Strategic Project Grant Final Report

Due date: **July 09, 2007**

Is your personal information correct?

_____ **Yes**
x **No** (please make the necessary corrections)

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Is the project information below correct?

_____ **Yes**
x **No** (please make the necessary corrections)

Project Title: Field-scale assessment of phytotechnologies applied to industrial and urban sites impacted with weathered hydrocarbons and inorganic contaminants

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1. Progress Towards Objectives/Milestones

1.1 To what extent were the objectives of the Strategic Project achieved? Please rate your answer on a scale from 1 to 7, where 1 means not at all, the mid-point means somewhat, and 7 means to a great extent. Indicate your response by placing an “x” under the number that best reflects your point of view. Please enter only one “x”.

Not at All			Somewhat			To a Great Extent
1	2	3	4	5	6	7
					x	

1.2 Using approximately 7 pages, please provide in the box below:

- ***a brief description of the overall objectives of the research project as awarded;***
- ***a description of the progress made towards these objectives as a result of the grant;***
- ***a description and justification for any deviations from the original objectives;***
- ***a description of the scientific and/or engineering significance of the results; and***
- ***a brief discussion of the potential benefits to Canada.***

The overall objective of this strategic research program was to assess the efficacy of plant-based bioremediation technologies for enhancing the degradation of weathered PHCs (alone or in combination with inorganic contaminants) in soils, at the field-scale and in a range of locations.

The specific, short-term objectives of the proposed research were to:

- establish field research sites in Saskatchewan (Carlyle) and Alberta (Bruderheim) to assess and demonstrate the utility of phytoremediation as a means of reducing PHC levels in oil-contaminated soils to environmentally acceptable endpoints;
- establish field sites in urban environments (Saskatoon, Kelvington, Kamsack, Young, and/or Hendon, SK) to assess and demonstrate the efficacy of phytoremediation as a clean-up tool for oil- and mixed-contaminant sites in urban communities;
- develop and use molecular techniques and DNA micro-array technology to monitor microbial community dynamics in response to contaminants, and identify catabolic activities of PHC-degrading, root associated microbial communities in field soils;
- assess tree and plant root growth patterns and dynamics at PHC contaminated field sites.
- produce botanical surveys of mixed contaminant (weathered hydrocarbons, salts & metals) sites in Alberta and Saskatchewan and identify salt- and metal-accumulator plants for use in phytoremediation trials;
- conduct greenhouse studies to identify salt- and metal-tolerant plants able to degrade PHCs;
- conduct a cost benefit analysis of phytoremediation at the field-scale; and
- conduct RTDF and other field-site tours and workshops for stakeholders.

Sub-project #1: Field Sites.

1a. Establish field research sites in Saskatchewan (Carlyle) and Alberta (Bruderheim) to assess and demonstrate the utility of phytoremediation as a means of reducing hydrocarbon levels in oil-contaminated soils to environmentally acceptable endpoints [Nancy McCrea & Darin Richman, Research Technicians; Adam Gillespie, Research Associate].

Cooperative trials to test the use of vegetation to enhance treatment of surface soils contaminated with weathered petroleum hydrocarbons (PHCs) were initiated in 2002 at a site provided by Talisman Energy Inc. (Carlyle, SK; Site L) and in 2003 at a site provided by Husky Energy (Bruderheim, AB; Site M). Experimental protocols were adapted from those developed by the *Phytoremediation Action Team* of the *USEPA Remediation Technologies Development Forum*, and are described below. In addition to the field-scale assessments, these sites were used to support the activities of various sub-projects. Both sites were selected in consultation with members of the *U of S* phytoremediation research team, the PTAC–Phytoremediation Steering Committee, and Environment Canada. Project milestones are summarized in Table 1.

Table 1. Milestones and timelines.

Sampling Event	Timing & Purpose	Analysis	– Completion Date – Site L	Site M
t_0	Characterize site variability	TPH & PHC-fractions, PAHs; total metals, salinity	03/11/01	28/05/03
$t_{1.1}$	After tillage but before planting: – determine fertilizer requirements	Fertility testing*; TPH & PHC-fractions	23/05/02	10/06/03
$t_{1.2}$	End of 1st growing season: – assess PHC degradation	TPH & PHC-fractions, plant & microbial assessments§,	22/10/02	01/10/03
$t_{2.1}$	Start of 2nd growing season: – determine fertilizer requirements	Fertility testing*	05/05/03	09/06/04
$t_{2.2}$	End of 2nd growing season: – assess PHC degradation	TPH & PHC-fractions, plant & microbial assessments§	22/10/03	14/10/04
$t_{3.1}$	Start of 3rd growing season: – determine fertilizer requirements	Fertility testing*	25/05/04	11/06/05
$t_{3.2}$	End of 3rd growing season: – assess PHC degradation and plant uptake	TPH & PHC-fractions, plant & microbial assessments§	06/10/04	19/09/05
$t_{4.1}$	Start of 4th growing season: – determine fertilizer requirements	Fertility testing*	06/06/05	NA
$t_{4.2}$	End of 4th growing season: – assess PHC degradation and plant uptake	TPH & PHC-fractions, plant & microbial assessments§	17/10/05	NA

* Includes available N, P, & K; micronutrients, soluble organic carbon, pH, electrical conductivity, CEC, particle size analysis (at t_1 only). §FAME, CLPP, MPN of TPH degraders.

NA Not applicable; work at the Bruderheim site was completed after the 3rd growing season and the site was decommissioned.

√ Completed.

The Carlyle Site (Site L). The site consisted of an excavated flare pit soil constructed into a raised bed (30-m × 40-m × 0.45-m). Research plots (6.5-m × 6.5-m) were established in the raised bed; four treatments were compared in a randomized complete block experimental design with four replications. Treatments included (1) an unplanted, unfertilized control; (2) an unplanted, fertilized control; (3) a standard cool-season grass/legume mixture composed of a combination of creeping red fescue, yellow sweet clover, and perennial ryegrass; and (4) a locally optimized treatment that included tall wheatgrass, Nuttall's salt meadow grass, Altai wild rye, and yellow sweet clover. Each trial was monitored for four growing seasons, with soil sampling conducted at planting and at the start and end of each growing season. Soils were sampled at two depths (0–15 cm and 15–45 cm) and were analysed according to the schedule described in Table 1.

Sampling at the time the site was established (t_0) yielded a mean TPH concentration of 5551 mg kg^{-1} (0–45 cm). At the end of the fourth growing season (October 2005) TPH concentrations averaged across the site had been reduced to 1101 mg kg^{-1} in the surface (0–15 cm) soil and 1942 mg kg^{-1} in the sub-surface (15–45 cm) soil. Reductions in PHC concentrations in the surface material generally occurred more rapidly in the amended treatments, with the best results generally observed in the unplanted plots. In the subsurface material, reductions in PHC concentrations (and numbers of PHC-degrading micro-organisms) were generally greater in the planted treatments. These results reflect several factors including (i) the high degree of spatial variability associated with PHC distribution at the site; (ii) competition between the plants and microbes for nutrients, especially N & P; (iii) changes in plant species composition as the best phytoremediator plants were generally less competitive than other, less effective plant species; and (iv) the limited volume of true 'rhizosphere soil' that develops under field conditions (as opposed to the rather large volume of 'rhizosphere soil' that develops in pot experiments).

Nevertheless, as a result of reaching their remedial target, the surface material was removed from the plots and returned to the pit. The site was then replanted as a demonstration site, using a revised protocol that included the use of single plant species rather than plant mixes (see Sub-Project #2) and the addition of a slow release organic fertilizer to address nutrient competition between the plants and microbes.

The Bruderheim site (Site M). The site consisted of a raised bed (74-m × 17-m × 0.45-m) constructed with oil-impacted soil from a tank battery. Research plots (6.1-m × 6.1-m) were established in the raised bed; four treatments were compared in a randomized complete block experimental design with four replications. Treatments included (1) an unplanted, unfertilized control; (2) an unplanted, fertilized control; (3) a standard cool-season grass/legume mixture composed of a combination of creeping red fescue, yellow sweet clover, and perennial ryegrass; and (4) a locally optimized treatment that included slender wheat grass, western wheatgrass, Altai wild rye, red clover, and Nuttall's salt meadow grass. Each trial was monitored for three growing seasons, with soil sampling conducted at planting and at the end of each growing season. Soils were sampled at two depths (0–15 cm and 15–45 cm) and were analysed according to the schedule described in Table 1.

Sampling at the time the site was established (t_0) yielded a mean TPH concentrations of 2148 mg kg^{-1} at 0–15 cm and 1939 mg kg^{-1} at 15–45 cm. At the end of the third growing season (October 2005) TPH concentrations averaged across the site had been reduced to 815 mg kg^{-1} in the surface (0–15 cm) soil and 701 mg kg^{-1} in the subsurface (15–45 cm) soil. Reductions in PHC concentrations were generally greater in the plots amended with fertilizer and compost; but, as at the Carlyle site, no significant plant effect was observed. However, reductions in the F3 (C16–C34) and F4 (C34-C50) fractions generally occurred more rapidly in plots seeded with the site-specific plant mix (Trt 4) than those seeded with the standard (RTDF) plant mix (Trt 3). Remedial targets were reached and the site decommissioned in 2005.

1b. Establish field sites in urban environments (Saskatoon, Kelvington, Kamsack, Young, and/or Hendon, SK) to assess and demonstrate the efficacy of phytoremediation as a clean-up tool for oil- and mixed contaminant sites in urban communities.

Urban field sites were established at Kelvington, Kamsack, and Hendon, SK. The Kamsack site (a former refinery, decommissioned in 1976) was established in support of *Sub-project No. 5* (*Modeling the spatial & temporal variability of contaminants*). The Hendon site (a former bulk distribution site) was established in support of *Sub-project No. 3* (*Root dynamics*) and was unique in that it involved the use of trees for both plume control and phytoremediation. The Kelvington site supported work under *Sub-Projects 2, 3 & 5* during the first two years of the program. As these projects matured, however, this site was no longer needed and the work at Kelvington ceased. At the same time, an opportunity arose to assess the potential of phytoremediation as a clean-up tool for tailings pond water produced during crude oil extraction from the Athabasca oil sands in Fort McMurray, AB (see Sub-project #6).

Sub-project #2: Microbial Diversity Studies.

2a. Develop and use molecular techniques and DNA micro-array technology to monitor microbial community dynamics in response to contaminants, and to identify catabolic activities of PHC-degrading root associated microbial communities in field soils [Lori Phillips; NSERC PGS-A recipient (2003), PGS-D recipient (2005); Ph.D. program to be completed in Summer 2007].

The overall purpose of this study was to elucidate plant-microbe interactions that facilitate hydrocarbon degradation in the rhizosphere. Although it is known that hydrocarbon degradation is carried out primarily by root-associated micro-organisms, little is known about the mechanisms by which specific plants stimulate the degradation potential of these microbial communities. To assess microbial community shifts in response to different plant stimuli, both known and newly developed microbiological assays were utilized. Standard culture-dependent analyses included TSA heterotrophic community enumeration, most-probable-number of hydrocarbon degrader assays, and BIOLOG™ assessment of community structure. Molecular analyses included community structure assessment by DGGE, taxonomic assessment by sequencing analysis, and functional diversity assessment by standard and quantitative PCR analysis of genes involved in hydrocarbon degradation. DNA extraction protocols, Q-PCR techniques, specific gene probes, and preliminary micro-array procedures were developed and optimized for the soils and plant material under investigation.

Growth chamber assessment of the impact of mixed and single plant treatments on microbial communities. The primary objective of this study was to determine the impact of mixed plant treatments and individual plant species that comprise the mix on indigenous microbial communities and on the overall degradation potential of soil from the Carlyle and Bruderheim sites. A growth chamber study was conducted in which the plants were grown in field soil from these sites for 4.5 months. In both soils it was found that single-species grass treatments were more effective than mixed plant treatments at reducing TPH levels. In Bruderheim soil, slender wheat grass and creeping red fescue facilitated 20% more TPH reduction than the mixed plant treatments; while in Carlyle soil, creeping red fescue facilitated up to 50% more degradation than mixed plant treatments. Reduced degradation potential in Bruderheim mixed treatments may be related to an overall shift in the metabolic competence of their rhizosphere communities, as the substrate utilization patterns of these microbial communities were significantly different than those of the individual plant species that comprised the mixes. Although heterotrophic and hydrocarbon-degrader populations were 10x higher in Bruderheim planted treatments than in the amended control, this did not appear to have a significant stimulatory effect on TPH degradation. Instead, the addition of fertilizer and manure amendments was likely the largest factor influencing TPH degradation, as the amended control treatment exhibited comparable degradation to the planted treatments while that of the non-amended control was significantly lower. In Carlyle soils, plant specific factors–both selective and non-selective–had a substantial impact on the degradation potential of indigenous hydrocarbon-degrading micro-organisms. Final hydrocarbon-degrader populations were higher, there was an increase in catabolic genotypes in specific planted treatments, and overall hydrocarbon degradation was enhanced in planted compared to un-planted soils. This enhanced potential was independent of the addition of amendments, as non-planted amended soil experienced a significant decrease in degradation potential. Reduced degradation in Carlyle mixed treatments was found to be related to the presence of alfalfa, which had a dominant and selective influence on microbial community structure and function.

Field assessment of the impact of mixed and single plant treatments on microbial communities. This study focused on determining whether the reduced degradation rates observed in mixed plant and alfalfa treatments under controlled conditions also occur in the field and to determine the probable role of endophytic (root interior) microbial communities in degradation responses. Micro-plots consisting of tall wheat grass, Altai wild rye (AWR), alfalfa, a mix of all plants, and non-planted amended controls were established at the Carlyle site in 2004 and were sampled over two growing seasons. Degradation trends in the first growing season were similar to those observed in the growth chamber, with AWR promoting greater than 50% TPH degradation whereas TPH levels in the alfalfa and the mixed treatments were reduced by less than 25%. Some of this discrepancy in degradation potential was explained by examining microbial population dynamics. While rhizosphere and endophytic degrader communities of most plants experienced significant declines during times of drought, those of AWR were more stable. During periods of drought AWR supported up to 100x more n-hexadecane degraders in its roots than other plants. Averaged over the two growing seasons these endophytic degrader populations were maintained at levels 10x higher than those of other plants. It is probable that the increased degradation seen in AWR treatments is related to its ability to act as a refuge and hence subsequent source for hydrocarbon degrader communities. As both plants and microbial communities mature, these discrepancies in degradation potential were reduced and cumulative TPH degradation increased in all treatments.

Degradation activity of endophytic bacteria associated with plants growing in hydrocarbon contaminated soil. The objective of this study was to determine the hydrocarbon degradation potential of mature endophytic communities. Three year old alfalfa, Altai wild rye, Nuttal's salt meadow grass, perennial rye grass, and tall wheat grass from the Carlyle site were assessed. All plants maintained large and active populations of aliphatic and total hydrocarbon degraders in their root tissue and catabolic genes associated with hydrocarbon degradation were widespread. The endophytic community structure of a given species however, was unique and showed no greater than 50% similarity with that of other species. Thus, although all plants exhibited some degradation potential, there were plant-specific differences in the presence of individual degrader populations and catabolic genotypes that resulted in increased hydrocarbon degradation by plants such as Altai wild rye.

Single grass treatments are initially more effective at stimulating hydrocarbon degradation in the sites under analysis. The addition of alfalfa into plant mixes had an initial repressive effect on hydrocarbon degradation potential, likely due to catabolite repression and/or competitive exclusion. Although discrepancies in overall hydrocarbon degradation potential are minimized as plants and their associated microbial communities mature, the endophytic communities do have an impact on the ability of plants to promote the degradation of specific types of hydrocarbons. All plants under analysis differed in their ability to maintain viable degrading populations in their roots and rhizosphere under varied environmental conditions. Altai wild rye exhibited superior degradation potential for saline-sodic flare-pit soil in part due to its ability to maintain high levels of rhizosphere and endophytic degrader communities during periods of environmental stress. Although no single factor emerged as the dominant reason why a given plant promotes increased PHC degradation, our findings will allow future researchers to better optimize plant selection for specific soil and environmental conditions.

2b. Bacterial inoculants for enhanced rhizodegradation of PHCs [Jennifer Fernet; M.Sc. program to be completed in Fall 2007].

The objectives of this research were to: (i) assess the effect of inoculation with the PHC-degrading micro-organisms *Sphingomonas yanoikuyae, Rahnella aquatilis,* and *Arthrobacter globiformis* on the germination of selected plant species; (ii) determine inoculant survival and colonization patterns on emerging roots; and (iii) assess the ability of the inoculants to produce enhanced PHC degradation in the rhizosphere.

The inoculants effectively colonized the seeds of selected grass species common to western Canada and, in general, had a neutral or positive effect on seed germination and seedling growth. As well, a soil-based assessment of survival and PHC-degradation by the selected inoculants in unplanted soil–carried out with or without a manure nutrient amendment–demonstrated that addition of the inoculants had a positive impact on the efficacy of PHC removal from the soil. Total petroleum hydrocarbon concentrations in the manure-amended and the non-amended *A. globiformis* treatments were reduced by ~45%, which was significantly greater than the 20% reduction observed for the control soil (i.e., no amendment or inoculant). Non-amended and manure-amended soils inoculated with either *S. yanoikuyae* or *R. aquatilis* also exhibited significantly greater reductions in TPH concentration (~33%) relative to the control.

Based on positive influence of the inoculant, the plant–bacteria pairs chosen for further study were perennial ryegrass (*Lolium perenne*) and creeping red fescue (*Festuca rubra)* with *S. yanoikuyae*. The efficacy of these plant–bacteria pairs for PHC removal in contaminated soil from the Bruderheim site was established in a growth chamber study. A sub-set of the Bruderheim soils also was spiked with a mixture of hexadecane, phenanthrene and pyrene to evaluate their performance under more toxic conditions. Inoculation with *S. yanoikuya* had a positive effect on root and shoot biomass of creeping red fescue in both spiked and non-spiked treatments. In the spiked treatment, inoculation resulted in an increase in root biomass; whereas in the non-spiked treatment, it was shoot biomass that increased in response to inoculation. Conversely, inoculation of perennial ryegrass had no effect on either root or shoot biomass production in the spiked and non-spiked soil treatments. In general, spiked soils receiving the inoculant, plant, or plant + inoculant exhibited increased rates of PHC degradation relative to the control soil. Creeping red fescue, alone and in combination with the inoculant, produced the greatest decrease in PHC concentration relative to the other plant-inoculant combinations. It also was demonstrated (Hynes et al., 2003) that inoculating white mustard (*Sinapis alba*) with *S. yanoikuyae* resulted in a 7-fold increase in phenanthrene degradation in soil.

The addition of plants and inoculants resulted in an increase in hydrocarbon degradation as compared to control soils, although the addition of vegetation alone had a comparable effect. A critical effect of inoculation was the increase in creeping red fescue root biomass at higher concentrations of contamination. This is important as increased root biomass means a larger effective rhizosphere which, in turn, should increase the volume of soil that can be remediated. Our results indicate that the use of specific plant-bacterial inoculant combinations can result in enhanced rhizodegradation of PHC contaminated soils.

Sub-project #3: Root Dynamics.

3a. Tree root growth patterns and dynamics at a PHC contaminated field site [Jeff Gunderson; completed M.Sc. in 2006].

This project examined root system dynamics for trees used in phytoremediation at sites contaminated with PHCs. The study used a minirhizotron system for *in situ* observations of root systems at a contaminated site near Hendon, SK. The project was initiated in the summer of 2003 with the objectives of quantifying: (i) the effects of petroleum hydrocarbon concentrations on the spatial and temporal patterns of fine root production in Griffin hybrid poplar (*P. deltoides x P. petrowskyana* cv. Griffin), and (ii) the effects of ectomycorrhizal colonization on hybrid poplar fine root dynamics and N and P uptake from diesel contaminated soil. Fine root production was stimulated by small amounts of hydrocarbon contamination in the field. Fine root production increased linearly up to approximately 500 mg kg^{-1} TPH, then remained constant as contamination levels increased. The stimulated production of fine roots by the presence of contamination should result in an enhanced rhizosphere effect and hence enhanced degradation. Under controlled conditions, colonization of hybrid poplar roots by the ectomycorrhizal fungus *Pisolithus tinctorius* stimulated fine root production in a diesel contaminated soil compared to non-colonized trees growing in the same soil. However, after 12 weeks of growth, diesel levels in the soils of the non-colonized treatments were lower (5 % of original levels remained) than levels in the soils of colonized treatments (6.7 % of original levels remained). The colonized trees sequestered more hydrocarbons in the roots than the non-colonized trees, perhaps protecting the hydrocarbons from direct degradation. In short, planting hybrid poplar on lightly contaminated soils should speed up remediation efforts. Inoculation of roots with an ectomycorrhizal fungi is not recommended.

3b. Role of root exudates in PHC degradation in the rhizosphere [Adam Gillespie; NSERC PGS–B recipient (2004); withdrew from the Ph.D. program in the fall of 2004] [Lori Phillips; NSERC PGS-A recipient (2003), PGS-D recipient (2005); Ph.D. program to be completed in Fall 2007].

The primary objective of this work was to investigate the link between rhizosphere chemistry and the rhizodegradation of PHCs. A method to collect rhizosphere soil in such a manner as to optimize the recovery of root exudates was developed. Likewise, analytical protocols were developed to extract and characterize root exudates from rhizosphere soil using solid phase and solvent extraction techniques coupled with gas chromatography to simultaneously extract, purify and identify organic acids, amino acids and phenolics. These methods were then used to characterize root exudation profiles in the rhizospheres of purple prairie clover (*Petalostemon purpureum*) and slender wheatgrass (*Agropyron trachycaulum*) grown in PAH-contaminated and non-contaminated soil. It was determined that there was a significant contaminant effect on root exudation, and that several compounds thought to be involved as signal molecules or contaminant analogues were present in the rhizospheres of plants grown in the presence of PAHs.

Having shown that hydrocarbon contaminants have a significant effect on root exudation patterns, Lori Phillips (Ph.D. candidate) examined whether specific root exudates could facilitate hydrocarbon degradation by increasing the prevalence of specific bacteria or by increasing catabolic gene expression in rhizosphere microbial communities. Exudates were collected from alfalfa (*Medicago sativa*) and Altai wildrye (*Leymus angustus*) grown aseptically both in the absence and presence of pyrene and phenanthrene. Bulk exudates and their class specific fractions (phenolics, organic acids, and amino acids) were amended to soil mineralization microcosms and the subsequent impact on degradation potential monitored. The addition of bulk root exudates initially repressed the mineralization of all hydrocarbons. Altai wildrye exudates released under hydrocarbon stress however, stimulated greater mineralization than other exudates. DGGE analysis revealed that this change in degradation potential was not linked to an increase in any single dominant microbial group, as no visible changes in community structure were observed. Instead, Q-PCR results indicated that genes associated with PAH degradation were reduced in all exudate-amended microcosms except those amended with exudates released by Altai wildrye under hydrocarbon stress. Over the short term, plant root exudates by themselves did not impact overall soil microbial community structure, though they did stimulate differential changes in the copy number of genes associated with hydrocarbon degradation. As the genes targeted in this study are found on plasmids, differences in the degradation potential between treatments is likely associated with changes in the transfer rates of these plasmid-encoded genes. This increased transfer may in turn be stimulated by the presence of specific low molecular weight organic acids. Moreover, our data indicate that the absolute gene copy number is a good indicator of hydrocarbon mineralization capability.

Sub-project #4: Remediation of Inorganics.

4a. Identify and evaluate salt-tolerant plants for the remediation of brine impacted sites [Shannon Gerrard; completed M.Sc. in 2006].

The objectives of this study were to identify plant species with the ability to accumulate salt from brine-contaminated systems and identify management strategies that maximize salt accumulation by plants. Brine-contaminated sites were surveyed to identify plant communities and soil properties controlling their distribution along a salinity gradient. In addition, 18 plant species were screened for sodium uptake in solution culture. Soil amendments (gypsum, calcium nitrate and humic acids) were evaluated for their ability to enhance sodium uptake from the soil. *Suaeda calceoliformis* and *Salicornia rubra* dominated on soils with high EC, soluble Mg^{2+} and SAR. Although *S. rubra* was the most abundant species occurring naturally on the brine contaminated sites, the low biomass productivity of this plant makes it an unlikely candidate for brine remediation. In laboratory screening studies, *S. calceoliformis* and *Distichlis spicata* were tolerant to 600 m*M* NaCl in solution and were moderately productive in these high salt concentrations. None of the soil amendments enhanced salt uptake by these two plant species. Recommended stand densities of 15 *D. spicata*, and 18 *S. calceoliformis* plants per m^2 should remove approximately 233 kg Na^+ ha^{-1} yr^{-1}.

4b. Investigation of metal-accumulator plants [Cory Sonntag; Ph.D. program to be completed in Fall of 2007].

The overall focus of this research was to identify plant proteins involved in uptake, translocation and storage of copper (Cu), a widespread inorganic contaminant released into the environment by mining, smelting and other human activities. We used a novel multidisciplinary approach that combines mass spectrometry (MS), proteomics, and synchrotron-based techniques in an effort to correlate spatial and temporal changes in protein expression and Cu speciation during metal uptake in the model plant *Arabidopsis thaliana* (*At*), for which the complete genome has been sequenced. Proteins play a key role in almost all biological processes, including the binding and transportation of metals in plants. Using 2-D gel electrophoresis (2-DE), or a combination of immobilized Cu(II)-ion affinity chromatography (Cu-IMAC) and 1-DE, followed by MS analysis and *At* sequence database searching, we identified copper-binding and other proteins that are differentially expressed in control (Columbia wild-type) and metal accumulating (man1-1 mutant) *At* plants exposed to Cu in the growth medium. The method was validated by detection of the oxidative stress-response protein superoxide dismutase (SOD) using both 1-DE and 2-DE workflows, the form of SOD that contains copper as a co-factor being found exclusively in the 1-DE samples, which were prepared using Cu-IMAC. A total of 2700 2-DE protein spots and 150 1-DE protein bands were analyzed, from which numerous proteins were identified as being differentially expressed in the roots and shoots of control and metal-accumulating *At* plants upon exposure to copper. These include metal transporters/chaperonins, enzymes involved in the biosynthesis of sulphur-containing (thiol) compounds thought to be involved in metal uptake and accumulation, and stress-response proteins.

To confirm uptake, transport and storage of Cu by proteins, we also attempted to use synchrotron techniques (XANES, EXAFS) to probe the molecular environment, or speciation, of Cu in wild-type and mutant *At* root and shoot tissues. These experiments were carried out at the advanced Photon Source (APS) synchrotron at the Argonne National Laboratory (IL, USA) in collaboration with Dr. Colleen Christensen and at the Canadian Light Source (CLS; Saskatoon). Data were acquired for the same tissues and time-points as for the Cu-IMAC/1-DE and 2-DE experiments, with XANES data suggesting reduction of Cu(II) in *At* roots during exposure to copper. This correlates with the differential expression of numerous proteins, as indicated by 2-DE gel image analysis. Synthesis of proteomic and synchrotron data is currently ongoing, and it expected to provide further insights regarding interactions between Cu and specific proteins, or protein classes, in plant tissues. Future plans include chemically specific imaging of Cu, S and other elements in whole plants during metal uptake experiments, for which beam-time has already been secured at the CLS.

This research has provided us with a unique new opportunity to identify the *At* genes that correspond to these proteins, and introduce or up-regulate these genes in related, high biomass species–some of which (e.g. *Brassica juncea*) are know to tolerate and accumulate metals–to enhance their potential as phytoremediator plants at copper-contaminated sites. The methods developed during this research can be extended to study and regulate plant uptake and accumulation of iron (Fe), nickel (Ni) and zinc (Zn) to enhance phytoextraction of these metals, or the nutritional value of certain crop species.

The results of this unique, multidisciplinary project will help us develop plants with enhanced phytoremediation capabilities (or nutritional properties) and ultimately, a cleaner environment and healthier food for Canadians.

Sub-project #5: Modeling the Spatial and Temporal Variability of Contaminants.

5a. Contaminant Variability [Carol Luca; NSERC PGS–A recipient (2004); completed M.Sc. program in 2005].

Site characterization–defining the nature and extent of the contamination–is the initial step in the remediation process. The objectives of this study were to (i) evaluate the use of fluorometry to determine concentrations of total petroleum hydrocarbons (TPH) in contaminated soils at an abandoned refinery near Kamsack, SK; and (ii) compare two geostatistical techniques for determining spatial patterns of PHC concentrations at the site. Work at the Kamsack site began in August 2003 and included an preliminary site characterization to determine the best sampling strategy. Soil samples were collected to a depth of 1.2 m and were extracted using simple shake extraction with either methanol or hexane:acetone as the solvent. Samples extracted with methanol were analyzed using a Turner Designs 10-AU field fluorometer; samples extracted using hexane:acetone were analyzed using standard (CCME) GC-FID techniques. The hexane:acetone shake-extraction had an extraction efficiency of 87–90% relative to the standard accelerated solvent extraction method and that PHC concentrations measured using the fluorometry and GC-FID methods were highly correlated (r = 0.755***). Data obtained using the reference (GC–FID) method were used with indicator kriging (IK) and sequential indicator simulation (SIS) to map the spatial patterns of the F2 (nC10–nC16) and F3 (nC16–nC34) PHC-fractions—using a 1-m × 1-m grid. The critical probability (CP) of individual grid cells exceeding remediation criteria also was determined. Results indicate that IK yielded a slightly greater estimate for the total area (6.3% > PF2; 0.8% > PF3) exceeding the CP levels than did SIS (4.5% > PF2; 0.6% > PF3). Although the differences between IK and SIS were small, IK appears to overestimate the true area requiring remediation.

Sequential indicator simulation was used to compare data sets obtained using the fluorometer and GC-FID methods. Both data sets yielded similar cumulative distribution functions and semivariograms, though the TPH maps were distinctly different. Specifically, analysis of the fluorometer data failed to identify areas of high contamination that were present in the map obtained using the GC-FID data. Consequently, attempts to estimate critical PHC-fractions using the fluorometer were unsuccessful–reflecting the fact that the fluorometer responds only to compounds that fluoresce (aromatics), while PHC contaminants consist of both aromatic and aliphatic compounds.

5b. Model development [Alexis McPherson; completed M.Sc. program in 2007].

The purpose of this study was to examine the effects of specific soil properties on the rate and amount bio/phyto degradation of PHC (weathered diesel and crude) contaminated soils in the laboratory under simulated field conditions. Specific objectives of the study were to: (i) examine PHC transfer and degradation processes involved in phytoremediation of contaminated field soils; (ii) compare phytoremediation of contaminated field soils with intrinsic bioremediation; and (iii) develop a rationally-based model that could be used as a starting point for a quantitative prediction of the rate of PHC removal. To realize these objectives a series of laboratory scale experiments were designed and carried out. The experiments reproduced pole planting of hybrid poplars into diesel contaminated field soils from a former bulk fuel station. The experiments were conducted in a closed and controlled environment over a 230 day period with numerous aspects of the system being monitored including volatilization of PHC from the tree and soil and microbial activity of the soil.

Monitoring data indicated that microbial degradation of the contaminant was by far the most influential degradation pathway, accounting for 98.1 to 99.3% of the mass removed for soils containing poplars. The monitoring data also indicated that the planted treatments produced significantly more PHC-degradation than the unplanted treatments. Indeed, contaminant degradation (microbial degradation + volatilization + phytovolatilization) from the soils containing poplars averaged 450 ± 186 mg PHC kg^{-1} soil versus only 110 ± 8 mg PHC kg^{-1} (microbial degradation + volatilization) from the unplanted soils. Below-ground biomass was the plant variable that yielded the best correlation with PHC degradation and, thus, was selected as the plant index used to develop a rationally-based model for quantitative prediction of the rate of PHC removal.

Sub-project #6: Aquatic phytoremediation systems.

Aquatic macrophytes as phytoremediators of naphthenic acids [Sarah Armstrong; Ph.D. program to be completed in Fall of 2007].

This study focused on (i) the phytotoxicity of naphthenic acids (NA) to the aquatic plants *Typha latifolia*, *Phragmites australis*, and *Scirpus acutus*; and (ii) plant effects on NA dissipation in aquatic systems. Differences in dissipation and phytotoxicity were initially evaluated for a commercially available mixture of NA standards (std-NA) and a mixture of NAs extracted from oil sands tailings pond water (os-NA) collected at Fort MacMurray, AB. The plants exhibited greater sensitivity to the std-NAs and there was evidence of NA sequestration within the plants. Although the os-NAs also produced a phytotoxic effect, there was no evidence to suggest NA sequestration within the plants. Experiments with the os-NAs also demonstrated that the protonated (non-ionized) NAs were more toxic than their deprotonated (ionized) analogs; though there was no evidence to suggest plant sequestration of either form. Differences among plant species were not observed. Experiments with *T. latifolia* also demonstrated that microbial community structures in the bulk solution, along the rhizoplane, and in endophytic niches varied in response to the different NA mixtures (i.e., std-NA *vs.* os-NA). These results suggest that root-associated bacteria may play a role in mitigating the phytotoxicity of NAs to wetland plants. As well, our results indicate that predictions of the environmental fate of os-NAs based on data obtained using std-NA mixtures as surrogates should be viewed with caution.

Sub-project #7: Technology Transfer

Conduct a cost benefit analysis of phytoremediation at RTDF and urban field sites.

No results to report (see Section 5.2).

Conduct RTDF and other field-site tours and workshops for stakeholders.

Several industry partners and selected government scientists toured the Carlyle site in the summer of 2003. Undergraduate students from the U of S (SLSC 412: *Integration and Application of Soil Science*) toured the field sites at Hendon and Kelvington in 2004 as part of a soil remediation module presented by Dr. Farrell. Plans for additional field tours and a workshop scheduled for 2006 were abandoned due to lack of funding (see Section 5.2). Summaries of this project are scheduled to be presented at the 4th International Conference on Phytotechnologies (24–26 September 2007, Denver, CO)–a biannual meeting of industry, government and university researchers that addresses the state-of-the-art in phytotechnologies.

Technology transfer also occurred through the dissemination of technical reports submitted to our co-operators and extension publications prepared for CAPP-ERAC (see Section 4).

2. Research Team

Please provide an overview of the participation in the project and scientific contributions for each member of the research team (principal investigator, co-investigators, collaborators, company and government scientists, research associates, postdocs, students, etc.).

The phytoremediation research team at the University of Saskatchewan consisted of Drs. Germida, Farrell, Knight, Si and Van Rees of the Department of Soil Science and Dr. Ian Fleming of the Department of Civil and Geological Engineering. Co-PIs from government labs included Drs. Andrew Ross (NRC PBI, Saskatoon), Charles Greer (NRC-BRI, Montreal), John Lawrence & John Headley (EC NWRI, Saskatoon). In all, 13 graduate students worked full- or part-time on this project: Lori Phillips (Ph.D. 2007TBC), Cory Sonntag (Ph.D. 2007TBC), Sarah Armstrong (Ph.D. 2007TBC), and Jen Fernet (M.Sc. 2007TBC); Alexis McFerson (M.Sc. 2007), Shannon Gerrard (M.Sc. 2006), Jeff Gunderson (M.Sc. 2006), Carol Luca (M.Sc. 2005), Adam Gillespie (Ph.D. withdrew 2005), Monique Wismer (M.Sc. 2004), Diana Robson (Ph.D.2003), Julie Roy (M.Sc. 2003) and Cindy Wall (M.Sc. 2003). Ms. Nancy McCrea worked as a research technician, in charge of the Carlyle and Bruderheim field sites, from 2003–2005; Nancy resigned in 2005 and was replaced by a Research Associate (Adam Gillespie). Another technician, Arlette Seib, worked part-time on the project conducting microbial analyses of field soil samples. Summer students were hired to assist with the field and lab programs in each year of the project: Mark Cooke (2006); Holly Kalyn (2005); Kim Kovacs, Cim Kneller, Kuni Niina & Curtis Sambrook (2004); Nyla Dubiel, Jola Pisz & Darin Richman (2003). A PDF (Brig Verma) worked on the project from August to December 2003 but then resigned to take a full-time position as a Research Scientist with the Alberta Research Council. Dr. Verma was replaced by Dr. Russ Hynes who worked as a Research Scientist on the project for 6 months before taking a position with AAFC.

The phytoremediation program hosted several visiting scholars at the University of Saskatchewan: Dr. Thomas Reichenauer and Ms. Nancy Stralis-Paavese (Ph.D. student) from Vienna (2003); Dr. Naeem Chudhary from Pakistan (2003); Dr. Alex Nogueira from the Federal University of Santa Catarina Florianopolis in Brazil (2003); Ms. Rossana Segreto (Ph.D. student), Department of Plant Biology, University of Torino, Italy (2005); Mr. Bernt Felderer (Ph.D. student), Department of Forest and Soil Sciences, BOKU–University of Natural Resources and Applied Life Sciences, Vienna, Austria; and Dr. Peter Leinweber, Institute for Land Use, University of Rostock, Rostock, Germany (2005). Dr. Leinweber also hosted one of our Ph.D. students (Adam Gillespie) in Rostock for three months in 2005.

Drs. Germida and Farrell are responsible for overall coordination of the project and liaison with industry and government partners. They also ensure linkage between field research and sub-projects providing graduate student training. Each sub-project is overseen by at least two co-PIs: Drs. Farrell and Germida coordinated Sub-project #1: Field-scale assessment of phytoremediation; Drs. Germida, Greer & Lawrence coordinated Sub-project 2: Microbial diversity studies; Drs. Knight & Van Rees coordinated Sub-Project #3: Root dynamics; Drs. Ross, Knight, & Farrell coordinated Sub-Project #4 Remediation of metals and salts; Dr. Si & Fleming coordinated Sub-project #5: Modeling the temporal and spatial variability of contaminants; and Drs. Germida & Headley coordinated Sub-project #6: aquatic systems.

Graduate students were typically co-supervised by two co-PIs: Sarah Armstrong (Germida & Headley), Jen Fernet (Germida & Lawrence), Shannon Gerrard (Knight & Farrell), Adam Gillespie (Farrell & Ross), Jeff Gunderson (Van Rees & Knight), Carol Luca (Si and Farrell), Lori Phillips (Germida & Greer), Cory Sonntag (Ross & Scoles) and Alexis McFerson (Fleming). All students have advisory committee meetings at least once a year; in addition to co-supervisors it was typical for other co-PIs to sit on these committees. Regular communication was maintained through email.

Annual "phytoremediation lunches" were held to provide the team with an update on milestones, budgets, and outputs and to outline project activities for the coming year. The students gave brief reports on their research activities to date and the fit within the project. As well, Drs. Germida and Farrell met annually with industry and government collaborators to provide research updates and discuss planned activities. Trevor Carlson/Kris Bradshaw (Fed. Co-op), Phil Langille/Maria Campbell (Talisman Energy) and Carol Engstrom (Husky Oil Operations Ltd) were actively involved in developing plans and setting up the field sites. Petroleum Technology Alliance Canada (Eric Lloyd; retired 2007) coordinated Technology Information Sessions where results of the project were reported. Dr. Terry McIntyre (Environment Canada) provided consultation on the project and linkage to the USEPA supported RTDF activities. Dr. Peter Kulakow (RTDF coordinator, Kansas State University) made a site visit (2004) and was involved in numerous discussions regarding analytical protocols and data analysis to ensure that our results were compatible with those from the United States RTDF sites.

3. Training

3.1 Please list **each** trainee (Undergraduate Students, Master's Students, Doctoral Students, Postdoctoral Fellows, Research Associates, Technicians …) on a separate line in the table below providing: a) the number of years they have been on the project, b) the percentage (%) of time each type of trainee spent on this project, and c) the percentage (%) of funding from this strategic grant. If a trainee is fully paid from other sources, type in 0 in the "% of funding from this grant" column.

Trainee- Specify type (e.g. M.Sc., Ph.D. etc) **(one trainee per line)**	**(a)** **Number of calendar years on the project**	**(b)** **% of research time spent on this project**	**(c)** **% of funding from this grant**
Lori Phillips (Ph.D.)[*‡]	4	100	10
Cory Sonntag (Ph.D.)[‡]	4	100	40
Sarah Armstrong (Ph.D.)[‡]	2	25	5
Adam Gillespie (Ph.D.)[†]	1	100	10
Jennifer Fernet (M.Sc.)[¶‡]	3	100	75
Jeff Gunderson (M.Sc.)	2.1	100	50
Shannon Gerrard (M.Sc.)	2.4	100	25
Carol Luca (M.Sc.)[*]	2	100	50
Alexis McPherson (M.Sc.)	2.5	100	50
Diana Bizecki-Robson (Ph.D.)	0.5	100	100
Adam Gillespie (M.Sc.)	1	100	80
Julie Roy (M.Sc.)	0.5	50	50
Cindy Wall (M.Sc.)	0.5	50	0
Monique Wismer (M.Sc.)	1.5	100	100
Brij Verma (PDF)	0.4	100	100
Russ Hynes (Research Scientist)	0.5	100	100
Adam Gillespie (Research Associate)[†]	1	75	25
Jay Booth (Research Associate)	0.3	100	100
Nancy McCrea (Research Associate)	3	100	60
Darin Richman (Technician)	3	15	0
Arlette Seib (Technician)	4	25	25
Mark Cooke (Undergraduate Assistant)	0.5	50	0
Nyla Dubiel (Undergraduate Assistant)	0.3	100	100
Jola Pisz (Undergraduate Assistant)	0.3	100	100
Darin Richman (Undergraduate Assistant)	1	50	25
Kim Kovacs (Undergraduate Assistant)	0.3	100	100
Cim Kneller (Undergraduate Assistant)	0.3	100	100
Kuni Nina (Undergraduate Assistant)	0.3	100	100
Curtis Sambrook (Undergraduate Assistant)	0.1	100	100
Holly Kalyn (Undergraduate Assistant)	0.5	75	75
Lissa de Freitas (Undergraduate Assistant)	0.4	100	100

[*] Ms. Phillips enrolled in the M.Sc. program in Sep. 2003 but was transferred into the Ph.D. program in Dec. 2004; Ms. Phillips was the recipient of a NSERC PGS-A (2003–2005) and PGS-D (2004–2007) scholarship; Ms. Luca was the recipient of a NSERC PGS-A scholarship (2004–2005).

[†] Mr. Gillespie was the recipient of a NSERC PGS-D (2004–2005). Mr. Gillespie withdrew from the graduate program for medical reasons in March 2005; however, he remained with the project as a research associate working for Dr. Farrell until re-entering the Ph.D. program (but on a different project) in March 2006.

[¶] Ms. Fernet was on a leave of absence from 01 Sep. 2004 to 30 Apr. 2005 and from 01 Sep. 2006 to 15 May 2007.

[‡] L. Phillips, C. Sonntag, S. Armstrong and J. Fernet are expected to defend their theses/dissertations in late summer/early fall 2007.

3.2 What type(s) of interaction did the highly qualified personnel (HQP) have with the partners during the project? (Select all that apply.)

_x_ HQP presented research results to the partners
_x_ HQP discussed the project directly with partners to obtain input
___ Partners jointly supervised thesis projects of HQP
___ HQP worked regularly in the partner's facilities
___ HQP did not interact with the partners
_x_ Other (Specify): HQP worked regularly at sites provided and monitored by the partners

3.3 To the best of your knowledge, please complete the following table on the employment of HQP involved in the project.

Type of HQP	# hired by partner	# hired by industry	# hired by government labs	# employed in academia (faculty)	# hired by other (specify)	# in academic training
Undergraduate Students	1	2	0	1*	0	5
Master's Students	0	2	2	0	3[†]	1
Doctoral Students	0	0	0	0	1[‡]	1
Postdoctoral Fellows	0	0	0	1	0	0
Research Associates	0	0	0	0	0	2
Technicians	1	0	0	0	2[¶]	0
Other (Research Scientist)	0	0	1	0	0	0

* M. Cooke was hired as a Research Technician by Dr. J.D. Knight in the spring of 2007.
[†] S. Gerard (M.Sc. 2006), J. Gunderson (M.Sc. 2006) and C. Wall (M.Sc. 2004) are employed as Research Associates at Texas A&M University, Noth Dakota State University, and the University of Saskatchewan, respectively.
[‡] D. Bizecki-Robson is employed as the Curator of Botany at The Manitoba Museum
[¶] D. Richman and A. Seib continue to be employed as Research Technicians at the University of Saskatchewan.

4. Dissemination of Research Results and Knowledge or Technology Transfer

4.1 Publications, conference presentations, etc.

Please provide the **number** of publications, conference presentations, and workshops arising from the research project supported by the grant in the table below.

_____ None to date

- OR -

	Number of publications, presentations…		
Status	Refereed Journal Articles	Conference Presentations/ Poster	Other (including Technical Reports, Non-Refereed Articles, etc.)
Accepted/Published	8	47	26
Submitted	2	2	4

4.2 Please provide the bibliographical reference data for the above publications, conference presentations and workshops under the corresponding headings specifying for publications whether they are submitted, accepted or published.

Refereed Journal Articles:

PUBLISHED:

J. Gunderson, J.D. Knight and K. Van Rees. 2007. Impact of ectomycorrhizal colonization of hybrid poplar on remediation of diesel contaminated soil. Journal of Environmental Quality, 36: 927-934.

L.A. Phillips, C.W. Greer and J.J. Germida. 2006. Culture-based and culture-independent assessment of the impact of mixed and single plant treatments on rhizosphere microbial communities in hydrocarbon contaminated flare-pit soil. Soil Biology and Biochemistry, 38:2823-2833.

D.B. Robson, J.J. Germida, R.E. Farrell and J.D. Knight. 2005. Hydrocarbon Tolerance Correlates with Seed Mass and Relative Growth Rate. Bioremediation Journal, 8:185-199.

R.K. Hynes, R.E. Farrell and J.J. Germida. 2004. Plant Assisted Phenanthrene Degradation by *Sphingomonas macrogoltabidus* as Assessed Using Solid-phase Microextraction (SPME). International Journal of Phytoremediation, 6:253-268.

D.B. Robson, J.D. Knight, R.E. Farrell and J.J. Germida. 2003. Natural Revegetation of Hydrocarbon-contaminated Soil in Western Canada. Canadian Journal of Botany, 82:22-30.

D.B. Robson, J.D. Knight, R.E. Farrell and J.J. Germida. 2003. Ability of Cold-Tolerant Plants to Grow in Hydrocarbon-Contaminated Soil. International Journal of Phytoremediation, 5:105-123.

J.J. Germida, C.M. Frick and R.E. Farrell. 2002. Phytoremediation of Oil Contaminated Soils. In A. Violante, P.M. Huang, J.-M. Bollag and L. Gianfreda (eds.), Soil Mineral-Organic Matter-Microorganism Interactions and Ecosystem Health, Pp. 169-186. Developments in Soil Science 28B. Amsterdam.

ACECEPTED:

Carol Luca, Richard Farrell and Bing Si. 2007. Assessing Spatial Distribution And Joint Uncertainty Of TPH-Fractions: Indicator Kriging And Sequential Indicator Simulation. Canadian Journal of Soil Science, *Accepted June 2007*.

SUBMITTED:

J. Gunderson, J.D. Knight and K. Van Rees. 2007. Hybrid poplar fine root production as affected by soil nutrients and hydrocarbon contamination. Bioremediation Journal, *Submitted May 2007*.

S.A. Armstrong, J.V. Headley, K.M. Peru, and J.J. Germida. 2007. Toxicity of oil sands naphthenic acids and dissipation from systems planted with emergent aquatic macrophytes. Journal of Environmental Science and Health Part A: Toxic/Hazardous Substances & Environmental Engineering, *Submitted May 2007*.

Note: another 10 publications are in various stages of preparation and are expected to be submitted within the next year.

Conference Presentation/Poster:

SUBMITTED CONFERENCE PRESENTATIONS:

R. Farrell, L. Phillips and J. Germida. 2007. Phytotechnology Applied to a Weathered Flare-pit Soil: Reflections on a Four-year Field Study, 4th International Conference on Phytotechnologies, 24–26 September, Denver, CO.

L. Phillips, R. Farrell, C. Greer, and J. Germida. 2007. Phytoremediation of Weathered Flare-pit Soil: Impact of Mixed and Single Plant Treatments on Hydrocarbon Degradation Potential. 4th International Conference on Phytotechnologies, 24–26 September, Denver, CO.

INVITED CONFERENCE PRESENTATIONS IN PUBLISHED ABSTRACTS:

Jim Germida and Rich Farrell. 2007. Reflections on a Four-year Field Study: Does Phytoremediation of Petroleum Impacted Soils Really Work? Canadian Society of Microbiology 57th Annual Meeting, 17–

20 June, Quebec, QC.

R. Farrell, N. McCrea and J. Germida. 2005. Field-scale Assessment of Phytotechnology Applied to Sites Impacted with Weathered Hydrocarbons. Proceedings of the 21st International Conference on Soils, Sediments & Water, 17–20 October, University of Massachusetts, Amherst, MA.

R.E. Farrell. 2002. Plant Phytoremediation of Hydrocarbon Contaminated Soils in Western Canada. Abstracts of the 52nd Annual Meeting of the Canadian Society of Microbiologists, S31, June 16-19, Saskatoon, Saskatchewan.

CONTRIBUTED CONFERENCE PRESENTATIONS IN PUBLISHED ABSTRACTS:

A. McPherson and I. Fleming. 2007. Monitoring Phytoremediation of Petroleum Hydrocarbon-Contaminated Soils in a Closed and Controlled Environment. The Ninth International In-Situ and On-Site Bioremediation Symposium. 7–10 May 2007, Baltimore, MD.

J.V. Headley, S.A. Armstrong, K.M. Peru, and J.J. Germida. 2007. Phytoremediation of oil sands naphthenic acids – evidence for selective uptake in cattails. 60th National Conference for the Canadian Water Resources Association, 25–28June, Saskatoon, SK.

L.A. Phillips, C.W. Greer, and J.J. Germida. 2007. The impact of plant root exudates on the hydrocarbon degradation potential of soil microbial communities. Canadian Society of Microbiology 57th Annual Meeting, 17–20 June, Quebec, QC.

L.A. Phillips, S.A. Armstrong, J.V. Headley, C.W. Greer, and J.J. Germida. 2007. Community structure and taxonomic analysis of micro-organisms associated with the roots of *Typha latifolia* growing in naphthenic acids. Canadian Society of Microbiology 57th Annual Meeting, 17–20 June, Quebec, QC.

S.A. Armstrong, J.V. Headley, K.M. Peru, D.W. McMartin, and J.J. Germida. 2007. Differences in phytotoxicity and dissipation between ionized and non-ionized oil sands naphthenic acids. 17th Society of Environmental Toxicology and Chemistry Europe Conference, 21–24 May, Porto, Portugal.

S.A. Armstrong, J.V. Headley, K.M. Peru, and J.J. Germida. 2007. Differences in phytotoxicity and dissipation between ionized and unionized oil sands naphthenic acids. 42nd Western Canada Trace Organic Workshop, 23–25 April, Calgary, AB.

C. Sonntag and A.R.S. Ross. 2006. Identification of Copper-binding Plant Proteins using Immobilized Metal-ion Affinity Chromatography, Gel Electrophoresis and LC-MS/MS. Proceedings of the 54th American Society for Mass Spectrometry (ASMS) Conference, Seattle WA.

J.L. Fernet, J.R. Lawrence and J.J. Germida. 2006. Plant bacterial inoculants for the remediation of hydrocarbon impacted soil. Canadian Society of Microbiologists Annual Meeting, 18–21 June 2006, London ON

J. Gunderson, J.D. Knight and K.C.J. Van Rees. 2006. The effect of mycorrhizal inoculation on hybrid poplar fine root dynamics in hydrocarbon contaminated soils. Canadian Society of Soil Science Annual Meeting, May 14-17 Banff, AB.

L.A. Phillips, R.E. Farrell, C.W. Greer, and J.J. Germida. 2006. Phytoremediation of flare-pit soil: The impact of mixed and single plant treatments on microbial hydrocarbon degradation potential. 13th International Petroleum Environmental Conference, San Antonio, TX.

L.A. Phillips, J.J. Germida, and C.W. Greer. 2006. Diversity and activity of hydrocarbon degrading bacteria in soil amended with plant root exudates. International Society of Microbiology 11th Symposium, Vienna, Austria.

L.A. Phillips, J.J. Germida, and C.W. Greer. 2006. Enhanced degradation activity by endophytic bacteria of plants growing in hydrocarbon contaminated soil. Canadian Society of Soil Science Annual Meeting, Banff, AB.

L.A. Phillips, J.J. Germida, and C.W. Greer. 2006. Diversity and activity of hydrocarbon degrading bacteria in soil amended with plant root exudates. Canadian Society of Microbiology 56th Annual Meeting, London, ON.

R.E. Farrell, J.J. Germida, N. McCrea, A.W. Gillespie, and L. Phillips. 2006. Field-scale assessment of phytoremediation at a site impacted with weathered hydrocarbons. Canadian Society of Soil Science Annual Meeting, Banff, AB.

S.A. Armstrong, J.V. Headley, K.M. Peru, and J.J. Germida. 2006. Uptake and toxicity of oil sands naphthenic acids in wetland plants. 27th Society of Environmental Toxicology and Chemistry Conference, 5–9 November, Montreal, QC.

S.A. Armstrong, J.V. Headley, K.M. Peru, and J.J. Germida. 2006. Fate of two different naphthenic acid mixtures in plants. 41st Western Canada Trace Organic Workshop, 23–26 April, Vancouver, BC.

C. Sonntag and A.R.S. Ross. 2005. Identification of Copper-binding Plant Proteins using LC-MS/MS.

18th Annual Tandem Mass Spectrometry Workshop, Lake Louise AB.

C. Sonntag, A.R.S. Ross, and C. Christensen. 2005. Localization and Speciation of Copper in Tissues of Two *Arabidopsis thaliana* Genotypes. 8th Annual Canadian Light Source Users' Meeting, Saskatoon SK.

L.A. Phillips, J.J. Germida, and C.W. Greer. 2005. Enhanced degradation activity by endophytic bacteria of plants growing in hydrocarbon contaminated soil. Canadian Society of Microbiology 55th Annual Meeting, Halifax, NS.

S. Gerrard, R.E. Farrell and J.D. Knight. 2005. Phytoremediation of Brine-Contaminated Soil with Salt-Tolerant Plants: A Screening Study. Proceedings of the 2005 Soils and Crops Workshop, February 17-18, Saskatoon, Saskatchewan. On CD.

S.A. Armstrong, J.V. Headley, K.M. Peru, J.J. Germida, and D.W. McMartin. 2005. Fate and toxicity of naphthenic acids in wetland plants. Research Poster: 26th Society of Environmental Toxicology and Chemistry Conference, 13–17 November, Baltimore, MD.

S.A. Armstrong, J.V. Headley, K.M. Peru, and J.J. Germida. 2005. Monitoring the phytodegradation of naphthenic acids using hydroponically grown cattails (*Typha latifolia*). 40th Western Canada Trace Organic Workshop, 2–4 May, Winnipeg, MB.

C. Sonntag, A.R.S. Ross, D.J.H. Olson, C. Christensen, S.J. Ambrose, and G. Scoles. 2004. Copper Speciation and Localization in Hyperaccumulating Plants. 7th Annual Canadian Light Source Users' Meeting, Saskatoon SK.

L.A. Phillips, C.W. Greer, and J.J. Germida. 2004. Microbial catabolic diversity in the rhizosphere of hydrocarbon-remediating plants. Canadian Society of Microbiology 54th Annual Meeting, Edmonton, AB.

J.J. Germida and R.E. Farrell. 2004. Field-scale Assessment of Phytotechnologies for Remediation of Industrial Sites Impacted with Weathered Hydrocarbons. Proceedings of the Cold Regions Engineering & Construction Conference 2004. 19 May 2004, Edmonton, AB. (on CD)

A.W. Gillespie, A.R.S. Ross, K.C.J. Van Rees and R.E. Farrell. 2004. Detection and Quantification of Organic Acids, Amino Acids and Phenolics in Root Exudates Using Gas Chromatography. Western Canada Trace Organic Residue Workshop, April 11-13, Saskatoon, Saskatchewan.

A.W. Gillespie, A.R.S. Ross, K.C.J. Van Rees, and R.E. Farrell. 2004. Does PAH Contamination Affect Root Exudation Patterns in Purple Prairie Clover and Slender Wheatgrass? Western Canada Trace Organic Residue Workshop, April, Saskatoon, Saskatchewan.

A.W. Gillespie, K.C.J. Van Rees and R.E. Farrell. 2003. Root Exudation Patterns of Plants Grown in Hydrocarbon Contaminated and Non-Contaminated Soils. 705 – p679. Canadian Society of Soil Science Meeting, August 10-13, Montreal, Quebec.

A.V. Nogueira, W.K. Ma, R.E. Farrell and J.J. Germida. 2003. Arbuscular Mycorrhizal Fungi Spores in Soils from a Phytoremediation Field Trial. 446 - p453. The Fourth International Conference on Mycorrhizae, August 10-15, Montreal, Quebec.

D.B. Robson, J.D. Knight, R.E. Farrell and J.J. Germida. 2003. Natural Revegetation of Hydrocarbon-Contaminated Soil in the Prairie Ecozone. Joint Annual Meetings of the Ecological Society of America and the Society for Ecological Restoration, August, Tucson, Arizona.

D.B. Robson, J.D. Knight, R.E. Farrell and J.J. Germida. 2003. Natural Revegetation of Hydrocarbon-Contaminated Soil in the Prairie Ecozone. Joint Annual Meetings of the Ecological Society of America and the Society for Ecological Restoration, August 4-9, Tucson, Arizona.

D.B Robson, J.D. Knight, R.E. Farrell and J.J. Germida. 2003. Natural Revegetation of Hydrocarbon-Contaminated Soil. NPSS - Native Plant Society of Saskatchewan Annual Meeting, February 20-22, Manitou Beach, Saskatchewan.

S.L. Luettgen, S.J. Ambrose, D.J.H. Olson, C. Sonntag and A.R.S. Ross. 2003. Speciation and Characterization of Dissolved Metal-binding Peptides by Electrospray MS and MS/MS. Proceedings of the 51st ASMS Conference, Montreal QC.

INVITED CONFERENCE PRESENTATIONS:

R.E. Farrell and J.J. Germida. 2007. Phytoremediation–Applications for the Remediation of Petroleum Hydrocarbons. PTAC 2007 Soils and Groundwater Forum, March 28th, Calgary, AB.

R.E. Farrell and J.J. Germida. 2006. Field-scale assessments of phytotechnologies applied to sites impacted with weathered hydrocarbons—Year 4 overview. PTAC 2006 Soils and Groundwater Forum, March 23rd, Calgary, AB.

R.E. Farrell. 2005. Phytotechnologies: Plant-based Systems for Remediating or Restoring Contaminated Sites. Saskatchewan Environmental Industry and Managers Association Soil

Contamination Workshop, November 13th, Saskatoon, SK.

R.E. Farrell and J.J. Germida. 2005. Field-scale Assessments of Phytotechnologies Applied to Sites Impacted with Weathered Hydrocarbons. PTAC 2005 Soils and Groundwater Research Forum for the Upstream Oil and Gas Industry, March 23, Calgary, AB.

R.E. Farrell and J.J. Germida. 2004. Field-scale Assessments of Phytotechnologies Applied to Sites Impacted with Weathered Hydrocarbons. PTAC 2004 Soils and Groundwater Research Forum, March 23–24, Calgary, AB.

R.E. Farrell and J.J. Germida. 2003. Phytoremediation. PTAC 2003 Soils and Groundwater Research Forum and Technical Review, May 14–15, Calgary, AB.

PRESENTATIONS AT CONFERENCES (Non-Invited):

J.L. Fernet. 2006. Clean-up on Aisle Four–Phytoremediation and Agriculture. 2006 Canadian Association of Agri-Retailers–Crop Management Forum, Saskatoon, SK.

R.E. Farrell, J.J. Germida, N. McCrea and A.W. Gillespie. 2006. Field-scale Assessment of Phtotechnology Applied to a Weathered Flare-pit Soil. Canadian Society of Soil Science Annual Meeting, May 14-17, Banff, AB.

S.A. Gerrard, R.E. Farrell and J.D. Knight. 2005. Phytoremediation of Brine-Affected Soil with Two Halophytic Species. Canadian Society of Soil Science Annual Meeting, May 15-18, Halifax, Nova Scotia.

C. Luca, R. Farrell and B. Si. 2004. Fluorometry for Determining Total Petroleum Hydrocarbons in Contaminated Soil. Annual Meetings of the American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, October 31-November 4, Seattle, Washington.

C.J. Luca, B.C. Si and R.E. Farrell. 2004. Contributing Area Predicts Soil Organic Carbon. Annual Meetings of the American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, October 31-November 4, Seattle, Washington.

Other (Including Technical Reports, Non-Refereed Articles, etc.):

TECHNICAL REPORTS:

R.E. Farrell. 2006. Field-scale Assessment of Phytoremediation of a Flare-Pit Soil in Carlyle, SK: Year 4 Overview. Technical Report submitted to Talisman Energy, Calgary, AB.

R.E. Farrell. 2006. Field-scale Assessment of Phytoremediation at a Former Tank Battery in Bruderheim, AB. Technical Report submitted to Husky Energy, Calgary, AB.

R.E. Farrell. 2006. Field-scale Assessment of Phytoremediation at a Former Tank Battery in Bruderheim, AB. Technical Report submitted to Alberta Energy & Utilities Board, March 2006.

Rich Farrell, Jim Germida, and Adam Gillespie. 2006. Field-scale assessment of phytotechnologies applied to sites impacted with weathered petroleum hydrocarbons: Carlyle, SK–2006 Field Activities. Technical Report submitted to Talisman Energy, Calgary, AB.

Rich Farrell, Jim Germida, and Adam Gillespie. 2005. Field-scale assessment of phytotechnologies applied to sites impacted with weathered petroleum hydrocarbons: Carlyle, SK–2005 Field Activities. Technical Report submitted to Talisman Energy, Calgary, AB.

R.E. Farrell. 2005. Summer 2005 Activities at Hendon, Kelvington & Wadena SK. Report submitted to Federated Co-Operatives, Ltd. Saskatoon, SK.

R.E. Farrell and J.J. Germida. 2005. Field-scale assessments of phytotechnologies applied to sites impacted with weathered hydrocarbons. 2004 Activities Report submitted to the Canadian Association of Petroleum Producers–Environmental Research Advisory Council, Calgary, AB.

R.E. Farrell and J.J. Germida. 2005. Field-scale assessments of phytotechnologies applied to sites impacted with weathered hydrocarbons. 2003–2004 Activities Report submitted to Environment Canada, Ottawa, ON.

J.J. Germida and R.E. Farrell. 2004. Effectiveness of plant species in promoting the degradation of TPH and BTEX compounds in soil. 2002–2003 Activities Report, Part 1 submitted to Environment Canada, Ottawa, ON.

J.J. Germida and R.E. Farrell. 2004. Field-scale assessments of phytotechnologies applied to sites impacted with weathered hydrocarbons. 2002–2003 Activities Report, Part 2 submitted to Environment Canada, Ottawa, ON.

R.E. Farrell and J.J. Germida. 2003. Effectiveness of plant species in promoting the degradation of TPH and BTEX compounds in soil. Technical report submitted to Environment Canada, Ottawa, ON.

EXTENSION MATERIALS:

R.E. Farrell. 2005. ERAC Project Update, Field-scale Assessment of Phytotechnologies Applied to Sites Impacted with Weathered Petroleum Hydrocarbons. Environmental Research Advisory Council, Calgary, AB.

R.E. Farrell. 2004. ERAC Project Update, Field-scale Assessment of Phytotechnologies Applied to Sites Impacted with Weathered Petroleum Hydrocarbons. Environmental Research Advisory Council, Calgary, AB.

R.E. Farrell. 2003. ERAC Project Update, Field-scale Assessment of Phytotechnologies Applied to Sites Impacted with Weathered Petroleum Hydrocarbons. Environmental Research Advisory Council, Calgary, AB.

THESES & DISSERTATIONS:

COMPLETED

A. McPherson. 2007. Monitoring Phytoremediation of Petroleum Hydrocarbon Contaminated Soils in a Closed and Controlled Environment. M.Sc. Thesis. Department of Civil & Geological Engineering, University of Saskatchewan, Saskatoon, SK (Fleming, supervisor).

J. Gunderson. 2006. The Effect of Hydrocarbon Contamination and Mycorrhizal Inoculation on Poplar Fine Root Dynamics. M.Sc. Thesis. Department of Soil Science, University of Saskatchewan, Saskatoon, SK (Knight & Van Rees co-supervisors).

S.A. Gerrard. 2006. Phytoremediation of Brine-Contaminated Soil with Halophytic Plants. M.Sc. Thesis. University of Saskatchewan, Saskatoon, SK (Farrell & Knight co-supervisors).

C.J. Luca. 2005. Characterizing the Spatial Distribution of Petroleum Hydrocarbons. M.Sc. Thesis. University of Saskatchewan, Saskatoon, SK (Farrell & Si co-supervisors).

A.W. Gillespie. 2004. Measurement of Root Exudation Patterns for Plants Grown in Petroleum Hydrocarbon-contaminated and Non-contaminated Soils. M.Sc. Thesis. University of Saskatchewan,

Saskatoon, SK (Farrell & Van Rees co-supervisors).
M.E. Wismer. 2004. In vitro Metabolism of Benzene by Plant Cell Suspensions. M.Sc. Thesis. University of Saskatchewan, Saskatoon, SK (Knight & Germida, co-supervisors).
D. Bizecki-Robson. 2003. Phytoremediation of hydrocarbon-contaminated soil using plants adapted to the western Canadian climate. Ph.D. Dissertation. (Knight & Germida co-supervisors).
J.L. Roy. 2003. Microbial Diversity Asociated with Roots of Plants Growing in Petroleum Contaminated Soils. M.Sc. Thesis. (Germida supervisor).
C.M. Wall. 2003. Production of a Granular Compost Carrier for Bacterial Inoculants. M.Sc. Thesis. (Farrell & Germida co-supervisors).

IN PROGRESS

C. Sonntag. 2007. Identification and Characterization of Metal-Binding Polypeptides for Enhanced Phytoremediation in Crucifers. Ph.D. Dissertation, Department of Plant Sciences, University of Saskatchewan (Ross & Scoles, co-supervisors; anticipated completion, Fall 2007).
L.A. Phillips. 2007. Characterization of Rhizosphere and Endophytic Bacteria Associated with the Roots of Plants Growing in Hydrocarbon Contaminated Soil. Ph.D. Dissertation, Department of Soil Science, University of Saskatchewan (Germida & Greer, co-supervisors; anticipated completion, Fall 2007).
S.A. Armstrong. 2007. Dissipation and Phytotoxicity of Oil Sands Naphthenic Acids in Aquatic Macrophytes. Ph.D. Dissertation, Environmental Toxicology, University of Saskatchewan (Headley & Germida, co-supervisors; anticipated completion, Fall 2007).
J.L. Fernet. 2007. Bacterial Inoculants to Enhance Phytoremediation of Hydrocarbon Contaminated Soil. M.Sc. Thesis, Department of Soil Science, University of Saskatchewan (Lawrence & Germida, co-supervisors; anticipated completion, Fall 2007).

4.3 How many of the papers and presentations listed above were co-authored by the partners?

0

4.4 **Patents and Licences**

Please provide the **number** of patents (applied for and granted) and licenses to date arising from the research project supported by the grant in the table below.

x **Not applicable**

- OR -

___ None Yet Filed/Granted

	Number of Patents or Licenses		
Description	**CANADA**	**U.S.**	**OTHER**
Patent Applications Filed			
Patents Issued			
Licenses or options			

4.5 Please provide details (titles, patent number…) about the above listed patents and licences under the corresponding headings.

Patent Applications Filed:

Not Applicable

Patents Issued:

Not Applicable

Licences or Options:

Not Applicable

4.6 Identify the tangible results obtained during the research project. (Select all that apply.)

_____ **Prototype/pilot**
_____ **New product**
_____ **New process**
_____ **Improved product**
__x__ **Improved process**
__x__ **Contribution to policy or regulation**
_____ **Other (specify)** ____________________________

Briefly describe these outcomes.

This research has led us to develop new protocols to optimze the phytoremediation process at sites impacted by weathered petroleum hydrocarbons. Whereas the standard protocols we used to establish the field sites depended on the use of multiple plant species to "enhance" the microbial diversity of root associated micro-organisms, our research showed that (i) the contaminant itself (in this case, PHCs) exerted the greatest control on these microbial communities; (ii) rhizodegradation of PHCs was generally greater in systems planted with a specific single plant species than in systems planted with mixed plant communities; (iii) specific plant-microbe combinations can yield enhanced rhizodegradation of PHcs (though issues surrounding the up-scaling of this technology remain to be solved); (iv) PHC-degrader communities are generally larger in planted systems than in unplanted systems (suggesting that a "plough-down" phase may result in enhanced distribution of these degrader organisms throughout the soil); (v) trees effectively remove water-soluble, light fraction (F1 & F2) hydrocarbons via phytovolatilization; (vi) geostatistical mapping techniques provide a means of identifying areas within a contaminated site that require remediation (thus reducing costs) or that, at the end of a remediation treatment, have achieved their environmental targets; and (vii) salt- and PHC-tolerant plants able to remove salts from brine contaminated soils were identified. As well, new insights into how plants tolerate and accumulate metals from contaminated soils are expected to help plant breeders develop high biomass plants with enhanced phytoremediation capabilities.

This research demonstrated that phytotechnologies can play an important role in future remediation strategies in Canada by providing critical 'field performance data' to assess its strengths and limitations, and determine appropriate uses of these newly emerging phytotechnologies. As such, it is expected that this research will help regulators at both the provincial and national levels make better informed decisions regarding the use of plants as an effective tool for on-site remediation. Together with information gathered at their sister sites in the United States (under the umbrella of the USEPA RTDF program), this information is expected to help drive future regulatory policies on both a national and international scale.

Significance to Canada: Our research is significant for Canada in a number ways. Foremost, is the identification of native plant species that contribute to the remediation of petroleum-impacted soil. In addition, the knowledge gained on how rhizosphere microorganisms interact with these native plant species to remediate petroleum hydrocarbons suggest that using rhizosphere bacteria as inoculants for selected plants may enhance phytoremediation systems. On the practical side, this research has demonstrated the importance of applying agronomic practices to help establish plants on petroleum impacted field soils. Another key outcome of this project is the training of a large number of highly qualified personnel, specifically graduate and undergraduate students, who have gone on to work on their advanced degrees or in jobs in industry or government labs. This represents an important investment in developing a cohort of young scientists capable of undertaking environmentally relevant research. Overall, our project provided an excellent opportunity for university and government scientists to collaborate with one another and industry partners. As a result, on-going research collaborations are established, and new opportunities for future research with industry partners on remediation of petroleum-impacted sites created. Finally, technology transfer to industry partners through this project provides them the opportunity to decide how best to utilize plant-based phytoremediation systems.

4.7 Prospects for the transfer of the results to the user sector

Describe how the results achieved are being transferred to the user sector and the prospects for their commercial/industrial exploitation or their use by other sectors (e.g., revising or formulating policy or regulations).

Information developed from this project is being transferred to the user sector (i.e., upstream oil and gas developers and environmental consultants) through meetings with our partners and meetings organized by supporting organizations (e.g., the annual Soil and Groundwater Forum organized by PTAC). Technology transfer also occurs through the dissemination of extension publications prepared for CAPP-ERAC (see Section 4.2). This information is also made available to the governments of Saskatchewan (through annual reports to Saskatchewan Agriculture & Food, which provides base funding to Drs. Farrell & Knight through the Strategic Research Program in Soil Biological Processes), Alberta (through their participation in the PTAC Phytoremediation Steering Committee), and Canada (through the participation of the Environmental Biotechnology Applications group at Environment Canada).

Information placed into the public domain via the internet (either by the co-PIs or from published abstracts and conference proceedings) has resulted in contacts with industry and government consultants regarding the use of plant-based remediation strategies for on-site clean-up of PHC-contaminated soils. As well, several industry organizations have initiated discussions regarding the outcomes of this project with an eye on exploiting plant-based systems for on-site remediation of rural and northern sites impacted by petroleum hydrocarbons. (see Section 9.2)

Summaries of this project will be presented at the 4th International Conference on Phytotechnologies (24–26 September 2007, Denver, CO)–a biannual meeting of industry, government and university researchers that addresses the state-of-the-art in phytotechnologies.

5. Problems Encountered

5.1 Identify the main problems encountered during the research project. (Select all that apply.)

__x__ **Technical or scientific problems**
_____ **Problems with direction of research or findings**
__x__ **Equipment and facilities**
__x__ **Staffing issues (including students)**
__x__ **Funding problems**
_____ **Partner withdrew from project**
_____ **Partner interaction issues**
_____ **Other (specify)** ________________________

- OR -

_____ No problems occurred during the research project

5.2 Briefly describe the main problems identified above and the steps taken to resolve each one.

Technical: As sub-Project #1 progressed, there was a significant change in the plant community composition in both the reference and site-specific treatments. It was decided (following consultations with various partners) that from a pratical perspective it would be best to let nature take its course and not disturb the plots. In hindsight, this was not the best solution; i.e., other studies both in the field and the greenhouse later found that the most competetive plant species were not necessarily the most effective phytoremediator plants (and that mixed plant communities generally were outperformed by monocultures).

Plans to develop an urban site (in Saskatoon or Regina) eventually were abandoned as it became clear that our objectives (research) and those of the city administrators (site clean-up) were not compatable. That is, there was an expectation that the University would accept responsibility for site clean-up upon completion of the research project–a condition we were not prepared to meet. At about the same time, work at the Kelvington site ceased as the sub-Projects using this site (#s 2, 3 & 5) had matured to the point where additional work at the site was no longer necessary. Moreover, it was determined that we were unlikely to see any appreciable treatment (i.e., plant) effects given the depth to the contaminant and the relatively short time scale involved. Thus, when an opportunity arose to assess the potential of phytoremediation for tailings pond water produced during crude oil extraction from the Athabasca oil sands (Fort McMurray, AB), we shifted some of the focus originally directed at Kelvington to Fort McMurray (see sub-Project #6). Dr. Headly's lab also performed all analyses associated with sub-Project #6.

Equipment: Initially, some problems were encountered with setting up the accelerated solvent extraction (ASE) unit and gas chromatography (GC) system for hydrocarbon analyses. Problems with the ASE unit involved some minor technical issues (primarily involving the need for special venting) of which we were unaware at the time of purchase. These were solved by having the manufacturer work with the University's Facilities Management Division. Problems with the GC involved the selection of proper columns and development of an appropriate temperature program for separation of the compounds of interest. These were solved by working closely with the manufacturer. To address some of the delays associated with these problems, Dr. Headley's lab at NWRI took over the sample analyses for sub-Project #5b.

Staffing: Whereas there was no shortage of either graduate students (applications continue to come in from potential students who want to work on phytoremediation) or undergraduates, we had some trouble finding a suitable PDF. Dr. Brij Verma was hired from Dr. Headley's lab in August of 2003, only to resign in December 2003 to accept a position with the Alberta Research Council. Dr. Verma was replaced by Dr. Russ Hynes (who was hired as a Research Scientist) who worked on sub-Project #2b and developed a solid phase microextraction (SPME) technique that was (and still is) used to screen potential plant-microbe systems for PHC degradation.

Funding: Total funding (cash + in-kind) from committed sources was about 17% less than anticipated, thus considerable time and effort were placed into obtaining additional funding to make up for these losses. We enjoyed considerable success in leveraging existing funds to overcome this shortfall and, in the end, total funding (cash + in-kind) received for the project exceeded the proposed budget by about 6%.

It should be noted that variations in the amount of cash committed and that received (-19%) were primarily a result of shifting priorities in the PERD program administered through Environment Canada, which entered a new funding phase during YEAR 3 of the project. The phytoremediation project did not fit into the new priorities and, hence, was not funded after the second year. Decreased funding from industry was essentially a reflection of changing oil prices and its impact on the remediation budgets of individual companies. (Note: many of our industry partners did not have research budgets; consequently, cash contributions to the project in any given year were generally dependent upon the amount of money available for on-site remediation at the various research sites.) These changes in funding also were reflected in the amount of in-kind support received (-16%) from these organizations. As with the cash contributions, we were able to overcome these shortfalls by leveraging existing funds.

6. Collaboration with Partners

6.1 Who initiated this strategic project?

__x__ **The university researcher**
_____ **The industry partner (if applicable)**
_____ **The government partner (if applicable)**
_____ **Other (Specify):** ______________________________

6.2 In what way were the partners directly involved in the project? (Select all that apply.)

_____ **Partners were not involved in the project apart from their financial and/or in-kind contribution**
__X__ **Partners were available for consultation**
__X__ **Partners provided facilites**
__X__ **Partners participated in the training**
__X__ **Partners discussed the project regularly with the university team**
__X__ **Number of meetings during the period covered by this report:__8__**
__X__ **Partners were involved in the research**

6.3 Describe the partners' involvement and comment on the collaboration.

We were very fortunate to work with an dedicated group of industry and government partners. Our industry partners provided us with access to field sites in Saskatchewan (Talisman Energy and Federated Co-Operatives Ltd.) and Aberta (Husky Energy). Indeed, site selection was made in consultation with our industry partners, the PTAC–Phytoremediation Steering Committee, and Environment Canada. Our partners were always available for consultation via phone and e-mail; as well, we held face-to-face meetings on an annual basis (either at our partners offices or at the annual Soil and Groundwater Forum organized by PTAC and held in Calgary every spring). Changes to the program were made only after consultation with the appropriate partners.

Our government partners (NRC and Environment Canada) provided access to analytical facilities in Saskatoon (NWRI and NRC-PBI) and Montreal (NRC-BRI), hosted a number of graduate students, and provided training to the students using these facilities. The students hosted at these laboratories found these research opportunities to be very positive experiences.

6.4 Was any cash committed to this project?

__x__ **Yes**
_____ **No**

6.5 Was any in-kind committed to this project?

__x__ **Yes**
_____ **No**

6.6 If any cash or in-kind was committed, please enter the amounts below, along with the amount of cash and in-kind that has been received (if any) to date. If no cash or in-kind was received, please enter "0". Where cash or in-kind was not committed enter "n/a".

	Amount Committed	Total Amount Received to Date
Cash	**429,000** 184,000 (U of S) 80,000 (Industry) 165,000 (EC/PERD)	**425,701** 200,500 (U of S) 67,201 (Industry) 80,000 (EC/PERD) 78,000 (CAPP-ERAC)
In-Kind	**831,692** 302,492 (NRC) 230,200 (EC/PERD) 299,000 (Industry)	**947,297** 258,192 (NRC) 197,200 (EC/PERD) 243,000 (Industry) 248,905 (Other)

6.7 Describe the in-kind received and explain variations between commitment and actual cash and in-kind contribution if applicable.

The in-kind received included cash from the University of Saskatchewan (College of Agriculture & Bioresources and the Departments of Soil Science and Civil & Geological Engineering) towards student tuition support and equipment purchases; from Industry (Talisman Energy and Federated Co-Operatives Ltd.) in support of work conducted at various filed sites in Saskatchewan; Environment Canada–Program for Energy Research and Development (funding was revisited on an annual basis, with the amount of funding received dependent upon Environment Canada's internal budgeting and changing priorities–as a result, the phytoremediation project received funding totaling $80k during the first two years of the project only); and the Canadian Association of Petroleum Producers–Environmental Research Advisory Council (as with the Environment Canada funding, CAPP-ERAC funding was revisited annually and was made available through an annual funding competition–Drs. Germida & Farrell were successful in attracting $78,000 in funding from CAPP-ERAC to support this project).

In-kind contributions from our partners also included time commitments for industry and government collaborators; set-up and maintenance of field sites; travel to field sites; Environment Canada funds to support the RTDF program (sub-Project #1a); use of the NRC-BRI Microarray Facility (sub-Project #2a), the NWRI analytical GC-MS facility (sub-Projects #s 5b & 6), and the NRC-PBI analytical Mass Spectrometry facility. In-kind support also includes salary for several technicians, research associates, and graduate* and undergraduate students that was provided by Drs. Farrell & Knight's SRP funding, funds from contract research, and by outside grants to several of the co-PIs.

Variations between the in-kind commitments and the actual contributions received reflect the facts that (i) a reallocation of funds for graduate student stipends was necessary after the project was changed from five years to four years; (ii) funding from industry and the federal governement was on an annual basis and was subject to shifting priorities that did not always work in our favor; (iii) activities at some of the FCL field sites were scaled back as some of the M.Sc. projects matured and priorities changed (see Section 5.2). At the same time, additional funding (not included in the original budget) was obtained from CAPP-ERAC, Talisman Energy (for additional work at the Carlyle site), and various other outside sources (salary support).

* Does not include scholarship monies paid directly to students.

7. Impact on Researcher

7.1 What impact has the project had on your teaching? (Select all that apply.)

_____ **No impact**

- OR -

_____ **Creation of new courses**
__x__ **New content for existing courses**
__x__ **Use of real world examples**
_____ **Guest lectures from partners**
__x__ **New equipment/Material**
_____ **Other (Specify:):** ______________________________

7.2 What impact has the project had on your research? (Select all that apply.)

__x__ **Influenced the direction to more industrial relevant topics**
__x__ **Opened up new opportunities for research beyond the original objectives**
_____ **Other (Specify):** ______________________________

8. Financial Information

An up-to-date Grants in Aid of Research Statement of Account (Form 300) must be provided for both the NSERC contribution and any partner contributions to this project. These should cover the full period of the project. Please forward them with your report if available, or ask your finance department to forward them directly to NSERC.

In addition, the following financial table must be completed.

8.1 What is the balance remaining at the end of the project?

$ 0.00

Budget Item	Total Budget	Actual Expenditures
Salaries and Benefits	**739,500**	**739,548**
a) Students	465,000	484,344*
b) Postdoctoral fellows	60,000	34,000†
c) Technical/professional assistants	138,500	143,965‡
d) Other (summer students)	76,000	77,239
Equipment or facility	**140,069**	**153,449**
a) Purchase or rental	128,769	138,257¶
b) Operation and maintenance costs	12,000	5,502
c) User fees	0	9,690‡
Materials and supplies	**133,500**	**130,780**
a) Materials and supplies	133,500	130,790
Travel	**54,000**	**35,870**
a) Conferences	26,000	20,649
b) Field work	24,000	6,221
c) Collaboration/consultation	4,000	9,000
Dissemination Costs	**20,000**	**20,000**
a) Publication costs	11,000	11,000
b) Other (specify)	9,000	9,000
Other (specify)	**0**	**0**
a)	0	0
b)	0	0
Totals	**1,087,069**	**1,079,657**

* Includes tuition support in the amount of $134k for students enrolled in the Departments of Soil Science and Civil & Geological Engineering. Does not include scholarship monies paid directly to students.

† Includes $24k in salary for a Research Scientist (Dr. R. Hynes) hired for six months as a replacement for the PDF.

‡ Partial salary for one Research Technician (Nancy McCrea) and one Research Associate (Adam Gillespie) provided from government and industry grants; supplemental salary for these positions was supplied by the Dep. of Soil Science.

¶ Includes $60k in matching funds from the College of Agriculture & Bioresources for equipment purchases. Also includes the rental of vehicle used for field work.

‡ Prior to operation of the ASE and GC-FID systems (i.e, during YEAR 1), soil samples from the Carlyle site were sent to a commercial lab for PHC analyses. After the in-house system became operational, a sub-set of samples from the Carlyle site was again sent to the commercial lab for analysis as a quality control check.

Please provide detailed explanations for any significant deviation from the budget.

The only significant deviation (>10%) from the budget occurred in travel expenses. This reflects the fact that (i) in many instances, students presenting papers/posters at conferences received travel awards either from the University of Saskatchewan or from the organizations sponsoring the conferences; (ii) the co-PIs generally supplemented their own travel to conferences with funds from other sources; and (iii) vehicle rental for the purposes of field work were catagorized as ‘rentals’ rather than as ‘travel’ by the University’s financial reporting system.

9. Future Plans

9.1 What links are you maintaining with the partners? (Select all that apply.)

____ **No contact with the partners**
x **Collaborating with the partners on the same research**
____ **Collaborating with the partners on other research**
x **Collaborating with other partners on the same research**
____ **Continuing the research without partners**

9.2 Please describe any follow-up or related work that will be undertaken as a result of this project, and who will be involved in this work (including partners).

Research at the Carlyle site has led to the site being redeveloped as a demonstration site, where new field protocols have been implemented to remediate the soil material remaining on-site. This work is being conducted in cooperation with our partners at Talisman Energy and their consultant, Strata Environmental Ltd., and is funded by Talisman Energy with in-kind support from Dr. Farrell's SRP grant.

The BC Grain Producers Association, has invited Dr. Farrell to participate in a B.C. Peace River region phytoremediation project to demonstrate the utility of plant-based remediation strategies for on-site remediation and restoration of soils impacted by weathered hydrocarbons. This project (which will be led by researchers at the University of Northern British Columbia) will build on the lessons learned from the U of SK phytoremediation project. The foundation for this project was developed at a meeting in Prince George, BC (Dec. 2006) between Dr. Farrell (representing the U of SK group), Dr. Mike Rutherford (representing UNBC) and Mr. Clair Langlois (representing the BCGPA). Interest in this project is high in both the agriculture and energy sectors. (Note: funding for this work is currently being sought.)

As well, our work with inorganics has led to a collaboration with Gord Androsoff (Prairie Plant Systems; Saskatoon, SK) for a revegetation project at the HBMSC copper/zinc mining complex in Flin Flon, MB. A pilot study was established in 2007 and is expected to lead to a NSERC-CRD funded project (with matching funds from HBMSC).

Several members of the phytoremediation research team are currently working (independently and in collaboration) on related projects in the oil sands region centered at Fort McMurray, AB.