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Distinguishing Natural vs Petroleum F3 Hydrocarbons in Oil Spill Impacted Muskeg Material

1.0 Background

Canada is rich in petroleum resources as demonstrated by the thousands of kilometers of pipelines in existence today. New technologies and routine monitoring can detect oil spills within days or weeks of the occurrence. In the case of older pipelines, historic spills may not be detected until pipeline retirement and removal procedures have begun. In each case, the impacted soils must be remediated in accordance with environmental regulations. The reduction of Total Petroleum Hydrocarbon (TPH) concentrations is a key component of oil spill remediation plans.

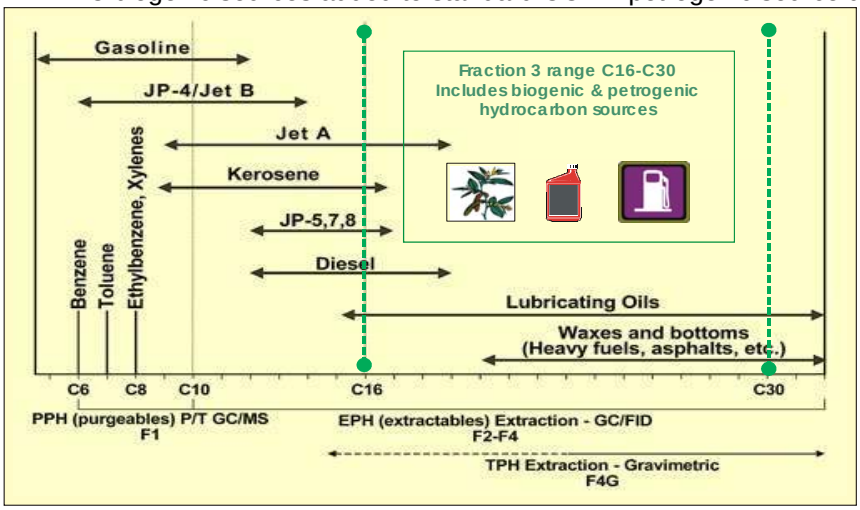
Current CCME Total Petroleum Hydrocarbon Analytical Protocols assume that all detectable hydrocarbons originate from petroleum sources (see Figure 1). In reality however, hydrocarbons can originate from ancient petroleum deposits as well as from newly biosynthesized plant and animal materials (Ref 1, 2, 4). Petrogenic (petroleum) hydrocarbons (PHCs) are produced over millions of years and are found in a variety of sources such as crude oil, gasoline, diesel, etc. In contrast, biogenic hydrocarbons (BHCs) are naturally biosynthesized during the life cycles of living organisms such as plants, insects, fish, birds and animals. Biogenic hydrocarbons naturally exist in a wide variety of mediums such as plant waxes, animal wastes, sewage sludge, etc.

Contaminated organically rich soils such as those found in muskegs, could routinely recover BHCs as well as PHCs. The natural background BHCs would falsely elevate the TPH concentrations under the current CCME TPH analytical protocols. This could create false exceedences of regulatory criteria, resulting in unnecessary and costly soil remediation/disposal requirements, in addition to unnecessary environmental disturbances.

CCME TPH Source Identification Methods

The CCME-PHC analytical methods categorize a wide range of petroleum products into Fractions 1, 2, 3 and 4. Figure 1 illustrates a standard CCME diagram that assumes all detectable hydrocarbons originate from petrogenic sources *only*. This would be a correct assumption in the case of Fractions 1 (C6-C10), 2 (C10-C16) and 4 (>C30). However, this assumption should not be made for Fraction 3 (C16-C30), which includes *both* petrogenic and biogenic hydrocarbon sources.

Figure 1: Petrogenic and biogenic hydrocarbon sources and carbon ranges
- F3 biogenic sources added to standard CCME petrogenic source diagram



2.0 RESEARCH PURPOSE AND SCOPE

The purpose of this research is to develop a new protocol for quantifying natural and petrogenic hydrocarbon concentrations in naturally organic materials. The research scope will include a fifteen-month microcosm contamination experiment focusing on chemical signatures and concentrations that are unique to various BHC and PHC sources. This data may then contribute to enhance the current CCME protocol by allowing BHCs and PHCs to be correctly identified in future environmental evaluations.

3.0 RESEARCH QUESTIONS

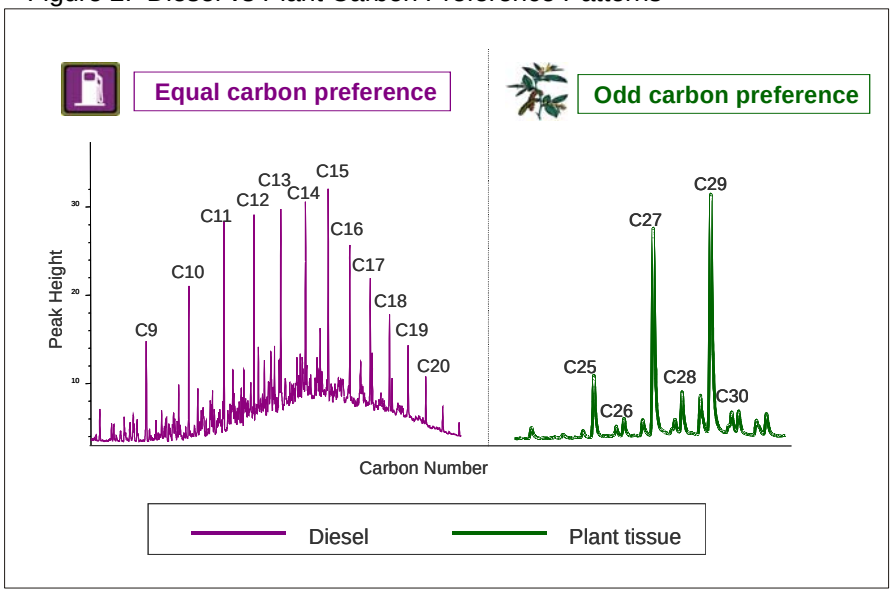
- *What are the current methods for identifying petrogenic and biogenic hydrocarbons?*

i) **Carbon Preference Index (CPI)**

The CPI is a ratio of the sum of odd-numbered hydrocarbons to the sum of even-numbered hydrocarbons. Hydrocarbons originating from biogenic sources have a predominance of odd-numbered carbon chains with CPI values >5-7. However, petrogenic hydrocarbons have a predominance of equal-numbered carbon chains with CPI values of >1.0.

- *CPI Limitations*: This method can only indicate *dominant* biogenic vs petrogenic hydrocarbon sources and cannot indicate separate concentrations. The CPI can be useful when minimal TPH criteria exceedences are dominated by biogenic sources. In cases such as these, the relatively lower petrogenic concentrations could be identified as not exceeding toxicity criteria. However, dominance of biogenic concentrations is irrelevant if high TPH concentrations exceed toxicity criteria by more than 50%. Even if the petrogenic concentrations were relatively lower than the biogenic concentrations, they could still be high enough to exceed toxicity thresholds.

Figure 2: Diesel vs Plant Carbon Preference Patterns



ii) **Biomarker Analysis**

Biomarker analysis utilizes molecular compounds that are unique to various hydrocarbon sources (see Table 1). For example, sterols are indicative of BHCs and hopanes are indicative of PHCs. Biomarker analysis is useful in determining the presence or absence of various BHC and PHC sources as well as in determining the age of a particular oil spill.

Limitations – Biomarker analysis does not provide a direct indication of BHC vs PHC concentrations.

Table 1: PHC and BHC molecular signature analysis

Biomarkers	Measurement Units	Source	Reference
Total Petroleum Hydrocarbons	ug/g	petrogenic and biogenic	3, 7, 14, 15
PAHs	ug/g	petroleum spillage and combustion. Vegetation fires.	3, 14
UCM	ug/g	Petrogenic	7
UCM/*n*-alkane ratio	Ratio >4	Lubricating oil	7
n-alkanes,CPI index			4, 14
Even/Odd *n*-alkanes	ug/g, Ratio <1	petrogenic, degraded organic matter, algae.	4, 14
Odd *n*-alkanes	ug/g, Ratio >1	plants, algae, bacteria, zooplankton, insects	4, 14
Branched Isoprenoids			
Phytane	ug/g, Ratio <1	petrogenic	7
Pristane	ug/g, Ratio >1	plants, algae, zooplankton	7
Phytol	ug/g	algae	13
Sterols			
24-methylcholesta-5,24(28)-dien-3β-o1	ug/g	algae	1, 8, 15
24-methylcholest-5-en-3β-o1	ug/g	plants, algae	1, 8, 15
24-ethylcholesta-5,22E-dien-3β-o1	ug/g	plants, algae	1, 8, 15
24-ethylcholest-5-en-3β-o1	ug/g	plants, algae	1, 8, 15
cholesta-5,22E-dien-3β-01	ug/g	plants, algae	6, 15
Ergosterol	ug/g	fungi	16
Hopanes			
17α-hopane	ug/g	petrogenic	2
21β-hopane	ug/g	petrogenic	2
Diploptene			
hop-22(29)-ene	ug/g	plants, bacteria	15
Plant Triterpenols			
β-amyrin (olean-12-en-3β-o1)	ug/g	plants	1, 15
α-amyrin (urs-12-en-3β-o1)	ug/g	plants	1, 15

- *How can existing biomarker analytical methods be used to develop new protocols for quantifying separate petrogenic and biogenic hydrocarbon concentrations when they are present in the same sample?*

  The proposed research experiments will quantify biomarker concentrations that are unique to individual BHC and PHC sources. For example, the presence of sterols, diplotene and plant Triterpenols are signature biogenic compounds, while hopanes are signature petrogenic compounds. Our study will quantify the PHC vs BHC biomarker concentrations within the microcosm soil samples. This data will then be used to develop a protocol for subtracting BHC concentrations from the F3 concentrations, in order to determine if a sample had truly exceeded PHC regulatory criteria.

4.0 BENEFITS TO INDUSTRY

The extent that an oil spill has spread into naturally organic soils and sediments could be better defined. Areas that were exposed to the oil spill could then be remediated, while areas that were not exposed would remain in their natural state. The resulting benefits pertain to bioremediation cost saving as well as minimizing disturbances to natural environments.

5.0 RESEARCH PLAN & METHODOLOGIES

5.1 How will this research build on existing knowledge?

Science can identify *dominant* hydrocarbon sources through Carbon Preference Indexing and biomarker analysis. However, these methods do not quantifying separate petrogenic and biogenic hydrocarbon concentrations when mixed into the same soil/sediment sample. We propose to develop a new hydrocarbon sourcing protocol that would combine existing knowledge with new research data to quantify BHC and PHC source concentrations in naturally organic materials.

5.2 Muskeg Microcosm Experimental Design

A fifteen-month contamination experiment will track the changing BHC and PHC biomarker concentrations in contaminated and uncontaminated muskeg materials. The chemistry data will be used to calculate the separate BHC and PHC concentrations from the beginning to the end of the contamination period. The petroleum treatments and sampling schedule is detailed in Figure 3 and Table 2.

Chemistry Analysis

All microcosm samples will be analyzed for the following parameters:

i) Total Petroleum Hydrocarbons (F1-F4)
ii) Biomarkers: sterols, branched Isoprenoids, hopanes, triterpenols, diplotene
iii) Alkylated PAH
iv) Carbon Preference Index
v) Total Organic Carbon
vi) Trace metals
vii) Bacteria and fungi

Biomarker Transition Chemistry Monitoring

BHCs and PHCs are subject to natural weathering and microbial degradation processes. Resulting changes in chemical compositions can significantly influence biomarker compositions (Wang, Z. 2004; Pollard, S.J.T. et al. 1999; Stout, S.A. et al. 1998). As a result, fresh vs aged petroleum can produce entirely different biomarker results. This experimental design will therefore include contamination experiments focusing on BHC and PHC weathering and degradation processes occurring over a fifteen-month study period.

Figure 3: Muskeg microcosm experimental design

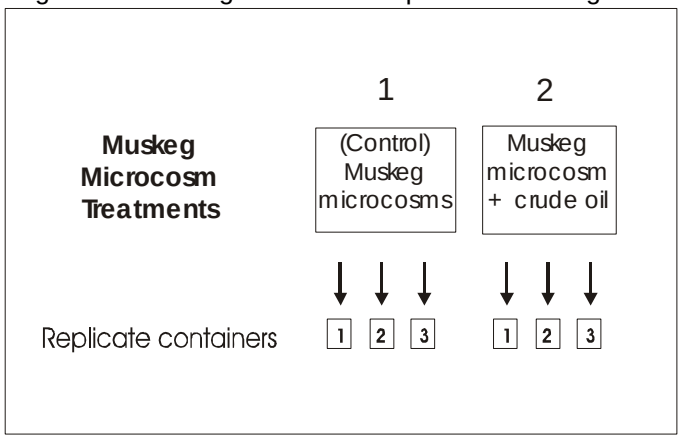


Table 2: 2007-2008 muskeg microcosm sample schedule

Number of Sample Events	Time (Days)	Approximate Months	Number of Samples
1	0	April 2007	6
2	7	April 2007	6
3	14	April 2007	6
4	30	April 2007	6
5	60	May 2007	6
6	120	July 2007	6
Freeze Microcosms	120-127	July 2007	0
7	140	August 2007	6
8	260	November 2007	6
Freeze Microcosms	260-267	November 2007	0
9	280	December 2007	6
10	400	March 2008	6
Freeze Microcosms	400-407	March 2008	0
11	420	April 2008	6
12	540	July 2008	6
Freeze Microcosms	540-547	July 2008	0
			Total = 72

Step #1: Literature Review (January-February 2007)

- Review all available journal articles, technical reports and data pertaining to crude oil contamination in muskeg environments. Focus will be placed on chemical changes created by weathering and microbial degradation processes.
- Apply relevant information to 2007 microcosm studies.

Step #2: Microcosm Baseline Analysis (March 2007)

- Submit separate muskeg core and crude oil samples for the following baseline analysis: TPH (F1-F4), biomarkers, alkyl PAH, CPI, TOC and trace metals.
- Quantify indigenous bacteria and fungi concentrations and dominant groups in separate muskeg core and crude oil samples. Produce separate bacteria and fungi broths from muskeg and oil samples. Submit broths for TPH, CPI, TOC and biomarker analysis.

Step #2: Muskeg Microcosm Preparation (March 2007)

- Establish six uncontaminated muskeg microcosms to be grown in the greenhouse laboratory.
- Grow microcosms in greenhouse for one month prior to petroleum exposure.

Step #3: Conduct Exposure Studies (April 2007-October 2007)

- Expose three of the six microcosms to a set concentration of crude oil.
- Collect core samples from all six containers in accordance with the Table 2 sampling schedule.

5.3 Data Analysis

Analysis of Variance (ANOVA) or simple comparison of means (and confidence intervals) to test for differences in response to variables among the BHC and PHC materials.

5.4 PHC and BHC concentration calculations

The baseline biomarker results will be used to evaluate the PHC vs BHC concentrations in the muskeg materials. The total petroleum hydrocarbon concentrations would then be broken into PHC and BHC concentrations to determine if the petroleum portion was above or below the CCME toxicity thresholds.

5.5 Apply research results to the CCME TPH Analytical Protocols

The ultimate goal of this research will be to enhance the current CCME TPH Analytical Protocols. This would be accomplished by incorporating BHC and PHC source distinctions into the existing methodologies and regulations.

6.0 BUDGETS

Table 3: 2007-2008 project costs

EXPENDITURE DESCRIPTIONS	Unit Costs	2007 Costs	2008 Costs
Laboratory Fees	**Costs/sample**		
2 Peat & Oil bacteria/fungi biomarker analysis and broth production	$5,056	$10,112	$0
Baseline & Microcosm Analytical Fees		*54 Microcosm & 2 Baseline Samples*	*18 Microcosm Samples*
Biomarkers, TPH (F1-F4), CPI, Alkylated PAHs	$1,000	$56,000	$18,000
HC extract	$35	$1,960	$630
Trace Metals	$42	$2,352	$756
TOC	$35	$1,960	$630
Bacteria & Fungi	$280	$15,680	$5,040
Subtotal		$88,064	$25,056
GST		$5,283.84	$1,503.36
Total lab costs		**$93,347.84**	**$26,559.36**
Equipment			
Muskeg Containers	$300 each	$1,800	$0
UV lights	$200 each	$1,200	$0
Redox meter	$600	$600	
Freezer facility	$1000/week	$2,000	$2,000
25% University Overhead		$24,737	$7,139
TOTAL		**$123,684.84**	**$35,698.36**

Table 4: ERAC 2007 funding budget

EXPENDITURE DESCRIPTIONS	FEES
60% of 2007 $123,684.84 budget	$74,210.90
PTAC Forum travel and accommodation expenses (assuming $600 air fair, 3 nights accommodations at $120/night and $40/day for meals)	$1,120
Contingencies	$2,000
TOTAL	**$77,330.90**

7.0 MILESTONES & DELIVERABLES

Table 5: 2007 hydrocarbon source fingerprinting research schedule

Research Activities	Timing
Transplant muskeg vegetation and peat into six microcosm containers in greenhouse laboratory. Monitor vegetation health and adjust conditions if necessary.	Feb.-March 2007
Analyze muskeg cores and crude oil for bacteria and fungi concentrations and identify dominant groups. Produce separate bacteria and fungi cultures for the core and oil samples. Identify molecular signatures for each culture through biomarker analysis.	Feb.- March 2007
Analyze unmixed muskeg core and crude oil samples for TPH (F1-F4), alkyl PAH, biomarkers, TOC, trace metals, bacteria and fungi.	Feb. 2007
Quantify TPH and F3 concentrations that naturally occur in peat and crude oil samples. Determine what volumes of crude oil will exceed CCME soil guidelines when mixed into the muskeg peat.	March 2007
Contaminate three of the six microcosms with unweathered crude oil	April 2007
Monitor microcosm chemistry patterns	April 2007- Aug. 2008
Data analysis and PHC vs BHC calculations	Feb. 2007- Feb. 2009
Submit progress report	May 2007
Prepare first year report	Aug.-Oct. 2007
Submit first draft	Nov. 2007
Submit first year final report	Dec. 2007

8.0 RESEARCH TEAM EXPERTISE & RELEVANT FUNDING.

Project Manager and PhD Candidate: Francine Kelly-Hooper, University of Waterloo

Francine Kelly-Hooper is an ecologist and water quality specialist with seventeen years of environmental consulting experience. Francine has owned Kelly Hooper Environmental Inc. for seven years, providing research services to government organizations throughout Canada.

Francine has recently completed the Canada-wide Stormwater Management Pond/Wetland Sediment Chemistry Field Sampling and Biomarker Analysis Research Study. The purpose of this study was to characterize biogenic and petrogenic hydrocarbons a 29 sites through the use of biomarker forensics techniques.

Relevant Funding:

Project Title: 2005 Canada-wide Stormwater Management Pond/Wetland Sediment Chemistry Field Sampling and Biomarker Analysis Research Study

- 22 participating municipalities → $203,500 cash contributions
- Ontario Ministry of Environment → $15,000 cash contributions
- Environment Canada Oil Spills Spills Research Laboratory → $53,000 inkind contributions
- Envirotest Laboratory → $5,000 inkind contributions

Selected Publications

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PhD SUPERVISOR – Dr. D.G. Dixon

Dr. Dixon is a Professor of Biology and Dean of Science at the University of Waterloo, Waterloo, Ontario. Dr. Dixon's research focuses on toxicology, specifically the modifying factors that influence exposure and effects as it pertains to environmental risk assessment and risk management. Dr. Dixon has been involved with oil sands research in Alberta since 1995 with studies focusing on the impacts of oil sands reclamation/tailings disposal waters on fish and benthic invertebrates. In addition to studies on whole reclamation/tailing disposal waters, other studies have examined the effects of the two major groups of organic constituents, namely polycyclic aromatic hydrocarbons and naphthenic acids. Recent work examined the effects of specific substituted PAHs and complex oil sands PAH extracts as well as the effects of UV on PAH toxicity on embryo-larval development. Dr. Dixon has also been involved with the re-evaluation of the toxicity of CCME petroleum hydrocarbon fraction 3, and interaction effects between fractions.

Relevant Funding

Project Title: A re-evaluation of the toxicity of CCME petroleum hydrocarbon fraction 3, and interaction effects between fractions.

- ERAC → $189,585 cash contributions for 2002-2004
- Imperial Oil → $15,000 cash contributions for 2002-2003
- NSERC → $38,000 cash contributions for 2002-2003

Selected Scientific Journal Publications

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Duxbury, C.L., D.G. Dixon and B.M. Greenberg. 1997. Effects of simulated solar radiation on the bioaccumulation of polycyclic aromatic hydrocarbons by the duckweed *Lemna gibba*. Environ. Toxicol. Chem. 16: 1739-1748. (22)

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Tetreault, G.R., M.E. McMaster, D.G. Dixon and J.L. Parrott. 2003. Physiological and biochemical responses of Ontario slimy sculpin (*Cottus cognatus*) to sediment from the Athabasca oil sands area. Wat. Qual. Res. J. Can. 38: 361-377.

LEAD RESEARCH CHEMIST - Dr. Zhendi Wang

Dr. Zhendi Wang is a senior research scientist and Head of Oil Spill Research of Environment Canada, working in the oil and toxic chemical spill research field. His specialties and research interests include: development of oil spill fingerprinting and tracing technology, environmental forensics of oil spill; oil properties, fate and behavior of oil and other hazardous organics in the environment; oil burn emission and products study; oil bioremediation; identification and characterization of oil hydrocarbons; and, spill treatment studies; applications of modern analytical techniques (such as GC, GC/MS, HPLC, LC/MS, SFE and SFC, and IC) to oil spill studies and other environmental science and technology.

Relevant Funding

- US EPA annual petroleum related research grants → $150,000

Selected Scientific Journal Publications

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9.0 PEER REVIEW

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Applicant: D. G. Dixon

Signature: _____

Date: _____