# Phytoremediation as a Strategy for Remote Contaminated Sites

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## Abstract

Earthmaster Environmental Strategies has successfully implemented plant growth promoting rhizobacteria (PGPR)-enhanced phytoremediation systems (PEPSystems®) for the remediation of crude petroleum hydrocarbon (PHC) contaminated soil at three sites located in northern Alberta. PEPSystems utilize soil bacteria coated onto the surface of seeds to facilitate better plant growth and greater production of abundant root biomass in impacted soils. This stimulates growth of endogenous rhizobacteria to enable degradation of PHCs, partitioning of contaminants out of the soil, and sequestration of salt into plant foliage. PEPSystems was first deployed at two sites near Edson in 2013 and at a third site in March of 2018 to treat historical oil and gas related contamination. PEPSystems successfully lowered PHC fraction F2 concentrations by 61% to comply with the Alberta remediation guideline value. PEPSystems also achieved decreases of 73% and 41 % of fraction F3 concentration in fine textured soil and coarse textured soils, respectively. These decreases were accurately predicted using previously established kinetic equations for fractions F2 and F3, confirming that, for phytoremediation sites located in western Canada, accurate PEPSystems remediation times can be predicted. Preliminary culturable bacteria amounts were shown to be consistent when comparing the bacteria in soil treatment layers to bacteria in stockpiled soil arising from successfully treated layers that had been removed. In addition, the presence of PHC did not appear to have a significant effect on bacteria amounts. A number of enhancements to PEPSystems has resulted in a cost effective and reliable way to remediate hydrocarbon impacted soil in an environmentally friendly low carbon output manner.

## 1 Introduction

Petroleum hydrocarbons (PHC) arising from oil and gas exploration and production are some of the most widespread persistent soil contaminants in Canada (CCME, 2008a), with fractions F2 (C10-16) and F3 (C16-34) being the most common in Western Canada. Soil contaminated with PHC arising from oil and gas exploration and production is a significant problem in Alberta. Abandoned wells (those no longer considered to be viable for use) are common in Alberta, numbering about 170,000 which represents 37% of all wells in the province (AER, 2020). Earthmaster estimates, based on over 20 years of oil and gas remediation experience, that approximately 25% of those sites require at least some remediation for contaminants such a salt and/or PHC (Murray et al., 2019). In addition to well site related contamination, the province has over 400,000 km of pipelines which experience an average of 600 pipeline release events per year involving PHC (Nikiforuk, 2017). From 2006-2010, the Alberta pipeline network leaked roughly ~27,700 $m^3$ of oil (Kheraj, 2012). Using a more environmentally friendly method for on-site remediation of PHC would negate the need for off-

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site landfill disposal of contaminated soil or use of other more environmentally taxing technologies such as thermal desorption, soil flushing, chemical oxidation, etc. (U.S. EPA, 2006; Caliman, 2011; Gerhardt et al., 2017).

Phytoremediation is the use of plants to extract, degrade, contain, and/or immobilize contaminants in the soil, water, or air (Gerhardt et al., 2009; Greipsson, 2011; Pilon-Smits, 2005). Plants are effective at remediating a number of compounds, including metals, organics, pesticides, PHC and PAHs (Salt et al., 1995; Salt et al., 1998; Pilon-Smits, 2005; Gerhardt, et al., 2017); however, contaminated environments can create stress in plants leading to decreased root and shoot biomass production and poor plant health. Co-localization of plant growth promoting rhizobacteria (PGPR) with plant roots can help reduce the stress the plant is under (Glick, 2003; Gerhardt et al. 2009; Khan et al., 2013; Tak et al., 2013; Gkorezis et al., 2016; Thijs et al., 2016; Gerhardt et al., 2017). The plant supports PGPR within the rhizosphere and many PGPR break down the stress ethylene precursor aminocyclopropane carboxylic acid (ACC) using ACC deaminase (Glick et al., 1999; Glick, 2010). Many PGPR also produce the plant hormone auxin (IAA, indoleacetic acid) which helps to promote growth (Patten and Glick, 2002). This relationship allows the plant to overcome the stress and facilitates production of large amounts of both plant and rhizosphere biomass, which leads to more efficient remediation of contaminated soil (Salt et al., 1998; Alkorta and Garbisu, 2001; Singh and Jain, 2003; Gerhardt et al., 2009; Cowie et al., 2010). The rhizosphere microbes are able to breakdown the PHC to non-toxic components and the plants are able to accumulate salts and metals into their tissues, removing them from the soil (Gerhardt et al., 2014; Chang et al., 2014).

PEPSystems® (PGPR- Enhanced Phytoremediation Systems) is a full scale commercial remediation system that utilizes plant growth in association with PGPR to remediate PHC and salt (Cowie et al., 2010; Gerhardt et al., 2009; Gerhardt et al., 2015; Greenberg et al., 2015; Gurska et al., 2009). This commercial technology was developed by Earthmaster, in collaboration with the University of Waterloo, and has been used to successfully remediate PHC contaminated sites across Canada. Using data from several phytoremediation sites across western Canada, predictive kinetic equations have been developed for fractions F2 and F3 degradation (Murray et al., 2019), allowing for the accurate prediction of time required for PEPSystems to remediate a PHC contaminated site based on the initial PHC contaminant level.

Here we report on phytoremediation at three remote and forested sites in northern Alberta where PEPSystems was deployed to treat PHC fractions F2 and F3 contaminated soil associated with oil and gas related activities. The phytoremediation activities and results for all three sites are discussed, and kinetic modeling of PEPSystems performance is reviewed as the basis for predicting the length of time required to remediate PHC contaminated soil at commercial/industrial sites. An examination of the bacteria populations and distribution in the different soil layers that were treated and within the previously treated stockpiled soil were examined. Recent enhancements in the PEPSystems technology for commercial phytoremediation applications are highlighted.

## 2 Materials and Methods

### 2.1. Plant Growth Promoting Rhizobacteria (PGPR)

PGPR were isolated previously from naturally occurring and common soil bacteria. Strains *Pseudomonas sp.* (UW3, GenBank Accession Number KF145175) (Glick et al., 1995; Chang et al., 2014) and *Pseudomonas sp.* (UW4, GenBank Accession Number CP003880) (Glick et al., 1995; Duan et al., 2013) were isolated as described previously (Gurska et al., 2009).

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PGPR strains (selected for high levels of 1-amino-cyclopropane-1-carboxylic acid (ACC) deaminase and indoleacetic acid (IAA)) were purified, cultured, and coated onto the surface of plant seeds using a Hege 14 commercial liquid seed treater (Wintersteiger, Austria).

### 2.2. PGPR-Enhanced Phytoremediation Systems (PEPSystems®)

Plant species were chosen for maximum biomass production, suitability for the growing area, and ease of treating and sowing. Three species were selected and consisted of annual ryegrass (*Lolium multiflorum*), perennial ryegrass (*Lolium perenne*), and tall fescue (*Festuca arundinacea)*. All seeds were purchased from commercial seed suppliers and were treated by the University of Waterloo or Earthmaster. Seedbeds were prepared using standard agricultural practices and PGPR-treated seed was planted using standard agricultural techniques. Plants were allowed to grow to maturity prior to end-of-season soil sample collection.

### 2.3. Field Sites

Three full scale phytoremediation field sites were in located a forested area in northern Alberta, northwest of Edmonton. Contained soil treatment areas were constructed on each site and permanent assessment points were established across the treatment areas such that soil sampling was conducted at the same locations over time to allow for more accurate temporal comparisons of data sets. The Site 1 treatment area was 13,000 $m^2$ in size and contained 10,000 $m^3$ of soil contaminated with PHC fraction F2 arising on a well site from a former drilling waste disposal area and an earthen pit where the source of contamination was unknown. The Site 2 treatment area was 11,500 $m^2$ in size and contained 11,000 $m^3$ of soil contaminated with PHC fraction F2 arising from two former drilling waste disposal areas, a disturbed area, and a wellbore area. The Site 3 treatment area was 2,700 $m^2$ in size and contained 6,000 $m^3$ of soil contaminated with PHC fractions F2 and F3 arising from buried contaminated soil on a well site.

Contaminated soil depth on the treatment areas ranged from 1.00 to 2.25 m; therefore, the soil was treated in consecutive soil lifts such that the successfully treated soil lift was removed to expose the next underlying contaminated soil layer that was then planted. Each soil lift was comprised of one (0.00-0.25 m) or two (0.00-0.25 and 0.25-0.50 m) soil layers.

Control areas consisting of uncontaminated soil were planted with PGPR treated seed and analyzed to account for biogenic organic compounds (BOC) not removed by the laboratory silica gel clean-up process during the laboratory PHC quantification analyses.

### 2.4. Soil Sampling and Assessment

Permanent assessment points were established across each treatment area. Site 1 contained 20 assessment points in the treatment area and four in the control area, Site 2 contained 14 assessment points in the treatment area and four in the control area, and Site 3 contained 7 assessment points in the treatment area. Each sample point represented a surface area of between 400 and 825 $m^2$. Soil samples consisted of a composite of three soil cores collected from within a 1 m radius of each permanent assessment point using a Dutch auger with a 7.5 cm cutting diameter. Depending on the contaminated soil depth, soil was sampled from a single treatment layer (0.00-0.25 m) or from two treatment layers (0.00-0.25 and 0.25-0.50 m). PHC concentrations for a treatment layer were reported as an average of all samples collected from all assessment points) from the layer ± standard error.

Soil samples collected for fractions F2 and F3 analyses were tightly packed into sterile 125 ml glass jars to minimize loss of organic vapours into the headspace. Samples were

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immediately stored on ice in an insulated cooler until delivery to the laboratory within designated time requirements. To monitor temporal performance of PEPSystems, end-of-season PHC levels at each assessment point and as an average were compared to those measured at the beginning of the growing season and to initial PHC levels (T=0) for a particular treatment layer to evaluate seasonal and overall remediation progress and efficiency.

Soil lifts (consisting of one or two soil layers) were removed (i.e. stripped) from the treatment area once PHC concentrations for a large majority of assessment points, that represented treatment grids, complied with remediation guideline values. The grids associated with permanent assessment points that had PHC concentrations that did not comply with remediation guideline values were not removed, were evenly spread across the entire treatment area and incorporated into the underlying soil layer to undergo additional phytoremediation. Successfully treated soil that was removed from the treatment area was placed into stockpiles and soil samples collected from the stockpiles were analyzed to reaffirm compliance with remediation guideline values.

### 2.5. PHC Analysis

PHC analyses for fractions F2 (C10-C16) and F3 (C16-C34) were conducted by a commercial third party analytical laboratory accredited by the Canadian Association for Laboratory Accreditation Inc. and/or the Standard Council of Canada for the individual required assays. PHC fractions were quantitated using analytical methods described in the CCME Canada Wide Standards for Petroleum Hydrocarbons (CCME, 2008b) following a double silica gel clean-up to remove BOC (Greenberg et al., 2012).

Laboratory soil test results were compared to the Alberta Tier 1 Soil and Groundwater Remediation Guidelines (AEP, 2019).

### 2.6. Bacteria Quantitation

Bacteria were isolated from soil samples as follows. Two g of soil was mixed with 20 mL of sterile 0.85% NaCl in a sterile container and placed on an orbital shaker for 30 minutes at room temperature (shaking speed of 1,000 rpm). A 10 fold dilution series of the culture was prepared and plated on TSA (Tryptone Soya Agar) plates (100 µL) to determine the number of colony forming units (cfu's) per gram of dry soil. The plates were placed in a 25°C incubator for 24 to 72 hours.

## 3 Results and Discussion

### 3.1. Phytoremediation of PHC

Full scale commercial deployment of PEPSystems was performed at three sites in northern Alberta. Alberta Tier 1 Remediation Guideline values (AEP, 2019) for Natural Land Use were used as per the applicable soil texture (i.e., coarse or fine) to determine the remediation endpoint. For all three sites, PHC fraction F2 was the major contaminant of concern. In addition, a small number of soil samples from all three sites also showed PHC fraction F3 concentrations that exceeded the Alberta remediation guideline value.

The remediation details for the sites are presented in Table 1. The timelines for sampling, stripping, and planting the soil were influenced by site conditions as well as client budgets and work approvals. The remediation summary for fraction F2 is provided in Table 2. The fraction F2 concentrations used to determine remediation amounts were the averages for all the assessment points on the applicable soil treatment layer, regardless of whether the samples had a

concentration that complied with or exceeded the remediation guideline value.

**Table 1: Summary of remediation timelines for PHC fraction F2 by PEPSystems at three sites in Northern Alberta.**

| Site | Lift # | Period | Sampling Dates | F2 Range (mg/kg) | F2 Average ± error (mg/kg) | Planting Dates |
|---|---|---|---|---|---|---|
| 1 | 1 | Oct2013-Mar2016 | Oct2013 | 66-830<br>60-1500 | 311±58<br>403±75 | Oct2013 |
| | | | Jul2014 | 18-1600<br>15-3100 | 271±77<br>430±148 | Jul2014 |
| | | | Sep2014 | 15-730<br>36-690 | 226±42<br>322±43 | |
| | | | Nov2015 | 11-590<br>13-1900 | 138±37<br>336±94 | overseed Aug2015 |
| | 2 | Oct2016-Dec2017 | Oct2016 | 200-520<br>190-1100 | 161±30<br>417±78 | Oct2016 |
| | | | Jul2017 | 11-1000<br>10-770 | 253±65<br>247±52 | |
| | 3 | Dec2017-Jan2019 | Dec2017 | 18-950 | 268±52 | Dec2017 |
| | | | Sep2018 | 50-457 | 113±21 | |
| | 4 | Jan2019-TBD | Jan2019 | 254-2840 | 584±161 | Jan2019 |
| 2 | 1 | Oct2013-Oct2016 | Oct2013 | 34-540<br>41-710 | 310±46<br>342±76 | Oct2013 |
| | | | Jul2014 | 26-380<br>14-380 | 174±30<br>204±34 | Jul2014 |
| | | | Sep2014 | 38-240<br>63-330 | 115±18<br>188±32 | |
| | | | Nov2015 | 29-240<br>34-300 | 68±18<br>126±23 | overseed Aug2015 |
| | 2 | Oct2016-Nov2017 | Oct2016 | 59-790<br>110-1300 | 270±78<br>333±102 | Oct2016 |
| | | | Jul2017 | 10-320<br>52-970 | 115±25<br>397±62 | |
| | 3 | Nov2017-Dec2018 | Nov2017 | 100-1200 | 283±69 | Nov2017 |
| | | | Oct2018 | 50-623<br>76-1050 | 190±54<br>317±72 | |
| | 4 | Dec2018-TBD | Dec2018 | 87-588 | 200±33 | Dec2018 |
| 3 | 1 | Mar2018-Dec2018 | Mar2018 | 110-200 | 161±61 | Mar2018 |
| | | | Oct2018 | 50-158<br>66-295 | 84±15<br>125±30 | |
| | 2 | Dec2018-TBD | Dec2018 | 60-212 | 106±20 | Dec2018 |

On average, PEPSystems achieved a 44% decrease in fraction F2 concentrations in the 0.00-0.25 m soil layer and a 39% decrease in fraction F2 in the 0.25-0.50 m soil layer before the layers were removed from the treatment areas and placed in stockpiles. The results show that the

two soil layers remediate at approximately the same rates, suggesting that the bacteria and roots in PEPSystems at these sites extended to at least 0.50 m in depth. When the average fraction F2 concentrations, for the treated soil layers that were removed and stockpiled, were compared to T0 concentrations of the corresponding treatment layers making up the stockpiled soil, the % reduction averaged 61%, demonstrating that remediation continued in the stockpiled soil following active soil layer treatment via PEPSystems.

**Table 2: PHC fraction F2 remediation by PEPSystems at three sites in Northern Alberta. Results are presented as % decrease in F2 for all samples in the layer.**

| Site | Period | # Lifts | Layer Depth (m) | Average % remediation* |
|---|---|---|---|---|
| 1 | Oct2013-Oct2019 | 4 | 0.00-0.25 | 30 |
| | | | 0.25-0.50 | 29 |
| | | | stockpile | 66 |
| 2 | Oct2013-Oct2019 | 4 | 0.00-0.25 | 55 |
| | | | 0.25-0.50 | 50 |
| | | | stockpile | 73 |
| 3 | Mar2018-Oct2019 | 2 | 0.00-0.25 | 49 |
| | | | stockpile | 69 |
| | | Overall | 0.00-0.25 | 44 |
| | | | 0.25-0.50 | 39 |
| | | | stockpile | 61 |

* from T0 for each individual layer

**Table 3: PHC fraction F3 remediation by PEPSystems at three sites in Northern Alberta. Results are presented as % decrease in F3 concentration for samples with F3 exceedances only.**

| Site | Soil Texture | Guideline Value (mg/kg) | # Samples | Range (mg/kg) | Layer Depth (m) | Average % Remediation* |
|---|---|---|---|---|---|---|
| 1 | fine | 1300 | 2 | 1500-2030 | 0.00-0.25 | 78 |
| 2 | fine | 1300 | 6 | 1400-1900 | 0.00-0.25 | 68 |
| | | | 1 | 2400 | 0.25-0.50 | 66 |
| 3 | coarse | 300 | 2 | 317-338 | 0.00-0.25 | 34 |
| | | | 3 | 330-444 | 0.25-0.50 | 45 |
| | | | | Overall | fine | 73 |
| | | | | | coarse | 41 |

*for 1 growing season

The remediation summary for fraction F3 is provided in Table 3. Due to the small number of samples at each site that had fraction F3 concentrations that exceeded the remediation guideline value, the average % reduction was calculated using only those samples and did not include all of the samples in the entire soil layer as was done for fraction F2. On average, PEPSystems achieved a 73% diminishment of fraction F3 concentration in fine textured soil and a 41% diminishment in coarse textured soil. All decreases were achieved in a single growing

season.

### 3.2. Kinetics of Phytoremediation

The kinetics of remediation was analyzed by comparing the actual time for remediation to predicted time. Results are summarized in Tables 4 and 5. Previous work done by Murray et al. (2019) showed that, using PEPSystems remediation results obtained for several sites across western Canada, kinetic equations could be developed to allow for the prediction of time to phytoremediate fractions F2 and F3 based on starting concentrations. The accuracy of the kinetic models was evaluated using remediation results from the three northern Alberta sites.

Applying the equation developed for fraction F2 using the average soil treatment layer starting concentrations and the applicable remediation guideline value (AEP, 2019), the predicted length of time and the associated number of growing seasons to achieve remediation were determined for each soil layer (Table 4). This was compared to the actual amount of time and number of growing seasons required to achieve remediation for each layer. In almost all cases, the predictive equation was accurate in determining the number of growing seasons required. Exceptions included lift #2 layer (0.00-0.25 m) for both Sites 1 and 2. For these particular soil layers, remediation was allowed to continue for an additional growing season to address the remediation requirements of the underlying soil layer (0.25-0.50 m).

**Table 4: Predicted and actual times for PHC fraction F2 remediation by PEPSystems at three sites in Northern Alberta.**

| Site | Lift # | Depth (m) | T0 (mg/kg) | Guideline value (mg/kg) | Predicted | | Actual | |
|---|---|---|---|---|---|---|---|---|
| | | | | | # years | # growing seasons | # years | # growing seasons |
| 1 | 1 | 0.00-0.25 | 311 | 150 | 1.2 | 2 | 2.3 | 2 |
| | | 0.25-0.50 | 403 | | 1.6 | 2 | 2.3 | 2 |
| | 2 | 0.00-0.25 | 161 | | 0.1 | 1 | 1.2 | 2 |
| | | 0.25-0.50 | 417 | | 1.6 | 2 | 1.2 | 2 |
| | 3 | 0.00-0.25 | 268 | | 0.9 | 1 | 1.8 | 1 |
| | 4 | 0.00-0.25 | 548 | | 2.2 | 1 | 0.9 | 1 |
| 2 | 1 | 0.00-0.25 | 310 | 150 | 1.2 | 2 | 2.3 | 2 |
| | | 0.25-0.50 | 342 | | 1.3 | 2 | 2.3 | 2 |
| | 2 | 0.00-0.25 | 270 | | 0.9 | 1 | 1.2 | 2 |
| | | 0.25-0.50 | 333 | | 1.3 | 2 | 1.2 | 2 |
| | 3 | 0.00-0.25 | 283 | | 1.0 | 1 | 1.8 | 1 |
| | 4 | 0.00-0.25 | 200 | | 0.5 | 1 | 0.9 | 1 |
| 3 | 1 | 0.00-0.25 | 161 | 150 | 0.1 | 1 | 0.75 | 1 |
| | 2 | 0.00-0.25 | 106 | | 0 | 0 | 1.00 | 1 |

Applying the equation developed for fraction F3 (Murray et al., 2019) and using the average starting concentrations for those samples that exceeded the remediation guideline value, the predicted length of time and the associated number of growing seasons to achieve remediation of the fraction F3 contaminated samples were determined for each soil layer (Table 4). All of the soil layers had their fraction F3 concentrations decrease to below remediation

guideline values in a length of time similar to what was predicted using the kinetic model.

**Table 5: Predicted and actual times for PHC fraction F3 remediation by PEPSystems at three sites in Northern Alberta.**

| Site | # Samples | Depth (m) | T0 (mg/kg) | Guideline value (mg/kg) | Predicted | | Actual | |
|---|---|---|---|---|---|---|---|---|
| | | | | | # years | # growing seasons | # years | # growing seasons |
| 1 | 2 | 0.00-0.25 | 1765 | 1300 | 1.3 | 2 | 0.5 | 1 |
| 2 | 6 | 0.00-0.25 | 1598 | 1300 | 0.9 | 1 | 0.7 | 1 |
| | 1 | 0.25-0.50 | 2400 | | 2.6 | 3 | 0.7 | 1 |
| 3 | 1 | 0.00-0.25 | 328 | 300 | 0.4 | 1 | 1.0 | 1 |
| | 2 | 0.00-0.25 | 395 | | 1.1 | 1 | 1.0 | 1 |

The results for both of the kinetic models are accurate in determining the time required to remediate fractions F2 and F3. It is important to note that the equations are based on elapsed time and include the time in winter months where soil is frozen and remediation is unlikely to occur. For example, a soil layer planted in October 2013 and stripped in April of 2015 would have an actual elapsed time of 1.4 years encompassing 1 growing season; however, the predictive equation for fraction F2 would have estimated the length of time to reach the remediation goal at 0.75 years or 1 growing season.

### 3.3. Contribution of BOC

Organic matter from plant tissues (mostly from plant sterols and terpenoids) can interfere with PHC quantitation (Kelly-Hopper, 2013a; Kelly-Hooper, et al., 2013b). This can be a significant problem when analyzing soil samples where PEPSystems has been deployed; therefore, a double silica gel column clean-up method was developed previously to further remove BOC from soil samples to facilitate the determination of biogenic vs. petrogenic (PHC) material, particularly for fraction F3 (Greenberg et al., 2012). This method removes almost all of the BOC (unlike the in situ silica gel method), is specific for BOC, and does not remove PHC.

Control areas were established at Sites 1 and 2, outside of the bermed treatment areas. Due to space constraints, a control area was not established at Site 3. The control areas consisted of clean soil where PEPSystems was deployed and each area was assessed using four permanent assessment points. Soil layers were not removed from the control areas over the course of the project (unlike the treatment areas); however, the plant material was stripped from the soil surface to accommodate the planting of seed for the upcoming growing season. The results of fractions F2 and F3 analyses for control samples analyzed using the silica gel cleanup method are provided in Table 6. Most of the fraction F2 analyses were below the lower limit of detection for the quantitative assay. Small amounts of fraction F3 were detected in the control area samples for Site 2. Given that the control areas are established outside of the bermed and contained treatment areas, the moisture levels may be significantly different than the treatment areas. Site 2 is in an area that contains significantly more water than Site 1, is a north facing slope and is in a forested area which restricted the amount of sunlight. Plant growth on the Site 2 control area has historically been exceptional when compared to the treatment area (data not shown); therefore, it is likely the increased moisture available to the control area plants has resulted in increased amounts of organics accumulating in the soil over the length of the project.

The results provided for the control area sample analyses suggest that the silica column clean up method is removing a large majority of BOC from the samples, allowing for accurate determination of petrogenic hydrocarbons. The small amounts of fraction F3 detected in samples are minor and would not significantly affect sample assessment.

**Table 6: Evaluation of biogenic organic compounds (BOC) in fraction F2 and F3 analyses for samples collected from control areas. Results are presented as an average of all of samples analyzed from the control area during treatment of the corresponding lift.**

| Site | Corresponding Lift # | BOC Control Area | |
|---|---|---|---|
| | | F2 (mg/kg) | F3 (mg/kg) |
| 1 | 1 | <LLOD | <LLOD |
| | 2 | <LLOD | <LLOD |
| | 3 | <LLOD | 69 |
| | 4 | <LLOD | <LLOD |
| 2 | 1 | 11.6 | 97 |
| | 2 | <LLOD | 70 |
| | 3 | <LLOD | <LLOD |
| | 4 | <LLOD | 91 |

LLOD - lower limit of detect for the assay

### 3.4. Preliminary Quantitation of Culturable Bacteria

A preliminary quantitation of culturable bacteria was completed on soil samples collected from treatment lift #5 for Sites 1 and 2 and lift #2 for Site 3, and compared to bacteria levels in the soil stockpiles. The soil stockpiles were created from successfully treated soil layers that had been removed from the treatment areas and stockpiled alongside. The stockpiles contained a significant amount of organic material from the aboveground plant biomass that had not been removed from the soil layer prior to stripping and stockpiling. The treatment lifts that were evaluated were the bare underlying soil layers arising from removal of the previous overlying lift, and contained roots and bacteria that had penetrated from the soil layers above.

The results of the preliminary bacteria quantitation are presented in Table 7. There were no statistically significant differences in the bacteria amounts found in the underlying bare soil treatment layer and the treated stockpiled soil. This suggested that the bacteria amounts in the treated soil stockpiles remained at levels comparable to the treatment area that had just had the active plant growth removed. This is also apparent when older stockpiles (lift #3 from 2018) are compared to newer stockpiles (lift #4 from 2019) (Table 7), suggesting that undisturbed stockpiles retain their culturable bacteria content for a significant amount of time following the termination of active plant growth. This was expected as remediation of PHC continued in the stockpiles over time (Table 2 and data not shown). The data also shows that underlying soil layers retained bacteria from the treatment layer above.

**Table 7: Preliminary culturable soil bacteria levels following remediation by PEPSystems at three sites in Northern Alberta.**

| Site | Soil source | Depth (m) | # Sample points | Average cfu/g x$10^6$ ± S.E. | Range cfu/g x$10^6$ |
|---|---|---|---|---|---|
| 1 | lift #3 stockpile | grab | 5 | 11.6 ± 7.1 | 2.9 - 39.9 |
| | lift #4 stockpile | grab | 10 | 5.7 ± 1.0 | 0.6 - 10.8 |
| | lift #5 | 0.00-0.25 | 20 | 14.6 ± 4.1 | 1.4 - 89.1 |
| | control area | 0.00-0.25 | 4 | 8.2 ± 5.0 | 0.8 - 22.8 |
| 2 | lift #3 stockpile | grab | 6 | 17.7 ± 5.2 | 7.0 - 40.4 |
| | lift #4 stockpile | grab | 10 | 19.3 ± 2.2 | 12.0 - 30.7 |
| | lift #5 | 0.00-0.25 | 17 | 19.7 ± 3.9 | 3.1 - 55.2 |
| | control area | 0.00-0.25 | 4 | 22.9 ± 9.1 | 6.9 - 42.2 |
| 3 | lift #1 stockpile | 0.00-0.25 | 10 | 10.5 ± 1.9 | 0.3 - 17.3 |
| | lift #2 | 0.00-0.25 | 7 | 6.8 ± 3.1 | 1.4 - 21.5 |

Bacteria content was also assessed to determine if the concentration of PHC affected the levels of bacteria in the soil. PHC is known to be a food source for the bacteria (Cowie et al., 2010); therefore, the bacteria content for soil containing less than the Alberta remediation guideline value of 150 mg/kg fraction F2 was compared to the bacteria content of soil samples containing more than 150 mg/kg fraction F2. There was no statistically significant difference between the amount of culturable bacteria in the samples containing higher vs. lower concentrations of fraction F2 (Figure 1). This suggested that the levels of F2 in the soil samples neither aids nor inhibits microbial growth in the soil. Studies with diesel fuel contaminated soil have shown that there is decline in species diversity and that a few species such as *Pseudomonas* dominate the bacterial community (van Dorst et al., 2014). Saadoun et al. (2008) found that PHC can cause a decrease in numbers and diversity of soil bacteria but that *Pseudomonas* prevails. Given these findings by others, a change in overall bacteria numbers was not expected since *Pseudomonas* species are being added as part of PEPSystems.

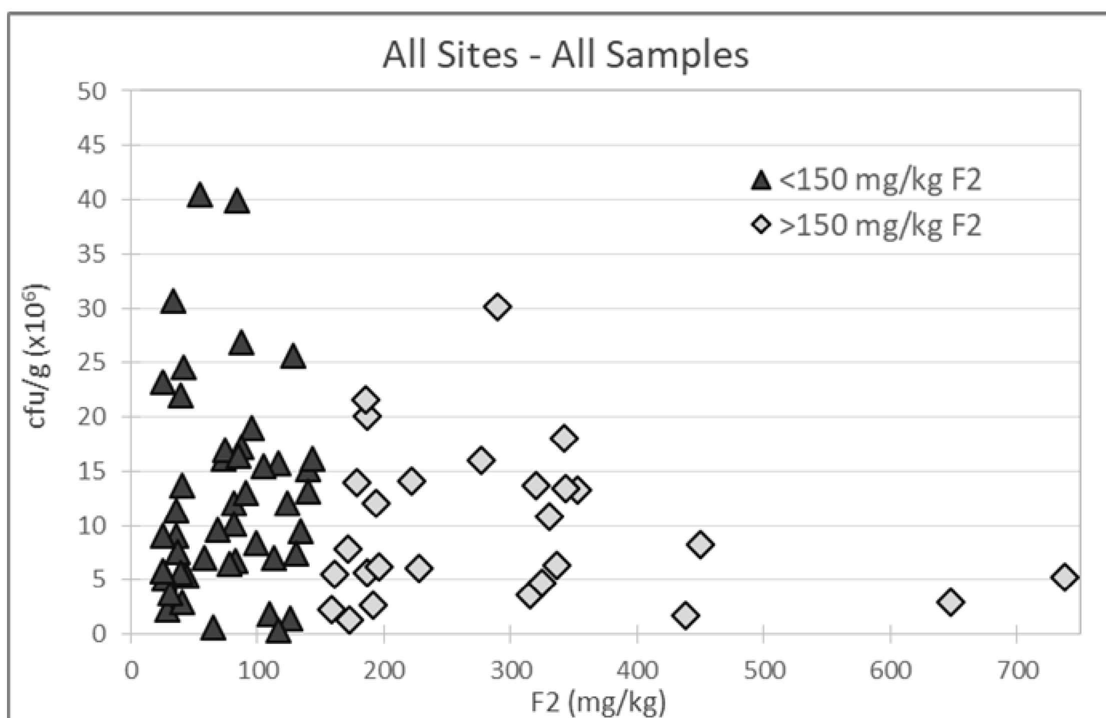


**Figure 1: Culturable soil bacteria levels vs. PHC fraction F2 concentration. Bacteria amounts (cfu/g x $10^6$) were plotted against the PHC fraction F2 concentration (mg/kg) in the soil sample. Results represent the bacteria levels for all samples collected for PHC analyses from all three sites. Different symbols were used to distinguish the samples with PHC fraction F2 concentrations below Alberta remediation guideline values (<150 mg/kg) or above.**

### 3.5. Recent Enhancements

Large scale commercial applications of PEPSystems have been conducted for 15 years. Recently, several enhancements have been made to the technology to improve its efficiency and predictability. Kinetic modeling (Murray et al., 2019) has improved and now provides a predictability to PEPSystems based remediation. The mix of plant species has been refined to provide maximum biomass production and winter planting is routinely done on remote sites where access in spring is delayed due to saturated soil and poor access. Future enhancements will focus on microbial management as the bacterial populations on phytoremediation sites are better understood.

## 4 Conclusions

PEPSystems was deployed at three sites in northern Alberta for the purposes of remediating PHC fractions F2 and F3 from contaminated soil in a series of treatment lifts over five growing seasons. Laboratory analyses of successfully remediated soil stockpiles following removal from the treatment area showed a decrease in fraction F2 concentrations of 61% to comply with the Alberta remediation guideline value. PEPSystems also achieved a 73% decrease of fraction F3 concentrations in fine textured soil and a 41% decrease in coarse textured soil, over the course of one growing season, to comply with the guideline value. These diminishments in PHC concentrations were accurately predicted using previously established kinetic equations for fractions F2 and F3 confirming that for phytoremediation sites located in western Canada where PEPSystems is used for remediation, accurate remediation times can be calculated. These

studies also confirm that adoption of the laboratory double silica gel column clean-up method adequately removes biogenic organic carbon for accurate quantitation of true petrogenic hydrocarbon levels. Culturable bacteria amounts were assessed and were shown to be consistent between treatment lifts and stockpiled soil. The presence of PHC at the levels studied did not appear to have a significant effect on bacteria numbers.

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