Sustainable remediation of petroleum hydrocarbons using phytotechnologies

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Executive Summary

This project used the selected phytotechnologies of rhizodegradation and biochar amendment in greenhouse treatability trials to remediate CCME PHC and PAHs in Alberta soils. Soil was collected from the Alberta site and the bulk soil was analyzed for particle size, PHCs and metals. Of the three coolers collected, one was determined to be contaminated with heavy hydrocarbons (CCME PHC F3-F4) and PAHs, the second was clean and was used as a control, and the third contained low levels of hydrocarbons. Germination experiments indicated that both alfalfa (*Medicago sativa*) and yellow clover (*Melilotus officinalis*) germinated successfully in the soils. A greenhouse experiment was set up to determine if these two species could remediate the hydrocarbons with and without biochar. All three soils were used; the third soil was spiked with 1% diesel to determine applicability of the remediation technique to a wider range of hydrocarbons.

Plant growth and CCME PHC soil concentrations were monitored four times over 136 days for alfalfa and at Day 51 for clover. Results indicated that biochar did enhance shoot growth of both plant species in control soil and the soil spiked with diesel. By day 136 of the trial, significant reductions were obtained in CCME fractions F2, F3 and F4, and in both weathered and unweathered soils in the soils planted with alfalfa. By day 52, significant remediation in the diesel spiked soils with clover was demonstrated. In the short time frame of this study, biochar did not enhance the rhizodegradation of PHCs, however over a longer time period there is potential for improved degradation with the addition of biochar. Significant remediation of the PAHs in the soils was demonstrated by Day 136 in the alfalfa planted soils. Assessment of the microbial activity in the soils indicated that biochar may improve soil quality. Biochar did enhance plant growth in two of the Alberta soils, and hence has potential to improve greenhouse gas reductions in subsequent field trials. Biochar also showed promise in reducing the immediate toxicity of recent hydrocarbon spills by reducing its bioavailability.

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(Project team: Dr. Allison Rutter, Dr. Barbara Zeeb and Dr. Darko Matovic)

Final Report

Prepared for Dr Susan Wood-Bohm, Biological GHG Management Program, CCEMC

Introduction

Petroleum hydrocarbons (PHCs) are one of the most widespread soil contaminants in Canada and consist of a wide range of organic compounds found in, or derived from, geological sources such as oil, coal and bitumen. PHCs can cause a wide variety of problems related to their toxicity, mobility and persistence. They are generally released to the environment as complex mixtures containing thousands of compounds, in varying proportions. The Canada-Wide Standard for Petroleum Hydrocarbons in Soil was established by the Canadian Council for Ministers of the Environment (CCME) in 2008. This standard prescribes analysis of PHCs in four specific fractions (F1: C6 to C10; F2: >C10 to C16; F3: >C16 to C34 and F4: C34+). Where, for example, F1 includes all extractable hydrocarbons that have a boiling point between the normal straight chain hydrocarbons nC_6 (hexane) and nC_{10} (decane). Polycyclic aromatic hydrocarbons (PAHs) are a particularly toxic group of PHCs. They are persistent organic compounds that are mutagenic, carcinogenic and do not degrade easily under natural conditions. For these reasons, the United States Environmental Protection Agency (EPA) has listed 16 PAHs as priority pollutants (El-Shahawi et al., 2010).

Currently, management of PHC (and PAH)-contaminated sites varies considerably across Canada. The traditional remediation strategies for dealing with these sites are physical-chemical methods which involve excavation of the soil followed by transport to an off-site treatment facility or to a hazardous waste disposal site. These methods, although expedient, are typically energy-intensive and very costly. Furthermore they may inadvertently affect the environment in other adverse ways through spills during transport, and they disrupt the local ecosystem by removing or destroying the native soil. Increasingly,

contaminated site owners, government legislators, and environmental consultants are turning to more 'green' technologies which additionally create new green spaces and decrease CO_2 emissions.

Previous studies have shown that the most significant mechanism for removal of PHCs in vegetated contaminated soils is microbial degradation in the rhizosphere (Noori et *al,* 2013; Hall et *al*, 2011). This important mechanism can be exploited in the phytotechnology known as 'rhizoremediation' or 'rhizodegradation'. Simultaneous research has shown that biochar (a 'green' carbon amendment produced via the pyrolysis of organic matter) has significant potential to serve as a mechanism to decrease the bioavailability of contaminants in soil, reducing their risk to environmental and human health, and at the same time improving soil quality and decreasing CO_2 emissions (Denyes et al., 2012, 2013).

This project involved characterizing PHC-contaminated soils, and carrying out a series of greenhouse treatability studies using native plant species. The treatability studies employed two phytotechnologies to remediate soil from a selected PHC-contaminated site in Alberta. Specifically, the combined approach of rhizodegradation with the addition of biochar) was investigated. Ultimately, the goal of this work is to determine how phytotechnologies in conjunction with biochar can best interact to lower carbon emissions and optimize contaminated site remediation.

Methods

Site Visit

Meeting were held with the site owners in Calgary on May 26th, 2014. Allison Rutter (School of Environmental Studies, Queen's University), Barbara Zeeb (Chemistry and Chemical Engineering, Royal Military College of Canada) and Darko Matovic (Mechanical and Materials Engineering, Queen's University) attended the meeting. The site owners and stake holders presented additional information on the site, and a presentation on the work proposed in this report was made by Drs. Rutter, Zeeb and Matovic.

After the meetings, a tour of the contaminated site was led by two of the stakeholders. Three coolers were filled with soils from three different locations on the site. The locations were based on previous knowledge provided by the site owners on the locations of known or suspected PHC-contaminated soils. Dr. Susan Wood-Bohm (Executive Director, Biological GHG Management) and some of the stakeholders

assisted with the filling of the coolers which were lined with clear plastic (Photograph 1). Photograph 2 shows the area from which cooler 3 was filled. The three filled coolers which were brought back to Kingston by Drs. Zeeb and Rutter are shown in Photograph 3. In addition to filling the coolers at the site, the indigenous vegetation was observed by Dr. Zeeb to facilitate the selection of plant species to use in the greenhouse studies. Subsequent to this initial site visit, Dr. Matovic attended an additional stakeholder meeting in Calgary in July 2014, and in October 2014, Dr Rutter presented preliminary work on this project at the Alberta Innovates BioSolutions CCEMC conference "Building the business case" conference in Edmonton (October 1-2, 2014).

Photograph 1: Filling the coolers with soil from the Calgary site.

Photograph 2: Cooler 3 was filled from this area of the Calgary site.

Photograph 3: The three filled coolers ready to be transported back to Kingston for characterization.

Analysis for particle size, PHCs, PAHs and metals

After the analysis of the bulk soils as received for PHC, PAH (polycyclic aromatic hydrocarbon) and lead , soils in each of the three coolers were dried, sieved to 2 mm and homogenized according to the "One-dimensional Japanese Slab-Cake" sampling method (Pitard, 1993). These soils were analyzed to confirm

the PHC, PAH and metals concentrations. In addition, particle size distribution (ASTM International D5158-98 Standard Test Method) was performed. The soil samples were analyzed for petroleum hydrocarbons according to the CCME (Canadian Council for Ministers of the Environment) Reference Method (2001). Selected samplings were also analyzed for PAHs and a standard suite of 30 elements (metals). The PAHs were analyzed using a method based on USEPA 8270d: Semi-volatile Organic Compounds by Gas Chromatography/ Mass Spectrometry (GC/MS). The metals analyses were conducted at the beginning and end of the experiment and was based on USEPA Method 200.7 Trace Elements in Water, Solids, and Biosolids by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). All analyses were carried out at the Queen's University Analytical Services Unit which is accredited by the Canadian Association for Laboratory Accreditation (CALA) for tests listed in the scope of accreditation.

Germination Tests

Based on observation of vegetation growing naturally at the contaminated site in Alberta, and on previous studies of plants that have been shown to enhance PHC degradation, four plant species (alfalfa - *Medicago sativa*; white clover - *Trifolium repens;* yellow clover - *Melilotus officin*alis, and ox-eye daisy - *Leucanthemum vulgare*) were selected for germination tests. Germination test were carried out according to the seed germination testing method outlined by Solaiman *et al.* (2012). Filter paper and potting soil was used as a positive control. Seeds were added to 10 g of soil wetted with 15 mL of water and exposed to a 14:10 h (day:night) fluorescent photoperiod at 27 °C. All tests were carried out in triplicate. After seven days, the number of seeds that germinated in each treatment was recorded.

Planting

Pots, with and without biochar, were set up with alfalfa (*Medicago sativa*) and yellow clover (*Melilotus officinalis*) using the three coolers of soil obtained from Alberta. Cooler 1 soil was naturally contaminated with the highest levels of PHCs and PAHs. Cooler 2 was contaminated below federal and provincial guidelines, and was hence used as a control. Cooler 3 was spiked with 1% diesel. Pots with no biochar contained 1000 g of soil. Pots with biochar contained 970 g of soil and 30 g of biochar, and were well mixed by manually shaking for one minute to ensure even distribution.

Sampling and Monitoring

Soils planted with alfalfa were sampled in triplicate on day 1, day 24, day 62, day 101 and day 136. Plants were also monitored by measuring the length of a single shoot and then gently removing the entire plant from the pot. Root and shoot weights were subsequently recorded and the plant samples were frozen. A soil sample from the root area of each plant was collected and kept refrigerated until analysis. Yellow clover was planted at a later date, sampled on day 51 and monitored as described above. Unplanted control pots were prepared and sampled in the same manner as the alfalfa and clover soils.

Statistics

Statistical analysis of the CCME PHC and growth data was performed using S+ version 8.2 (Tibco Software In., USA). One way analysis of variance (ANOVA) was performed using a significance level of $\alpha = 0.05$, followed by a *post hoc* Tukey comparison. Data were tested for normality using the Kolmogorov-Smirnov test using a p value of 0.5, and all non-normal data was log transformed.

Community Level Physiological Profiling

Community level physiological profiling (CLPP) provides information relating to mixed microbial function and functional adaptations over space and time. Heterotrophic microbial communities are compared and classified based on sole carbon source utilization patterns (CSUPS) gathered using BIOLOG™ microplates (Weber and Legge, 2010). In essence, this is a technique that allows for the characterization of soils based on the microorganisms living in the soil. In this study, BIOLOG™ ECO plates containing 31 carbon sources and a control well, in triplicate, were used.

A suspended mixed microbial sample (set to an optical density of 0.19 at 420 nm) was obtained by adding the individual soil samples to 100 mL of phosphate buffer (10 mM with 8.5 g/L NaCl) and orbitally shaking at 100 rpm for 3 hours. Suspensions representing a single sample were then inoculated into each of the 96 wells (125 µL per well) on a BIOLOG™ ECO plate and the absorbance was read at 595 nm at periodic intervals for seven days.

CLPP data was analyzed using average well colour development (AWCD), substrate richness, substrate diversity, and principal components analysis (PCA) using carbon source utilisation patterns (CSUPs). AWCD refers to the absorbance value (corrected by the blank well) averaged for all 31 wells giving an assessment of overall catabolic activity.

$$AWCD = \frac{1}{31}\sum_{i=1}^{31}\left(A_i - A_0\right)$$

Where

AWCD – average well colour development

A_i – absorbance reading of well *i*

A_0 – absorbance reading of the blank well (inoculated, but without a carbon source).

Substrate richness is a measure of the number of different carbon sources utilised by a microbial population, and is calculated as the number of wells with a corrected absorbance greater than 0.25 AU. Diversity is expressed here in terms of the Shannon index A single time point (67 hours) was selected for the evaluation of all plate data based on a combination of greatest variance between well responses and least number of absorbance values above 2 (as these are above the linear absorbance range). The data was assessed for normality, homoscedasticity, and linear correlations between variables yielding a recommended Taylor power law transform for principle components analysis. Principal components were extracted and ordinations created from the covariance matrix of the data using Statistica 8.0. Following an ANOVA, a *post hoc* Tukey comparison was performed to assess differences in metabolic responses (activity, richness, diversity) based on the type of soil contamination (i.e. cooler 1, 2 or 3) and vegetated (alfalfa) versus non-vegetated treatments.

Results and Discussion

Soil Characterization

The reports of analyses for the bulk soils 'as received' are presented in Appendix A. Briefly, analysis of Cooler 1 (Sample 1; Appendix A) before sieving indicated levels of PHCs at 8000 ppm. Cooler 2 (Samples 2A to 2D; Appendix A) contained tar and therefore additional samples were analyzed for PHCs to characterize the tar. Sample 2A is the bulk soil and indicated no significant amounts of PHCs. Cooler 3 (Sample 3; Appendix A) had low concentrations of PHCs (200 ppm). The PAH analysis indicated the same trend with Cooler 1 having the highest concentrations, followed by cooler 3, with the lowest concentrations in Cooler 2. Significant lead contamination (1700 ppm) was also found in Cooler 1.

Following homogenization and sieving, the three coolers were re-analyzed and these results are summarized in Table 1 below (full results are in Appendix B). PHC and PAH results are similar, however

a reduction in the concentrations for Cooler 1 was noted, possibly due to volatilization of the F1 and F2 PHC fractions.

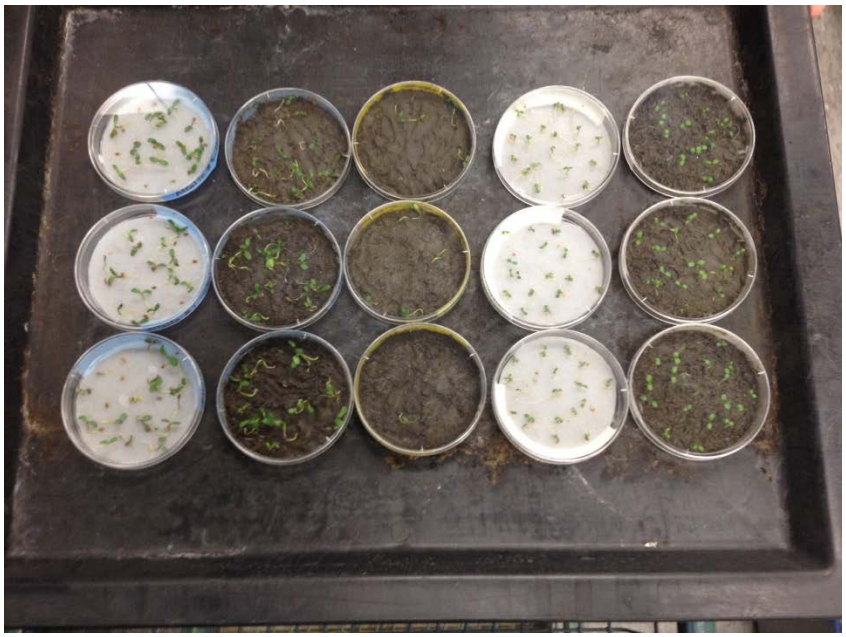
Given the results in Table 1 and Appendices A and B, the greenhouse experiments were designed as follows. Cooler 1 was used as is, soils in cooler 2 were used as is, as a control (to determine plant growth in uncontaminated soils from the site), and cooler 3 was spiked with diesel. By spiking the soil it was possible to determine how well the combination of rhizodegradation and biochar would work on different concentrations and types of PHC contamination. Both PAH and PHC concentrations were monitored during the greenhouse trials.

Table 1: Results of soil analyses from the three sieved homogenized coolers.

Cooler	CCME PHC (ppm)	PAH (total) (ppm)
1	1140 (F3-F4)	13.8
2	<60 ppm	0.2
3	380	2.9

Germination

Germination experiments indicated that of the four species tested, alfalfa (*Medicago sativa)* and yellow clover (*Melilotus officinalis*) were the most appropriate species for germination in the Alberta soils (Photograph 4; Figure 1).



Photograph 4: Germination experiment with filter paper (controls) and Alberta soils.

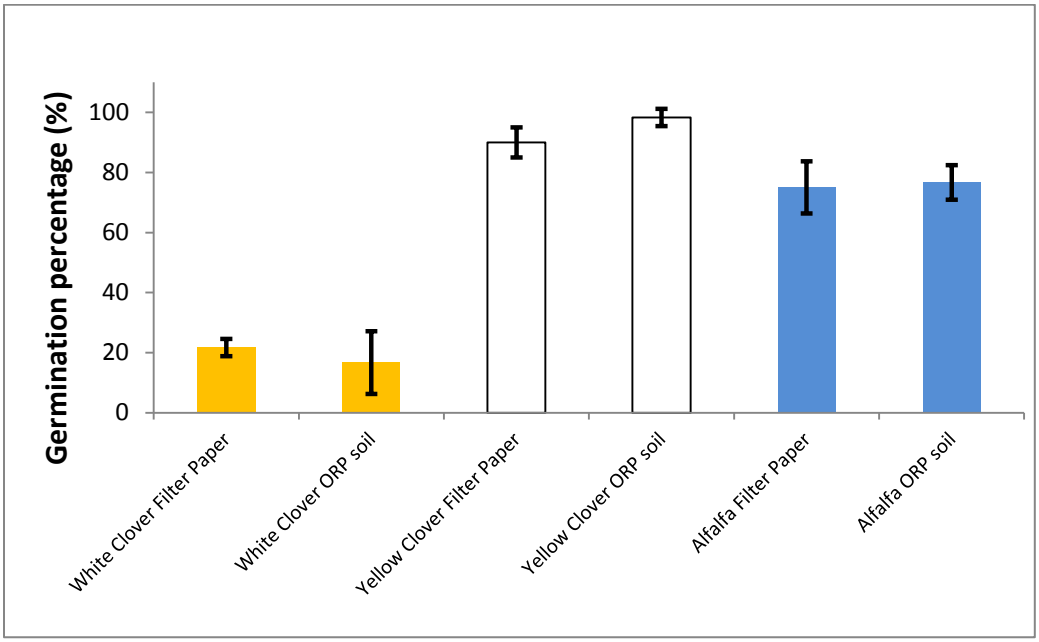


Figure 1: Results from the germination tests.

Plant Growth

Yellow clover (Photograph 5) and alfalfa (Photograph 6) grew well in all three soils. Shoot length was measured on representative plants (Photograph 7). Root and shoot weights of alfalfa at Day 136 (i.e. harvest 4) are plotted in Figure 2. Despite the addition of 1% diesel to cooler 3, there was no significant difference in plant growth between the three soils at this harvest.

At Day 101, in cooler 2 soil (i.e. clean industrial soil), the addition of 3% biochar significantly increased shoot weight. There was no significant difference in plant growth in cooler 1 soil (i.e. F2-F3 & PAH contamination) or cooler 3 soil (i.e. spiked with 1% diesel) with the addition of biochar. At Day 136, the addition of 3% biochar significantly increased root and shoot weights in Cooler 2 (clean industrial) soils (Figure 2). There was no significant difference in plant growth in cooler 1 soil (F2-F3 & PAH contamination), however in cooler 3 soil (1% diesel spike), shoot growth was significantly higher with the addition of biochar.

Shoot and root weights for yellow clover at Day 51 are shown in Figure 3. The addition of 3% biochar significantly increased the clover shoot weights in the diesel spiked soil. Data for all four harvests of alfalfa and harvest 1 of clover are included in Appendix C of this report. Variability of shoot length was high, and therefore that data is not included.

Photograph 5: Yellow clover at day 45.

Photograph 6: Alfalfa at day 129.

Photograph 7: Measuring shoot length of alfalfa.

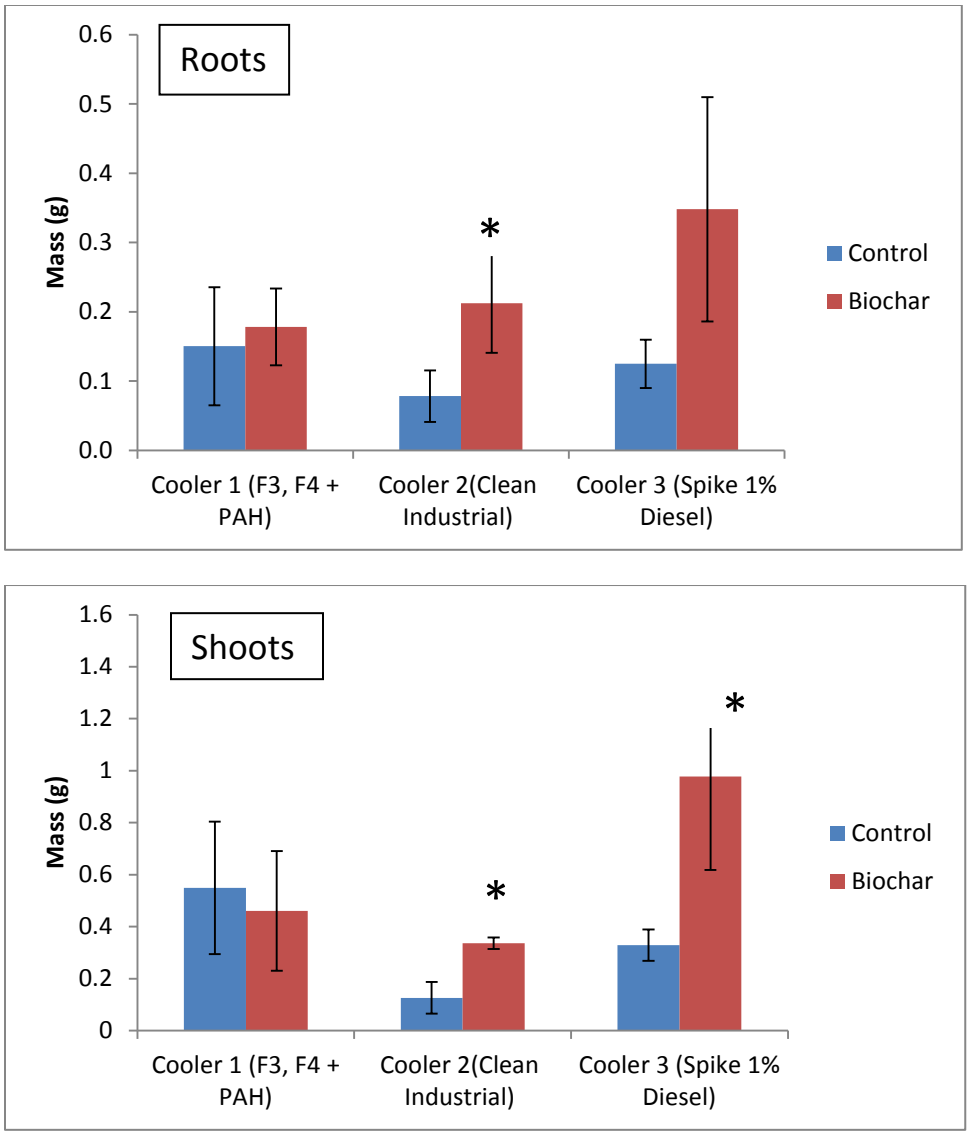


Figure 2: Growth data for alfalfa roots and shoots at Day 136 (Harvest 4) for all three soils.

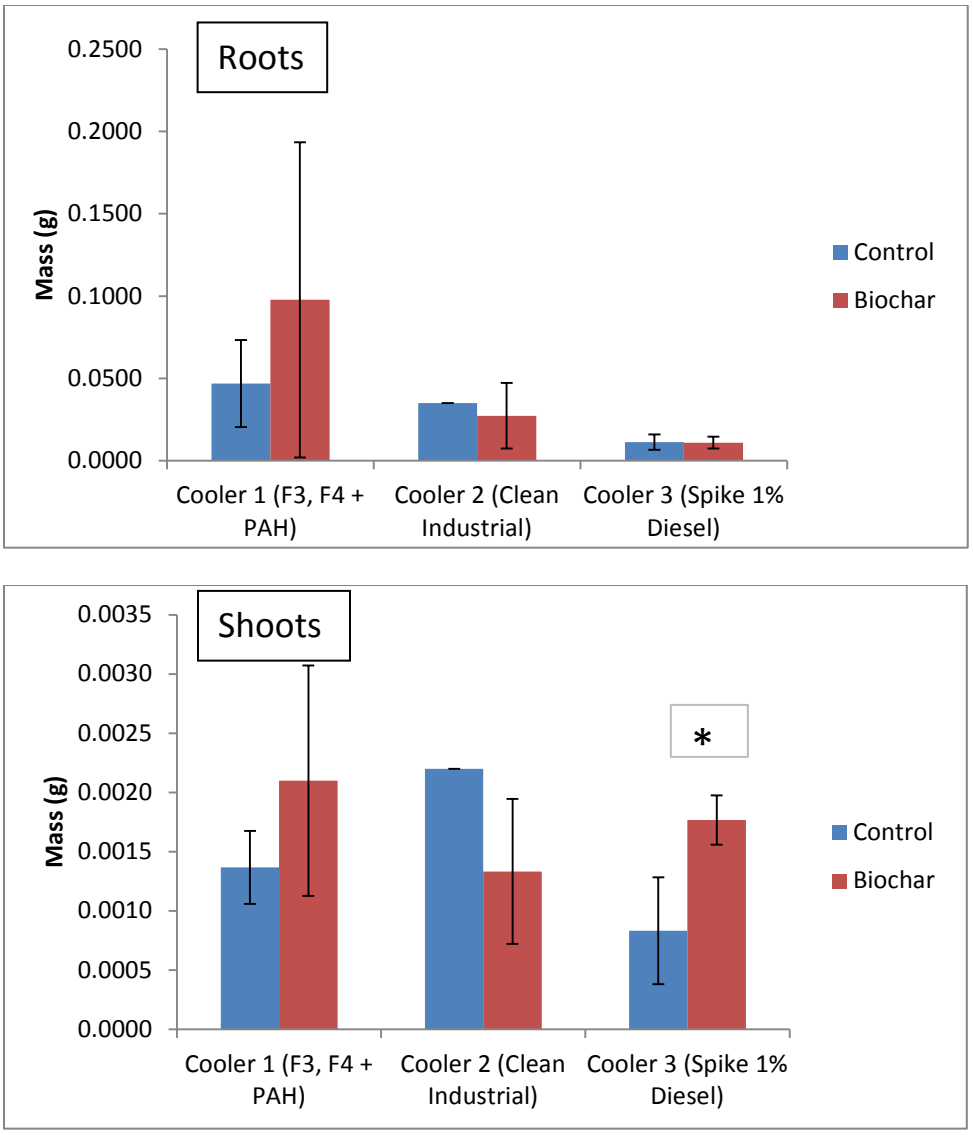


Figure 3: Growth data for clover roots and shoots at Day 136 (Harvest 4) for all three soils

CCME PHCs in Soils - Alfalfa trials

Analytical reports for all four fractions of CCME petroleum hydrocarbons can be found in Appendix D. Cooler 1 soils, containing PHC fractions F3 and F4, showed a significant reduction in PHCs 136 days after planting alfalfa. Between harvest 1 (Day 26) and harvest 5 (Day 136), total PHCs were reduced from 1860 to 916 ppm in the pots without biochar and from 1840 to 1150 ppm with 3% biochar indicating reductions of 51% and 38%, respectively. PHC concentrations for the individual F3 and F4 fractions are plotted in Figure 4. Although no changes were observed in the first three harvests, the graphs illustrate that both F3 and F4 are reduced significantly by the fourth harvest (Day 136) despite the highly weathered nature of the soil. The apparent increase at Day 101 is reflective of the inherent variability in biological data and is largely caused by the heterogeneity of PHC contamination in the soils, despite thorough mixing. There was no significant difference between PHC concentrations in soils which contained 3% biochar and those which contained no biochar.

As cooler 3 soil was spiked with 1% diesel, CCME PHC fractions F2 and F3 predominate in this soil. Between day 26 and day 136, total PHC CCME was reduced from 6360 to 4147 ppm in the pots with 3% biochar and from 7015 to 2465 ppm in the pots without biochar indicating reductions of 35% and 65%, respectively. PHC fractions F2 and F3 are plotted in Figure 5. For both fractions, only the soils without the 3% biochar addition showed a significant difference from the initial harvest. Biochar and activated carbon have been widely used to sorb contaminants in sediments and more recently in soils (Denyes *et al.* 2012). Sorption of the hydrocarbons to the biochar may limit their accessibility to the microbial community in the rhizosphere hence slowing remediation. Longer term studies are required to more fully explore and ultimately understand this mechanism. It is very important to note that this difference is significant only in the freshly spiked soils, not in those where PHCs have weathered over time. This indicates that biochar may serve to minimize PHC bioavailability, and hence reduce toxicity, in freshly contaminated soils.

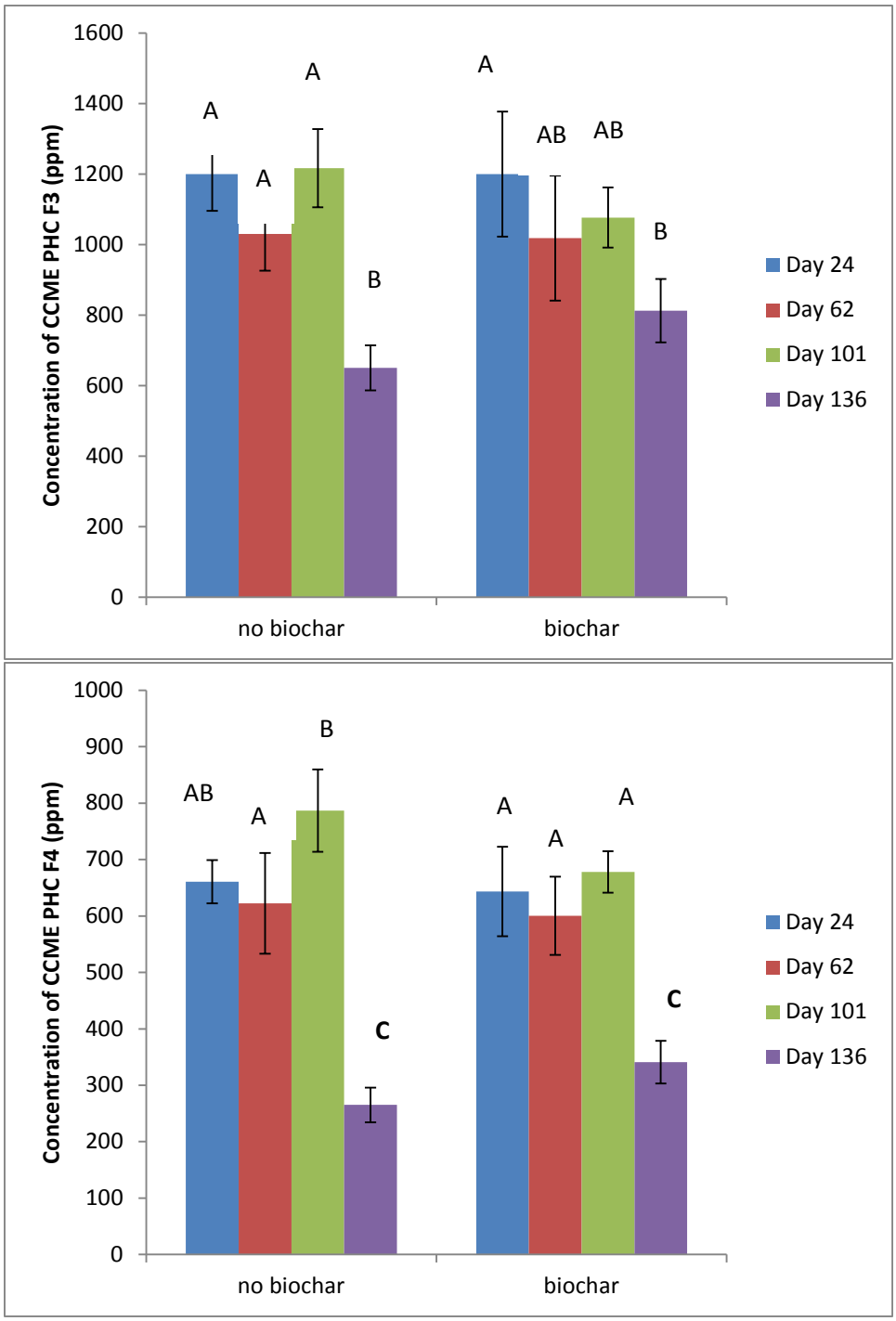


Figure 4: Concentrations of CCME PHC Fraction F3 (top) and F4 (bottom) in weathered F2-F3 & PAH contamination (i.e. cooler 1 soils) over five harvests in pots planted with alfalfa with and without 3% biochar. Different letters (A, B, C) indicate significant differences.

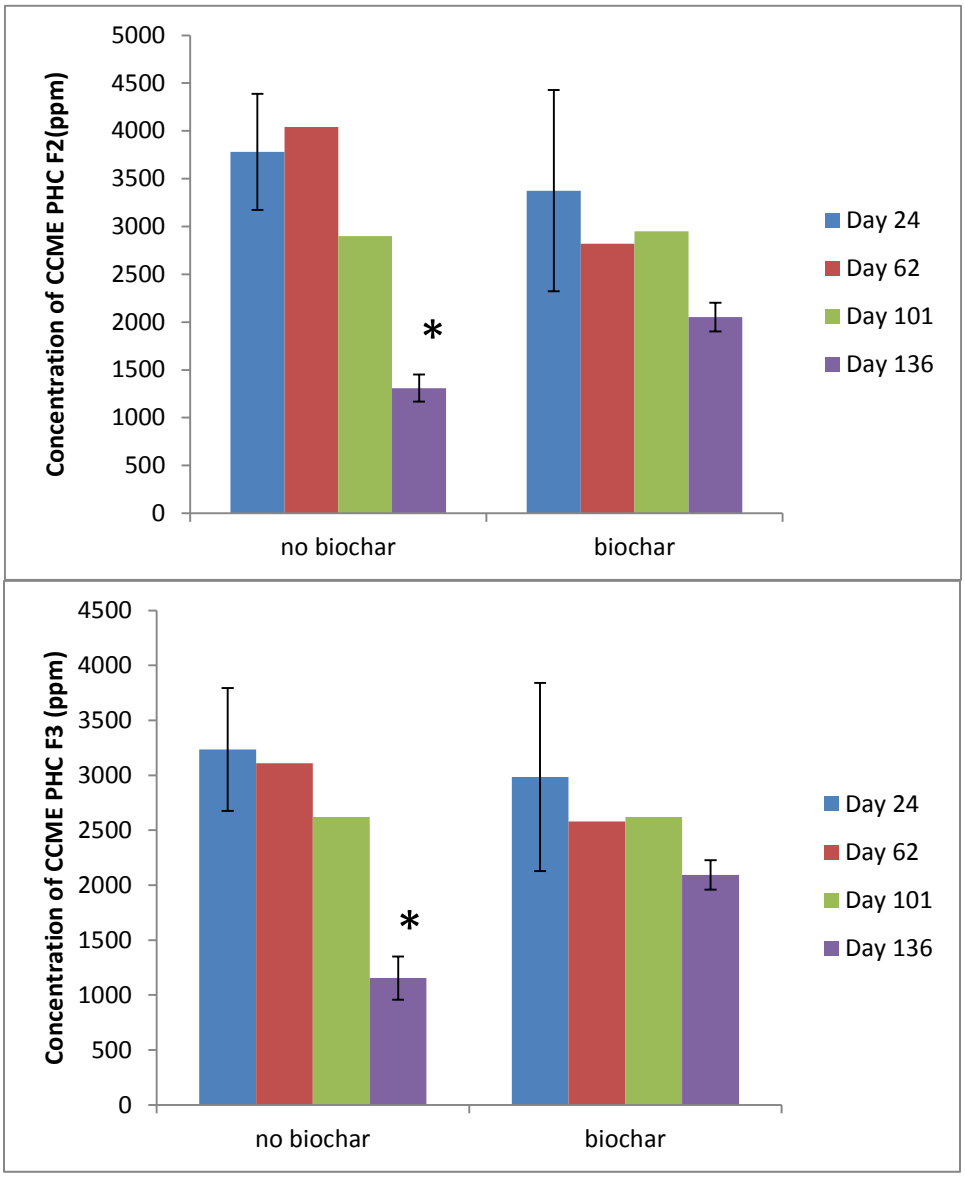


Figure 5: Concentrations of CCME PHC Fraction F2 (top) and F3 (bottom) in soils freshly spiked with diesel (i.e. cooler 3 soils) over five harvests in pots planted with alfalfa, with and without 3% biochar. Asterisk (*) indicates significant difference.

CCME PHCs in Soils- Clover trials

Yellow clover greenhouse trials were initiated later than alfalfa trials and only harvested on day 51 (to date). No significant reduction in the PHC fractions occurred over this time period in the weathered F2 & F3 and PAH (i.e. cooler 1) contaminated soils (Figure 6). This result is similar to the alfalfa results which

did not show reductions until day 136 in either fraction. In the freshly diesel spiked soils (i.e. cooler 3) however, significant reductions in both the F2 and F3 fractions occurred in pots with and without 3% biochar. This is a very promising result indicating that rhizodegradation of PHCs begins to occur within two months of planting yellow clover. This result is expected, as the lighter PHC fractions in the spiked soils, are expected to remediate more quickly than the heavier weathered fractions and PAHs in cooler 1. No differences were observed with the addition of biochar.

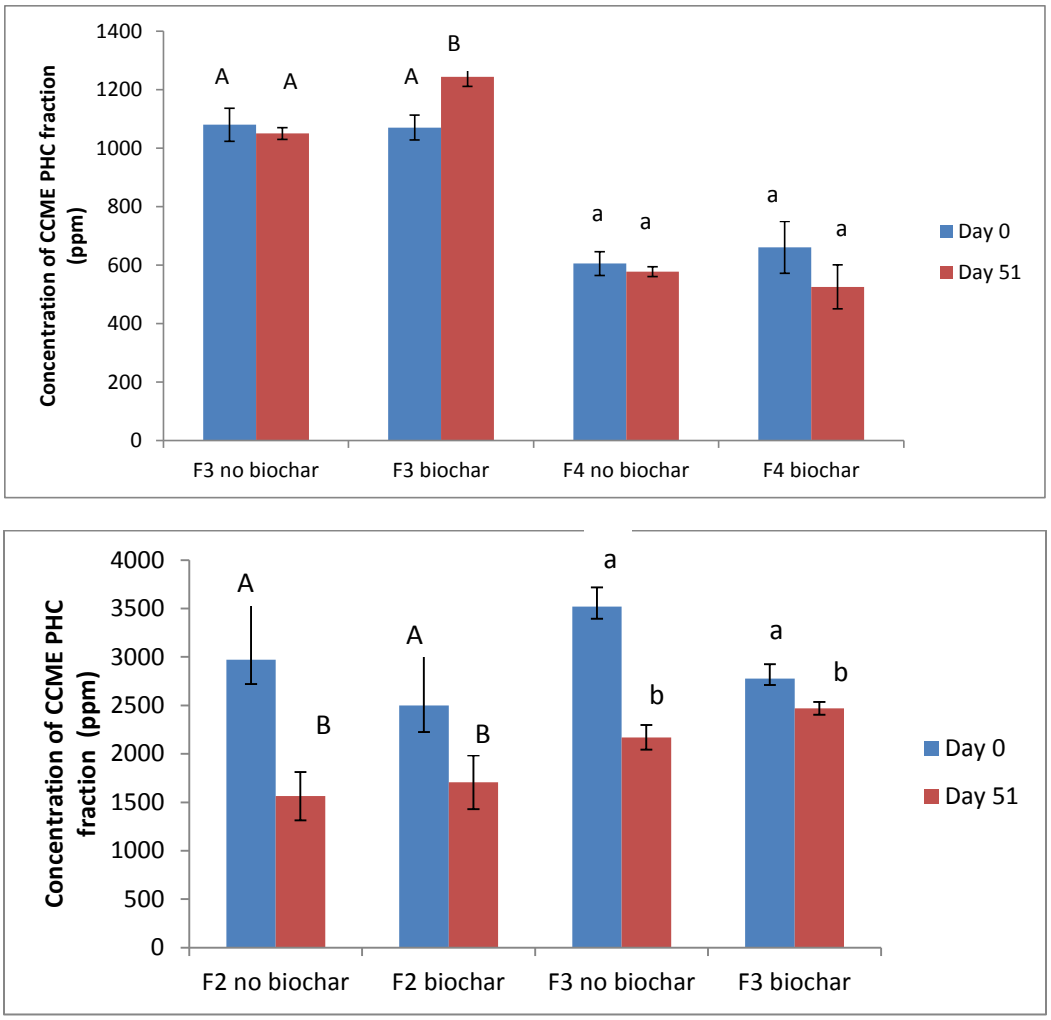


Figure 6: Concentrations of CCME F3 and F4 in weathered soils (i.e. cooler 1) (top) and F2 and F3 (i.e. cooler 3) (bottom) over 51 days in pots planted with clover with and without 3% biochar. Different letters (e.g. A, B,) indicate significant differences.

PAHs in Soils

Polycyclic aromatic hydrocarbon concentrations in weathered soils (i.e. cooler 1) at day 24 and day 136 are shown in Table 1. Analytical reports are in Appendix D. The total PAH content was reduced significantly from 18200 to 2600 ppb (a reduction of 86%), and all 16 US EPA priority pollutant PAHs were reduced. Similar results were obtained for cooler 1 soils amended with 3% biochar, however the presence of biochar (which itself contains PAHs) increased the heterogeneity of the samples and the results are not significant.

Table 2: PAH results from cooler 1 soils. Averages of triplicate samples from harvest 1 (Day 24) and harvest 4 (Day 136) are shown with standard deviations.

	Concentration at Day 24 (ppb)	Concentration at Day 136 (ppb)
Naphthalene	375±48	106±61
Acenaphthylene	20±5	<10
Acenaphthene	32±15	<10
Fluorene	68±21	<20
Phenanthrene	1220±280	251±236
Anthracene	144±20	34±32
Fluoranthene	1110±597	170±171
Pyrene	1670±477	239±239
Benzo(a)anthracene	1040±280	128±133
Chrysene	1690±285	230±236
Benzo(bkj)fluoranthene	2350±590	318±354
Benzo(a)pyrene	2190±214	289±299
Dibenzo(ah)anthracene	899±43	140±165
Indeno(123cd)pyrene	1420±147	168±181
Benzo(ghi)perylene	3980±219	517±552
Total PAH	18200±2980	2600±2680

Community Level Physiological Profiling

The results of CLPP analysis on the three industrial soils from Alberta are plotted to illustrate the effects of plant roots (Figure 7), and the amendment with 3% biochar (Figure 8). Soils for the CLPP analysis were harvested on Day 118 and only non-vegetated soils and those planted with alfalfa were used.

Presence of Plant Roots

In both un-amended (Figure 7A) and 3% (by weight) biochar amended soils (Figure 7B), AWCD, richness and diversity were higher in alfalfa planted treatments relative to the non-vegetated treatments, and increased from cooler 1 (F3, F4 and PAH) < cooler 2 (clean industrial) < cooler 3 (F3, F4 and PAH). These differences were significant (p < 0.05) for AWCD and species richness for un-amended and biochar amended soils in coolers 1 (F3, F4 and PAH) and 2 (clean industrial), and for diversity in biochar amended cooler 3 (spike 1% diesel). Thus the presence of plant roots appears to be having a positive effect on microbial activity, especially in the weathered industrial soils (coolers 1 and 2). This improvement in microbial activity will likely result in increased contaminant degradation in the rhizosphere.

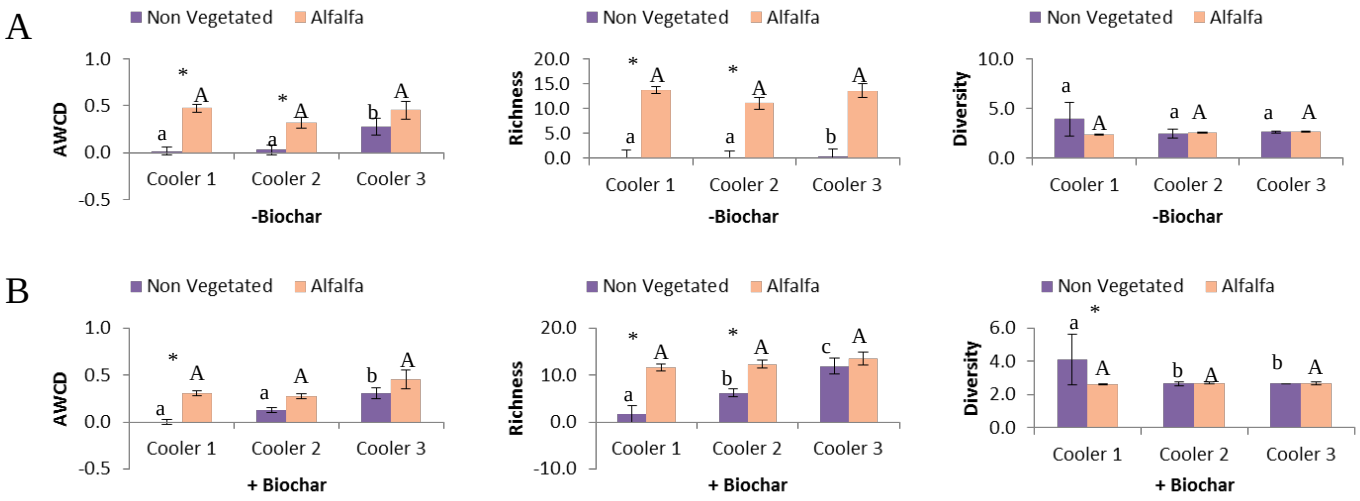


Figure 7. Average well colour development (AWCD), richness and diversity of non-vegetated and alfalfa planted treatments in A) un-amended soil and B) 3% (w/w) biochar amended soils. Values represent the mean, and error bars represent one standard deviation. Lower-case (non-vegetated) and upper-case (alfalfa planted) letters indicate statistically significant differences between coolers (i.e. soil contaminant type) (p < 0.05), and * indicate significant differences between vegetated and non-vegetated conditions

in the respective cooler ($p < 0.05$). Cooler 1 contains the industrial soil contaminated with F3, F4 and PAHs, cooler 2 contains clean industrial soil, and cooler 3 is soil spiked with 1% diesel.

Amendment with 3% Biochar

Biochar added at 3% (w/w) improved the AWCD, species richness and species diversity in the non-vegetated treatments (Figure 8A) suggesting that biochar offers improvements to all three soils. Only the increase in diversity in cooler 2 was significant. Similar results have been observed in intensely degraded PCB-contaminated Brownfield soils, where biochar restored the microbial community to that of a remediated site (Denyes et al., subm.). In the alfalfa treatments (Figure 8B), biochar did not have a positive effect, suggesting that the presence of plant roots has a larger relative contribution to the increases in microbial activity than amendment with biochar.

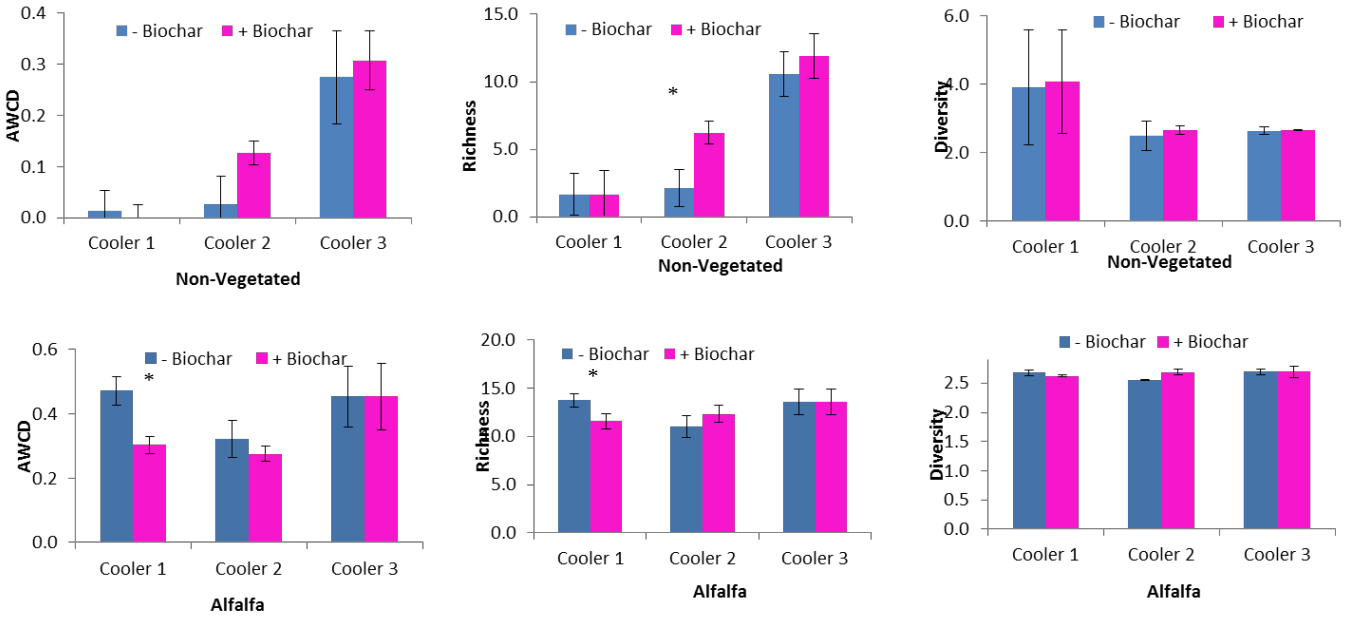


Figure 8. Average well colour development (AWCD), richness and diversity of un-amended and biochar amended soils A) without vegetation (i.e. non-vegetated) and B) planted with alfalfa. Values represent the mean, and error bars represent one standard deviation. * indicate significant differences between un-amended and biochar amended soils in the respective cooler ($p < 0.05$). Cooler 1 contains the industrial soil contaminated with F3, F4 and PAHs, cooler 2 contains clean industrial soil, and cooler 3 is soil spiked with 1% diesel.

Principal Components Analysis

Principal components analysis based on carbon source utilization patterns (CSUPs) revealed that the samples collected in both un-amended and 3% biochar amended soils from all three industrial soils planted with alfalfa grouped closely together, whereas the scatter was much larger with the non-vegetated samples (Figure 9). These results again indicate that the presence of plant roots appears to offer a larger relative contribution to increased microbial community than amendment with biochar.

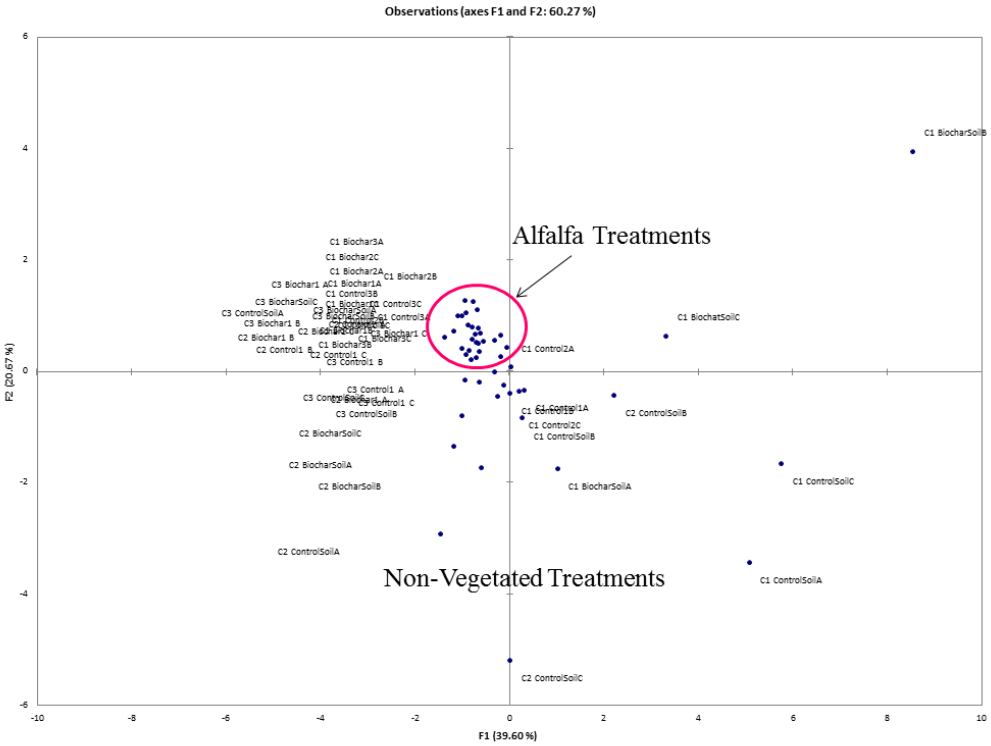


Figure 9. CLPP Taylor transformed (b=0.7803) principal components analysis results for all samples from non-vegetated and alfalfa treatments in un-amended and 3% biochar amended soils. The red circle shows the distinct grouping of soils planted with alfalfa.

Conclusions and Recommendations

The greenhouse treatability trials carried out in this project clearly demonstrated significant reductions in CCME PHC and PAH levels in the Alberta soils using the selected phytotechnologies of rhizodegradation and biochar amendment. Both alfalfa (*Medicago sativa*) and yellow clover (*Melilotus officinalis*) show promising results in less than six months. Significant reductions were obtained in CCME fractions F2, F3 and F4, and in both weathered and unweathered soils. In the short time frame of this

study, biochar did not enhance the rhizodegradation of PHCs, however over a longer time period there is potential for improved degradation with the addition of biochar. Biochar did enhance plant growth in two of the Alberta soils, and hence has potential to improve greenhouse gas reductions in subsequent field trials. Biochar also showed promise in reducing the immediate toxicity of recent hydrocarbon spills by reducing its bioavailability.

A follow-up field trial employing alfalfa and yellow clover is highly recommended. The success of the greenhouse trials detailed in this study, indicates that significant reductions in petroleum hydrocarbons in general, as well as polycyclic aromatic hydrocarbons can occur within a single growing season. A field trial will allow a full assessment of the potential GHG reductions. It is expected that the replacement of dig and treat remediation with phytotechnologies will reduce GHG emissions by approximately 50%. The further GHG reductions from biochar production and increased plant growth will be dependent on the production and source material of the biochar and plant species used. The field trial should incorporate at least two plant species and the use of locally produced biochar to enhance GHG reductions. Soils that are contaminated with PHCs in the top 30 cm would be most amenable to remediation using the plant species reported on in this study.

References

Canadian Council for Ministers of the Environment, (CCME). 2001. Reference Method for the Canada-Wide Standard for Petroleum Hydrocarbons in Soil - Tier 1 Method. Publication No. 1310, CCME, Winnipeg.

Denyes, M.J., Langlois V. S., Rutter, A. and Zeeb, B.A. The use of biochar to reduce soil PCB bioavailability to Cucurbita pepo and Eisenia fetida. *Science of the Total Environment,* 437: 76–82 (2012).

Denyes, M.J., Rutter, A., Zeeb, B.A. In situ application of activated carbon and biochar to PCB-contaminated soil and the effects of mixing regime. Environmental Pollution, 182, 201-208, doi: 10.1016/j.envpol.2013.07.016 (2013).

El-Shahawia, M.S., Hamzaa, A., Bashammakhb, A.S. and W.T. Al-Saggaf. An overview on the accumulation, distribution, transformations, toxicity and analytical methods for the monitoring of persistent organic pollutant. Talant 80: 1587-1597 (2010).

Hall, J., Soole, K., and Bentham, R. 2011. Hydrocarbon phytoremediation in the family Fabaceae – A Review. Int. J. Phytoremediation 13: 317-332.

Noori, A., Maivan, H., and Alaie, E. 2013. Leucanthemum vulgare flavonoid content during crude oil phytoremediation. Presentation at 10th International Phytotechnologies Conference, Syracuse, NY.

Pitard, F.F. 1993. The delimitation error. p.229-258. In F.F. Pitard (eds.) Pierre Gy's sampling theory and sampling practice- Heterogeneity, sampling correctness, and statistical process control. 2nd ed. CRC Press, Boca Raton, Fl.Pitard, 1993.

Solaiman, Z.M. Murphy, D.V., Abbott, L.K. Biochars influence seed germination and early growth of seedlings. *Plant Soil*. **353** (1-2), 273-287, doi: 10.1007/s11104-011-1031-4 (2012).

Weber, K.P. and Legge, R.L. Community-level physiological profiling in *Methods in Molecular Biology: Bioremediation*. S.P. Cummings (Ed.), The Humana Press Inc., New Jersey (2010), pp. 263–281

Appendix A: Laboratory Results for the soil as received

CCME PHC

PAH

Lead

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REPORT OF ANALYSIS

ASU # 15085
Client: Alberta Innovates

Report ID: ASU 15085 Alberta Innovates CCME
Date Submitted: 3-Jun-14
Date Testing Initiat 3-Jun-14
Date Reported: 19-Jun-14

Method: CCME TPH in Soil†
Matrix: Soil

FINAL REPORT

RESULTS

Sample ID	% water of Wet Soil	F1 C6-C10 mg/kg	F2 C10-C16 mg/kg	F3 C16-C34 mg/kg	F4 C34-C50 mg/kg	TPH SUM
Sample 1	8%	-	<10	5800	2390	8190
Sample 2A	23%	-	<10	14	<10	<30
Sample 2B	13%	<10	144	324	1010	1480
Sample 2C	4%	-	1980	3820	6680	12480
Sample 2D	20%	-	<10	<10	709	709
2D suppl	20%	-	<10	<10	449	449
Cooler 3*	13%	<10	<10	<10	200	200

- F1 fraction not assessed

†Complies with CWS PHC Tier 1 method

‡F4G gravimetric heavy hydrocarbons cannot be added to the sum

*Average of duplicate results

QUALITY CONTROL

Quality Criteria:

1) nC6 and nC10 response factors within 30% of response for toluene;
2) nC10, nC16 and nC34 response factors within 10% of average;
3) C50 response factors within 70% of nC110 + nC16 + nC34 average;
4) linearity of calibrations standard with 15%

	F1 C6-C10 mg/kg	F2 C10-C16 mg/kg	F3 C16-C34 mg/kg	F4 C34-C50 mg/kg
Blank	<10	<10	<10	<10
Cooler 3 duplicate	<10 ; <10	<10 ; <10	<10 ; <10	218 ; 181
Control	140	166		
Control Target	152	169		

COMMENTS

F4G Fraction not assessed
Chromatogram descended to baseline by the C50 retention time
Fractions include BTEX, Napthalene and PAHs
Analysis holding times for samples were met
Results relate only to the items tested

Prepared by: Paula Whitley

Authorized by:

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REPORT OF ANALYSIS

ASU #: 15085
Client: Alberta Innovates

Report ID: ASU 15085 Alberta Innovates Lead in Soil
Date Submitted: 3-Jun-14
Date Tested: 4-Jun-14
Date: 5-Jun-14

Method: Metals by ICP-OES
Matrix: Soil

Results relate only to the items tested

Sample	Lead ug/g	
Sample 1	1700	
Sample 2A	23	*
Sample 2B	12	
Sample 2D	20	
Sample 3	120	
Blank	<10	
MESS-3	21	
Sample 2A	23	
Sample 2A	23	

* Average result of duplicates

Prepared by: Paula Whidden

Authorized by: A. Rutter

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REPORT OF ANALYSIS

ASU #	15085	**Report ID:**	ASU 15085 Alberta Innovates PAH
Client:	Alberta Innovates	**Date Submitted:**	3-Jun-14
		Date Reported:	21-Aug-14
Method:	PAH by GC/MS	**Matrix:**	Soil

FINAL REPORT
RESULTS in ppb

Compound	Sample #1	Sample # 2A	Cooler # 3*
Naphthalene	348	12.8	50.9
Acenaphthylene	58.7	<10	<10
Acenaphthene	200	<10	33.7
Fluorene	79.9	<10	43.0
Phenanthrene	1150	38.9	610
Anthracene	169	11.9	99.1
Fluoranthene	537	42.3	603
Pyrene	1100	39.6	528
Benzo(a)anthracene	1060	35.2	539
Chrysene	1070	37.2	373
Benzo(bkj)fluoranthene	553	21.1	223
Benzo(a)pyrene	2280	26.8	501
Dibenzo(ah)anthracene	478	<10	<10
Indeno(123cd)pyrene	929	<10	247
Benzo(ghi)perylene	3660	<10	554

Prepared by: Paula Whidley Authorized by:

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Compound	Blank	Control	Control Target	Cooler # 3	Cooler # 3 DUP
Naphthalene	<10	362	400	53.3	48.4
Acenaphthylene	<10	561	400	<10	<10
Acenaphthene	<10	419	400	46.3	21.0
Fluorene	<10	487	400	56.6	29.3
Phenanthrene	<10	541	400	733	487
Anthracene	<10	403	400	118	80.8
Fluoranthene	<10	548	400	722	485
Pyrene	<10	550	400	625	431
Benzo(a)anthracene	<20	632	400	624	454
Chrysene	<10	490	400	430	316
Benzo(bkj)fluoranthene	<10	340	400	262	184
Benzo(a)pyrene	<10	429	400	628	375
Dibenzo(ah)anthracene	<10	248	400	<10	<10
Indeno(123cd)pyrene	<10	324	400	292	203
Benzo(ghi)perylene	<10	417	400	588	520

* Average results of duplicates
Results relate only to the items tested

Appendix B: Laboratory Results for the sieved homogenized soils

CCME PHC

PAH

Metals

Particle Size distribution

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Tel: 613 533-2642 Fax: 613 533-2897

ASU #	15085	**Report ID:**	ASU 15085 30 Elements-S1
Client:	Alberta Innovates	**Date Submitted:**	3-Jun-14
		Date Tested:	27-Jan-15
		Date:	28-Jan-15
Method:	Metals by ICP-OES	**Matrix:**	Soil

Report of Analysis

Results relate only to the items tested: Results in ug/g

Sample	Cooler 1 Sample 1	Cooler 1 Sample 2*	Cooler 2	Cooler 3
Ag	<2.0	<2.0	<2.0	<2.0
Al	6900	6800	9200	8700
As	5.6	5.9	6.5	5.5
B	<20	<20	<20	<20
Ba	170	180	250	210
Be	<4.0	<4.0	<4.0	<4.0
Ca	93000	92000	55000	66000
Cd	<1.0	<1.0	<1.0	<1.0
Co	<5.0	<5.0	5.3	<5.0
Cr	<20	<20	<20	<20
Cu	26	26	14	19
Fe	12000	12000	12000	13000
K	1200	1200	1500	1400
Mg	16000	16000	10000	10000
Mn	300	300	320	330
Mo	<2.0	<2.0	<2.0	<2.0
Na	140	140	320	180
Ni	14	14	16	14
P	510	520	620	560
Pb	2000	1800	13	110
S	610	580	610	320
Sb	<10	<10	<10	<10
Se	<10	<10	<10	<10
Sn	<2.0	<2.0	<2.0	<2.0
Sr	74	75	66	69
Ti	45	52	58	100
Tl	<1.0	<1.0	<1.0	<1.0
U	<10	<10	<10	<10
V	21	21	25	26
Zn	54	54	61	79

* Average result of duplicate analysis; All Mess-3 results within acceptable lab control limits

Prepared by: Paula Whidley Authorized by:

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Laboratory QA/QC

Sample	Blank	Control	Control Target	MESS-3 Found	MESS-3 Expected
Ag	<2.0	1.1	1.2	<2.0	<2.0
Al	<50	3.0	3.0	16000	20000
As	<1.0	4.0	4.0	17	18
B	<20	2.0	2.0	-	-
Ba	<5.0	5.9	6.0	310	350
Be	<4.0	3.0	3.0	<4.0	<4.0
Ca	<100	5.1	6.0	12000	14000
Cd	<1.0	4.1	4.0	<1.0	<1.0
Co	<5.0	8.1	8.0	12	12
Cr	<20	4.1	4.0	31	36
Cu	<5.0	8.0	8.0	30	31
Fe	<50	16	16	30000	35000
K	<20	15	15	3900	4900
Mg	<20	6.2	6.0	12000	13000
Mn	<1.0	16	16	310	300
Mo	<2.0	2.9	3.0	2	2.1
Na	<75	16	15	9600	11000
Ni	<5.0	8.2	8.0	36	37
P	<20	29	30	1000	1000
Pb	<10	40	40	22	19
S	<25	29	30	1600	1700
Sb	<10	2.8	3.0	<10	<10
Se	<10	3.0	3.0	<10	<10
Sn	<2.0	2.8	3.0	<2.0	<2.0
Sr	<5.0	3.1	3.0	63	64
Ti	<10	2.7	3.0	-	-
Tl	<1.0	3.1	3.0	<1.0	<1.0
U	<10	2.0	2.0	<10	<10
V	<10	3.1	3.0	77	84
Zn	<15	15	15	120	130

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Sample	Cooler 1 Sample 2	Cooler 1 Sample 2
Ag	<2.0	<2.0
Al	6800	6800
As	5.8	6.0
B	<20	<20
Ba	180	180
Be	<4.0	<4.0
Ca	97000	86000
Cd	<1.0	<1.0
Co	<5.0	<5.0
Cr	<20	<20
Cu	26	27
Fe	12000	12000
K	1200	1200
Mg	16000	16000
Mn	300	300
Mo	<2.0	<2.0
Na	140	140
Ni	13	14
P	520	530
Pb	1700	1800
S	580	590
Sb	<10	<10
Se	<10	<10
Sn	<2.0	<2.0
Sr	77	73
Ti	46	58
Tl	<1.0	<1.0
U	<10	<10
V	21	21
Zn	54	55

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REPORT OF ANALYSIS

ASU # 15085
Client: Alberta Innovates

Method: CCME TPH in Soil †

Report ID: ASU 15085 Alberta Innovates CCME-2
Date Submitted: 3-Jun-14
Date Testing Initiate 23-Sep-14
Date Reported: 26-Sep-14
Matrix: Soil

FINAL REPORT
RESULTS

Sample ID	% water of Wet Soil	F1 ** C6-C10 mg/kg	F2 C10-C16 mg/kg	F3 C16-C34 mg/kg	F4 C34-C50 mg/kg	TPH SUM
Cooler 1-1	4%	-	27	913	235	1180
Cooler 1-2	4%	-	22	831	251	1100
Cooler 2	1%	-	<20	<20	<20	<60
Cooler 3*	1%	-	<20	123	259	382

** F1 fraction not assessed
† Complies with CWS PHC Tier 1 Method
‡ F4G gravimetric heavy hydrocarbons cannot be added to the sum
* Average of duplicate results
Detection limit increased in samples due to interferences

QUALITY CONTROL
Quality Criteria:

1) nC6 and nC10 response factors within 30% of response for toluene;
2) nC10, nC16 and nC34 response factors within 10% of average;
3) C50 response factors within 70% of nC110 + nC16 + nC34 average;
4) linearity of calibrations standard with 15%

	F1 ** C6-C10 mg/kg	F2 C10-C16 mg/kg	F3 C16-C34 mg/kg	F4 C34-C50 mg/kg
Blank	-	<10	<10	<10
Cooler 3 duplicate	-	<20 ; <20	117 ; 128	250 ; 268
Control	-	165		
Control Target	-	161		

COMMENTS
F4G Fraction not assessed
Chromatogram descended to baseline by the C50 retention time
Fractions include BTEX, Napthalene and PAHs
Analysis holding times for samples were met
Results relate only to the items tested

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Authorized by: A. Nutter

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REPORT OF ANALYSIS

ASU #	15085	**Report ID:**	ASU 15085 Alberta Innovates PAH-2
Client:	Alberta Innovates	**Date Submitted:**	3-Jun-14
		Date Reported:	26-Sep-15
Method:	PAH by GC/MS	**Matrix:**	Soil

FINAL REPORT
RESULTS in ppb

Compound	Cooler 1 Sample1	Cooler 1 Sample 2	Cooler 2	Cooler 3*
Naphthalene	529	514	21.8	54.7
Acenaphthylene	17.4	15.7	<10	<10
Acenaphthene	41.6	36.0	<10	18.0
Fluorene	71.3	58.0	<10	20.0
Phenanthrene	1500	1430	16.0	353
Anthracene	201	242	<10	82.7
Fluoranthene	842	991	<10	440
Pyrene	1440	1520	18.5	437
Benzo(a)anthracene	990	784	<20	242
Chrysene	1150	1160	16.6	241
Benzo(bkj)fluoranthen	598	605	<10	132
Benzo(a)pyrene	1720	1920	20.2	291
Dibenzo(ah)anthracene	551	374	<10	57.0
Indeno(123cd)pyrene	1140	884	21.0	165
Benzo(ghi)perylene	3480	2850	58.0	403

Prepared by: Paula Whitley Authorized by:

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Compound	Blank	Control	Control Target	Cooler 3	Cooler 3 Duplicate
Naphthalene	<10	445	400	53.5	56.0
Acenaphthylene	<10	403	400	<10	<10
Acenaphthene	<10	265	400	20.0	16.0
Fluorene	<10	291	400	21.7	18.3
Phenanthrene	<10	359	400	340	366
Anthracene	<10	365	400	94.2	71.3
Fluoranthene	<10	406	400	394	487
Pyrene	<10	434	400	428	446
Benzo(a)anthracene	<20	505	400	226	258
Chrysene	<10	411	400	241	241
Benzo(bkj)fluoranthen	<10	678	800	129	135
Benzo(a)pyrene	<10	493	400	332	251
Dibenzo(ah)anthracene	<10	490	400	68.4	45.6
Indeno(123cd)pyrene	<10	546	400	187	143
Benzo(ghi)perylene	<10	461	400	455	352

* Average results of duplicates
Results relate only to the items tested

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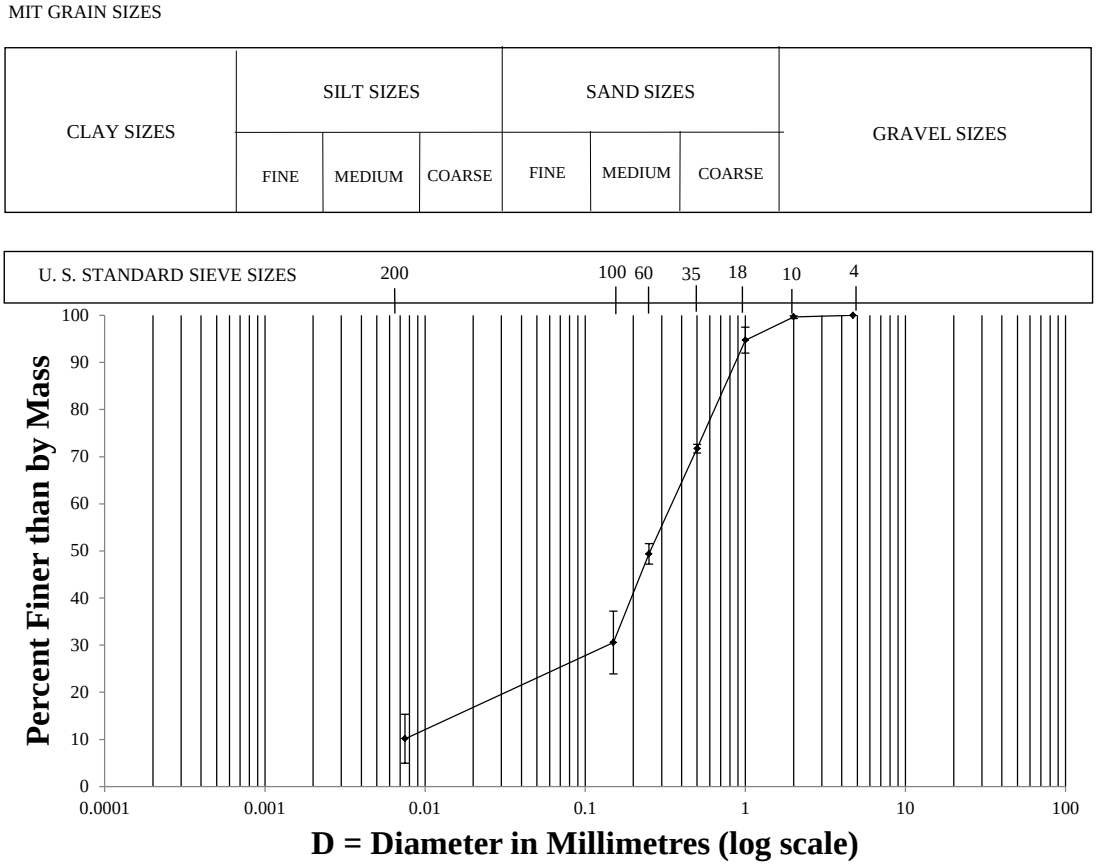
ASU # 15085
Client: Alberta Innovates

Report ID: ASU 15085 Alberta Innovates psd-1
Date Submitted: 3-Jun-14
Date Testing Initiated: 3-Jun-14
Date Reported: 19-Jun-14

REPORT OF ANALYSIS

Matrix: Soil

Particle Size Distribution Curve for Cooler 1



Prepared by: Paula Whitley

Authorized by:

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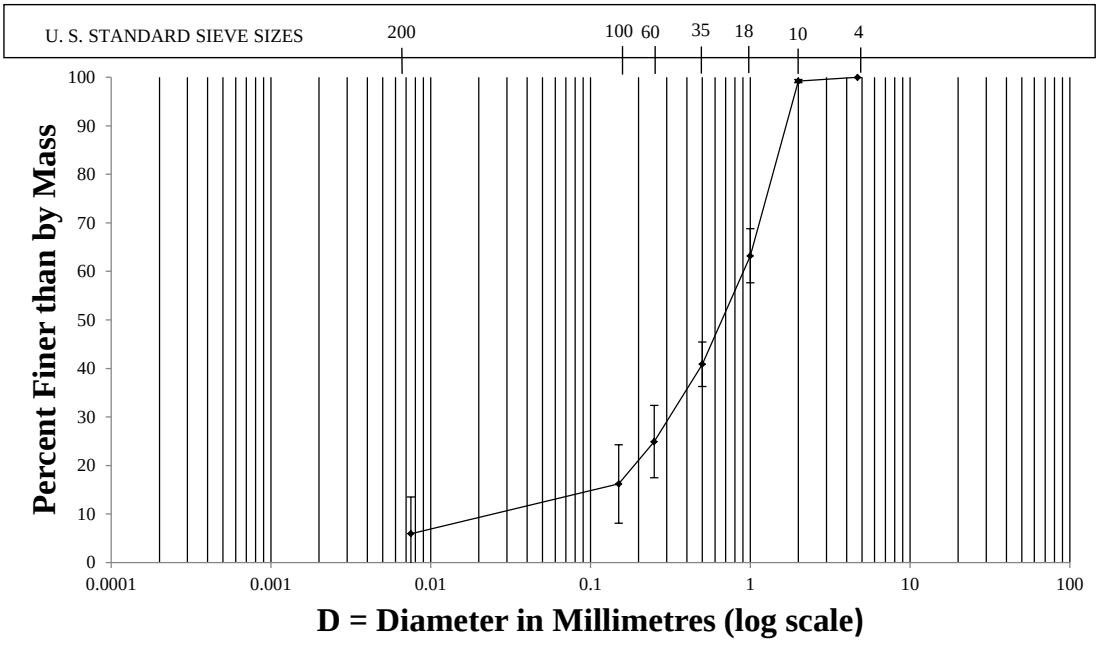
Particle Size Distribution Curve for Cooler 2

MIT GRAIN SIZES

CLAY SIZES	SILT SIZES			SAND SIZES			GRAVEL SIZES
	FINE	MEDIUM	COARSE	FINE	MEDIUM	COARSE	



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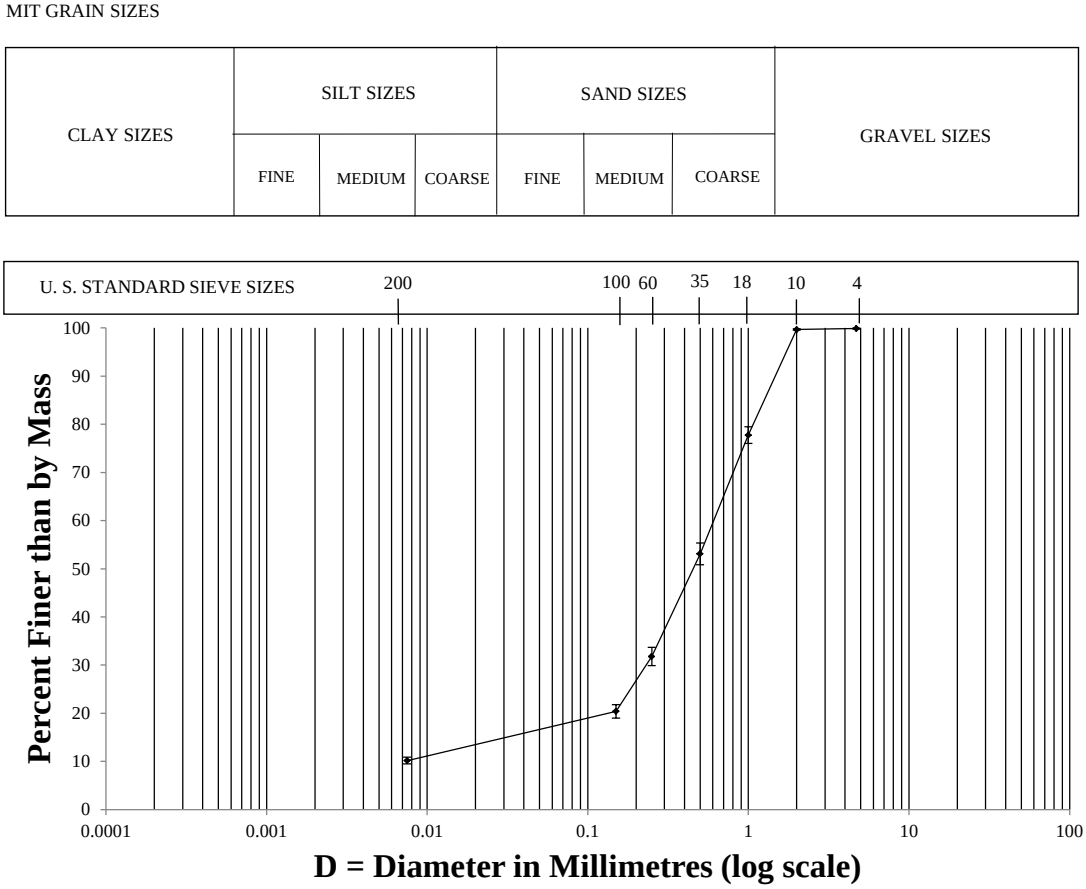
Particle Size Distribution Curve for Cooler 3



Appendix C: Growth data for Alfalfa and Clover

Alfalfa

Cooler 1

		Shoots		Roots	
Harvest 1		Average	SD	Average	SD
	Control	4.41E-03	1.88E-03	8.11E-04	2.80E-04
	Biochar	5.42E-03	3.03E-03	6.78E-04	1.99E-04

		Shoots		Roots	
Harvest 2		Average	SD	Average	SD
	Control	1.45E-01	2.15E-01	4.02E-03	2.29E-03
	Biochar	2.56E-01	2.76E-01	8.48E-03	7.42E-03

		Shoots		Roots	
Harvest 3		Average	SD	Average	SD
	Control	2.60E-01	1.32E-01	7.16E-02	2.54E-02
	Biochar	3.18E-01	1.74E-01	8.33E-02	3.56E-02

		Shoots		Roots	
Harvest 4		Avg	SD	Avg	SD
	Control	5.49E-01	2.55E-01	1.50E-01	8.52E-02
	Biochar	4.60E-01	2.30E-01	1.78E-01	5.56E-02

Cooler 2

		Shoots		Roots	
Harvest 1		Average	SD	Average	SD
	Control	3.07E-03	8.62E-04	7.33E-04	1.15E-04
	Biochar	9.80E-03	5.91E-03	9.00E-04	2.65E-04

		Shoots		Roots	
Harvest 2		Average	SD	Average	SD
	control	1.36E-01	7.80E-02	1.14E-02	8.45E-03
	biochar	4.12E-02	1.51E-02	3.77E-03	2.45E-03

		Shoots		Roots	
Harvest 3		Average	SD	Average	SD
	control	1.26E-01	3.98E-02	3.44E-02	1.75E-02
	biochar	2.70E-01	5.89E-02	1.35E-01	5.32E-02

		Shoots		Roots	
Harvest 4		Avg	SD	Avg	SD
	Control	1.26E-01	6.05E-02	7.85E-02	3.72E-02
	Biochar	3.36E-01	2.17E-02	2.12E-01	7.17E-02

Cooler 3

		Shoots		Roots	
Harvest 1		Average	SD	Average	SD
	Control	5.63E-03	7.27E-03	6.33E-04	5.77E-05
	Biochar	1.30E-03	3.00E-04	9.67E-04	2.52E-04

		Shoots		Roots	
Harvest 2		Average	SD	Average	SD
	control	8.67E-02	2.69E-02	6.50E-03	4.23E-03
	biochar	6.22E-02	3.59E-02	3.27E-03	2.18E-03

		Shoots		Roots	
Harvest 3		Average	SD	Average	SD
	control	2.62E-01	1.22E-01	2.20E-01	2.45E-01
	biochar	3.93E-01	8.97E-02	2.53E-01	3.22E-01

		Shoots		Roots	
Harvest 4		Avg	SD	Avg	SD
	Control	3.29E-01	6.05E-02	1.25E-01	3.50E-02
	Biochar	9.77E-01	3.60E-01	3.48E-01	1.62E-01

Clover

Cooler 1

		F3		F4	
		Average	SD	Average	SD
TO	**Control**	1.08E+03	5.66E+01	6.05E+02	4.09E+01
	Biochar	1.07E+03	4.21E+01	6.61E+02	8.89E+01
Day 51	**Control**	1.05E+03	2.00E+01	5.78E+02	1.69E+01
	Biochar	1.24E+03	3.21E+01	5.26E+02	7.48E+01

Cooler 3

		F2		F3	
		Average	SD	Average	SD
TO	**Control**	2.97E+03	6.93E+02	3.52E+03	1.98E+02
	Biochar	2.50E+03	5.37E+02	2.78E+03	1.48E+02
Day 51	**Control**	1.56E+03	2.50E+02	2.17E+03	1.27E+02
	Biochar	1.71E+03	2.76E+02	2.47E+03	6.56E+01

Appendix D: Laboratory Results for CCME PHC, PAHs and metals in soils during the greenhouse trial

Analytical Services Unit
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ASU #	15085	**Report ID:**	ASU 15085 Alberta Innovates 30 Element
Client:	Alberta Innovates	**Date Submitted:**	9-Mar-15
		Date:	24-Mar-15
Method:	Metals by ICP-OES	**Matrix:**	Soil

Results relate only to the items tested.

Total Metals Results in ug/g

Sample	C1	C2	C3*	C1 B	C2 B
Ag	<2.0	<2.0	<2.0	<2.0	<2.0
Al	7500	10000	9200	7500	10000
As	5.6	6.3	5.5	6.1	6.5
B	<20	<20	<20	<20	<20
Ba	170	270	210	180	260
Be	<4.0	<4.0	<4.0	<4.0	<4.0
Ca	73000	50000	62000	69000	51000
Cd	1.3	<1.0	<1.0	<1.0	<1.0
Co	<5.0	5.9	<5.0	<5.0	5.8
Cr	<20	<20	<20	<20	<20
Cu	27	14	23	28	15
Fe	13000	14000	14000	13000	14000
K	1300	1800	1600	1300	1800
Mg	17000	11000	12000	15000	11000
Mn	310	330	340	310	340
Mo	<2.0	<2.0	<2.0	<2.0	<2.0
Na	160	330	190	150	330
Ni	14	17	14	14	16
P	510	620	520	510	600
Pb	2000	14	110	2100	17
S	610	590	300	610	550
Sb	<10	<10	<10	<10	<10
Se	<10	<10	<10	<10	<10
Sn	<2.0	<2.0	2.5	<2.0	<2.0
Sr	71	64	67	68	65
Ti	45	78	87	56	83
Tl	<1.0	<1.0	<1.0	<1.0	<1.0
U	<10	<10	<10	<10	<10
V	21	27	24	21	26
Zn	60	69	93	63	68

* averaged results of duplicate analysis

Prepared by: Paula Whitley Authorization:

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Sample	C3 B	C3 control Jan 30th	C3 control Jan 30th B	C1 control Jan 30th	C1 control Jan 30th B
Ag	<2.0	<2.0	<2.0	<2.0	<2.0
Al	10000	9800	11000	8200	7800
As	5.5	6.0	5.4	5.9	8.1
B	<20	<20	<20	<20	<20
Ba	210	200	640	170	170
Be	<4.0	<4.0	<4.0	<4.0	<4.0
Ca	60000	59000	62000	73000	73000
Cd	<1.0	<1.0	<1.0	<1.0	<1.0
Co	5.1	<5.0	5.3	5.1	<5.0
Cr	<20	<20	<20	<20	<20
Cu	19	20	22	31	29
Fe	14000	13000	13000	14000	13000
K	1600	1700	1800	1400	1400
Mg	11000	12000	12000	16000	15000
Mn	350	310	360	320	300
Mo	<2.0	<2.0	<2.0	<2.0	<2.0
Na	190	340	350	250	270
Ni	15	14	14	14	13
P	520	510	1100	540	530
Pb	110	94	100	2000	2100
S	290	350	370	640	700
Sb	<10	<10	<10	<10	<10
Se	<10	<10	<10	<10	<10
Sn	<2.0	<2.0	<2.0	<2.0	<2.0
Sr	72	65	201	71	71
Ti	120	120	150	89	84
Tl	<1.0	<1.0	<1.0	<1.0	<1.0
U	<10	<10	<10	<10	<10
V	26	25	27	24	23
Zn	84	83	87	63	61

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Sample	C2 control Jan 30th	C2 control Jan 30th B	H4C1-1-A	H4C1-2-A	H4C1-3-A
Ag	<2.0	<2.0	<2.0	<2.0	<2.0
Al	10000	10000	7700	7400	7600
As	15	5.9	5.8	5.5	5.6
B	<20	<20	<20	<20	<20
Ba	220	240	180	180	180
Be	<4.0	<4.0	<4.0	<4.0	<4.0
Ca	48000	50000	73000	71000	71000
Cd	<1.0	<1.0	<1.0	<1.0	<1.0
Co	6.0	5.8	<5.0	<5.0	<5.0
Cr	<20	<20	<20	<20	<20
Cu	16	17	32	29	29
Fe	14000	14000	13000	13000	12000
K	1700	1800	1200	1200	1200
Mg	10000	11000	16000	15000	16000
Mn	330	340	300	300	310
Mo	<2.0	<2.0	<2.0	<2.0	<2.0
Na	350	320	200	200	200
Ni	16	16	14	13	13
P	590	600	530	520	500
Pb	15	15	2100	2000	2100
S	670	580	570	630	550
Sb	<10	<10	<10	<10	<10
Se	<10	<10	<10	<10	<10
Sn	<2.0	<2.0	<2.0	<2.0	<2.0
Sr	62	65	72	72	68
Ti	91	98	86	69	81
Tl	<1.0	<1.0	<1.0	<1.0	<1.0
U	<10	<10	<10	<10	<10
V	26	27	23	22	23
Zn	67	66	61	59	59

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Sample	H4C1-1-A-B*	H4C1-2-A-B	H4C1-3-A-B	H4C2-A	H4C2-B
Ag	<2.0	<2.0	<2.0	<2.0	<2.0
Al	7800	8200	8500	12000	12000
As	6.4	6.5	7.2	7.2	7.0
B	<20	<20	<20	<20	<20
Ba	180	190	190	290	310
Be	<4.0	<4.0	<4.0	<4.0	<4.0
Ca	72000	75000	74000	56000	53000
Cd	1.1	<1.0	1.2	<1.0	<1.0
Co	5.1	5.0	5.3	6.4	6.8
Cr	<20	<20	<20	<20	<20
Cu	31	30	40	18	18
Fe	13000	13000	14000	15000	15000
K	1400	1400	1400	1800	1800
Mg	16000	16000	16000	12000	12000
Mn	350	320	340	360	370
Mo	<2.0	<2.0	<2.0	<2.0	<2.0
Na	250	220	210	250	270
Ni	14	14	15	18	18
P	510	530	500	610	620
Pb	2200	2100	2100	17	16
S	640	610	580	460	570
Sb	<10	<10	<10	<10	<10
Se	<10	<10	<10	<10	<10
Sn	<2.0	<2.0	<2.0	<2.0	<2.0
Sr	71	72	76	70	72
Ti	75	91	85	110	110
Tl	<1.0	1.2	<1.0	<1.0	<1.0
U	<10	<10	<10	<10	<10
V	23	24	25	31	32
Zn	62	63	62	71	73

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Sample	H4C2-C	H4C2-A-B	H4C2-B-B*	H4C2-C-B	H4C3-A
Ag	<2.0	<2.0	<2.0	<2.0	<2.0
Al	11000	11000	13000	12000	9900
As	6.6	6.8	7.2	7.1	5.8
B	<20	<20	<20	<20	<20
Ba	270	260	280	28/0	220
Be	<4.0	<4.0	<4.0	<4.0	<4.0
Ca	51000	47000	51000	51000	70000
Cd	<1.0	<1.0	<1.0	<1.0	<1.0
Co	6.6	5.6	6.4	6.5	5.3
Cr	<20	<20	<20	<20	<20
Cu	20	19.1	22.3	22	26
Fe	15000	14000	15000	15000	17000
K	1700	1900	1900	1800	1400
Mg	12000	10000	12000	12000	13000
Mn	370	340	360	370	360
Mo	<2.0	<2.0	<2.0	<2.0	<2.0
Na	240	240	510	290	230
Ni	19	16	18	18	15
P	600	540	990	600	560
Pb	16	15	16	17	98
S	570	460	600	530	330
Sb	<10	<10	<10	<10	<10
Se	<10	<10	<10	<10	<10
Sn	<2.0	<2.0	<2.0	<2.0	2.5
Sr	68	64	72	70	85
Ti	90	110	120	120	120
Tl	1.0	<1.0	<1.0	1.3	<1.0
U	<10	<10	<10	<10	<10
V	30	29	33	31	26
Zn	70	65	72	71	89

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Sample	H4C3-B	H4C3-C	H4C3-A-B	H4C3-B-B	H4C3-C-B
Ag	<2.0	<2.0	<2.0	<2.0	<2.0
Al	11000	10400	12800	11000	11300
As	12	5.1	5.6	6.8	7.1
B	<20	<20	49	<20	<20
Ba	230	220	270	220	240
Be	<4.0	<4.0	<4.0	<4.0	<4.0
Ca	65000	67000	67000	66000	61000
Cd	<1.0	<1.0	<1.0	<1.0	<1.0
Co	5.7	5.0	5.6	5.3	5.9
Cr	<20	<20	<20	<20	<20
Cu	26	23	29	24	38
Fe	16000	13000	15000	14000	16000
K	1600	1600	1700	1600	1700
Mg	12000	12000	12000	12000	13000
Mn	360	350	380	340	380
Mo	<2.0	<2.0	<2.0	<2.0	<2.0
Na	210	200	770	270	380
Ni	16	14	15	14	21
P	520	510	610	510	570
Pb	110	97	120	97	100
S	470	300	370	320	370
Sb	<10	<10	<10	<10	<10
Se	<10	<10	<10	<10	<10
Sn	<2.0	<2.0	7.2	2.8	2.7
Sr	77	82	120	79	74
Ti	140	140	226	170	150
Tl	<1.0	<1.0	<1.0	<1.0	<1.0
U	<10	<10	<10	<10	<10
V	29	28	31	30	29
Zn	90	80	83	82	85

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Laboratory QA/QC

Sample	Blank	Blank	MESS-3	SS-2
Ag	<2.0	<2.0	<2.0	<2.0
Al	<50	<50	16000	17800
As	<1.0	<1.0	17	89
B	<20	<20	-	-
Ba	<5.0	<5.0	300	250
Be	<4.0	<4.0	<4.0	<4.0
Ca	<100	<100	13000	130000
Cd	<1.0	<1.0	<1.0	2.1
Co	<5.0	<5.0	12	16
Cr	<20	<20	29	43
Cu	<5.0	<5.0	30	220
Fe	<50	<50	31000	26000
K	<20	<20	4200	4400
Mg	<20	<20	12000	13000
Mn	<1.0	<1.0	290	570
Mo	<2.0	<2.0	<2.0	2.8
Na	<75	<75	9900	840
Ni	<5.0	<5.0	34	58
P	<20	<20	970	750
Pb	<10	<10	23	130
S	<25	<25	1500	2200
Sb	<10	<10	<10	<10
Se	<10	<10	<10	<10
Sn	<2.0	<2.0	<2.0	4.6
Sr	<5.0	<5.0	58	220
Ti	<10	<10	-	1400
Tl	<1.0	<1.0	<1.0	<1.0
U	<10	<10	<10	<10
V	<10	<10	71	52
Zn	<15	<15	130	490

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Sample	C3	C3	H4C1-1-A-B	H4C1-1-A-B
Ag	<2.0	<2.0	<2.0	<2.0
Al	9300	9100	7600	8000
As	5.8	5.2	6.0	6.8
B	<20	<20	<20	<20
Ba	200	210	180	170
Be	<4.0	<4.0	<4.0	<4.0
Ca	64000	59000	72000	72000
Cd	<1.0	<1.0	1.0	1.2
Co	5.1	<5.0	5.1	5.0
Cr	<20	<20	<20	<20
Cu	25	20	32	30
Fe	15000	13000	13000	13000
K	1600	1600	1300	1400
Mg	12000	12000	16000	15000
Mn	360	310	350	350
Mo	<2.0	<2.0	<2.0	<2.0
Na	190	200	240	250
Ni	14	14	14	14
P	520	510	490	520
Pb	110	110	2200	2100
S	300	290	620	670
Sb	<10	<10	<10	<10
Se	<10	<10	<10	<10
Sn	2.6	2.3	<2.0	<2.0
Sr	69	66	70	71
Ti	85	90	64	86
Tl	<1.0	<1.0	<1.0	<1.0
U	<10	<10	<10	<10
V	24	23	23	24
Zn	100	86	63	62

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Sample	H4C2-B-B	H4C2-B-B
Ag	<2.0	<2.0
Al	13000	12000
As	7.5	6.8
B	<20	<20
Ba	290	280
Be	<4.0	<4.0
Ca	51000	51400
Cd	<1.0	<1.0
Co	6.3	6.4
Cr	<20	<20
Cu	22	22
Fe	15000	15000
K	2000	1800
Mg	12000	12000
Mn	360	360
Mo	<2.0	<2.0
Na	520	500
Ni	18	18
P	1400	600
Pb	16	16
S	650	540
Sb	<10	<10
Se	<10	<10
Sn	<2.0	<2.0
Sr	72	71
Ti	130	120
Tl	<1.0	<1.0
U	<10	<10
V	34	32
Zn	71	73

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REPORT OF ANALYSIS

ASU #	15085	Report ID:	ASU 15085 Alberta Innovates CCME-5
Client:	Alberta Innovates	Date Submitted:	5-Jan-15
		Date Testing Initiated:	10-Mar-15
		Date Reported:	17-Mar-15
Method:	CCME TPH in Soil †	Matrix:	Soil

RESULTS

Sample ID	% water of Wet Soil	F1 ** C6-C10 mg/kg	F2*** C10-C16 mg/kg	F3 *** C16-C34 mg/kg	F4*** C34-C50 mg/kg	TPH SUM
Cl H1 C1-1-a	15%	-	<20	1030	597	1630
Cl H1 C1-2-a	10%	-	<20	1070	571	1640
Cl H1 C1-3-a	15%	-	<20	1050	565	1615
Cl H1 C1-1-a B	23%	-	<20	1220	474	1690
Cl H1 C1-2-a B	18%	-	<20	1230	491	1720
Cl H1 C1-3-a B	27%	-	<20	1280	612	1890
Cl H1 C2-a	14%	-	<20	50	<20	<60
Cl H1 C2-a B	19%	-	<20	73	<20	73
Cl H1 C2-b B*	19%	-	<20	<20	<20	<60
Cl C1 control	14%	-	<20	1120	634	1750
Cl C1 B control	18%	-	<20	1100	724	1820
Cl H1 C2-c B	19%	-	<20	<20	<20	<60
Cl H1 C3-a	18%	-	1390	2280	<20	3670
Cl H1 C3-b	19%	-	1850	2030	<20	3880
Cl H1 C3-c	17%	-	1450	2200	<20	3650
Cl H1 C3-a B	25%	-	1500	2530	<20	4030
Cl H1 C3-b B	21%	-	2020	2480	<20	4500
Cl H1 C3-c B*	20%	-	1600	2400	<20	4000
H4 C1 control	14%	-	<20	1040	576	1620
H4 C1 B control	19%	-	<20	1040	598	1640
H4 C2 control	16%	-	<20	<20	<20	<60
H4 C2 B control	17%	-	<20	<20	<20	<60
H4 C3 control	15%	-	3460	3380	<20	6840
H4 C3 B control	25%	-	2880	2880	<20	5760
Cl C2 control	10%	-	<20	<20	<20	<60
Cl C2 B control	15%	-	<20	<20	<20	<60
Cl C3 control	10%	-	2480	3660	<20	6140
Cl C3 B control*	10%	-	2120	2670	<20	4790

* Average of duplicate results
** F1 fraction not assessed
*** Detection limit increased due to interferences
† Complies with CWS PHC Tier 1 method
‡ F4G gravimetric heavy hydrocarbons cannot be added to the sum

QUALITY CONTROL

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Quality Criteria: 1) nC6 and nC10 response factors within 30% of response for toluene;
2) nC10, nC16 and nC34 response factors within 10% of average;
3) C50 response factors within 70% of nC110 + nC16 + nC34 average;
4) linearity of calibrations standard with 15%

	F1 C6-C10 mg/kg	F2*** C10-C16 mg/kg	F3*** C16-C34 mg/kg	F4*** C34-C50 mg/kg
Blank	-	<10 ; <10 ; <10	<10 ; <10 ; <10	<10 ; <10 ; <10
Cl H1 C2-b B duplicate	-	<20 ; <20	<20 ; <20	<20 ; <20
Cl H1 C3-c B duplicate	-	1460 ; 1740	2350 ; 2440	<20 ; <20
Cl C3 B control duplicate	-	2000 ; 2230	2690 ; 2640	<20 ; <20
Control	-		161 ; 142	
Control Target	-		171	
Control	-		131	
Control Target	-		158	

COMMENTS
F4G Fraction not assessed
Chromatogram descended to baseline by the C50 retention time
Fractions include BTEX, Napthalene and PAHs
Analysis holding times for samples were met

Prepared by: Paula Whidden Authorized by: A. Netten

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REPORT OF ANALYSIS

ASU #	15085	Report ID:	ASU 15085 Alberta Innovates CCME-6
Client:	Alberta Innovates	Date Submitted:	19-Mar-15
		Date Testing Initiated:	19-Mar-15
		Date Reported:	23-Mar-15
Method:	CCME TPH in Soil†	Matrix:	Soil

FINAL REPORT

RESULTS

Sample ID	% water of Wet Soil	F1 C6-C10 mg/kg	F2 C10-C16 mg/kg	F3 C16-C34 mg/kg	F4 C34-C50 mg/kg	TPH SUM
Cl-H1 C2-A	17%	**	<20	<20	<20	<60
Cl-H1 C2-B	18%	**	<20	<20	<20	<60
Cl-H1 C2-C*	19%	**	<20	<20	<20	<60

* Average of duplicate results
** F1 fraction not assessed
*** Detection limit increased due to interferences
† Complies with CWS PHC Tier 1 method
‡ F4G gravimetric heavy hydrocarbons cannot be added to the sum

QUALITY CONTROL

Quality Criteria: 1) nC6 and nC10 response factors within 30% of response for toluene;
2) nC10, nC16 and nC34 response factors within 10% of average;
3) C50 response factors within 70% of nC110 + nC16 + nC34 average;
4) linearity of calibrations standard with 15%

	F1 C6-C10 mg/kg	F2 C10-C16 mg/kg	F3 C16-C34 mg/kg	F4 C34-C50 mg/kg
Blank	-	<10	<10	<10
Cl-H1 C2-C duplicate	**	<20 ; <20	<20 ; <20	<20 ; <20
Control	-		158	
Control Target	-		158	

COMMENTS

F4G Fraction not assessed
Chromatogram descended to baseline by the C50 retention time
Fractions include BTEX, Napthalene and PAHs
Analysis holding times for samples were met

Prepared by: Paula Whidden Authorized by: A. Netten

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REPORT OF ANALYSIS

ASU #	15085	Report ID:	ASU 15085 Alberta Innovates PAH-6
Client:	Alberta Innovates	Date Submitted:	9-Mar-15
		Date Reported:	26-Mar-15
Method:	PAH by GC/MS	Matrix:	Soil

FINAL REPORT
RESULTS in ppb

Compound	H4C1-1-A	H4C1-1-A B*	H4C1-2-A	H4C1-2-A B	H4C1-3-A
Naphthalene	175	437	83.4	140	59.1
Acenaphthylene	<10	25.9	<10	<10	<10
Acenaphthene	14.3	38.6	<10	<10	<10
Fluorene	29.4	93.8	<10	18.4	<10
Phenanthrene	524	1530	116	322	113
Anthracene	70.7	176	14.2	46.7	17.2
Fluoranthene	367	1090	69.1	295	72.4
Pyrene	514	1820	112	391	90.4
Benzo(a)anthracene	282	1400	55.2	246	48.1
Chrysene	501	1940	104	388	83.2
Benzo(bkj)fluoranth	726	2800	134	482	93.1
Benzo(a)pyrene	631	2930	155	357	80.6
Dibenzo(ah)anthrac	330	1240	55.2	160	34.4
Indeno(123cd)pyrer	376	1670	77.5	182	49.3
Benzo(ghi)perylene	1150	5510	268	580	133

Prepared by: Paula Whidden Authorized by: A. Mutter

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Compound	H4C1-3-A B	H4C2-A	H4C2-B	H4C2-C	H4C2-A B
Naphthalene	288	14.9	<10	23.6	<10
Acenaphthylene	19.8	<10	<10	<10	<10
Acenaphthene	19.2	<10	<10	<10	<10
Fluorene	46.2	<10	<10	<10	<10
Phenanthrene	749	<10	<10	<10	<10
Anthracene	96.1	<10	<10	<10	<10
Fluoranthene	429	<10	<10	<10	<10
Pyrene	670	<10	<10	<10	<10
Benzo(a)anthracene	428	<20	<20	<20	<20
Chrysene	695	<10	<10	<10	<10
Benzo(bkj)fluorantl	918	<10	<10	<10	<10
Benzo(a)pyrene	1210	<10	<10	<10	<10
Dibenzo(ah)anthrac	443	<10	<10	<10	<10
Indeno(123cd)pyrei	503	<10	<10	<10	<10
Benzo(ghi)perylene	1680	<10	<10	<10	<10

Compound	H4C2-B B*	H4C2-C B	H4C3-A	H4C3-B	H4C3-C
Naphthalene	<10	<10	20.4	67.7	50.8
Acenaphthylene	<10	<10	24.8	57.4	41.8
Acenaphthene	<10	<10	33.8	98.9	68.1
Fluorene	<10	<10	151	514	323
Phenanthrene	<10	<10	368	889	555
Anthracene	<10	<10	17.4	49.3	27.8
Fluoranthene	<10	<10	96.2	81.1	49.2
Pyrene	<10	<10	135	164	134
Benzo(a)anthracene	<20	<20	32.6	20.2	14.6
Chrysene	<10	<10	44.1	30.1	24.3
Benzo(bkj)fluorantl	<10	<10	37.2	28.8	25.5
Benzo(a)pyrene	<10	<10	20.3	15.7	12.0
Dibenzo(ah)anthrac	<10	<10	<10	10.6	<10
Indeno(123cd)pyrei	<10	<10	16.1	14.0	11.4
Benzo(ghi)perylene	<10	<10	34.1	38.1	26.2

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Compound	H4C3-A B	H4C3-B B	H4C3-C B	CL H1C1-1-A	CL H1C1-1-A B
Naphthalene	533	345	225	239	215
Acenaphthylene	92.5	58.8	25.8	13.2	15.3
Acenaphthene	122	85.9	40.3	20.1	22.3
Fluorene	649	444	259	46.9	50.5
Phenanthrene	982	688	436	998	894
Anthracene	59.5	41.9	28.6	113	91.3
Fluoranthene	122	48.5	39.3	775	828
Pyrene	274	177	131	1290	1220
Benzo(a)anthracene	33.8	13.2	11.2	982	867
Chrysene	55.9	21.7	15.7	1220	1140
Benzo(bkj)fluorantl	52.8	22.5	18.8	2020	1980
Benzo(a)pyrene	29.6	14.7	16.9	1730	1820
Dibenzo(ah)anthrac	16.0	12.8	<10	944	1090
Indeno(123cd)pyrer	21.4	14.5	<10	1300	1240
Benzo(ghi)perylene	47.1	35.1	24.9	3610	3170

Compound	CL H1C1-2-A*	CL H1C1-2-A B	CL H1C1-3-A	CL H1C1-3-A B	CL H1C2-A B
Naphthalene	279	259	273	275	29.5
Acenaphthylene	17.0	16.2	23.9	14.8	<10
Acenaphthene	23.0	23.2	25.3	24.3	<10
Fluorene	54.8	48.8	46.6	55.4	<10
Phenanthrene	1020	999	954	1120	19.7
Anthracene	126	110	139	156	<10
Fluoranthene	767	776	622	969	10.8
Pyrene	1320	1350	1140	1590	21.3
Benzo(a)anthracene	1120	1070	956	1280	<20
Chrysene	1280	1260	1070	1480	21.2
Benzo(bkj)fluorantl	2030	2010	1670	2230	25.8
Benzo(a)pyrene	1990	1690	1690	2010	16.2
Dibenzo(ah)anthrac	1140	994	1060	1250	<10
Indeno(123cd)pyrer	1410	1240	1190	1520	14.6
Benzo(ghi)perylene	3670	3320	3220	3840	31.2

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Compound	CL H1C2-B B	CL H1C2-C B	CL H1C2-A	CL H1C2-B	CL H1C2-C
Naphthalene	<10	34.1	<10	<10	<10
Acenaphthylene	<10	<10	<10	<10	<10
Acenaphthene	<10	<10	<10	<10	<10
Fluorene	<10	<10	<10	<10	<10
Phenanthrene	16.4	16.4	<10	<10	<10
Anthracene	<10	<10	<10	<10	<10
Fluoranthene	10.0	14.0	<10	<10	<10
Pyrene	16.3	20.1	10.8	<10	<10
Benzo(a)anthracene	<20	<20	<20	<20	<20
Chrysene	<10	17.1	10.8	<10	<10
Benzo(bkj)fluorantl	33.6	21.0	20.3	25.2	<10
Benzo(a)pyrene	15.5	11.6	12.0	<10	<10
Dibenzo(ah)anthrac	<10	<10	12.7	<10	<10
Indeno(123cd)pyrei	<10	<10	11.5	<10	<10
Benzo(ghi)perylene	17.0	21.1	21.7	<10	<10

Compound	CL H1C3-A *	CL H1C3-B	CL H1C3-C	CL H1C3-A B	CL H1C3-B B
Naphthalene	34.1	40.4	64.7	1270	1600
Acenaphthylene	26.1	22.7	33.4	74.6	158
Acenaphthene	17.7	21.7	27.5	79.7	164
Fluorene	48.1	52.9	71.3	467	774
Phenanthrene	142	100	230	1260	1690
Anthracene	21.3	23.2	49.1	86.0	85.4
Fluoranthene	103	46.0	136	148	195
Pyrene	222	159	344	345	542
Benzo(a)anthracene	51.9	<20	70.2	48.7	58.4
Chrysene	67.2	26.8	81.7	55.9	74.0
Benzo(bkj)fluorantl	85.5	41.3	113	73.4	81.4
Benzo(a)pyrene	47.7	24.0	57.8	42.5	40.5
Dibenzo(ah)anthrac	29.1	11.4	47.9	28.7	28.3
Indeno(123cd)pyrei	37.7	19.4	55.6	37.2	42.7
Benzo(ghi)perylene	83.5	55.4	143	77.0	97.4

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Compound	CL H1C3-C B
Naphthalene	2150
Acenaphthylene	125
Acenaphthene	159
Fluorene	962
Phenanthrene	2290
Anthracene	145
Fluoranthene	300
Pyrene	700
Benzo(a)anthracene	103
Chrysene	126
Benzo(bkj)fluoranth	152
Benzo(a)pyrene	82.4
Dibenzo(ah)anthrac	57.3
Indeno(123cd)pyrer	66.0
Benzo(ghi)perylene	174

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Laboratory QA/QC

Compound	Blank	Blank	Blank	Blank
Naphthalene	<10	<10	<10	<10
Acenaphthylene	<10	<10	<10	<10
Acenaphthene	<10	<10	<10	<10
Fluorene	<10	<10	<10	<10
Phenanthrene	<10	<10	<10	<10
Anthracene	<10	<10	<10	<10
Fluoranthene	<10	<10	<10	<10
Pyrene	<10	<10	<10	<10
Benzo(a)anthracene	<20	<20	<20	<20
Chrysene	<10	<10	<10	<10
Benzo(bkj)fluoranth	<10	<10	<10	<10
Benzo(a)pyrene	<10	<10	<10	<10
Dibenzo(ah)anthrac	<10	<10	<10	<10
Indeno(123cd)pyrer	<10	<10	<10	<10
Benzo(ghi)perylene	<10	<10	<10	<10

Compound	Control	Control	Control	Control	Control Target
Naphthalene	376	289	238	268	400
Acenaphthylene	450	363	347	275	400
Acenaphthene	327	269	245	199	400
Fluorene	378	329	262	246	400
Phenanthrene	369	353	322	297	400
Anthracene	298	277	263	272	400
Fluoranthene	300	320	297	285	400
Pyrene	290	309	296	273	400
Benzo(a)anthracene	352	334	339	311	400
Chrysene	312	337	291	271	400
Benzo(bkj)fluoranth	692	623	643	580	800
Benzo(a)pyrene	312	248	280	246	400
Dibenzo(ah)anthrac	328	275	321	254	400
Indeno(123cd)pyrer	352	298	344	259	400
Benzo(ghi)perylene	356	256	279	251	400

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Compound	H4C1-1-A B	4C1-1-A B duplica	H4C2-B B	H4C2-B B duplicate
Naphthalene	376	499	<10	<10
Acenaphthylene	24.2	27.5	<10	<10
Acenaphthene	35.6	41.6	<10	<10
Fluorene	80.6	107	<10	<10
Phenanthrene	1380	1680	<10	<10
Anthracene	142	210	<10	<10
Fluoranthene	885	1300	<10	<10
Pyrene	1580	2050	<10	<10
Benzo(a)anthracene	1180	1620	<20	<20
Chrysene	1660	2210	<10	<10
Benzo(bkj)fluoranth	2270	3320	<10	<10
Benzo(a)pyrene	2400	3460	<10	<10
Dibenzo(ah)anthrac	1140	1330	<10	<10
Indeno(123cd)pyrer	1370	1970	<10	<10
Benzo(ghi)perylene	4890	6120	<10	<10

Compound	CL H1C1-2-A	H1C1-2-A duplic	CL H1C3-A	L H1C3-A duplicate
Naphthalene	269	288	31.9	36.3
Acenaphthylene	16.1	17.9	26.5	25.6
Acenaphthene	17.9	28.0	15.7	19.6
Fluorene	53.0	56.6	38.5	57.8
Phenanthrene	951	1090	124	161
Anthracene	116	136	25.9	16.8
Fluoranthene	728	806	84.5	122
Pyrene	1270	1360	218	227
Benzo(a)anthracene	1030	1210	35.3	68.5
Chrysene	1230	1330	54.1	80.2
Benzo(bkj)fluoranth	1870	2180	68.4	103
Benzo(a)pyrene	1720	2250	38.6	56.9
Dibenzo(ah)anthrac	972	1300	26.5	31.6
Indeno(123cd)pyrer	1270	1550	34.5	40.9
Benzo(ghi)perylene	3430	3910	77.5	89.6

* Average results of duplicates
Results relate only to the items tested

Appendix E: Calculation of Greenhouse Gas Reductions

The project encompassed targeted greenhouse experiments involving native plants and biochar amendments to soil in order to determine the efficacy of using these joint phytotechnologies to remediate petroleum hydrocarbon (PHC)-contaminated soils in Alberta. GHG reductions are expected once the technology is moved to the field. Given that the scope of this project only involved greenhouse trials the GHG impact can only be based on referring to the literature.
When this technology is moved to the field, we be able to demonstrate the mitigation of greenhouse gases in three ways; - i) by revegetating large tracts of contaminated land, ii) by adding biochar into the soil, and by iii) minimizing or completely avoiding energy intensive remediation measures.

Revegetation of PHC impacted land
The process of phytoremediation will naturally include revegetation of the PHC-contaminated land. Planting some species of poplars and other woody plants have not only been shown to help rhizoremediate PHCs, but have also been demonstrated to significantly mitigate GHG emissions (e.g. Cook & Hesterberg 2013).

We plan to intersperse hybrid poplars (HP) with grasses proven to rhizoremediate PHCs, to ensure not only remediation of the hydrocarbons but also a long term GHG reduction as the vegetation in the impacted areas gradually mature. It must be emphasized that this form of phytoremediation (i.e. rhizoremediation) does not require harvesting of the plant matter at any time. In Alberta, the Canadian Wood Fiber Centre (CWFC) within NRCan is spearheading the development of tools and management regimes for short-rotation woody crops including hybrid poplar (HP) in partnerships with the provincial government and industrial sector. CWFC HP demonstration plots in different parts of the province have been found to yield an aboveground mean annual increment (MAI) of 13.6-20 m^3 ha^{-1} yr^{-1} at 4-8 years age, equivalent to biomass production of 7.6-11 Mg DM ha^{-1} y^{-1}. In comparison, growth rates of natural trembling aspen and mixed wood (mainly aspen and spruce) stands are very low with an aboveground MAI of about 1 m^3 ha^{-1} yr^{-1} for aspen and 1.2 m^3 ha^{-1} yr^{-1} for mixed wood stands at maturity at 60-80 years (Andersonn et al., 2012).

Addition of biochar into the soil
Biochar is a carbon rich by-product produced from the thermal decomposition of organic matter under very low oxygen concentrations at relatively low temperatures (<700^{o}C). Although the synthesis of biochar mirrors the ancient industrial technology for producing charcoal, biochar is different in that it is produced with the intent of being applied to soil as a means of sustainably sequestering carbon and improving soil function. Adding biochar to soils can be described as a means for sequestering atmospheric carbon dioxide (CO_2). Biochar decomposes much more slowly than fresh plant material and thus the rate of CO_2 released back into the atmosphere is also much slower. This diverts carbon from the rapid biological cycle into a much slower biochar cycle (Roberts et al., 2009). Therefore, addition of biochar to soils is a carbon sink, and can function in the mitigation of climate change.

While our greenhouse studies will use biochar produced in Ontario, once we move to field trials in Alberta we will use biochar produced in Alberta. Our approach to selection and development

of the source biomass and its conversion methods will be based on life cycle assessment (LCA) of available sources, using standard LCA tools and the detailed, stand-based carbon management methodology based on the CBM-CFS3 software tools.

Phytotechnologies as an alternative to energy intensive remediation strategies

Remediation of contaminated sites has obvious environmental benefits, but the remediation itself can cause environmental impacts. Impacts differ among technologies, and are likely to be greater at remote sites than in more populated areas due to transport of materials over long distances. Environmental life cycle assessment (LCA) can quantify the overall environmental burdens of treatment systems, and assist in selecting the most environmentally efficient approaches. Although we are unaware of any LCA involving rhizoremediation of PHC-contaminated soil, in one study, the environmental performance of three different treatment options were compared, using LCA, for remediation of a remote diesel-contaminated site (Sanscartier et al. 2010). The study focused on impacts associated with the remedial activities. On-site *ex–situ* bioremediation and *in-situ* treatments were found to have far less environmental impacts than off-site treatment, with transportation being the main contributor to overall pollution. In terms of materials used and emissions to the air, on site bioremediation was found to have <50% of the impact of transporting the contaminated soil off site for treatment or containment. There are clear parallels that can be drawn in using vascular plants to rhizoremediate PHCs and using microbes to bioremediate diesel on-site, rather than excavating the soils and shipping them to an off-site facility. Both of these 'green' technologies avoid transporting soil off site, and rhizoremediation has the added benefit of not requiring any excavation of the soil. Based on this study, we expect GHG emissions to be reduced by ~50% by using phytoremediation in place of *ex-situ* technologies.

Calculations of Expected Overall GHG Reductions in the field trials

Since calculations can only be based on research papers we have estimated a conservative GHG reduction of 50% once this technology is moved into the field. This is based on an estimate using a combination of the three ways this project will reduce GHGs ie i) by revegetating large tracts of contaminated land, ii) by adding biochar into the soil, and by iii) minimizing or completely avoiding energy intensive remediation measures.

Andersonn, J.A., Armstrong, G.W., Luckert, M.K., Adamowicz, W.L., 2012. Optimal zoning of forested land considering the contribution of exotic plantations. Mathematical and Computational Forestry & Natural-Resource Sciences 4, 92-104.

Cook, R. and Hesterberg, D. 2013. Comparison of trees and grasses for rhizoremediation of petroleum hydrocarbons. Int. J. Phytoremediation 15: 844-860.

Roberts, K.G., Brent A. Gloy, B.A, Joseph S., Scott, N.R. and Lehmann, J. 2009. Life Cycle Assessment of Biochar Systems: Estimating the Energetic, Economic, and Climate Change Potential. Environ. Sci. Technol. 2010, 44 (2), pp 827–833

Sanscartier, D., Reimer, K., Zeeb, B.A, and George, K. 2010. Management of hydrocarbon-contaminated soil through bioremediation and landfill disposal at a remote location in Northern Canada. Can. J. Civ. Eng. 37(1): 147-155.