Phytoremediation of Petroleum and Salt Impacted Soils: A Scientifically-Based Innovative Remediation Process

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Abstract

We have successfully developed and implemented advanced phytoremediation systems for removal of petroleum hydrocarbons (PHC), polycyclic aromatic hydrocarbons (PAH) and salt from soils. The plant growth promoting rhizobacteria (PGPR) enhanced phytoremediation systems (PEPS) we deploy provide large amounts of root biomass in impacted soils, which promotes growth of rhizosphere microorganisms. The root and rhizosphere biomass facilitate rapid partitioning of contaminants out of the soil, and their subsequent uptake and metabolism by microbes and/or plants. PEPS result in degradation of PHC and PAH in soil, and the production of large amounts of biomass for sequestration of salt into plant foliage. We have successfully performed > 25 full-scale deployments of PEPS. PHC and salt remediation to Tier 1 criteria have been achieved at several of these sites. Not only are these 'green' solutions effective for remediation of impacted sites, but the costs for PEPS are less than half the costs associated with landfill disposal. From 2007 to 2011, we utilized PEPS at 17 sites in Alberta, British Columbia, the Northwest Territories, Manitoba, Ontario and Quebec for PHC remediation. We averaged 33 % remediation per year of weathered PHC from soil (mostly F3 and F4). At 7 sites, we met Tier 1 criteria, and at the remaining 10 sites, we are well on our way to achieving remediation goals. We are now performing research to optimize analytical laboratory CCME PHC quantification methods to ensure accurate measurement of soil PHC levels at phytoremediation sites. We are also using Tier 2 toxicity end-points at a research level to assess when the soils become non-toxic during a PEPS deployment. Our work shows that PEPS is broadly deployable at a wide variety of PHC impacted sites (including sites that have barite as a co-contaminant), with a time frame of 2 to 3 years to complete remediation. Beginning in 2009, we initiated full scale deployments of PEPS at 10 salt impacted sites in Saskatchewan, Alberta and the Northwest Territories. PGPR greatly enhanced plant performance on the salt impacted soils, resulting in excellent plant growth on soils with EC_e levels up to 25 dS/cm. Furthermore, the plants (both grasses and cereals) take up sufficient amounts of salt to make phytoremediation feasible. Notably, we have already achieved salt remediation to regulatory targets at two of the sites. The innovative 'green' PEPS technologies described above are based on procedures that have been scientifically proven and are effective at full-scale field levels when deployed by qualified scientists.

1 Introduction

Large amounts of contaminants, including petroleum hydrocarbons (PHC) and salt, have been released into the environment as a result of industrial processes. The persistence of PHC and salt in soils at thousands of sites in Canada necessitates the development of environmentally responsible, cost-effective and efficient remediation technologies. Many strategies have been employed to remediate organic and inorganic contaminants from impacted soils (Chaudhry et al., 2005; Susarla et al., 2002; Daugulis, 2001). Methods such as physical removal of soil to landfill, soil washing, land farming and use of biopiles for soil remediation have been used (Schnoor, 2002). These strategies have met with different levels of success and can be high in cost. The development of cost-effective, in situ techniques for remediation of PHC and salt impacted soils is a high priority for the upstream oil and gas industry, as well as other environmental and economic sectors (Greenberg, 2006; Glass, 1999; Salt et al., 1998; Pilon-Smits, 2005). This has led to pressure to develop cost-effective and reliable in situ remediation methods such as phytoremediation (Schnoor, 2002; Gerhardt et al., 2009).

Phytoremediation is the use of plants to extract, degrade, contain, and immobilize chemicals from the soil (U.S. EPA, 2000; Glick, 2003, Gerhardt et al., 2009). The inability to generate sufficient biomass in bioremediation applications is addressed by use of plants, which support microbial organisms within the rhizosphere, allowing for increased rates of remediation (Salt et al., 1998; Alkorta and Garbisu, 2001; Singh and Jain, 2003, Gerhardt et al., 2009; Cowie et al., 2010). Phytoremediation, using a variety of plant species, has been successfully employed to remediate numerous organic contaminants including pesticides, PAH, PCB, PHC and explosives (Lunney et al., 2004; Mattina et al., 2003; Singh and Jain, 2003; Meagher, 2000; Huang et al., 2004a, 2004b, 2005; Gurska et al., 2009). Plants have also been used to remediate metals and salt from soil by sequestering the salt into the foliage and then removing the foliage from the impacted site (Gerhardt et al., 2006; Greenberg et al., 2011).

If phytoremediation can be carried out on site, environmentally damaging and expensive processes, such as land filling, can be minimized (Greenberg et al., 2008a; Huang et al., 2009; Gurska et al., 2009; Pilon-Smits, 2005). Phytoremediation of PHC holds great promise: 1) in contrast to microbial bioremediation, it provides sufficient biomass for acceptable rates of remediation; 2) it results in degradation of PHC in the soil; 3) it is applicable to any site where plant growth can be achieved; 4) it can be applied at remote sites; 5) it is < 50 % of the cost of many other remediation strategies; 6) it is environmentally responsible. We have developed plant growth promoting rhizobacteria (PGPR) enhanced phytoremediation systems (PEPS) that effectively degrade PHC (Huang et al., 2004b; Greenberg et al., 2008a; Huang et al., 2009; Gurska et al., 2009; Cowie et al., 2010) and sequester salt into foliage (Greenberg et al., 2008b; Greenberg et al., 2011). Two benefits of these remedial strategies are the alleviation of plant stress by the PGPR and metabolism of PHC by the PGPR (Glick et al., 1998; Glick 2003; Gerhardt et al., 2009). This leads to substantial amounts of root and microbial biomass in the soil, providing a sink which allows for rapid partitioning of PHC out of the soil, and their subsequent metabolism within the rhizosphere. We have shown that the PHC are degraded in situ in the rhizosphere of the impacted soils (Gurska et al., 2009; Cowie et al., 2010). This was

shown by 3 lines of evidence: 1) Isotope analysis showed that the PHC are metabolized to fatty acids and mineralized to CO_2; 2) GC/MS and HPLC analyses showed that the chemicals do not accumulate in the plants and that specific PHC compounds are degraded; 3) Soil microbial analyses during PEPS usage showed that naturally-occurring, petroleum-consuming microbe populations increased by two orders of magnitude due to plant growth. In the case of salt, the high levels of biomass provide a sink for the salt to migrate to the above ground portions of the plants. Plants that have been used with positive results include annual ryegrass, perennial ryegrass, oats, fescue, wheatgrass, barley, timothy grass, alfalfa and brome grass. PEPS have been successfully used for several full scale remediations in British Columbia, Alberta, the Northwest Territories, Saskatchewan, Manitoba, Ontario and Quebec (Greenberg, 2006; Greenberg et al., 2008a; Huang et al., 2009; Gurska et al., 2009; Greenberg, 2011).

Over the past six years we have successfully performed full scale field-level PHC remediations with PEPS (Greenberg, 2006; Greenberg et al., 2008a; Huang et al., 2009; Gurska et al., 2009), culminating in meeting generic Tier 1 targets at four sites in Alberta, one site in British Columbia and one site in Manitoba and one site in Quebec. At these sites, generic Tier 1 targets were met using the Canadian Council of Ministers of the Environment (CCME) analytical method and site closure was achieved after one or three years of treatment. For salt remediation we have shown that plants take up enough salt to make phytoremediation feasible, and we have met remediation targets at two sites (Greenberg et al., 2011).

Although we have clearly shown that PHC can be degraded in soils using PEPS (Gurska et al., 2009; Cowie et al., 2010), at some sites accumulation of phytogenic hydrophobic material due to plant growth can confound the results of the standard PHC analytical methods accepted by environmental regulations. Due to the complexity of PHC, PHC have been classified by the CCME into fractions: F1, C6 – C10; F2, C10 – C16; F3, C16 – C34; F4, C34 – C50 (CCME, 2008). A problem is that many root-derived compounds are natural analogs of the constituents of PHC that are being remediated (Holden and Firestone, 1997; Kalita, 2006). This is not surprising since a good deal of petroleum was primarily derived from ancient plant material. Unfortunately, the plant contribution to the organic material in soil can obfuscate sample analyses because of the structural similarities to compounds being remediated (Calvin, 1980; Holden and Firestone, 1997; Kalita, 2006). Techniques have been developed to distinguish petrogenic materials from such biogenic organic compounds (BOC). However, these methods have primarily been used for chemical fingerprinting to assess liability after accidental releases of PHC (Stout et al., 2001). We have found that if proper controls and analytical methods are performed, the gas chromatography/flame ionization detector (GC/FID) method of PHC analysis supported by the CCME (CCME, 2008) can be used more effectively to assess the progress and completion of phytoremediation. Such optimization of the CCME method is making phytoremediation an even more fully viable technology at a broader range of PHC impacted sites.

Here we report on PHC and salt remediation using PEPS. The sites we have worked at are described and the amounts of remediation we have observed are summarized. We also report on recent research to characterize and quantify PHC and BOC in soil during phytoremediation. This is allowing us to distinguish petrogenic

material from BOC. We have also compared data from advanced analytical methodologies (GC/MS) and accepted risk assessment toxicity tests with the CCME PHC remediation data.

2 Materials and Methods

2.1 Plant Growth Promoting Rhizobacteria (PGPR)

Three strains of PGPR, *Pseudomonas* spp UW3, *P. putida* UW4 and *P. corrugata* CMH3, were used in PEPS to promote plant growth and increase tolerance to petroleum and/or salt (Glick, 2003, 2004; Hontzeas et al., 2004; Huang et al., 2004a; Greenberg et al., 2008b). All strains are classified as Biosafety Level 1 (the safest possible level, posing almost no risk to the environment). All strains are naturally occurring and express 1-amino-cyclopropane-1-carboxylic acid (ACC) deaminase, an enzyme that consumes the precursor to ethylene, a plant stress hormone. They also synthesize indoleacetic acid (an auxin), which promotes root cell growth of the host plants (Patten and Glick, 2002). Prior to seed treatment, the PGPR were cultivated in tryptic soy broth at room temperature (Gurska et al., 2009). A bacterial suspension in distilled water ($OD_{600} = 2$) was distributed evenly onto the seeds, using a batch seed treater (Hege, Wintersteiger, Austria) (Gurska et al., 2009).

2.2 Field Sites

Full scale remediations were performed from 2007 to 2011 at PHC and salt impacted sites in Canada (Tables 1 and 2). Most sites were impacted with PHC and salt from upstream oil and gas activities. At the beginning of each deployment PHC were generally in the range of 0.5 to 1 % (approximately 60 % F3 at most sites). At the salt sites, ECe was generally in the range of 5 to 20 dS/m.

2.3 PGPR-Enhanced Phytoremediation Systems (PEPS)

The field deployments of PEPS were as described previously (Gurska et al., 2009). At each site, the soil was tilled and fertilized prior to planting seeds. All seeds were treated with PGPR. In most cases the plant seed mix was tall fescue (*Festuca arundinacea*), annual ryegrass (*Lolium multiflorum*), and perennial ryegrass (*Lolium perenne*). In some cases, tall wheatgrass, oats, red fescue or slender wheatgrass were used in the seed mixture. Seeds were purchased from appropriate seed suppliers (e.g., Ontario Seed Company, Waterloo, ON, Canada). Seeds were planted with a drill seeder or a broadcaster followed by harrowing. Plants were allowed to grow for the entire plant growing season (100 d to 150 d). In most cases, supplemental irrigation was not supplied.

2.4 PHC Extraction and Analysis

PHC levels in field soils were determined gravimetrically and by GC-FID (Gurska et al., 2009). Soil samples were collected and stored at 4ºC until analysis. For gravimetric analysis, soil samples were air dried at room temperature in the dark. The soil was extracted by ultrasonication using hexane/acetone (1:1 v:v) (U.S. EPA, 1998). Extracts were dried by completely evaporating the solvent under a gentle stream of nitrogen gas. The amount of extracted PHC was then determined gravimetrically. To determine levels of CCME fractions 1 to 4, soil samples were analyzed by Maxxam Analytics by GC-FID (CCME, 2008). Salt levels in soil were

determined in-house for ECe and samples were sent to various analytical laboratories to measure ECe, Cl^-, Na^+ and SAR (Greenberg et al., 2008b). As well, plant foliage samples were sent to various analytical labs for determinations of Cl^- and Na^+ levels in the tissue. To analyze for BOC in the soil extracts, GC/MS analyses were performed by Maxxam Analytics. Plant toxicity assays for Tier 2 endpoints were performed according to Environment Canada protocols.

3 Results and Discussion

3.1 Phytoremediation of PHC

PHC remediation has been carried out at more than 25 field sites. These were full scale deployments of PEPS. The sites are in British Columbia, Alberta, the Northwest Territories, Manitoba, Ontario and Quebec. Examples are given in Table 1. Most sites had CCME F2 and F3 above criteria. Note, for sites in BC, EPH(C10-19) and EPH(C19-32) are used instead of F2 and F3, respectively. This is due to provincial regulations specific to BC. Tier 1 criteria have been met at 7 sites (examples of 5 sites are given in Table 1). On average, we observed about 33 % remediation per year. All CCME fractions are remediated, with F2 being remediated faster than F3 and F4 (F3 and F4 are remediated at about the same rate). Note, F4 data are not presented here because at all sites F4 levels were below Tier 1 criteria before PEPS were deployed. The sites that have been remediated to Tier 1 criteria took 1 to 3 years to remediate. The sites in progress are moving towards Tier 1 criteria at rates similar to those of the completed sites. We anticipate that these sites will be remediated to Tier 1 criteria in 1 to 2 more years. In 2012, we will begin deploying PEPS at 5 to 10 new sites.

Table 1: PHC remediation at several full scale sites. The PHC fractions requiring remediation at each site are given. The sites listed below are in British Columbia, Alberta and Quebec. Note: in British Columbia, EPH(C10-19) and EPH(C19-32) are used instead of F2 and F3, respectively.

Average PHC Remediation at Several PEPS Sites					
Site	**Analysis**	**Date**	**Average (mg/kg)**	**% Remediation**	**Notes**
Completed Sites					
Edson	CCME F3	Spring 2007	1500	33.33%	5 of 10 sample points above Tier 1 criteria
	CCME F3	Fall 2008	1000		All sample points met Tier 1 criteria
Hinton 2	CCME F3	Spring 2007	900	44.44%	6 of 15 sample points above criteria
	CCME F3	Fall 2008	500		All sample points met Tier 1 criteria
Dawson 1	EPH(C10-19)	Spring 2009	6500	91.54%	12 of 12 sample points above Tier 1 criteria
	EPH(C10-19)	Fall 2011	550		1 of 12 sample points above Tier 1 criteria
	EPH(C19-32)	Spring 2009	2500	72.00%	11 of 12 sample points above Tier 1 criteria
	EPH(C19-32)	Fall 2011	700		All sample points met Tier 1 criteria
Peace River	F3	Spring 2007	900	78.89%	4 of 11 sample points above Tier 1 criteria
	F3	Fall 2008	190		All sample points met Tier 1 criteria
Quebec City	F3	Spring 2009	550	49.09%	3 of 3 sample points above criteria
	F3	Fall 2009	280		All sample points met Tier 1 criteria
Sites in Progress					
Hinton 1	CCME F2	Spring 2010	1100	77.27%	10 of 10 sample points above Tier 1 criteria
	CCME F2	Fall 2010	250		6 of 10 sample points above Tier 1 criteria
	CCME F3	Spring 2010	3200	56.25%	9 of 10 sample points above Tier 1 criteria
	CCME F3	Fall 2010	1400		3 of 10 sample points above Tier 1 criteria
Swan Hills	CCME F2	Spring 2009	1400	78.57%	8 of 8 sample points above Tier 1 criteria
	CCME F2	Fall 2010	300		4 of 8 sample points above Tier 1 criteria
	CCME F3	Spring 2009	2550	64.71%	7 of 8 sample points above Tier 1 criteria
	CCME F3	Fall 2010	900		1 of 8 sample points above Tier 1 criteria
Dawson 2	EPH(C10-19)	Spring 2009	6500	46.15%	15 of 15 sample points above Tier 1 criteria
	EPH(C10-19)	Fall 2011	3500		8 of 15 sample points above Tier 1 criteria
	EPH(C19-32)	Spring 2009	700	42.86%	3 of 15 sample points above Tier 1 criteria
	EPH(C19-32)	Fall 2011	400		All sample points met Tier 1 criteria
Dawson 3	EPH(C10-19)	Spring 2009	7000	81.43%	11 of 12 sample points above Tier 1 criteria
	EPH(C10-19)	Fall 2011	1300		5 of 15 sample points above Tier 1 criteria
	EPH(C19-32)	Spring 2009	3500	57.14%	12 of 12 sample points above Tier 1 criteria
	EPH(C19-32)	Fall 2011	1500		6 of 12 sample points above Tier 1 criteria
Beaver River	EPH(C10-19)	Spring 2010	1600	25.00%	8 of 20 sample points above Tier 1 criteria
	EPH(C10-19)	Fall 2010	1200		6 of 20 sample points above Tier 1 criteria
	EPH(C19-32)	Spring 2010	850	35.29%	8 of 20 sample points above Tier 1 criteria
	EPH(C19-32)	Fall 2010	550		3 of 20 sample points above Tier 1 criteria

3.2 Phytoremediation of Salt

We have deployed PEPS at full scale for remediation of 10 salt impacted sites. We began work on full scale salt sites in 2009. The sites are in Saskatchewan, Alberta and the Northwest Territories. At two sites, remediation goals were met (one site in Alberta and one site in the Northwest Territories). We have observed that the ECe drops at a rate of approximately 15 % per year (Table 2). The amount of salt taken up by the foliage (grass leaves) is approximately 30 g/kg plant dry weight. We have found that this amount of salt assimilation by the plants accounts for the drop in ECe. On a weight basis, the plants take up more Cl^- than Na^+. The amount of NaCl removed from a field per crop harvest is 150 kg/ha, which is enough to remediate a site with an ECe of 10 to 15 dS/m in about 5 years.

Table 2: Typical salt remediation data for salt impacted sites treated with PEPS. Based on 10 sites where PEPS has been applied at full scale and 2 research sites where PEPS was applied. The sites were in Alberta, the Northwest Territories and Saskatchewan.

Parameter	Value
Annual Drop in EC_e	10% to 20%
NaCl Uptake into Foliage	29 g/kg dry weight
Na:Cl ratio in plant foliage (weight basis)	25:75
NaCl Removed From Project Sites in Foliage	150 kg/ha
Change in EC_e Accounted for by Foliar Uptake of Salt	95 %

3.3 Analysis of Soil Extracts for PHC and BOC

We have found at some sites where PEPS have been deployed, BOC can interfere with accurate analysis of PHC. It is recognized in the CCME method that soil extracts may contain BOC, and it allows for clean-up procedures to remove BOC from soil extracts. With Maxxam Analytics, we have optimized the CCME PHC analytical method to remove as much of the BOC as possible. We have done this with a double Si gel column clean-up method (the soil extract is passed through 2 Si gel columns). We compared no clean-up to clean-up with an in situ Si gel step (activated Si gel is added to the soil extract) and clean-up with a double Si gel column step (Figure 1). With no clean-up, a large amount of BOC is evident in the extract. In a typical CCME gas chromatogram for PHC analysis, the BOC immediately follows the unresolved complex mixture (UCM) that represents PHC (Figure 1). When an in situ Si gel clean-up is performed, about half of the BOC are removed. When a double Si gel column clean-up is employed, almost all of the BOC are removed. We also tested the efficacy of a single Si gel column and found that it did not remove all of the BOC (data not shown). It should be noted that the magnitude of the UCM is not altered by the Si gel clean-up steps, indicating that the PHC in the extracts are not removed by the Si gel clean-up.

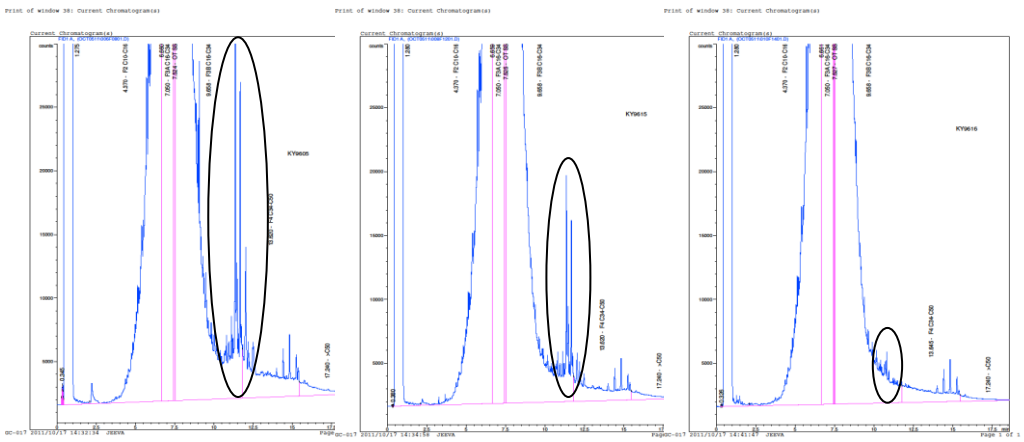

Figure 1: CCME analysis of PHC by GC-FID. Following extraction with n-hexane/acetone, the sample was back extracted with water to remove the acetone. The sample was divided into three aliquots. One aliquot was then left untreated, one aliquot was subjected to an in situ Si gel clean-up and one aliquot was subjected to a double Si gel column clean-up. The samples were then analyzed by GC-FID. The BOC in the gas chromatograms are circled.

To determine if any PHC were removed from the samples with the Si gel clean-up, GC-MS analyses were performed. Total ion MS analyses and selective ion MS analyses were performed on extracts with no clean-up, clean-up with an in situ Si gel step, and clean-up with a double Si-gel column step (Figure 2). In the total ion MS analyses, it can be observed that neither of the clean-up steps affect the magnitude of the UCM. However, in the total ion mode and in the selective ion mode, it was observed that specific compounds were removed by the Si gel clean-up processes. The double Si gel column clean-up almost quantitatively removed these compounds. We have analyzed several of these compounds and all have been found to be BOC (e.g., plant sterols and terpenoids) (Figure 2). Thus, we are confident the enhanced BOC method employing a double Si gel column clean-up step, is accurately reporting PHC soil levels without a large interference from BOC. We are now using Fourier Transform-MS to further confirm that only BOC are removed from the soil extracts.

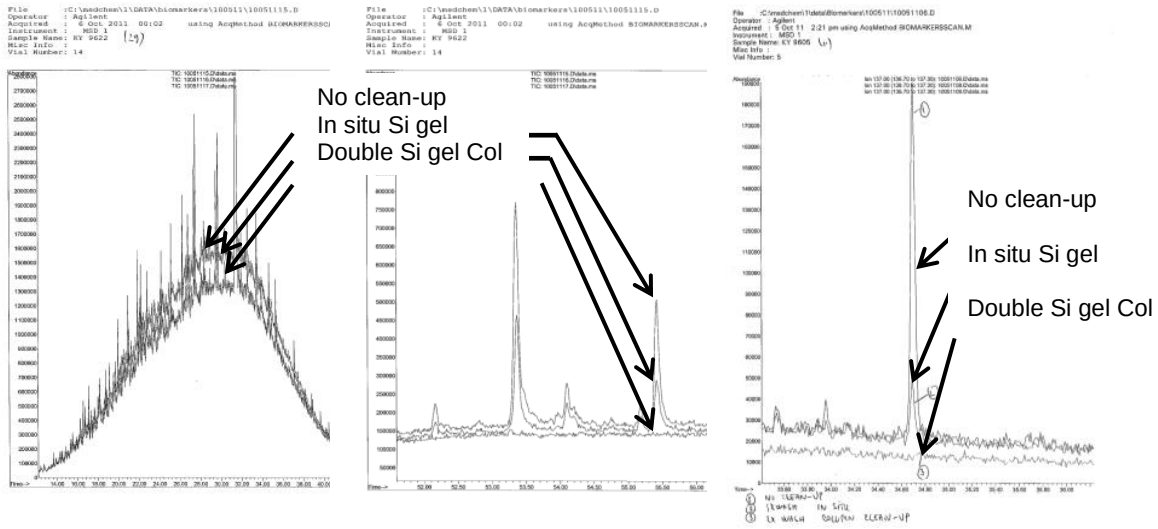

Figure 2: GC-MS analyses of CCME soil extracts. The clean-up processes are the same as in Figure 1. Total ion (left and middle panels) and selective ion (right panel) ($m/e^- = 137$) scans are shown. Compounds shown to be removed by the double Si gel column clean-up were all BOC (e.g., plant terpenoids and plant sterols).

3.4 Effect of Soil F3 Levels on Tier 2 Toxicity Assays

We have begun work on toxicity testing to meet Tier 2 criteria following phytoremediation. This will allow the option of achieving site closure based on Tier 2 risk assessment criteria. We are performing toxicity tests with plants and springtails (Colembola). Intial findings for the plant toxicity tests are provided in Figure 3. The plant toxicity tests are based on Environment Canada protocols. The plant species used were cucumber, barley and northern wheatgrass. The endpoints were percent cotyledon emergence, root and shoot length and weight. All of the data are presented as a function of F3 concentration (Figure 3) (note: the same results were observed as a function of F2 and F4, data not show). The soils came from various sites listed in Table 1. We have found that in most cases, passing toxicity tests were observed. Strikingly, toxicity does not correlate with F3 levels. This implies that plant toxicity is not being driven by PHC levels in the soil. We speculate that failing toxicity tests are caused by poor soil quality.

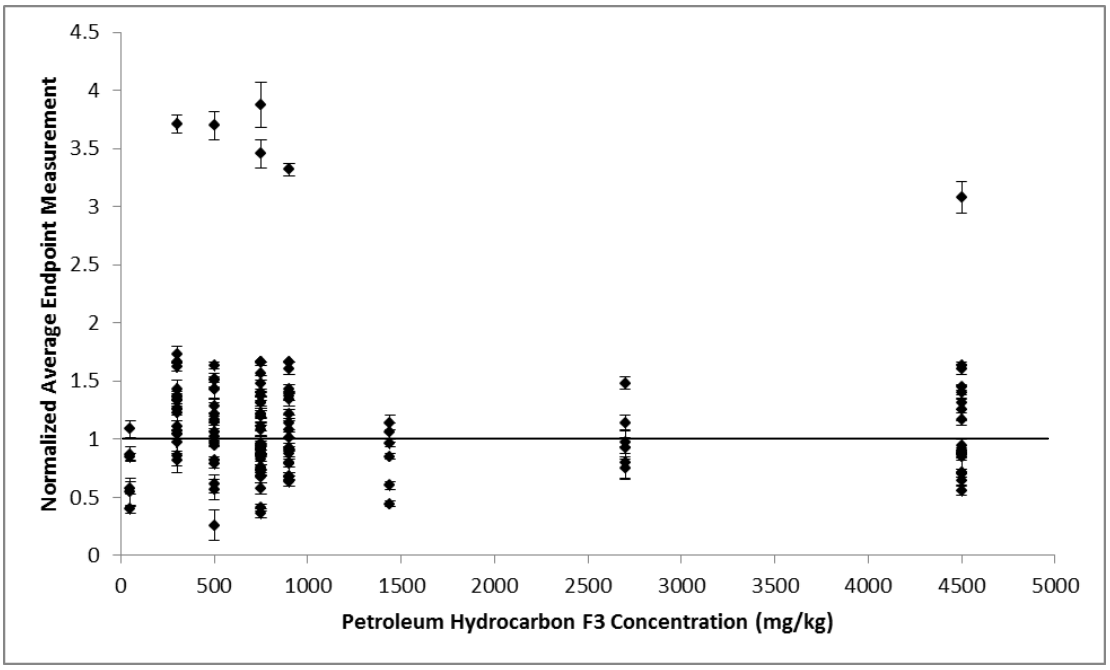

Figure 3: Plant toxicity tests of soils from various sites listed in Table 1. Tests followed Environment Canada toxicity test protocols. Plant species used were cucumber, barley and wheatgrass. End points were percent cotyledon emergence and root and shoot length and weight. All data were normalized to a passing score of 1, and then plotted on the same graph as a function of F3 concentration in the soil.

4 Conclusions

PEPS have been deployed at several sites for PHC and salt remediation. Using PEPS, we have reached Tier 1 criteria at 7 PHC impacted sites and 2 salt impacted sites. At the PHC impacted sites, we observed an average remediation rate of 33 % per year. The PHC are degraded in the rhizosphere. At the salt sites, we have observed an average annual drop in ECe of 15 % per year. The drop in ECe is accounted for by the amount of salt taken up into the leaf tissue. The CCME PHC method has been optimized to remove BOC without affecting the PHC levels in the soil extracts. GC-MS analyses have been used to show that only BOC are removed by the double Si gel column clean-up method. Finally, toxicity testing is being used to define Tier 2 remediation goals.

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