. Plot maintenance will occur at 3–4 week intervals throughout the growing season, this will include hand weeding the vegetated plots (Treatments 3 & 4) and spraying to control weed populations in the unvegetated plots

(Treatments 1 & 2) and along the berms and alleyways. Soil sampling and plant assessments for T=2 will occur in the fall of 2003.

Site M, AB. Work at the site will include (i) constructing the raised bed and collecting grid samples (T=i) to characterize the spatial variability of the contaminant; (ii) establishing the experimental plots, including the addition of amendments (compost) and fertilizers; (iii) seeding the Treatment 3 & 4 plots; (iv) routine maintenance at 3–4 week intervals; and (v) soil sampling and plant assessments at the end of the 1st growing season (T=1).

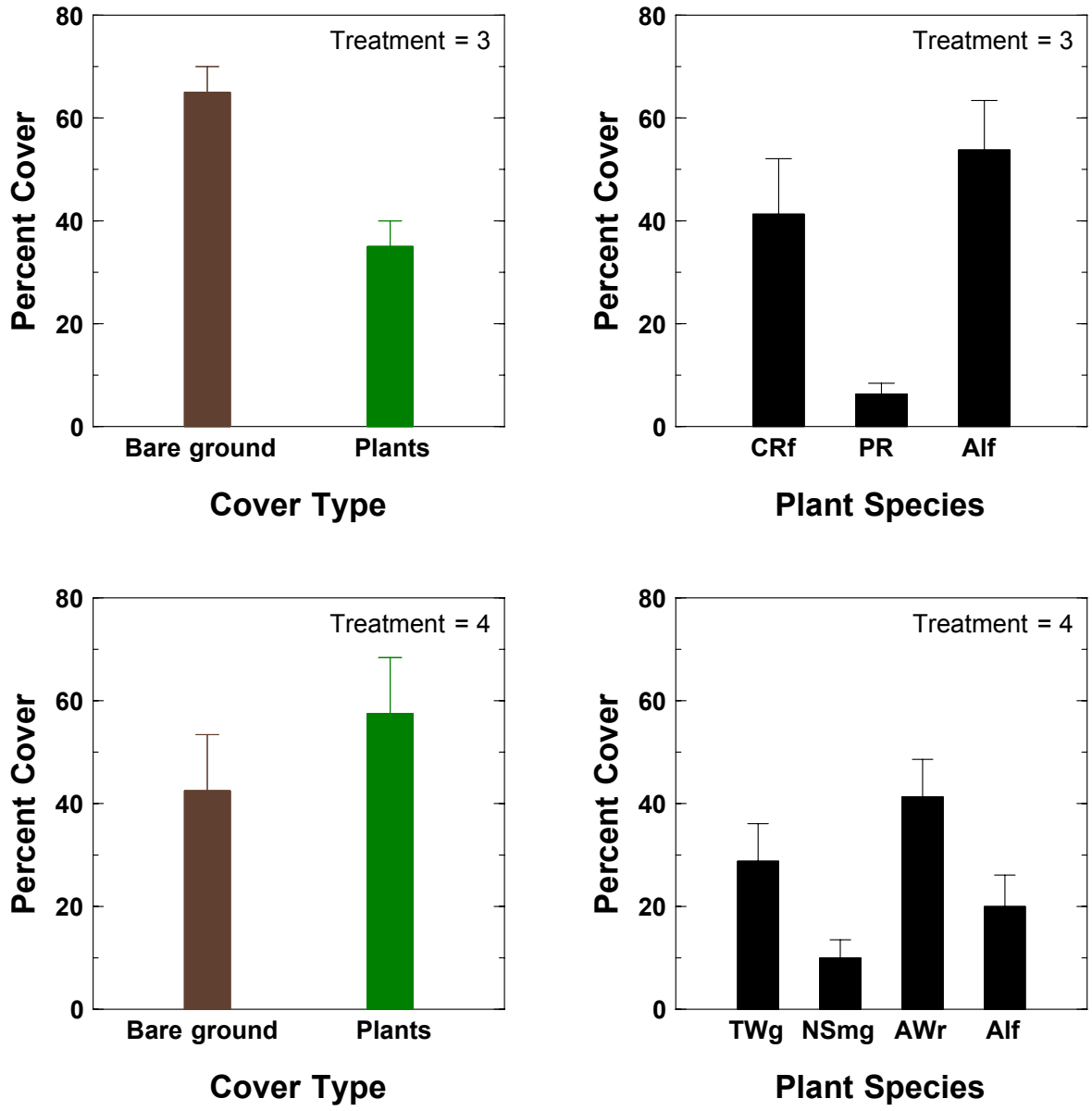

Figure 1. Percent cover and species composition in the planted treatments at the Saskatchewan site.

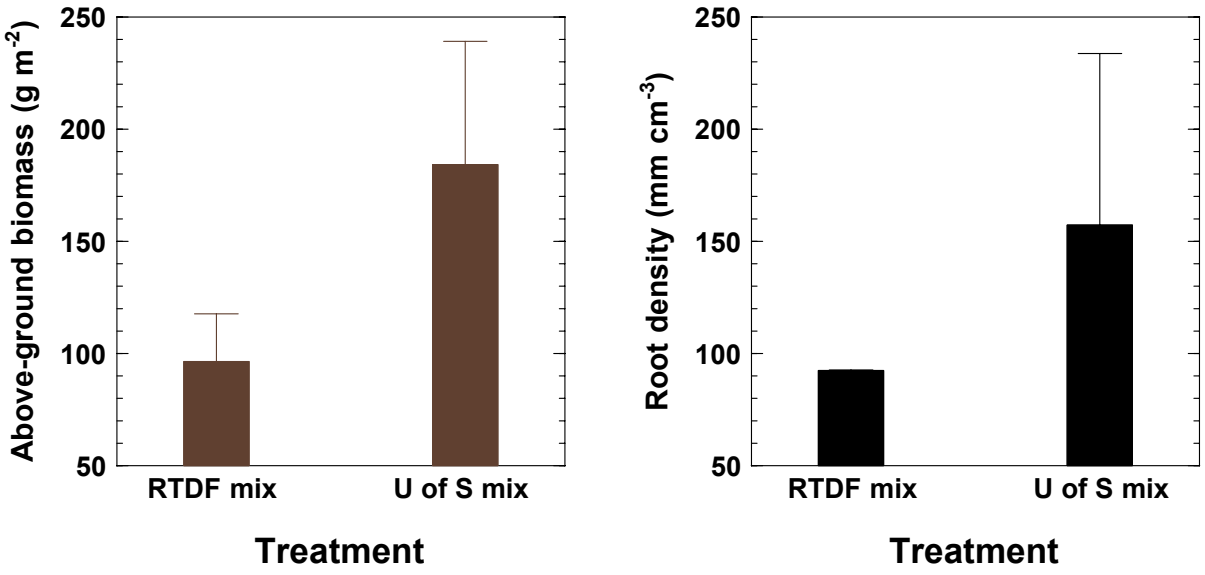

Figure 2. Above-ground biomass production and root density in the planted treatments at the Saskatchewan site.