F2:F3b Ratio and BOC-Adjusted PHC F3 Approach to Resolving False Detections of Crude Oil and Diesel Drilling Waste in Clean Soils and Manure Compost

by

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I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

ABSTRACT

The Canadian Council of Ministers of the Environment (CCME) endorsed the *Reference Method for the Canada-Wide Standard (CWS) for Petroleum Hydrocarbons (PHC) in Soil – Tier 1 Method* in 2001. The purpose of the CWS is to provide laboratories with analytical methods for producing accurate and reproducible PHC soil chemistry analysis results. CWS PHC concentrations are reported according to the following carbon ranges/fractions: F1 (C6-C10), F2 (C10-C16), F3 (C16-C34) and F4 (>C34). The *Canada-wide Standards for Petroleum Hydrocarbons (PHC) in Soil* provide generic toxicity guidelines for the each of the four PHC fractions. The CWS PHC extraction solvents inadvertently co-extract natural biogenic organic compounds (BOC) from organic soils. BOCs, such as waxes and fatty acids, are produced by living organisms such as plants, animals and microbes. PHC analysis of highly organic clean soils and manure compost can cause false exceedences of the F3 soil toxicity guidelines. This thesis presents a new mathematical Tier 2 approach to resolving biogenic interferences through the use of biogenic versus petrogenic Gas Chromatography - Flame Ionization Detector (GC-FID) chromatogram patterns produced by the CWS PHC Tier 1 method. This approach is based on the results of four studies: i) 300-day crude oil contaminated peat and sand microcosm experiment; ii) 300-day diesel drilling waste contaminated manure compost and sand microcosm experiment; iii) PHC analysis of 14 light to heavy crude oils and iv) Canadian background PHC soil field survey. These studies determined that the clean soils and compost had F3 ranges that were dominated by the F3b sub-fraction range (C22-C34). In contrast, the F3a (C16-C22) and F3b sub-fraction ranges were evenly distributed in the 14 fresh light to heavy crude oils. The diesel drilling waste

was strongly dominated by the F3a sub-fraction range. The second important trend was that F2 concentrations were non-detectable or slightly detectable in all of the clean soils and compost samples. In contrast, F2 concentrations were strongly prevalent in all of the crude oils and in the diesel drilling waste. F2 and F3b concentrations were applied to the F2:F3b ratio, which identified PHC absence in the clean materials (<0.10 ratio) and PHC presence ($\geq$0.10 ratio) in the contaminated materials. The %F3a:%F3b distributions were applied to the BOC-adjusted PHC F3 calculation, which estimated true PHC F3 concentrations in the clean and contaminated soils and manure compost. The combination of these two approaches provided an accurate and efficient solution to resolving false detections of crude oil and diesel PHCs and false exceedences of F3 soil toxicity guidelines by in clean soils and compost.

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BOC:	biogenic organic compounds
CCME:	Canadian Council of Ministers of the Environment
CFU:	colony forming units
CWS:	Canada-wide standards
DCM:	dichloromethane
F1:	CWS PHC Fraction 1 carbon range C6-C10
F2:	CWS PHC Fraction 2 carbon range C10-C16
F3:	CWS PHC Fraction 3 carbon range C16-C34
F4:	CWS PHC Fraction 4 carbon range >C34
F3a:	sub-fraction carbon range C16-C22
F3b:	sub-fraction carbon range C22-C34
GC-FID:	gas chromatography-flame ionization detector
PAH:	Polycyclic aromatic hydrocarbon
PHC:	petroleum hydrocarbons
TOC:	total organic carbon
UCM:	unresolved complex mixture

CHAPTER 1

General Introduction

INTRODUCTION

"Petroleum hydrocarbon" (PHC) is a general term, which refers to highly complex and variable organic compounds composed primarily of carbon and hydrogen. Crude oil is a naturally occurring form of PHCs, which is extracted from underground reserves. Refineries distill crude oil into a wide range of energy products, which are used to fuel transportation systems, heat indoor environments and power industrial operations. Crude oil is also used as a feedstock material in the production of many everyday items such as clothing, shoes, toys, plumbing, cosmetics, foods and pharmaceuticals (APPE 2012).

PHCs releases into the environment pose risks to human and environmental health. These risks include: fire and explosion hazards; carcinogenicity and toxicity; offensive odours, tastes and appearances; and interferences of water and nutrient uptake by plants (CCME 2008a). It is estimated that14,173 PHC contaminated soil sites exist throughout Canada (ELM 2006; Sanscartier et al. 2009; TBS 2011; FCM 2012).

Crude Oil Formation

Crude oil is naturally formed over millions of years from the ancient buried remains of marine plants and animals. Plants and animals biosynthesize a wide range of biogenic organic compounds (BOCs) such as lipids, carbohydrates, proteins and nucleic acids (Figure 1.1A) (Cooper 2000). All four BOC groups include compounds containing carbon, hydrogen and oxygen. The lipid group is unique in that it also includes biogenic PHCs such as straight chain *n*-alkanes, branched alkanes and alkenes. Carbohydrates, proteins and nucleic acids include additional elements such as nitrogen, phosphorus and sulfur. Lipids and proteins are respectively, the primary and secondary source materials for crude oil

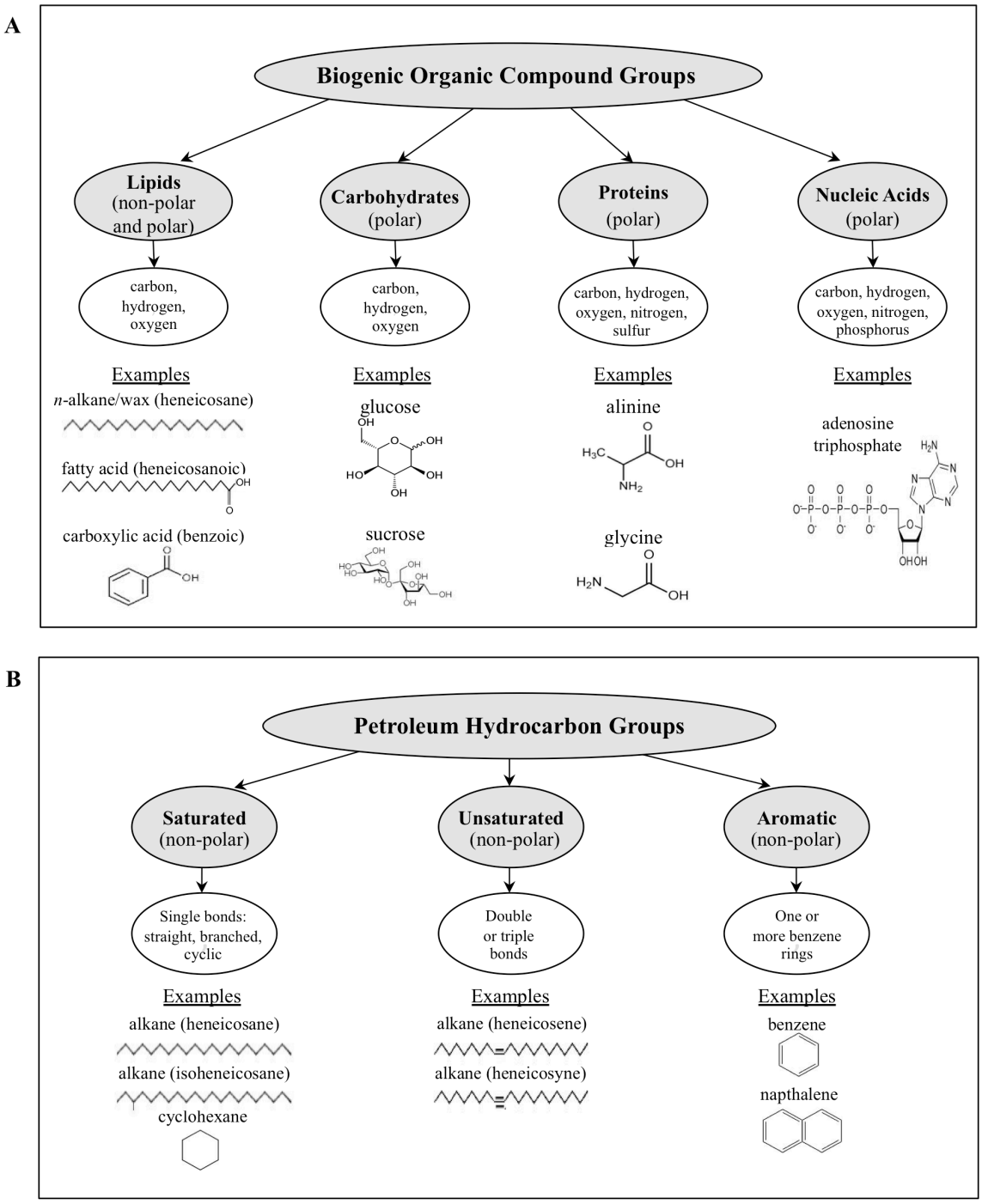

Figure 1.1. (A) Biogenic organic compound groups, elements and polarities (B) Petroleum hydrocarbon groups, elements and polarities.

formation (Evans and Felbeck 1983). Over time, dead plant and animal remains settled to the sea floor and accumulated in deep layers that were gradually buried under heavy silt

and sand deposits. The combined effects of pressure, heat and bacterial decomposition stripped away virtually all elements except for carbon and hydrogen to form PHCs (Hunt 1979). PHCs are classified into three distinctive groups (Figure 1.1B): i) Saturated (single bonds) – straight chain *n*-alkanes, branched alkanes and cyclic alkanes; ii) Unsaturated (double or triple bonds) – alkenes and alkynes, respectively; and iii) Aromatics – one or more benzene rings, which are defined as conjugated/alternating double bonds. Compounds with two or more bonded benzene rings are referred to as polycyclic aromatic hydrocarbons (PAHs). Small amounts (<5%) of oxygen, sulfur and nitrogen heteroatoms and trace amounts of metals may be present as well (Wang and Stout 2007).

CWS PHC Tier 1, 2 and 3 Risk Management Approaches

The *Canada-wide Standards for Petroleum Hydrocarbons (PHC) in Soil* (CCME 2008a) is a site-specific ecological and/or human health risks assessment approach. PHC CWS risk management options are identified through a site characterization studies. Basic site characterization data would include: size, location, built environment, land use, proximity to surface water and drinking water supplies, depth to groundwater, human receptors, ecological receptors, primary exposure pathways, soil texture, contaminant characterization, contamination delineation, depth to contamination and distances to points of exposure/compliance.

CWS PHC contamination site assessment data is applied through a three-tier risk evaluation framework. Each tier provides equal levels of protection, with increasing levels of precision provided from Tier 1 to Tier 2 to Tier 3. Tier 1 uses generic numeric screening soil quality guidelines to evaluate soil contaminant concentration risks. The guidelines are

applied according to site specific soil types (fine or course) groundwater potability and the following land use categories: agricultural, residential, parkland, commercial and industrial (CCME 2008a). The Tier 1 guidelines are based on conservative assumptions regarding site, receptor and contaminant factors to ensure that remediation will meet environmental objectives. Tier 2 involves site-specific recalculations of the Tier 1 generic guidelines where assumptions and/or exposure scenarios do not apply. Tier 2 level is based on site-specific parameters and/or pathway/receptor modification or elimination. Tier 3 involves the completion of a site-specific risk assessment study and the development of a risk management plan. Tier 3 is based on site-specific receptors, pathways and contaminants. The risk management plan should outline the controls that will be necessary to preserve the assumptions used in the establishment of the Tier 3 objectives. These controls may include engineered systems, designed to limit exposure via one or more exposure pathways through physical means such as barriers, and/or controls designed to limit exposure through land and water use restrictions. They may also involve remediation or natural attenuation.

CWS PHC Soil Quality Guidelines

The CWS PHC Tier 1 soil guidelines are based upon the likelihood that petroleum constituents within four carbon ranges/fractions could produce potential environmental and/or human health risks. Fraction 1 (F1) encompasses the C6-C10 range and consists of volatile aromatic and aliphatic hydrocarbons. Fraction 2 (F2) encompasses the semi-volatile C10-C16 range and Fraction 3 (F3) encompasses the C16-C34 range. Both F2 and F3 are composed of aromatic and aliphatic hydrocarbons. Fraction F4 encompasses the >C34

range and differs from the other fractions because it has low aromaticity and also contains small amounts of heteroatoms (nitrogen, sulfur, oxygen) (CCME 2008b).

The fraction-specific PHC soil guidelines were developed through a weight of evidence approach. Fraction-specific and whole product toxicity testing used Federated Crude Oil, which was vacuum distilled into the F1, F2, F3 and F4 carbon ranges. The four fractions include all light to heavy carbon range PHC products (Figure 1.2). Toxicity tests were conducted to assess the acute and chronic toxicological effects associated with the exposure of soil invertebrates and plants to each fraction (CCME 2008b). CWS PHC Tier 1 generic soil quality guidelines were developed for F1, F2, F3 and F4 (Table 1.1) (CCME 2008a). The soil guidelines apply to direct contact exposure pathways for different land use classes and soil types. The soil guidelines do not specify limits for target PAHs, as they have their own set of guidelines (CCME 2010a).

Table 1.1. Tier 1 CWS generic guidelines (mg/kg) for PHCs in surface soils[a]

Land Use	Soil Texture[b]	Fraction 1 C6-C10	Fraction 2 C10-C16	Fraction 3 C16-C34	Fraction 4 >C34
Agricultural/ Residential/ Parkland	Coarse-grained soil	30	150	300	2,800
	Fine-grained soil	170	150	1,300	5,600
Commercial/ Industrial	Coarse-grained soil	240	260	1,700	3,300
	Fine-grained soil	170	230	2,500	6,600

[a]Most stringent Tier 1 soil criteria for potable groundwater protection. Less stringent criteria may be applied at the discretion of regulatory agencies (CCME 2008a).
[b]Coarse sand and gravel, median grain size of >75 µm; Fine silt and clay, median grain size of <75 µm.

The CWS PHC soil quality guidelines are based on site-specific land use, mineral soil texture and proximity to potable groundwater. The "fine-grained" soil category includes silt and clay with a median grain size of <75 µm and the "coarse-grained" soil category includes sand and gravel with a median grain size of >75 µm. The most stringent

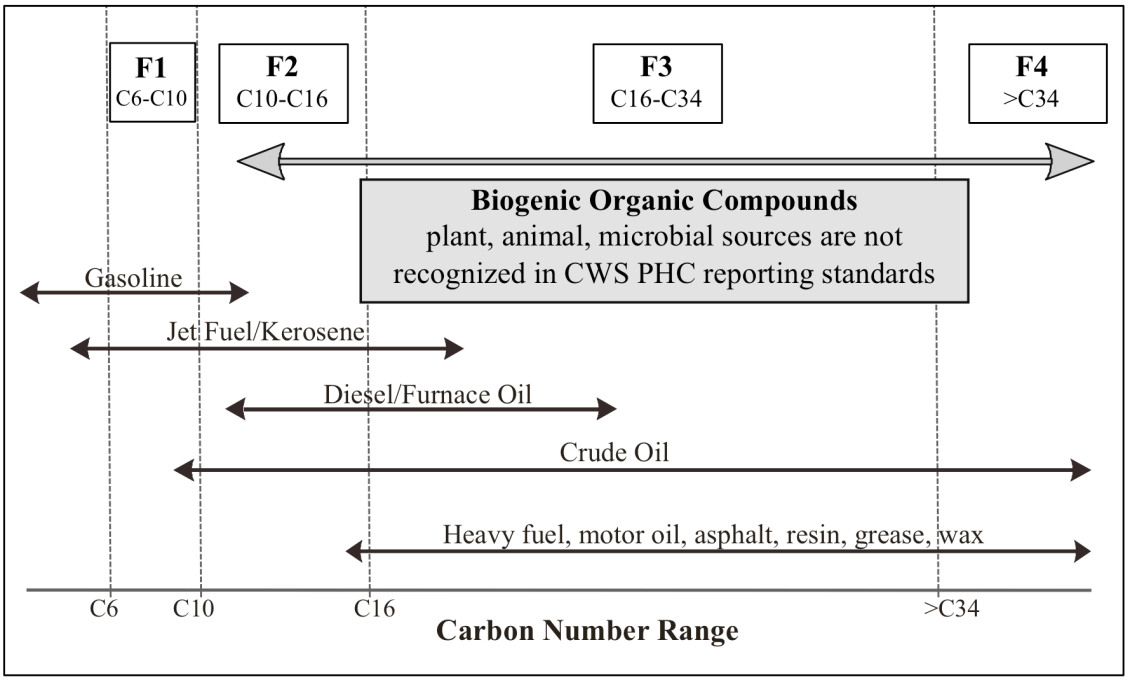

Figure 1.2. Petroleum hydrocarbon products organized by F1, F2, F3 and F4 carbon number ranges. (Wang et al. 2007b; CCME 2008a)

guidelines apply to coarse-grained surface soils in agricultural/residential/ parkland areas with potable groundwater uses. Organic peat soils have very low mineral content and do not therefore fit either the fine or coarse soil categories. Regulatory requirements for adherence to either the fine or coarse soil category guidelines in peatlands are site-specific, depending on potential risks of released PHCs entering nearby surface water and/or groundwater systems.

CWS PHC Tier 1 analytical standards and BOC interference issues

The CWS PHC Tier 1 analytical standards use hexane, acetone and dichloromethane (DCM) organic solvents to extract and isolate PHCs occurring within the F2, F3 and F4 carbon ranges. However, these solvents inadvertently co-extract biogenic organic compounds (BOCs) associated with Fractions 2, 3 and 4 in soils and manure

compost as fresh and decayed plant and animal organic matter. BOC interferences, created by organic peat soils and manure compost, are recognized by the CCME (CCME 2001a). The following section explains the causes and solutions to this problem.

Reference Method for the Canada-Wide Standard (CWS) for Petroleum Hydrocarbons (PHC) in Soil – Tier 1 soil chemistry analytical method

The *Reference Method for the Canada-Wide Standard (CWS) for Petroleum Hydrocarbons (PHC) in Soil – Tier 1 Method* (CCME 2001a) was endorsed in 2001 by the Canadian Council of Ministers of Environment, with the exception of the province of Quebec. The CWS PHC standards provide a standardized analytical method for the quantification of PHC concentrations in soils. The purpose of the CWS PHC analytical standards is to reduce variability and uncertainties related to soil sample extraction, purification, quantification and reporting processes among Canadian laboratories. The analytical methods are applicable to soils that have been contaminated by crude oils and refined petroleum products occurring within the F1, F2, F3 and F4 carbon ranges. The following discussion focuses specifically on the methods of extraction and analysis F2, F3 and F4 because these fractions are most susceptible to BOC interferences.

CWS PHC Soil Extraction Methods : Non-polar and polar compound separations

The CWS PHC soil analysis uses a mixture of polar and non-polar organic solvents to extract organic compounds in the F2, F3 and F4 carbon ranges. The term “polarity” refers to electronegativity differences between two atoms bound covalently to each other. PHC compounds are composed only of non-polar carbon and hydrogen bonds (Figure

1.1B). It is however important to note that non-polarity strengths are highest in saturate compounds, intermediate in unsaturated compounds, and lowest in aromatic compounds (Hawthorne et al. 2000). Non-polarity strength also decreases with increasing molecular weight, meaning that smaller PHC molecules are more non-polar than larger PHC molecules (Guerin 1999). In contrast, almost all BOC compounds have polar bonds due to the presence of oxygen and other elements such as nitrogen, sulfur and phosphorus (Figure 1.1A). However, non-polar *n*-alkanes, branched alkanes and alkenes are also present in biogenic materials such as plant waxes, insect cuticals, animal feces and microbial biofilms (Kumari 1986; Volkman et al. 1992; Nelson et al. 2004; Samuels et al. 2008; Golebiowski et al. 2011). It is important to note that BOCs can have wide ranges of polarity strengths as well. For example, long chain fatty acids, can have both polar and non-polar components within the same molecule (Ruiz-Gutiérrez and Pérez-Camino 2000).

The CWS PHC analytical standards allow a Soxhlet heat apparatus or other equivalent or better apparatus to be used for extraction processes (CCME 2001a). A 5 g dry weight or greater soil sample is mixed into a 50:50 hexane:acetone organic solvent solution, which indiscriminately extracts all non-sequestered carbon-containing compounds from the sample (Figure 1.3A). The non-polar hexane (C_6H_{14}) component extracts non-polar compounds, while the polar acetone $(CH_3)_2CO$ component extracts polar compounds.

The sample extract may be treated with sodium sulphate and a water backwash step for the purpose of acetone removal. A polar compound removal step is then conducted by pouring the extract through 5g of highly polar silica gel (SiO_2). The purpose of this step is to irreversibly retain polar BOCs within the silica gel. A 50:50 mixture of hexane and moderately polar DCM (CH_2Cl_2) is also poured through the silica gel in order to ensure that

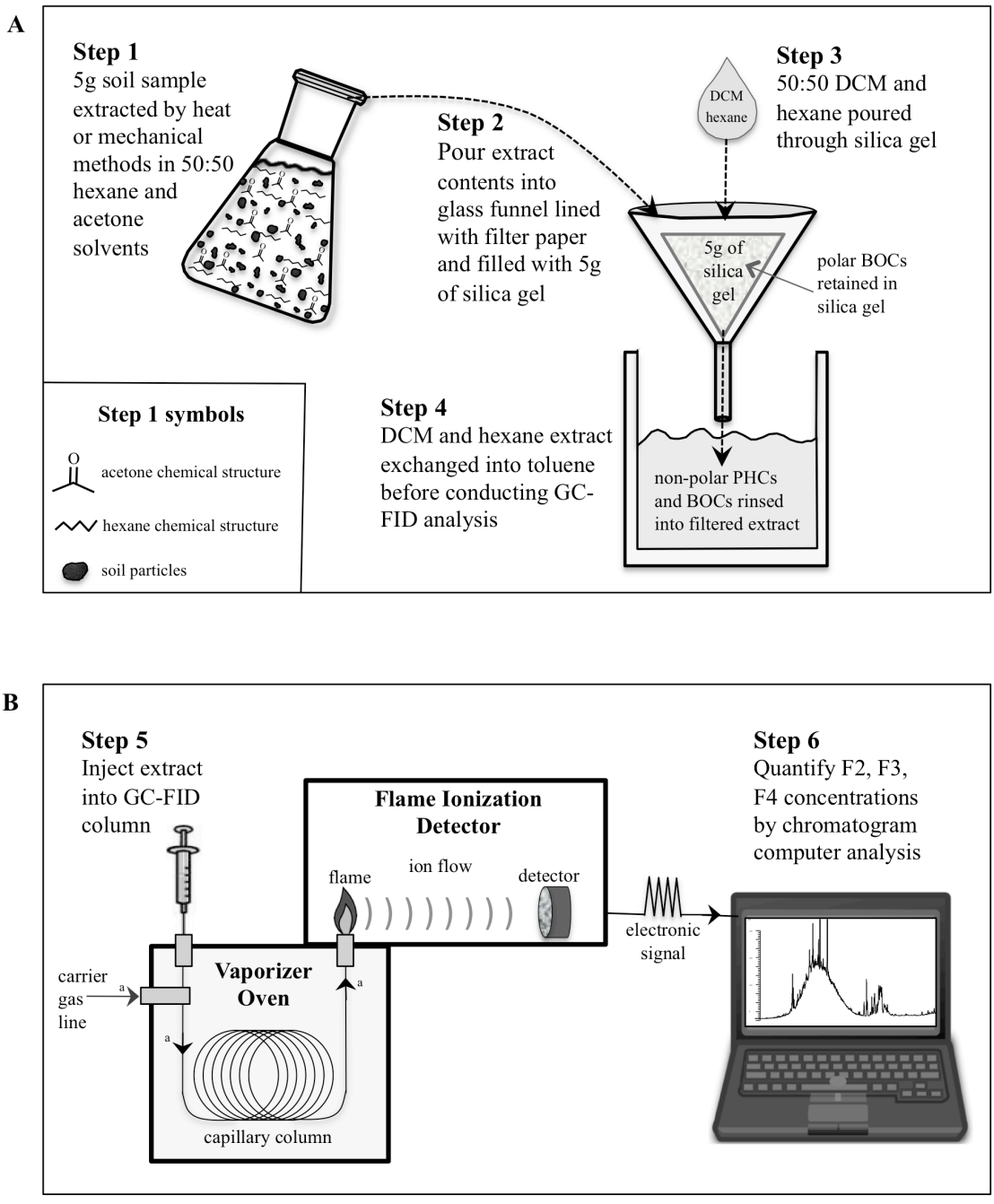

Figure 1.3. CWS PHC extraction and Gas Chromatography Flame-Ionization Detection (GC-FID) analysis. **(A)** Soil extraction and silica gel polar compound treatment. Dashed arrows represent poured extracts and solvents. DCM (dichloromethane). **(B)** GC-FID data analysis. [a]Arrows indicate directional flow of gas.

less non-polar PAHs are rinsed into the final PHC extract as well. A polar aromatic toluene (C_7H_8) holding solvent is added to the extract, which is then evaporated to remove hexane

and DCM prior to analysis.

Gas Chromatography Flame Ionization Detector Analysis

Gas Chromatography (GC) is a term that describes the group of analytical separation techniques used to quantify volatilized organic substances in the gas phase. Flame Ionization Detection (FID) non-selectively detects analytes by measuring an electrical current generated by electrons from burning carbon particles. Gas Chromatography Flame Ionization Detectors (GC-FID) combine both technologies to quantify F2, F3 and F4 concentrations in PHC soil sample extracts.

GC-FID instruments consist of an extract injection port, a mobile inert gas phase, a stationary phase contained within a heated separation column, a flame ionization detector and a data recording system (Figure 1.3B). The injected extract is vapourized into a gas, which is carried through a long coiled tube or "column" by a nonreactive carrier gas such as nitrogen or helium. The mobile phase is a chemically inert gas, which carries the extracted molecules through the column stationary phase. The column is internally coated with a polar material, such as poly(dimethylsiloxane), in order to minimize interactions of the non-polar extract with the column walls. The column is housed inside an oven, which gradually heats the extract so that the lightest and most volatile compounds elute from the column first, while the heaviest and least volatile compounds elute last. Eluted compounds enter the FID where they undergo combustion in a hydrogen/synthetic air flame. Voltage is applied across the flame and the resulting ion flow is detected as a current. The voltage from the detector is proportional to the number of molecules passing through the detector at certain times, which is used to calculate the carbon range compositions and concentrations

in the sample.

GC-FID Chromatograph Analysis

The GC-FID detector transmits signals to a computer system which analyzes and plots the signals as a chromatogram image (Figure 1.3B). Detector response (abundance) is plotted on the y-axis and retention time (minutes) on the x-axis (Figure 1.4). This provides a spectrum of peaks and unresolved complex mixture (UCM) patterns representing the compounds and their concentrations in a sample. PHC F2, F3 and F4 GC-FID analysis can use internal and/or external chemical standards which have retention times that are identical to C10, C16, C34 and C50 carbon numbers and corresponding F2, F3 and F4 carbon ranges. Known concentrations of internal standards can be spiked into each sample or external standards can be spiked into a reference blank for comparison to the entire group of extracts. A mathematical integration function is used to calculate the F2, F3 and F4 areas for each sample chromatogram, which are directly proportional to concentrations.

BOC Interferences in PHC Soil Analysis Samples

BOC interferences occur when non-petrogenic organic compounds are co-extracted from organic materials such as peat and compost. One cause of BOC interferences is that solvents used in the CWS PHC soil extraction and cleanup procedures inadvertently extract non-polar and partially polar BOC compounds as well. The second cause of BOC interferences pertains to silica gel oversaturation by high BOC concentrations, which are naturally present in peat soils and manure compost. Silica gel oversaturation issues can be correlated to total organic carbon (TOC) soil content, which is a measure of the amount of

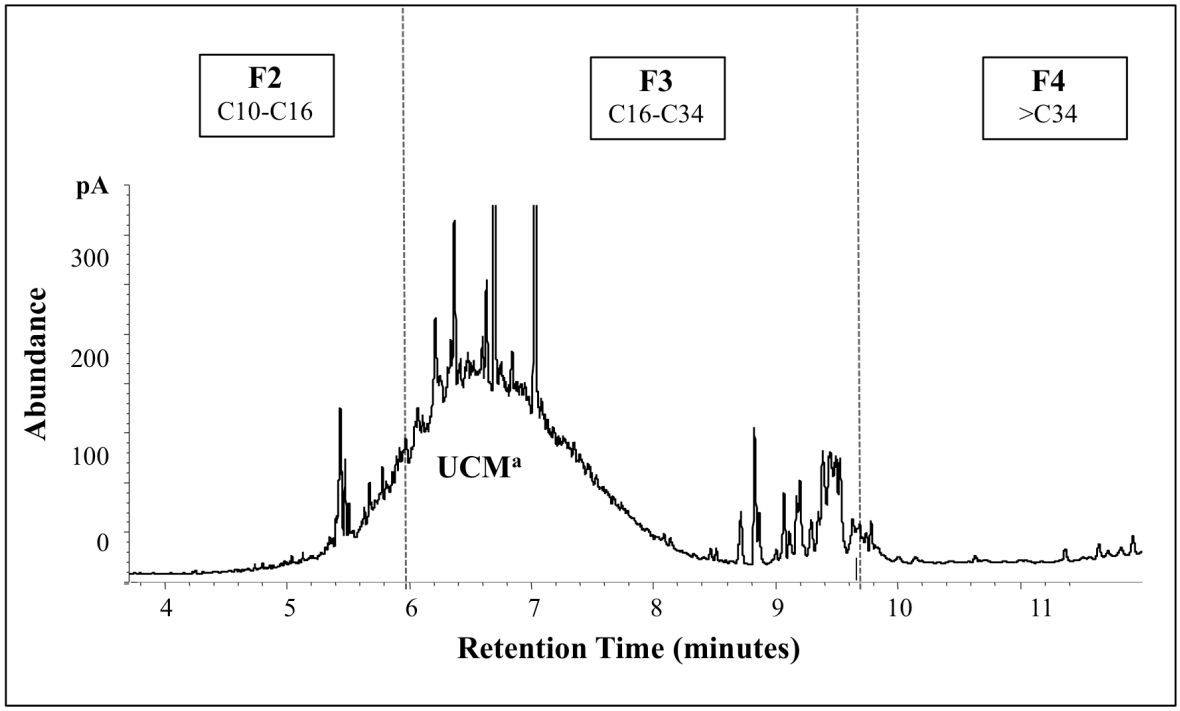

Figure 1.4. Example GC-FID chromatogram of CWS PHC F2, F3 and F4 carbon ranges; diesel spike manure compost. [a]Unresolved Complex Mixture, uniformly shaped UCMs are characteristic of PHC products.

bound organic carbon originating from living and decayed organisms. The CWS PHC polar cleanup standard was originally developed and validated for mineral and loam soils with less than 5% TOC. The CWS PHC standards allow a maximum 5g of silica gel to be used for the removal of polar biogenic compounds, which is generally suitable for soils with less than 5% TOC. However, organic peat soils and manure compost have much higher TOC levels of up to 60% (Szajdak et al. 2007; Li et al. 2004; Zhou et al. 2005) and 38% (Chapter 3), respectively. The standard amount of silica gel becomes saturated and cannot retain high BOC concentrations in peat soils and manure compost. Consequently, unretained BOCs pass through the silica gel and dissolve into the PHC extract. This results in elevated F3 and F4 concentrations, with elevated F2 concentrations possible as well. BOCs can cause false exceedances of the CWS PHC F3 soil quality guidelines (Chapters 2, 3 and 4). The issue of

background BOC interferences is especially significant when considering that peat soils cover approximately 1.1 million km^2 (12%) of the Canadian landscape (Kettles and Lacelle 2000; Tarnocai 2006).

Existing Solutions to Resolving BOC Interferences

- Background soil comparisons, Tier 2 quantitative approach

The CWS PHC Tier 2 background approach to resolving BOC interferences is to subtract F2, F3 and F4 concentrations in clean background soils from concentrations in contaminated soils. The Canada-wide Standard for Petroleum Hydrocarbons (PHC) in Soil, User Guidance document (CCME 2008c), states the following: ``*Certain soil samples, particularly soils with a high natural organic carbon content (such as peats) or soils that have been remediated with manure may give a 'false positive' result when analyzed. Specifically, laboratory results for F2, F3 and F4 may be falsely elevated. If there is reason to suspect that soils may have a high organic carbon content, it may be beneficial to collect samples for organic carbon content analysis from background soils, and to analyze background soils for PHC concentrations*. The Tier 2 background comparison approach is however limited for several reasons. In the best-case scenario, contaminated soil could be collected directly from the spill site, with clean soil samples easily accessed from the surrounding lands. However, natural variations in clean parent material, depth and hydrologic regimes can create wide ranges of F2, F3 and F4 concentrations (Alberta Environment 2010). In a worst-case scenario, comparable background soils would not exist in remediation facilities that treat contaminated soil mixtures delivered from many different locations over periods of years.

Existing Solutions to Resolving BOC Interferences

- Forensic Analysis, Tier 2 presence versus absence approach

Several existing forensic analytical techniques were first developed decades ago and are still used today for petroleum exploration and contaminated soil evaluation purposes (Hunt 1979; Volkman et al. 1992; Wang et al. 2012). The following analytes were used in this study to evaluate the presence versus absence of PHCs in selected samples: i) Petroleum biomarkers (e.g. steranes, hopanes, etc.) are environmentally persistent petrogenic organic compounds, whose chemical structures originally derive from ancient biological sources such as plants, algae and/or microorganisms. ii) Polycyclic Aromatic Hydrocarbons (PAHs) are associated with PHCs and consist of fused aromatic rings composed of carbon and hydrogen atoms. Alkylated and non-alkylated PAHs indicate presence versus absence of spilled petroleum products in a range of refined and unrefined forms. They are also used to identify pyrogenic PHCs originating from point or non-point atmospheric deposition sources. iii) Unresolved Complex Mixture (UCM) can be visually described as a "hump" (Figure 1.4), which appears on gas chromatograms between the solvent baseline and the resolved peaks baseline. The UCM appears because GC-FID analysis cannot resolve thousands of hydrocarbon compounds that are present in crude oil. PHC sources have visually pronounced and uniformly shaped UCMs, while non-contaminated BOC sources have relatively smaller and non-uniformly shaped UCMs. iv) Carbon Preference Index (CPI) refers to the ratio of odd carbon numbered *n*-alkanes to even carbon numbered *n*-alkanes in the C21-C34 carbon range. The CPI values for most PHC sources are close to the value of one (unity). In contrast, non-contaminated living and

decayed materials (e.g. tissues, waste, etc.) are dominated by odd *n*-alkanes, resulting in higher CPI values that may range from two to twelve. Mixtures of PHCs and BOCs may have a range of intermediate CPI values.

Petroleum biomarker and PAH (alkylated and non-alkylated) analysis are especially effective at identifying PHC spill sources as well as presence versus absence in soils. However, biomarker and alkylated PAH analysis requires highly specialized analytical expertise, testing equipment and methods that are not widely offered by commercial laboratories. Large-scale application of these forensic techniques can therefore be significantly limited by time and cost factors. Additionally, these methods cannot quantify authentic PHC concentrations in contaminated organic soils and/or compost.

Literature Review of Diesel and Crude Oil Weathering Patterns in Contaminated Soil

Natural weathering refers to biological, chemical and physical processes that change the composition, toxicity, availability and partitioning of PHCs after they have been released into the environment. PHC weathering processes in contaminated soils primarily include: volatilization, dissolution, adsorption, absorption, photo-oxidation and biodegradation (Brassington KJ and Hough RL 2007). Volatilization, dissolution, adsorption and absorption change PHC compositions by physically partitioning molecules into different phases. Photo-oxidation and biodegradation cause chemical changes to PHC molecules. Volatilization occurs when low molecular weight PHC molecules are converted from a liquid phase to a gas phase and are lost to the atmosphere (Labud et al. 2007). Dissolution occurs when water soluble PHC compounds dissolve into the water phase (Harayama et al. 1999; O'Reilly K and Thorsen W 2010). Low molecular weight aromatic

compounds are more soluble than low molecular weight aliphatic compounds. Water solubility generally declines with increasing molecular weight. PHCs that dissolve into the water phase of moist soils would be detectable by the CWS PHC soil extraction standards. However, PHCs that dissolve into the overlying water layer would only be detected by water analysis methods. A Canadian method has not yet been approved for F2-F4 analysis of water samples. However, analysis of petroleum hydrocarbons (PHCs) in ground water must be conducted in accordance with the CWS PHC soil standards (CCME 2008c). Adsorption occurs when PHC molecules attach to soil particle surfaces (Theng et al. 2001). Absorption occurs when PHC molecules enter into soil micropores (Chaîneau et al. 2000; Sanscartier et al. 2010). CWS PHC soil extraction solvents are designed to desorb PHCs from contaminated soil particles. Photo-oxidation involves the chemical breakdown of PHC molecules by sunlight (Dutta TK and Harayama S 2000; Prince et al. 2003). Aromatic components react to light and are therefore sensitive to photo-oxidation. Aliphatic components do not significantly absorb sunlight are therefore not sensitive to photo-oxidation. Biodegradation involves the chemical breakdown of PHC molecules by microorganisms (Dutta TK and Harayama S 2000; Prince et al. 2003). Photo-oxidized and biodegraded compounds that include carbon would be detected by the CWS PHC soils analysis standards.

Weathering rates are affected by environmental conditions and the chemical and physical characteristics of the PHC product. Optimal weathering occurs in aerobic soils with neutral pH and an ambient air temperature of 22°C (Dibble and Bartha 1979; Tarnocai 2006; Kroetsch et al. 2011). Volatilization, dissolution, adsorption and absorption processes begin as soon as the PHCs are exposed to the environment. The fastest and greatest

physical changes in freshly spilled diesel and crude oil are caused by the volatilization of light molecular weight compounds in the F2 (C10-C16) carbon range (Harayama et al. 1999). Diesel volatilizes at a faster rate than crude oil (Labud et al. 2007). Predicting volatilization in contaminated soils is complicated by several factors. For example, volatilization is highest in sand, which is highly porous, highly permeable and has a low density. In contrast, volatilization is lower in organic soils, which have lower porosity, lower permeability and higher density (Labud et al. 2007). Volatilization of PHCs is generally highest in surface soils as compared to sub-soils (Serrano et al. 2006).

Compounds that are not subject to rapid volatilization or photo-oxidization may undergo microbial biodegradation. Biodegradation rates are fastest for n-alkanes in the C10-C25 carbon range (Harayama et al. 1999). Longer n-alkane chains exist as hydrophic solids and consequently are difficult to degrade due to their poor water solubility and bioavailability. Branched chain alkanes and cycloalkanes also degrade at relatively slower rates. High molecular weight aromatic and cycloparaffinic structures are the most resistant to biodegradation. Diesel has a carbon range of C8 to C24 as compared to crude oils, which have a wider carbon range of C8 to greater than C50. The lighter carbon range in diesel allows for complete degradation within short periods of time. Crude oil is composed of heavier and more recalcitrant compounds. Consequently, diesel tends to degrade more completely and within shorter time periods as compared to crude oils (Fingas 1997). In both cases however, GC-FID chromatogram patterns show that resolved peaks are prominent in fresh diesel and crude oil. Resolved peaks are primarily composed of n-alkanes, which are the first components to degrade. Recalcitrant components are detected as the unresolved complex mixture (UCM) (Figure 1.4). This compositional change is

visually apparent in freshly contaminated versus weathered sample chromatograms. Fresh diesel and crude oil chromatograms have prominent resolved peaks, which originate from n-alkanes. In contrast, resolved peaks are virtually absent in highly weathered diesel and crude oil. The elimination of easily weathered n-alkanes in diesel and crude oil PHCs generate chromatogram patterns that are dominated by the UCM with a virtual absence of resolved peaks (Brassington et al. 2007; Killops and Al-Juboori 1990).

Research Objectives

The primary objective of this study was to determine if Tier 1 CWS PHC GC-FID chromatogram signature patterns could be used to develop a simple, cost-effective and accurate Tier 2 approach to resolving BOC interference issues in clean soils and manure compost. Standard PHC GC-FID chromatogram signature patterns for F2 (C10-C16), F3 (C16-C34) and F4 (>C34) and new sub-fractions F3a (C16-C22) and F3b (C22-C34) were used to develop this method. Chapters 2, 3 and 4 demonstrate that F3a and F3b signature patterns were identified in the clean soils and compost versus the PHC spiked soils and compost. The F2:F3b PHC presence versus absence ratio and the BOC-adjusted F3 PHC calculation were developed during this study. The scientific basis for the application of the F2:F3b PHC presence versus absence ratio and the BOC-adjusted F3 PHC Tier 2 approach was developed using PHC GC-FID chromatogram signature patterns for two different PHC contamination experiments, in addition to a survey of different crude oil types and a survey of background soils from various regions in Canada. Chapter 2 presents the results of a 300-day microcosm experiment, in which peat and sand soils were spiked with fresh Federated crude oil, bacteria and nutrients and analyzed for PHCs on days 0, 150 and 300.

The chemical analyses of 14 light to heavy crude oils are presented in Chapter 2. Chapter 3 presents the results of a 300-day microcosm experiment, in which manure compost and sand soils were spiked with diesel drilling waste, bacteria and nutrients and analyzed for PHCs on days 0, 150 and 300. Chapter 4 presents the results of a Canadian background soil survey. Each chapter demonstrates how GC-FID chromatogram PHC and BOC signature patterns and carbon ranges were used to mathematically resolve falsely elevated PHC concentrations and soil quality guideline exceedances in peat soils and manure compost.

CHAPTER 2

PHC analysis of peat soil spiked with crude oil: A new mathematical GC-FID approach to resolving false detections of petroleum hydrocarbons in clean peat soils

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OVERVIEW

The Canadian Council of Ministers of the Environment (CCME) *Reference Method for the Canada-Wide Standard (CWS) for Petroleum Hydrocarbons (PHC) in Soil* provides standardized and analytical procedures measuring PHC fractions in soil for site remediation purposes. However, the CWS PHC chemistry analytical methods inadvertently co-extract natural biogenic organic compounds (BOCs), (e.g. waxes, fatty acids, sterols, etc.), which enrich organic soils such as peat. Co-extracted BOCs are misidentified as PHCs, which can result in false exceedances of the Tier 1 PHC F3 soil quality guidelines. This microcosm experiment used CWS PHC Tier 1 soil extraction and Gas Chromatography Flame Ionization Detector (GC-FID) chromatogram analysis to develop a new Tier 2 mathematical approach to resolving the problem of false PHC detections in clean peat soils. This 300-day microcosm experiment compared carbon range fractions F2 (C10-C16), F3 (C16-C34), F4 (>C34) and sub-fractions F3a (C16-C22) and F3b (C22-C34) in Federated crude oil spiked peat, spiked sand, clean peat and clean sand. These carbon ranges were studied in 14 light to heavy crude oils as well. This new Tier 2 approach uses two calculations, referred to as the F2:F3b ratio and the BOC-adjusted PHC F3 calculation. The experimental results determined the following: i) the F2:F3b ratio threshold of 0.10 indicated PHC absence (<0.10) in clean peat and PHC presence ($\geq$0.10) in crude oil spiked peat and spiked sand; and ii) the BOC-adjusted PHC F3 calculation provided accurate estimates of authentic PHC concentrations and soil quality guideline exceedances in clean and spiked peat. Adoption of this new Tier 2 approache could minimize unnecessary ecological disruptions of thousands of peatlands throughout Canada, while also saving millions of dollars in unnecessary site remediation costs. Although this approach

specifically addresses Canadian standards, it is relevant to other countries that face the problem of false PHC detections in clean organic soils as well.

INTRODUCTION

Conventional crude oil is a naturally occurring hydrocarbon-based liquid that is formed over millions of years from the buried remains of plants, animals and microbes (Hunt 1979). Canada is the seventh largest producer of conventional crude oil in the world (NEB 2011). Approximately 2 million m^3 (14.5 million barrels) of conventional crude oil were produced in Canada during the period of 1998 to 2011 (NEB 2011). Crude oil is pumped from underground reserves and transported by ships, trucks and/or pipelines to oil refineries where it is converted into a wide range of products (i.e., heating and transportation fuels, motor oils, asphalt, etc.). Although spill prevention is a key component of crude oil exploration, extraction and transportation activities, spills can potentially occur during any of these activities. Causes of crude oil spills include drilling-well blowouts, shipping/trucking accidents and pipeline failure. Pipeline corrosion can cause spills, with and estimated pipeline failure rate of 1.7 releases per 1,000 km of pipeline. In Canada, the 36,033-km pipeline network extends across a wide variety of soil types. The majority of the oil pipeline (50% or 18,125 km) extends through Alberta (CAPP 2009), with 20% or 132,370 km^2 of the total land area composed of peat soils (Alberta Environment 2003). The Canada Oil and Gas Operations Act requires that reasonable measures be taken to stop spills and to repair or remedy any resulting conditions that pose risks to life, health, property and/or the environment (Department of Justice Canada 2012).

The Canadian Council of Ministers of the Environment (CCME) *Reference Method for the Canada-Wide Standard (CWS) for Petroleum Hydrocarbons in Soil* (CCME 2001a) is based on a risk assessment approach to site remediation. The standards can be applied at three risk assessment levels or "Tiers". Tier 1 is based on generic numerical standards corresponding to four land uses (Chapter 1, Table 1.1). Exceedances of the Tier 1 soil guidelines may lead to detailed site-specific evaluations at the Tier 2 and/or Tier 3 levels. The research presented in this study supports the use of a new Tier 2 approach to resolving false exceedences of the Tier 1 soil quality guidelines

The CWS PHC soil standards are organized into the following fractions with the associated equivalent carbon numbers in brackets: F1 (C6-C10), F2 (C10-C16), F3 (C16-C34) and F4 (>C34). F1 comprises the aliphatic and volatile aromatic PHCs. F2 comprises primarily the semi-volatile PHCs. Both F2 and F3 fractions contain aromatic and aliphatic hydrocarbons. The F4 fraction has low aromaticity and contains small amounts of polar nitrogen, sulfur and oxygen heteroatoms.

The Tier 1 CWS for PHC in soil quality guidelines apply to the F1, F2, F3 and F4 carbon ranges (Chapter 1, Table 1.1). These generic soil guidelines are based on risk management of environmental and human health exposures to PHC concentrations for each fraction. Tier 1 risk management considers site-specific conditions including: land use, mineral soil texture and proximity to potable groundwater. However, organic peat soils do not fit either the fine or coarse soil categories because they have very low mineral content. Regulatory discretion is used in the selection of the most appropriate soil guidelines for subsequent tiers where site-specific factors are taken into consideration. For example, the most stringent coarse soil guideline may be applied to contaminated peat sites located in

higher risk areas with potable drinking water and/or nearby surface water systems that would allow off-site migration of PHC contaminants.

The CCME provides a reference analytical method for generating accurate and reproducible PHC soil chemistry results (CCME 2001a). Detailed descriptions of these methods are discussed in Chapter 1. Briefly, the CWS PHC Tier 1 analytical method uses hexane, acetone and dichloromethane (DCM) solvents for the PHC extraction process. However, these solvents inadvertently co-extract biogenic organic compounds (BOCs) originating from fresh and decayed plant, animal and microbial soil matter.

The CWS PHC solution to BOC interferences is to analyze comparable clean background soils and to subtract those concentrations from the contaminated soil concentrations. This approach can however be problematic where natural variations in background soil parent materials, depths and hydrologic regimes produce variable PHC results (Alberta Environment 2010). For example, remediation facilities may mix contaminated soils from multiple locations. Comparable background soils would not exist for this type of scenario.

Introduction Chapter 1 provides a detailed discussion of how PHC forensic analysis methods (e.g. biomarkers, alkylated PAHs, etc.) can resolve BOC interference issues by confirming PHC absence in soils (Wang and Stout 2007). Although biomarker and PAH analysis provide excellent tools for determining PHC presence versus absence, they require highly specialized chemistry analysis expertise and materials that can be cost-prohibitive and time consuming for large-scale applications. Although UCM analysis is also a valuable tool, it is limited for the reasons that it depends on the subjective opinions of chemistry professionals regarding the visual characteristics of UCM chromatogram patterns.

In this study, CWS PHC Tier 1 Gas Chromatography - Flame Ionization Detector (GC-FID) chromatograms were used to mathematically identify false PHC detections in clean peat and to estimate authentic PHC concentrations in contaminated soils on a Tier 2 basis. This new approach requires one additional integration step to quantify concentrations and percentages of sub-fractions F3a (C16-C22) and F3b (C22-C34). The F3a and F3b data were used to calculate BOC-adjusted PHC F3 concentrations in peat samples. In addition, F2:F3b ratios of less than 0.10 indicated PHC absence in clean peat and ratios of $\geq$0.10 indicated PHC presence in crude oil spiked peat and sand. This approach was developed through the results of a 300-day crude oil contaminated peat and sand microcosm experiment. F2, F3, F4, F3a and F3b concentrations and GC-FID chromatogram patterns were recorded for Day 0, Day 150 and Day 300 microcosm samples, in addition to a survey of 14 light to heavy crude oils as well. These data provided the basis for this new Tier 2 mathematical approach to resolving false PHC F3 detections and soil quality guideline exceedances in clean peat soils.

MATERIALS AND METHODS

Microcosm experiment design

The microcosm study was conducted indoors at the ALS Environmental laboratory, located in Waterloo, Ontario, Canada. The microcosms were designed to simulate saturated peatland conditions and to promote weathering and biodegradation of peat spiked with fresh crude oil. The microcosms consisted of 70 L rectangular (30 cm x 35 cm x 66 cm) glass tanks fitted with full spectrum lighting to simulate 24-hour sunlight exposure. Each tank held soil depths of approximately 15 cm with a 2 cm overlay of deionized water that

was maintained throughout the experiment. Aerobic conditions were maintained by placing 5 cm long aeration stones under the substrate in each tank. The laboratory facility air temperature was maintained at 22°C. These environmental conditions were maintained for 24 hours per day for the entire 300-day experiment period.

Microcosm soil types

The soils used in the microcosm experiment included silica sand as a control and two sources of peat, which are described as follows: i) processed peat (P1) purchased from a commercial landscape supplier (collected from a bog located in northern Ontario, Canada); ii) natural peat (P2) collected from a fen in Lakeland Provincial Park, located in northern Alberta. The natural peat was collected along with the overlying vegetation layer, stored on ice in coolers and shipped to Waterloo where the vegetation was removed and temporarily held in glass tanks.

The sand and peat soils were manually homogenized prior to submitting samples to the Environment Canada Oil Spill Research Laboratory (Ottawa, Ontario, Canada) for baseline forensic analysis. The following forensic tools were used to identify the presence versus absence of PHCs in the peat and sand (Wang et al. 2007; Wang et al. 2012): i) petroleum biomarkers; ii) polycyclic aromatic hydrocarbons (PAHs); iii) unresolved complex mixture (UCM); and iv) carbon preference index (CPI). The forensic analysis results for this study indicated the absence of liquid PHCs. It did however indicate the presence of trace pyrogenic PAHs (non-alkylated) in the peat samples, likely originating from atmospheric deposition.

The processed peat (P1) and natural peat (P2) had high TOC levels of 45.0% and 35.2% by weight, respectively. The natural peat had a neutral pH of 6.7. In contrast, the processed peat (P2) had an acidic pH of 4.2, which was below the optimal microbial biodegradation range of 4.5-7.5 (Dutta and Harayam 2000; Kroetsch et al. 2011). Calcium carbonate was added to the processed peat, to increase the pH to a neutral value of 7.0.

Silica sand was used to monitor crude oil degradation in the absence of detectable organic matter. The sand, purchased from Anachemia Science (Richmond, British Columbia), was pre-washed with deionized water and dried. The sand had a neutral pH of 7.5, 0.0% TOC by weight and non-detectable bacteria levels (<10,000 colony forming units (CFU)/g). Nutrient levels were non-detectable for total phosphorus (<50 mg/kg), nitrate (<1.0 mg/kg), nitrite (<1.0 mg/kg) and potassium (<100 mg/kg). On Day 0, nutrients and bacteria were added to the sand treatments at similar concentrations that were detected in the natural peat. The purpose of these amendments was to promote similar crude oil degradation processes between the inorganic sand and the organic natural peat. Potassium phosphate and sodium nitrate were added to the sand at concentrations of 950 mg/kg, phosphorus, 140 mg/kg potassium and 9.2 mg/kg nitrate. Nitrite was non-detectable in the natural peat and was therefore not added to the sand. Bacteria from the natural peat was cultured in inorganic agar broth by GAP Laboratories (London, Ontario, Canada), concentrated and added to selected sand microcosms at concentrations of 4.6×10^7 CFU/g, similar to the total aerobic plate count of the natural peat (3.7×10^7 CFU/g). Gram-negative, aerobic *Burkholderia sp*. was identified as the dominant bacteria in the natural peat, based on a heterotrophic plate count.

Federated crude oil description and microcosm soil spiking procedures

Fresh whole Federated crude oil was shipped from Alberta by Imperial Oil. The crude oil was stored in amber glass bottles with Teflon lined lids and kept at 4°C prior to use in the microcosm study. Federated crude was the same oil used to generate the CWS PHC Tier 1 soil quality guidelines (CCME 2008c). Federated crude is a light, sweet oil with a sulfur content of 0.34%. Density and viscosity at 15°C are 0.8298 g/mL and 5 cP, respectively. Pour point and flash point are -22°C and -26°C, respectively.

The following protocol was used to spike the highly contaminated processed peat (sP1), moderately spiked natural peat (sP2) and the moderately spiked sand (sS). Soil moisture content was first determined to calculate the desired concentration as mg of crude oil per kg dry weight of soil. Soil was evenly spread onto 60 cm x 30 cm aluminum pans at a depth of 4 cm. A glass syringe was used to uniformly spread whole crude oil across the soil surface. The spiked soil was then transferred to an aluminum bowl and homogenized with an electric mixer. In order to ensure consistency, all of the spiked soils and clean soils were stored in food-grade plastic bags at -20°C prior to the start of the experiment. Upon completion of the three-week soil spiking procedures, all of the frozen spiked soils and clean soils were thawed and placed into the microcosm tanks on the same day.

Microcosm Soil Treatments

The microcosm experiment consisted of 7 treatments conducted in triplicate. The treatments are described as follows: **C** - Control, untreated silica sand; S**1** - silica sand amended with bacteria and nutrients; **sS** - silica sand amended with bacteria and nutrients and spiked with a moderate nominal concentration of 2,942 mg/kg F2-F4 (1,300 mg/kg F3)

whole crude oil; **P1** – clean processed peat; **sP1** – processed peat spiked with a high nominal concentration of 19,608 mg/kg F2-F4 (10,000 mg/kg F3) whole crude oil; **P2** – clean natural peat; and **sP2** – natural peat spiked with a moderate nominal concentration of 2,942 mg/kg F2-F4 (1,300 mg/kg F3) whole crude oil. Overlying moss (*Drepanocladus aduncus*) and herbaceous plants originally harvested with the natural peat, were re-planted in treatments P2 and sP2. The sand treatments were used to monitor PHC levels in the absence of BOCs. Peat soils were contaminated with moderate and high crude oil concentrations to document false and authentic F3 CWS PHC F3 guideline exceedances on Day 150 and Day 300.

Microcosm Monitoring and Sampling Procedures

Soil samples were collected from the microcosm tanks on a monthly basis. Aluminum spoons were used to collect a 300 ml full-depth soil sample from the centre of each tank. The remaining soils left in each tank were then manually homogenized and left undisturbed until the next sampling period. Each sample was homogenized with an electric mixer and placed into amber glass jars with Teflon lined lids. All of the soil samples were stored at -20°C prior to PHC analysis.

Conductivity, pH and redox measurements were recorded at the time of sampling. A soil slurry was produced by measuring a 1:2 ratio of soil to deionized water for measurement of these parameters. The pH, conductivity and redox levels remained relatively constant during the entire 300-day study period. The pH levels were all within the neutral range of 6.5 to 8.5. Conductivity ranged from 0.293 to 0.628 dS/m, which is

considered to be an ecologically acceptable range (Alberta Environment 2010). The redox levels ranged from aerobic levels of +88 to +154 mV.

F2, F3, F4 PHC Soil Extraction and Analysis

The F2-F4 PHC soil extractions and GC-FID runs were conducted by ALS Environmental using materials and methods that were in compliance with the CCME Reference Method for the *Canada-Wide Standard (CWS) for Petroleum Hydrocarbons in Soil – Tier 1 Method* (CCME 2001a). All chromatograms were integrated by the author of this study. The lowest carbon number range, F1 (C6-C10), was not analyzed because the CCME user guidance document identifies biogenic interferences as occurring in the F2-F4 carbon range (CCME 2008c). All solvents and acids were trace/organic/pesticide grade and were purchased from Caledon Laboratories located in Georgetown, Ontario, Canada. Cleaning procedures are described as follows. Glassware and magnetic stir bars were washed with soap and hot water and rinsed with a 25% hydrochloric acid solution, followed by deionized water and oven dried at approximately 110 $^{\circ}$C ± for 1-2 hours. The Soxtec extraction filter cups were soaked in a beaker of DCM for four hours, removed from the beaker and dried in a fume hood.

Soxtec soil extraction quality assurance measures included one method blank and one duplicate sample for each group of twenty extracted samples or less. The acceptable method blank F2-F4 concentrations were <10 mg/kg F2, <50 mg/kg F3 and <50 mg/kg F4. The duplicate data quality objectives were <50% relative percent difference. A 10 g (wet weight) soil sample was mixed with a celite drying agent and then placed into a filter cup. All samples were spiked with the analytical surrogate o-terphenyl (2000 µg/mL in acetone;

Supelco, Mississauga, Ontario, Canada) to evaluate the extraction recovery objective of 60-120%. The sample in the spiked filter cup was packed into a Soxtec extraction thimble and placed onto an automated Soxtec extraction instrument. The thimble was submersed into a glass Soxtec cup, which held a 50:50 hexane:acetone solvent mixture and was boiled for a period of two hours.

The in-situ silica gel treatment, for the removal of polar BOCs, is described as follows. The Soxtec cup, which held the boiled soil extract, was placed into a fume hood. The 50:50 hexane and acetone extract was mixed with de-ionized water for acetone removal (5 times the volume of acetone used in the extraction) and the top hexane layer was decanted into a glass flask. DCM was added to the decanted hexane at a 50:50 ratio and was mixed with 5 g of silica gel for five minutes by a magnetic stir bar to remove polar BOCs. The DCM, hexane and silica gel mixture was poured through a Teflon funnel lined with filter paper (pre-rinsed with acetone and hexane) to physically separate the silica gel from the solvents. Toluene holding solvent was added to the beaker containing the solvents, which was placed onto a rotary evaporator in order to exchange the DCM and hexane to toluene. The evaporated 10 mL final extract was then transferred to a glass vial for GC-FID analysis.

F2-F4 PHC GC-FID Analysis Procedures

The Agilent 6890Ns GC-FID was equipped with an on-column injector and a 0.32 mm x 0.1 µm x 30 m capillary 100% poly(dimethylsiloxane) column and a flame ionization detector. External calibration standards, Restek CWS PHC calibration mix of C10, C16 and C34, ATSM D5442 C12-C60 linearity standard, and Accustandard FTRPH

Calibration/Window Defining Standard were purchased from Chromspec, located in Brockville, Ontario, Canada. Calibration by linear external standard technique used the average response factors of nC10/nC16/nC34. A solution of pentacontane (nC50) was used as a retention time and response factor standard for the C10 to C50 hydrocarbons. A five-point calibration curve (10, 50, 100, 250 and 500 µg/mL) was generated at the beginning of each analytical batch. Method detection limits were as follows: 10 mg/kg F2, 50 mg/kg F3 and 50 mg/kg F4. An external standard was used to identify the C22 peak for distinguishing the F3a and F3b carbon ranges. All concentrations were reported on a dry weight basis.

Survey of 14 light to heavy fresh crude oils

F2, F3, F4, F3a and F3b percentages and F2:F3b ratios were analyzed in 14 light to heavy fresh crude oils. The following eight crude oil samples were provided by Imperial Oil: Federated, Rainbow, Peace Sour, Peace Sweet, Pembina, Syncrude, Cook Inlet and Cold Lake. The crude oil samples were diluted in toluene and analyzed by ALS in accordance with the previously described protocol for F2, F3, F4, F3a and F3b GC-FID analysis. The Environment Canada Oil Spill Research Laboratory provided F2, F3, F4, F3a and F3b data for the following six fresh crude oils: South Louisiana, Arabian Heavy, Troll, Maya, IFO-180 and Imperial Heavy. The methods used to analyze these six crude oils are described in Wang et al 2003.

Statistical analysis

The R statistical software package (RDCT 2011) was used to calculate balanced two-way analysis of variance (ANOVA) F-test *p*-values for significant differences between Day 0 and Day 300 BOC-adjusted PHC F3 concentrations and also for each of the F2, F3a,

F3b, F4 carbon ranges. The triplicate soil sample data sets were not large enough to estimate mean significant differences. The F2, F3a, F3b and F4 p-values are reported as compositional data, where each variable is calculated as one component of the total group. The BOC-adjusted PHC F3 p-values are also reported as compositional data. The BOC-adjusted PHC F3 compositional p-values were calculated as components of the F2, F4 and BOC-adjusted PHC F3 group. The Day 0 and Day 300 F2, F3a, F3b and F4 concentrations were treated as individual components of one group and were used to calculate the F2, F3a, F3b and F4 compositional p-values. When data are expressed in the compositional form, the data must be modified to apply standard statistical methods (Aitchison 1986). The statistical analysis of compositional data requires special treatment by transforming the data based on logratios (Ecozcue and Pawlowsky-Glahn 2011). In this study, the data were transformed using the logcentred transform expressed as:

$$z_i = \log(x_i/g(x_D)) \quad (i = 1, \ldots, D), \qquad \text{(Eqn. 2.1)}$$

where $g(x_D)$ is the geometric mean of the composition

The control (C) and clean sand (S) data were not included in the statistical analysis because the F2, F3 and F4 concentrations were less than the following respective method detection limits: 10, 50 and 50 mg/kg. The F2 concentrations in the clean peat (P1, P2) were below the 10 mg/kg detection limit and were therefore calculated as half the detection limit (5 mg/kg).

R software was also used to run quantile-quantile plots and Shapiro-Wilk normal distribution tests on F3a and F3b percentage distributions for the fourteen light to heavy crude oils.

RESULTS AND DISCUSSION

The clean and spiked peat and sand treatments were monitored for changes in the following analytes on Day 0, Day 150 and Day 300: CWS PHC F2, F3, F4 and F2-F4 concentrations; F3a and F3b percentages; F2:F3b ratios; and BOC-adjusted PHC F3 concentrations. Mean data for the triplicate samples are presented in summary Table 2.1. Individual sample data are presented in appendix Table A-1.

Changes in F2, F3, F4 and F2-F4 concentrations over time

Clean sand and crude oil spiked sand

The F2, F3 and F4 concentrations on Day 0, Day 150 and Day 300 were non-detectable in the clean control sand (C) and in the sand mixed with nutrients plus bacteria (S) (data not shown). However, the F2, F3 and F4 concentrations were detectable in all of the moderately spiked sand samples (sS). The greatest decreases of F2, F3, F4 and F2-F4 concentrations occurred in the moderately spiked sand, with the greatest reduction occurring in the F2 range (Figure 2.1). The Day 0 mean F2 concentration of 435 mg/kg decreased by 83% on Day 150 and 93% on Day 300. The Day 0 mean F3 concentration of 1,077 mg/kg decreased by 59% on Day 150 and 82% on Day 300. The Day 0 mean F4 concentration of 327 mg/kg decreased by 46% on Day 150 and 70% on Day 300. The Day

Table 2.1: Day 0, Day 150 and Day 300 mean results for clean peat and crude oil spiked peat and sand F2, F3, F4, F3a, F3b concentrations, F3a and F3b percentages, F2:F3b ratios, BOC-adjusted PHC F3

Sample Days/ Analytes	**P1** Clean Processed Peat	**P2** Clean Natural Peat	**sP1**[a] Highly Spiked Processed Peat	**sP2**[b] Moderately Spiked Natural Peat	**sS**[b] Moderately Spiked Sand + Bacteria & Nutrients
Day 0					
F2-F4 (mg/kg)	3,933±900	2,076±545	17,613±1,819	3,408±418	1,839±82
F2 (mg/kg)[c]	<10	<10	3,535±279**	610±35**	435±25**
F3 (mg/kg)[c]	1,921±378**	1,235±344*	9,793±1,039**	1,953±261**	1,077±51*
F4 (mg/kg)[c]	2,007±521	836±211	4,285±534*	844±162	327±9
F3a (mg/kg)[c]	96±19	99±25	3,819±626**	508±23*	506±23*
F3b (mg/kg)[c]	1,825±363**	1,136±323*	5,974±431**	1,445±251**	571±29*
F3a (% of F3)[c]	5±1%	8±1%	39±3%	26±3%	47±1%[b]
F3b (% of F3)[c]	95±1%	92±1%	61±3%	74±3%	53±1%[b]
F2/F3b ratio[d]	0.00±0.00	0.00±0.00	0.60±0.01	0.43±0.07	0.76±0.01
PHC F3 (mg/kg)[e]	190±41	208±52	8,229±1,352**	1,070±50*	NC
Day 150					
F2-F4 (mg/kg)	2,368±352	1,524±81	13,281±827	2,304±598	692±49
F2 (mg/kg)	<10	<10	2,207±280**	319±62**	72±11
F3 (mg/kg)	1,168±198*	882±73*	7,648±452**	1,140±459*	442±38*
F4 (mg/kg)	1,196±172	637±72	3,425±122*	845±332	177±12
F3a (mg/kg)	47±12	88±7	2,447±301**	160±79	168±18
F3b (mg/kg)	1,121±189*	794±66*	5,201±152**	980±381*	274±20
F3a (% of F3)	4±1%	10±0%	32±2%	14±1%	38±1%
F3b (% of F3)	96±1%	90±0%	68±2%	86±1%	62±1%
F2/F3b ratio	0.00±0.00	0.01±0.00	0.43±0.04	0.35±0.12	0.26±0.02
PHC F3 (mg/kg)[g]	134±31	232±19	6,524±793**	418±208*	NC
Day 300					
F2-F4 (mg/kg)	3,320±810	1,548±126	10,161±609	2,328±146	324±153
F2 (mg/kg)	<10	<10	1,339±130**	252±25**	29±7
F3 (mg/kg)	1,579±355**	831±55*	5,876±288**	1,295±109*	197±104
F4 (mg/kg)	1,736±461	711±74	2,945±217*	781±27	98±42
F3a (mg/kg)	95±25	50±4	1,528+72**	155±13	61±35
F3b (mg/kg)	1,484+338**	781±54*	4,348±226**	1,140±96*	136±69
F3a (% of F3)	6±1%	6±1%	26±1%	12±0%	31±2%
F3b (% of F3)	94±1%	94±1%	74±1%	88±0%	69±2%
F2/F3b ratio	0.00±0.00	0.00±0.00	0.31±0.01	0.22±0.02	0.25±0.11
PHC F3 (mg/kg)[h]	288±81	170±13	4,864±231**	501±42*	NC

[a]sP1 - whole crude oil nominal spike concentration: F2-F4 = 19,608 mg/kg; F2 = 6,608 mg/kg; F3 = 10,000 mg/kg; F4 3,000 mg/kg.

[b]sP2 and sS - whole crude oil nominal spike concentration: F2-F4 = 2,942 mg/kg; F2 = 1,000 mg/kg; F3 = 1,500 mg/kg; F4 450 mg/kg.

[c]F2 (C10-C16), F3 (C16-C34), F3a (C16-C22), F3b (C22-C34), F4 (>C34).

[d]Non-detectable F2 concentrations calculated as 5 mg/kg (half of the 10 mg/kg detection limit)

[e]BOC-adjusted PHC F3 concentrations; Formula 2.1.

[f]The 47%F3a:53%F3b ratio in the spiked Sand (sS1) was used as the crude oil source in the Day 0 Equation 2.2 calculations.

[g]The 38%F3a:62%F3b ratio in the spiked Sand (sS1) was used as the crude oil source in the Day 150 Equation 2.2 calculations.

[h]The 31%F3a:69%F3b ratio in the spiked Sand (sS1) was used as the crude oil source in the Day 300 Equation 2.2 calculations.

*One asterisk indicates F2, F3, F4 and/or calculated PHC F3 concentration exceeded CWS PHC coarse soil guidelines

**Two asterisks indicate F2, F3, F4 and/or calculated PHC F3 concentration exceeded CWS PHC fine and coarse soil guidelines.

Mean ± standard deviation (n=3); NC - PHC F3 not calculated for spiked sand; Values reported on dry weight basis.

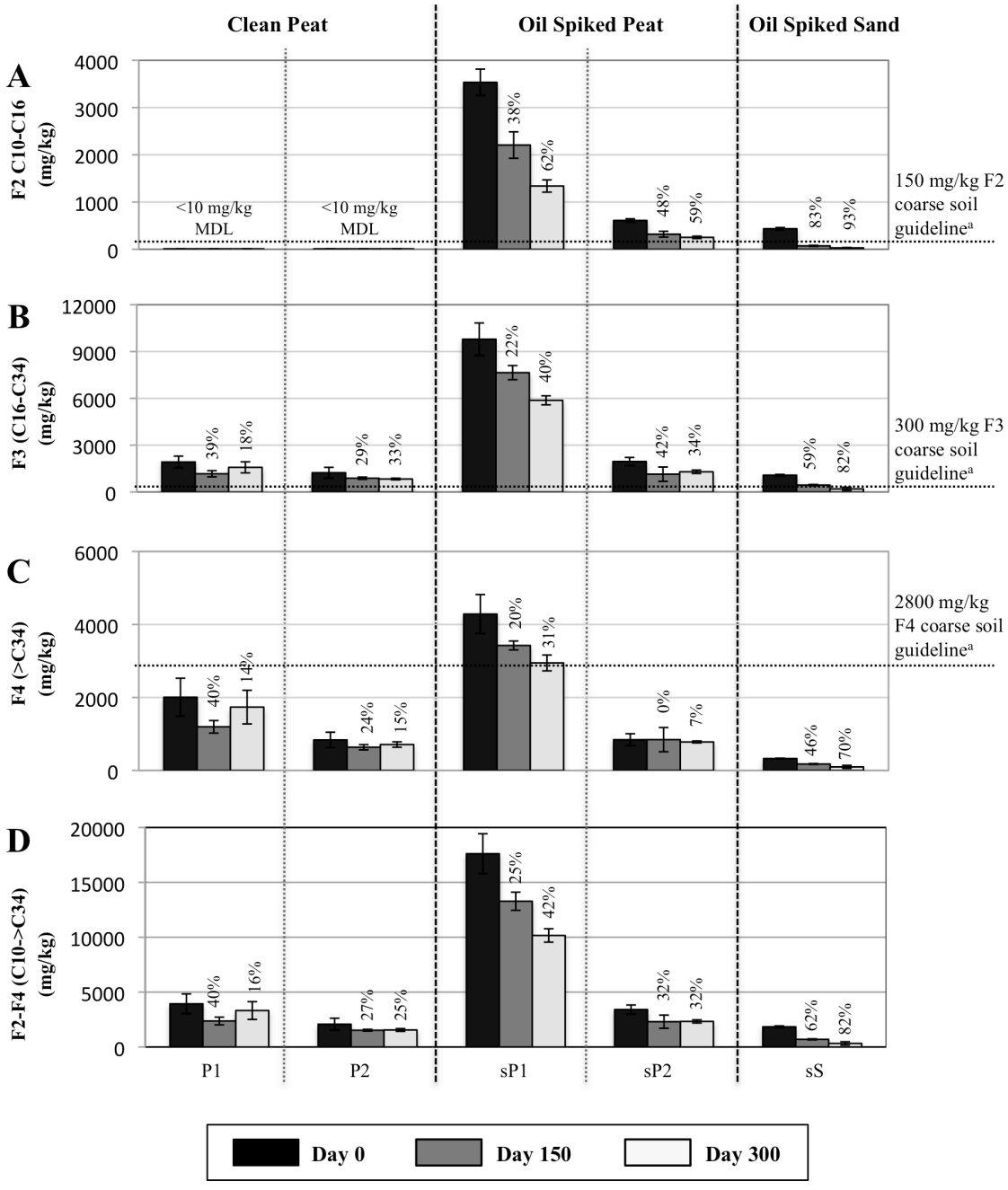

Figure 2.1. CWS PHC concentrations for F2 **(A)**; F3 **(B)**; F4 **(C);** and F2-F4 **(D)** in clean and crude oil spiked soils on Day 0, Day 150 and Day 300. Results are expressed as the mean of three replicate sample concentrations with standard deviation bars. Numbers above bars represent percentage decreases of mean concentrations on Day 150 and Day 300. [a]CWS PHC Tier 1 F3 coarse surface soil guideline for agricultural/residential/parkland land uses in potable groundwater conditions (CCME 2008a). MDL – method detection limit; P1 - clean processed peat; P2 - clean natural peat; sP1 - processed peat spiked with nominal 19,608 mg/kg F2-F4 crude oil; sP2 - natural peat spiked with nominal 2,942 mg/kg F2-F4 crude oil; sS - sand spiked with nominal 2,942 mg/kg F2-F4 crude oil.

0 mean F2-F4 concentration of 1,839 mg/kg decreased by 62% and 82% on Day 150 and Day 300 respectively. This was the largest F2-F4 decrease among all of the microcosms. To the best of the authors' knowledge, there are no published studies on CWS PHC F2, F3 and F4 degradation rates in crude oil spiked soils. However, Peressutti et al. (2003) reported TPH concentrations for a crude oil degradation study. In that study, sand was spiked once with a whole crude TPH concentration of 49,200 mg/kg, which degraded by a relatively smaller amount of 46% at the end of 390 days.

Clean peat and crude oil spiked peat

The F2 concentrations were less than the detection limit (10 mg/kg) in all of the clean processed peat (P1) and in the clean natural peat (P2) during the entire 300-day study (Figure 2.1). The Day 0 mean F3 concentration of 1,921 mg/kg in the processed peat was 39% on Day 150 and 18% lower on Day 300. The Day 0 mean F4 concentration of 2,007 mg/kg was 40% lower on Day 150 but 14% higher on Day 300. The Day 0 mean F2-F4 concentration of 3,933 mg/kg was 40% lower on Day 150, but 16% higher on Day 300. The Day 0 mean F3 concentration of 1,235 mg/kg in the clean natural peat (P2) was 29% lower on Day 150 and 33% lower on Day 300. The Day 0 mean F4 concentration of 836 mg/kg was 24% lower on Day 150 but 15% higher on Day 300. The Day 0 mean F2-F4 concentration of 2,076 mg/kg was 27% lower on Day 150 and 25% lower on Day 300. These decreases in the clean processed peat and clean natural peat may be attributed to the natural degradation of peat BOCs, which can occur under the optimal conditions of neutral pH, oxygenated water and 22°C ambient air temperature (Dibble and Bartha 1979; Tarnocai 2006; Kroetsch et al. 2011).

The Day 0 mean F2 concentration of 3,535 mg/kg in the highly spiked peat (sP1) was 38% lower on Day 150 and 62% lower on Day 300. The Day 0 mean F3 concentration of 9,793 mg/kg was 22% lower on Day 150 and 40% lower on Day 300. The Day 0 mean F4 concentration of 4,285 mg/kg was 20% lower on Day 150 and 31% lower on Day 300. The Day 0 mean F2-F4 concentration of 17,613 mg/kg was 25% lower on Day 150 and 42% lower on Day 300.

The Day 0 mean F2 concentration of 610 mg/kg in the moderately spiked peat (sP2) was 48% lower on Day 150 and 59% lower on Day 300. The Day 0 mean F3 concentration of 1,953 mg/kg was 42% lower on Day 150 and 34% lower on Day 300. The Day 0 mean F4 concentration of 844 mg/kg was 0% lower on Day 150 and 7% lower on Day 300. The Day 0 mean F2-F4 concentration of 3,408 mg/kg was 32% lower on Day 150 and remained at 32% on Day 300.

The crude oil spiked sand (sS) had comparatively larger reductions in the F2, F3 and F4 concentrations as compared to the spiked peat (sP1, sP2). This may be attributed to adsorption and/or partitioning effects, which occur in highly organic soils such as peat (Chaîneau et al. 2000; Sanscartier et al. 2010). PHC molecules become trapped in organic soil particle micropores, which are too small to permit physical access by microorganisms. Reduced PHC availability in organic soils could result in lower biodegradation rates in peat relative to the sand treatments. A previous study observed similar reductions in the CWS PHC F2, F3 and F4 carbon ranges for weathered diesel in fertilizer amended sandy gravel soils (Sanscartier et al. 2010). The Day 0 mean F2, F3 and F4 concentrations were 940 mg/kg, 210 mg/kg and 24 mg/kg, respectively. After 110 days, the mean F2, F3 and F4 concentrations were reduced by 82%, 71% and 63%, respectively. It is also important to

note that partitioning of water soluble PHC compounds may have occurred as well (González et al. 2013).

Compliance with CWS PHC F2, F3 and F4 soil quality guidelines

The F2 concentrations were non-detectable in both the clean peat and clean sand (Figure 2.1), which indicated the absence of PHCs. Correspondingly, the presence of F2 in all of the spiked soils indicated the presence of crude oil PHCs in the volatile carbon range. F2 concentrations in all of the spiked soils exceeded the CWS PHC F2 soil guideline of 150 mg/kg, with the exception of the moderately spiked sand (sS), which degraded to levels below the guideline by Day 300. These data indicate that elevated F2 concentrations in the spiked peat and sand soils were indicators of authentic crude oil PHC contamination.

In contrast, the F3 concentrations in the clean processed peat and clean natural peat were 2x higher than the CWS PHC 300 mg/kg F3 coarse soil guideline on Day 0, Day 150 and Day 300. BOCs in the F3 range were clearly associated with peat soils. These data demonstrate that false exceedances of the CWS PHC soil guideline by clean peat soils occurred only in the F3 carbon range. All highly spiked and moderately spiked peat F3 concentrations exceeded the CWS PHC F3 soil guideline on Day 0, Day 150 and Day 300. In contrast, the moderately spiked sand exceeded the F3 guideline on Day 0 and Day 150, but the concentration had degraded to below the guideline by Day 300.

Although BOCs in the F4 range were detected in the clean processed and natural peat, they did not exceed the CWS PHC F4 soil guideline of 2,800 mg/kg. The moderately spiked sand and peat did not exceed the F4 guideline at any time. However, the highly

spiked processed peat exceeded the PHC F4 guideline on Day 0, Day 150 and Day 300. Exceedance of the F4 guideline was therefore an indicator of authentic PHC contamination.

F2, F3, F4, F3a and F3b GC-FID Chromatogram Patterns

GC-FID chromatograms were used to quantify F2, F3 and F4 concentrations in accordance with CWS PHC soil analysis methods (CCME 2001a). This study also used GC-FID chromatograms to visually distinguish clean peat from crude oil spiked peat and sand, in addition to monitoring pattern changes. Figure 2.2 illustrates examples of Day 0 and Day 300 GC-FID chromatograms for fresh Federated crude oil, clean peat (P1, P2), crude oil spiked peat (sP1, sP2) and crude oil spiked sand (sS). The directly injected fresh crude oil chromatogram (Fig. 2.2A) illustrates dominance of the F2 range and relatively equal F3a and F3b sub-fraction distributions. These same PHC patterns were clearly present in the Day 0 spiked sand (Fig. 2.2D), but they were not present in the Day 300 spiked sand due to extensive PHC degradation. In contrast, the crude oil PHC patterns were absent in the Day 0 and Day 300 clean peat chromatograms (Figs. 2.2B and E), with non-detectable F2 and a strong dominance of the F3b sub-fraction range. PHC and BOC patterns were present, to varying degrees, in all of the highly spiked peat (Fig. 2.2C) and moderately spiked peat (Fig. 2.2F) chromatograms. Although degradation reduced the crude oil PHC patterns in the Day 300 spiked peat chromatograms, the peat BOC patterns remained virtually unchanged during the entire study.

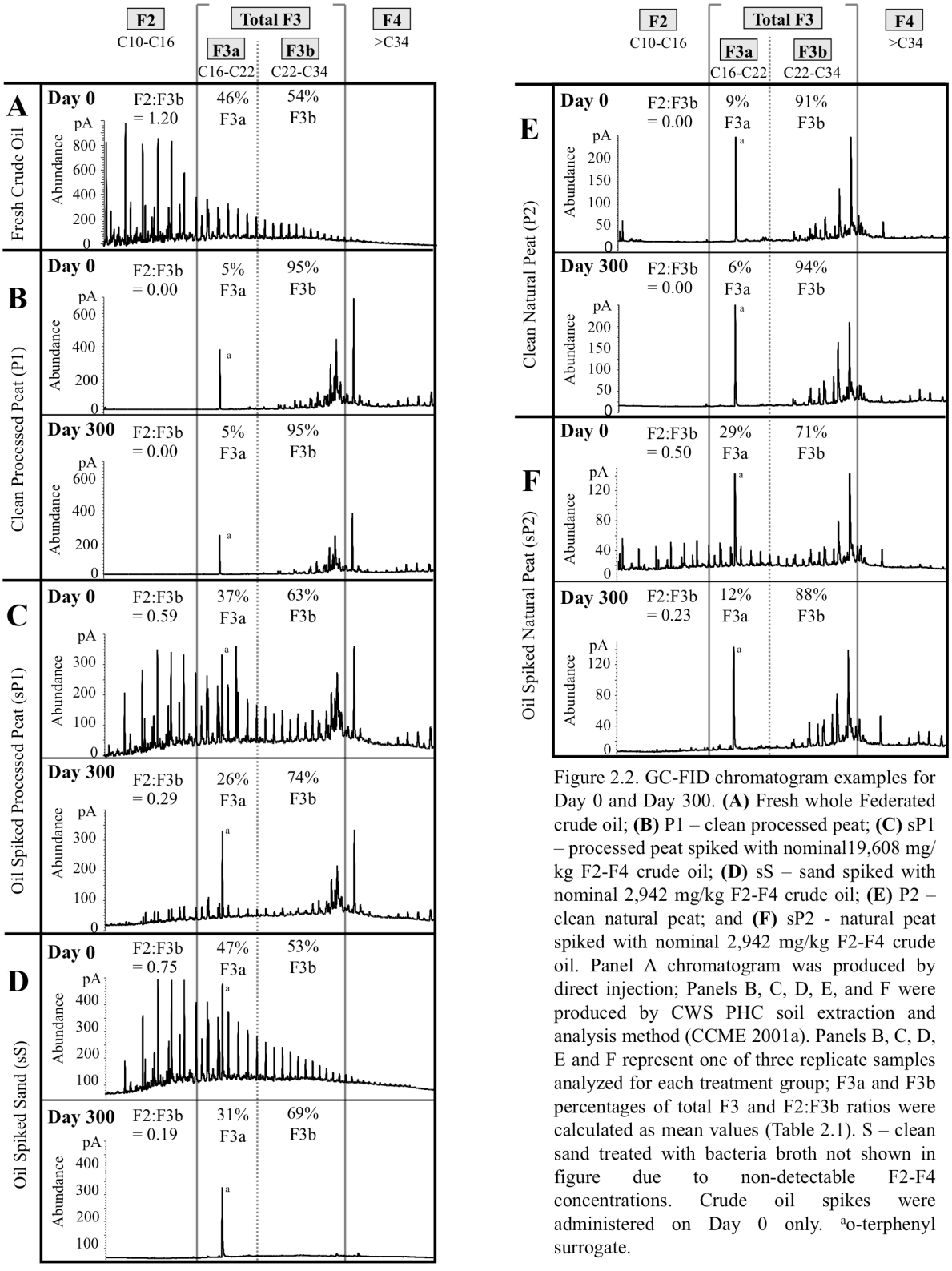

Figure 2.2. GC-FID chromatogram examples for Day 0 and Day 300. **(A)** Fresh whole Federated crude oil; **(B)** P1 – clean processed peat; **(C)** sP1 – processed peat spiked with nominal19,608 mg/kg F2-F4 crude oil; **(D)** sS – sand spiked with nominal 2,942 mg/kg F2-F4 crude oil; **(E)** P2 – clean natural peat; and **(F)** sP2 - natural peat spiked with nominal 2,942 mg/kg F2-F4 crude oil. Panel A chromatogram was produced by direct injection; Panels B, C, D, E, and F were produced by CWS PHC soil extraction and analysis method (CCME 2001a). Panels B, C, D, E and F represent one of three replicate samples analyzed for each treatment group; F3a and F3b percentages of total F3 and F2:F3b ratios were calculated as mean values (Table 2.1). S – clean sand treated with bacteria broth not shown in figure due to non-detectable F2-F4 concentrations. Crude oil spikes were administered on Day 0 only. [a]o-terphenyl surrogate.

The author is not aware of any other studies that have used F3a and F3b percentage distributions to distinguish PHCs from BOCs. However, Cermak et. al. (2010) investigated the toxicity of slightly different sub-fraction ranges F3a (C16-C23) and F3b (C23-C34) in fresh Federated crude oil to soil invertebrates and plants. The results determined that toxicities of crude oil F3a and F3b were not sufficiently different to recommend regulating hydrocarbons based on the two sub-fraction ranges.

Changes in F3a and F3b percentages over time

Figure 2.3 illustrates that the F3a and F3b percentages were relatively stable in the clean peat. The mean F3b percentages in the processed peat (P1) were 95%, 96% and 94% on Day 0, Day 150 and Day 300, respectively. The mean F3b percentages in the natural peat (P2) were 92%, 90% and 94% on Day 0, Day 150 and Day 300, respectively. In contrast, the F3b percentages in all of the spiked peat and sand treatments steadily decreased during the study period. The mean F3b percentages in the highly spiked peat (sP1) were 61%, 68% and 74% on Day 0, Day 150 and Day 300, respectively. The mean F3b percentages in the moderately spiked peat (sP2) were 74%, 86% and 88% on Day 0, Day 150 and Day 300, respectively. The mean F3b percentages in the moderately spiked sand (sS) were 53%, 62% and 69% on Day 0, Day 150 and Day 300, respectively. The distribution shifts toward the F3b range are partially attributed to the volatilization of PHC compounds in the F3a range (Killops and Al-Juboori 1990; Wang et al. 2011). They are also attributed to the combined short-term and long-term effects of photo-oxidation and biodegradation (Dutta and Harayam 2000; Prince et al. 2003; Wang et al. 2011).

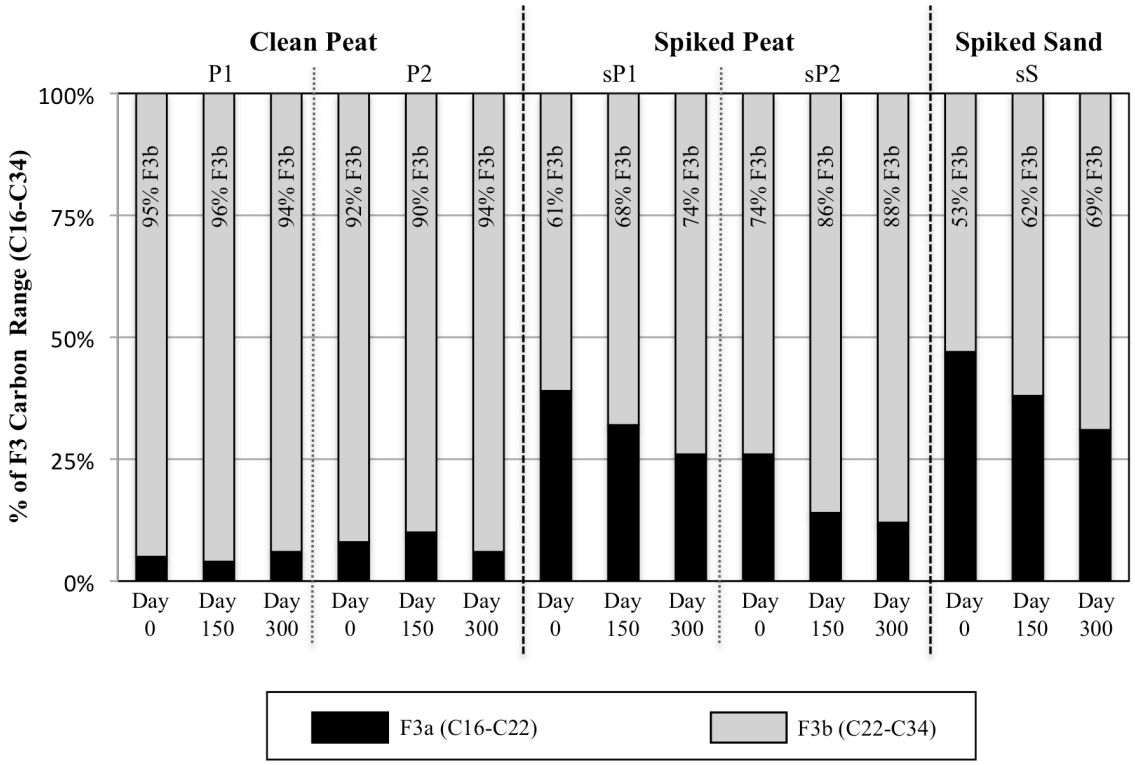

Figure 2.3. F3a (C16-C22) and F3b (C22-C34) percentage distributions of the F3 carbon range (C16-C34) on Day 0, Day 150 and Day 300. Results are expressed as the mean of three replicate sample concentrations. P1- clean processed peat; P2 - clean natural peat; sP1 - processed peat spiked with nominal19,608 mg/kg F2-F4 crude oil; sP2 - natural peat spiked with nominal 2,942 mg/kg F2-F4 crude oil; sS - sand spiked with nominal 2,942 mg/kg F2-F4 crude oil.

BOC-adjusted PHC F3 Calculation Description and Rationale

The BOC-adjusted PHC F3 concentration of a contaminated soil sample is defined as the sum of the measured PHC F3a concentration plus the calculated PHC F3b concentration (Formula 2.1). Formula 2.1 was used to calculate the PHC F3b concentration and the BOC-adjusted PHC F3 concentrations for the Day 0, Day 150 and Day 300 sampling periods. Please note that the following section of this chapter explains the rationale for using the crude oil spiked sand F3a and F3b percentages as the crude oil source in this calculation.

BOC-adjusted PHC F3 concentration (mg/kg) (Formula 2.1)

= measured F3a (mg/kg) + calculated F3b (mg/kg)

= a + (b/c x a)

a = measured F3a concentration in peat sample

b = measured %F3b of total F3 in crude oil spiked sand

c = measured %F3a of total F3 in crude oil spiked sand

This conservative approach is based on the premise that measured F3a concentrations can be used to estimate F3b concentrations in a contaminated soil sample, but only if the F3a and F3b percentages in the crude oil contamination source are known. For example, the chromatogram presented in Figure 2.4A illustrates that fresh crude oil had relatively equal percentages of F3a and F3b (46%:54%). In contrast however, the clean peat (Fig. 2.4C) was strongly dominated by the F3b range (95%). Peat spiked with Federated crude oil (Fig. 2.4B) had an F3b percentage of 61%. An example calculation is provided for crude oil spiked peat from the data presented in Figure 2.4A and B. While the measured F3 concentration was 10,591 mg/kg, the BOC-adjusted PHC F3 calculation determined that the authentic PHC F3 concentration from the crude oil spike was 9,464 mg/kg.

BOC-adjusted PHC F3 (mg/kg)

= measured F3a (mg/kg) + calculated F3b (mg/kg)

= 4,448 + (0.54/0.46 x 4,448)

= 9,464 mg/kg

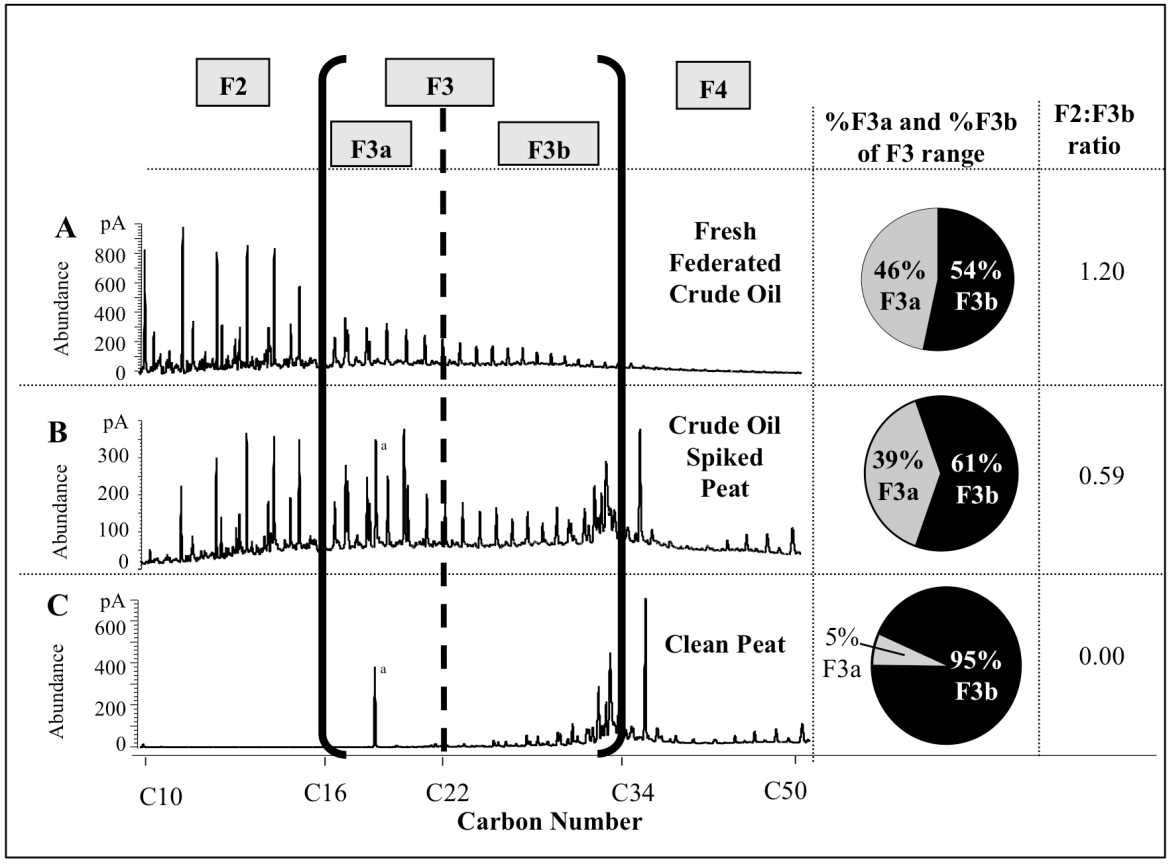

Fig. 2.4. Example GC-FID chromatograms of PHC F2 (C10-C16), F3 (C16-C34), F4 (>C34), sub-fractions F3a (C16-C22) and F3b (C22-C34); F3a and F3b percentage pie charts; and F2:F3b ratios. **(A)** Fresh crude oil; **(B)** Peat spiked with fresh whole Federated crude oil - nominal F3 =10,000 mg/kg, measured F2 = 3,656 mg/kg, measured F3a = 3,966 mg/kg, measured F3b = 6,204 mg/kg; and **(C)** Clean peat– measured F2 <10 mg/kg; measured F3a = 111 mg/kg; measured F3b = 2,221 mg/kg. The clean peat F2:F3b ratio calculation used an F2 concentration of 5 mg/kg, which was half of the F2 detection limit. [a]o-terphenyl surrogate.

Rationale for using spiked sand F3a and F3b percentages to calculate the BOC-adjusted PHC F3 concentrations in clean peat and crude oil spiked peat

Comparisons of the GC-FID chromatograms confirmed that there was a loss of the lightest F2 carbon range in the crude oil spiked sand (Fig. 2.2D), which did not occur in the directly injected fresh crude oil (Fig. 2.2A). The majority of this loss likely occurred by volatilization as the crude oil spiked sand was vigorously mixed under a fume hood during the contamination procedure. However, the spiked sand and spiked peat treatments were

prepared by identical contamination and mixing procedures and they were also exposed to identical environmental conditions and extraction methods. Therefore, the Day 0, Day 150 and Day 300 spiked sand provided the best tool for quantifying the fresh and degraded crude oil PHC patterns in the absence of detectable BOC interferences. The F3a and F3b percentages in the Day 0, Day 150 and Day 300 spiked sand were used as the representative crude oil sources for calculating the BOC-adjusted PHC F3 concentrations in the clean and spiked peat samples (Formula 2.1).

BOC-adjusted PHC F3 concentrations

All of the BOC-adjusted PHC F3 concentrations in the highly spiked peat (sP1) and moderately spiked peat (sP2) exceeded the CWS PHC F3 300 mg/kg coarse soil guideline (Figure 2.5). The total measured F3 concentrations in these spiked samples were therefore identified as authentically exceeding the CWS PHC F3 soil guideline. In contrast, the total measured F3 concentrations in the clean processed peat (P1) and clean natural peat (P2) exceeded the guideline, while the BOC-adjusted PHC F3 concentrations were below the guideline. The total F3 concentrations in these clean peat samples were identified as falsely exceeding the CWS PHC F3 soil guideline.

F2:F3b PHC presence versus absence ratios

The F2:F3b ratio is the measured F2 concentration divided by the measured F3b concentration. Low F2:F3b ratios of less than 0.10 indicated PHC absence in clean

peat, while high ratios of greater than or equal to 0.10 indicated PHC presence in

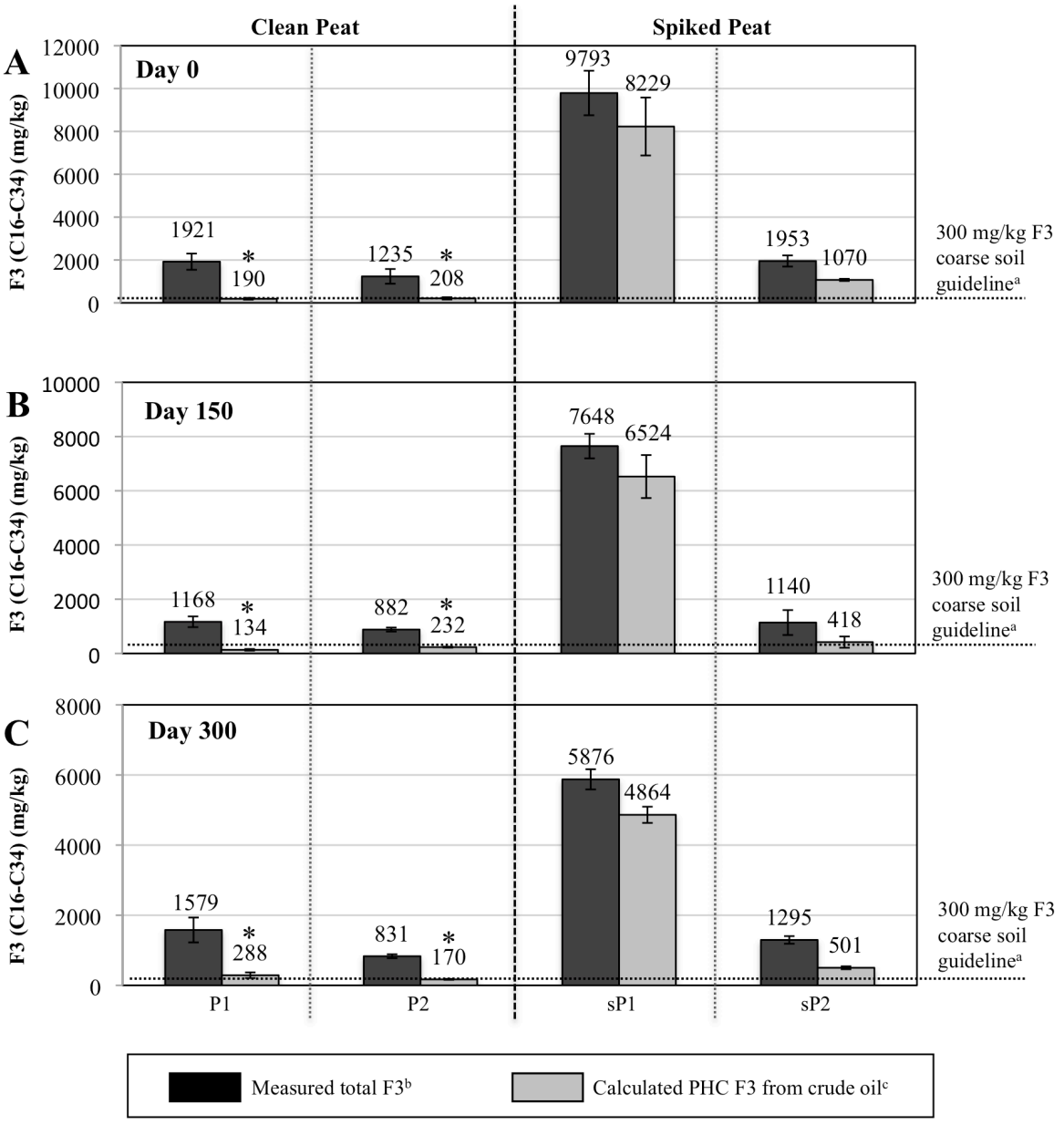

Fig. 2.5. Measured F3 and calculated BOC-adjusted PHC F3 concentrations in clean and crude oil spiked peat on: **(A)** Day 0; **(B)** Day 150; and **(C)** Day 300. Results are expressed as the mean of three replicate sample concentrations with standard deviation bars. [a]CWS PHC Tier 1 F3 coarse surface soil guideline for agricultural/residential/parkland land uses in potable groundwater conditions (CCME 2008a). [b]Total F3 measured by CCME CWS PHC method (CCME 2001a). [c]BOC-adjusted PHC F3 concentration from crude oil calculated by Formula 2.1. *Asterisk indicates concentrations that did not exceed the F3 soil guideline. P1 - clean commercial peat; P2 - clean natural peat; sP1 - commercial peat spiked with nominal 19,608 mg/kg F2-F4 (10,000 mg/kg F3) crude oil; sP2 - natural peat spiked with nominal 2,942 mg/kg F2-F4 (1,300 mg/kg F3) crude oil; sS - sand spiked with nominal 2,942 mg/kg F2-F4 (1,300 mg/kg F3) crude oil.

crude oil spiked peat and sand. The F2:F3b ratios in the clean peat samples were at least one order of magnitude lower than the ratios in all of the crude oil spiked peat and sand samples (Figure 2.6). All of the clean peat F2:F3b ratios were less than 0.10, while all of the crude oil spiked peat and sand samples had ratios of greater than 0.10. The F2:F3b ratio threshold value of 0.10 identified clean peat soils that had falsely exceeded the F3 soil guideline, while also identifying authentically contaminated peat and sand.

The Day 0, Day 150 and Day 300 mean F2:F3b ratios were 0.00 in all of the clean processed peat (P1), but ranged from 0.00 to 0.01 in the clean natural peat (P2). The Day 0, Day 150 and Day 300 mean F2:F3b ratios in the highly spiked peat (sP1) were 0.60, 0.43 and 0.31, respectively. The Day 0, Day 150 and Day 300 mean F2:F3b ratios in the moderately spiked peat (sP2) were 0.43, 0.35 and 0.22, respectively. The Day 0, Day 150 and Day 300 mean F2:F3b ratios in the moderately spiked sand (sS) were 0.76, 0.26 and 0.25, respectively.

The F2:F3b ratios were lowest in the Day 150 and Day 300 crude oil spiked sand, though three of the nine samples had F3 concentrations that did not exceed the 300 mg/kg soil guideline. These consistently low ratios occurred because the sand did not contain measurable natural organic matter, which is necessary to increase the F3b portion of the ratio. The most important points to be made from these data are: i) The F2:F3b ratios were greater than 0.10 in all crude oil spiked peat and spiked sand samples, regardless of F3 concentrations, TOC levels and/or weathering stages; and ii) All of the clean peat samples falsely exceeded the 300 mg/kg CWS PHC F3 soil guideline, but all of the F2:F3b ratios were less than 0.10.

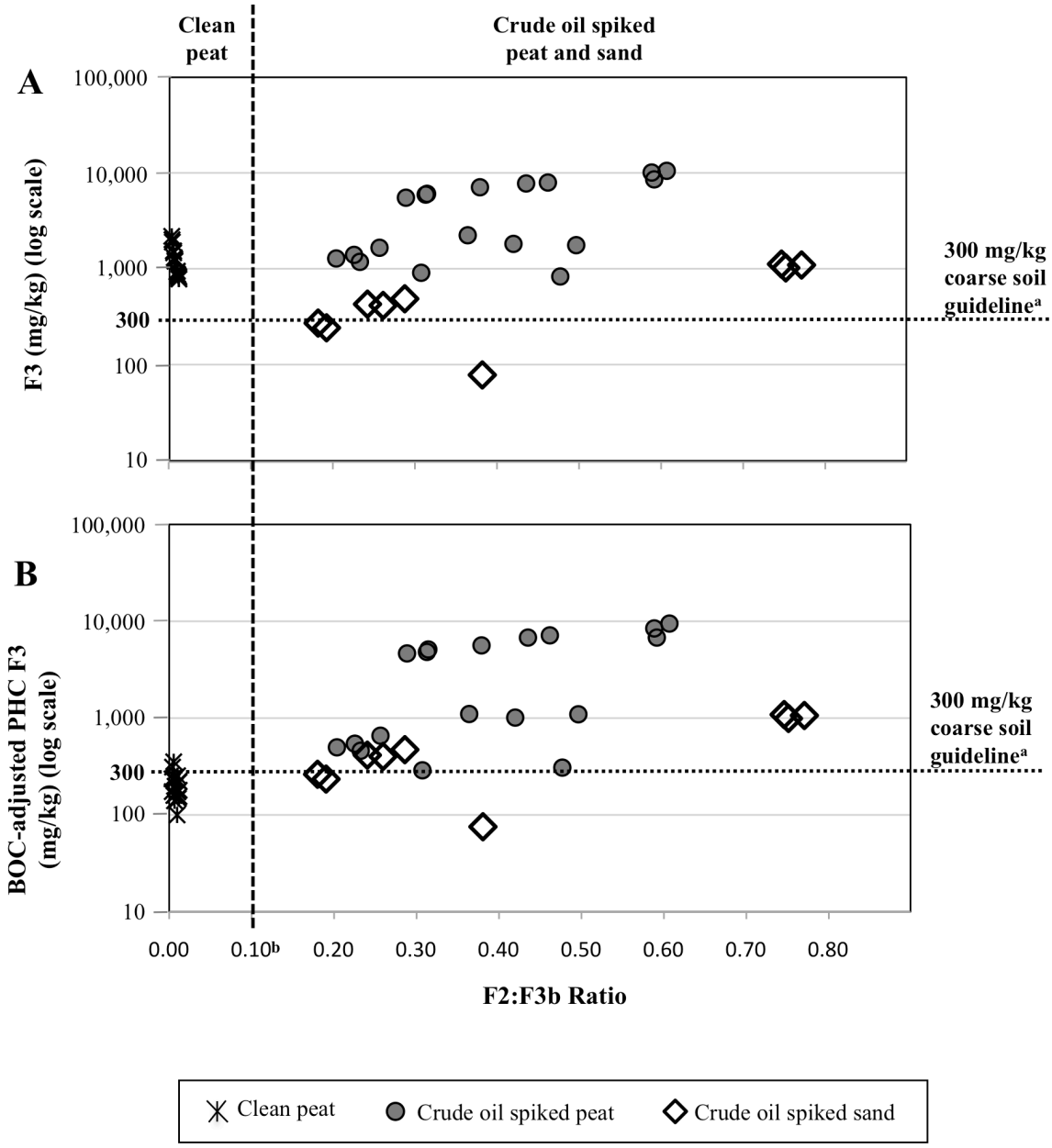

Figure 2.6. Comparison of Day 0, Day 150, Day 300 clean peat to crude oil spiked peat and sand. **(A)** Measured F3 concentrations and measured F2:F3b ratios; and **(B)** Calculated BOC-adjusted PHC F3 concentrations and measured F2:F3b ratios. [a]CWS PHC Tier 1 F3 coarse surface soil guideline for agricultural/residential/parkland land uses in potable groundwater conditions (CCME 2008a). [b]All clean peat samples had F2:F3b ratios less than 0.10. All crude oil spiked peat and sand samples had F2:F3b ratios greater than 0.10.

Combined F2:F3b ratios and BOC-adjusted PHC F3 concentrations

Combining the F2:F3b ratios with the BOC-adjusted PHC F3 concentrations strengthened the mathematical approach to identifying false PHC F3 exceedances in clean

peat soils. Figure 2.6A illustrates that the measured PHC F3 concentrations in all of the clean peat samples exceeded the 300 mg/kg coarse soil guideline. However, Figure 2.6B illustrates that the BOC-adjusted PHC F3 concentrations were below the guideline, with the exception of a slight exceedances by two of the clean processed peat samples (Appendix A: Table A-1, samples P1-B, P1-C, Day 300). Although these clean samples slightly exceeded the guideline with concentrations of 312 mg/kg and 354 mg/kg, they was still identified as non-contaminated by their low F2:F3b ratios of 0.00. This approach was also useful for evaluating one of the moderately spiked peat samples (Appendix: Table A-1, sample sP2-C, Day 150), with a BOC-adjusted PHC F3 concentration of 289 mg/kg. Although this was the only spiked peat sample that did not exceed the CWS PHC F3 guideline, the high F2:F3b ratio of 0.31 confirmed that PHCs were present in this sample.

Tier 2 decision process for determining if a peat sample location should be excluded or included within a soil remediation zone at a crude oil and/or diesel contaminated site

The F2, F3, F4, F3a and F3b data were used to develop a decision tree for determining if a soil sample location should be included or excluded from a soil remediation zone at a crude oil and/or diesel contaminated site (Chapter 5, Figure 5.3).

Question 1 (CWS PHC Tier 1): Do the F2 and/or F4 concentrations exceed the soil quality guidelines?

- "Yes" – The soil is PHC contaminated and requires remediation. (All of the highly spiked peat and moderately spiked peat samples exceeded the F2 guideline, which would therefore require remediation. Only the highly spiked peat exceeded the F4 guideline, which indicated that remediation would be required.).

- "No" – Proceed to Question 2. (All of the clean peat samples had non-detectable F2 concentrations. The F4 concentrations were well below the guideline).

Question 2 (CWS PHC Tier 1): Does the F3 concentration exceed the CWS PHC 300 mg/kg F3 soil guideline?

- "No" – Sample location would not require remediation.
- "Yes" – Proceed to Question 3 (All of the measured F3 concentrations in the clean peat exceeded the F3 guideline).

Question 3: Does the crude oil contamination source have an F2:F3b ratio greater than or equal to the 0.10 PHC presence threshold value?

- "Yes" – Proceed to Question 4 (The Federated crude oil used in this experiment did have an F2:F3b ratio of greater than or equal to 0.10).
- "No" – Do not proceed because crude oil contamination source does not meet carbon range requirements necessary for the F2:F3b evaluation and/or BOC-adjusted PHC F3 soil evaluation.

Question 4: Does the sample GC-FID pattern match the crude oil spike source and/or the clean background peat?

- "Yes" – Proceed to Question 5 (All of the sample GC-FIDs matched the Federated crude oil spike source and/or the clean peat/sand).
- "No" – The sample must be excluded from the decision tree process because it may be contaminated by a non-crude oil PHC product.

Question 5 (CWS PHC Tier 2): Is the F2:F3b ratio greater than or equal to the 0.10 PHC presence threshold value?

- "No" – The sample location would not require remediation. (False F3 guideline exceedances in the clean peat samples were resolved by the low F2:F3b ratios, which were all less than 0.02. All of the spiked peat and sand samples had high ratios of greater than 0.17.).
- "Yes" – proceed to Question 6

Question 6 (CWS PHC Tier 2): Does the BOC-adjusted F3 concentration exceed the CWS PHC 300 mg/kg F3 soil guideline?

- "No" – The sample location would not require remediation.
- "Yes" – Proceed to Question 7 (Only one of the clean peat samples had a BOC-adjusted F3 concentration that exceeded the F3 soil guideline).

Question 7 (CWS PHC Tier 2): Does the biomarker and/or PAH analysis verify the presence of crude oil PHCs?

- "No" – The sample location would not require remediation.
- "Yes" – Sample location would require remediation

F2, F3, F4, F3a and F3b percentages and F2:F3b ratios in light to heavy fresh crude oils

The survey of 14 fresh crude oils included the following analysis: i) F2, F3 and F4 percentages of the total F2 to F4 carbon range (Figure 2.7A); ii) F3a and F3b percentages of the total F3 carbon range (Figure 2.7B); and iii) F2:F3b ratios (Figure 2.7C). The crude oils ranged from the lightest oil with an F2 percentage of 34% (Rainbow) and the heaviest oil with an F2 percentage of 17% (Cold Lake). The predominant fraction was F3, with percentages ranging from 51% (Federated) to 73% (IFO-180).

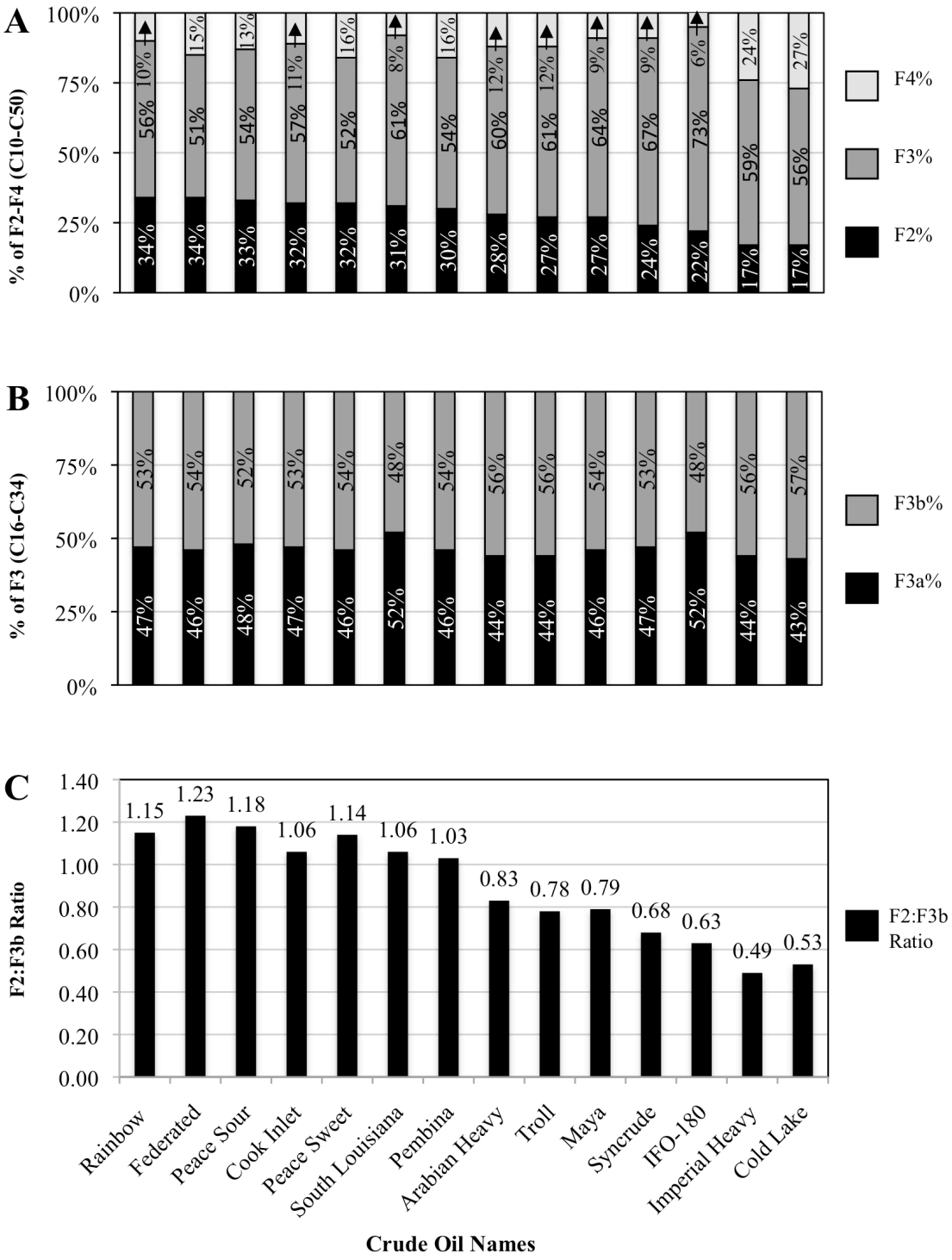

Fig. 2.7. PHC carbon range distributions in light (Rainbow) to heavy (Cold Lake) fresh crude oils. **(A)** Percentages of F2 (C10-C16), F3 (C16-C34) and F4 (>C34) within the F2 to F4 range (C10-C50); **(B)** Percentages of F3a (C16-C22) and F3b (C22-C34) sub-fractions within the F3 range; and **(C)** F2:F3b ratios.

The quantile-quantile and Shapiro-Wilk analysis determined that the F3a and F3b percentages in the sample group were normally distributed. The F3a percentages ranged from 43% (Cold Lake) to 52% (South Louisiana and IFO-180), with an average of 47%. The similar F3a and F3b percentages in the other thirteen fresh crude oils indicate that the BOC-adjusted PHC F3 approach developed using Federated crude oil could be applied to other crude oils as well. The F2:F3b ratios were highest in the lighter crude oils and lowest in the heavier crude oils, with a range of 1.23 (Federated) to 0.49 (Imperial Heavy). The ratios in all of the crude oils were one to two orders of magnitude higher than the ratios in the clean peat, which ranged from 0.00 to 0.01. These data indicate that the F2:F3b ratio could indicate PHC absence versus presence in clean and crude oil contaminated soils.

Statistical analysis results

The Day 0 and Day 300 ANOVA F-test *p*-values were calculated as two compositional groups. The The F2, F3a, F3b and F4 concentrations were treated as individual components of one group and were used to calculate the F2, F3a, F3b and F4 compositional *p*-values. The F2, F3 and F4 concentrations were treated as components of the BOC-adjusted PHC F3 group that were used to calculate the BOC-adjusted PHC F3 compositional *p*-values. *p*-values less than the critical value of 0.05 were identified as significantly different, while values greater than 0.05 were not significantly different. F2 p-values are not reported for the clean processed peat and clean natural peat due to non-detectable F2 concentrations. All of the F2, F3a, F3b, F4 and BOC-adjusted PHC *p*-values were strongly significant in the highly spiked peat and moderately spiked peat (Table 2.2). The F2 p-values were 0.0003 in the highly spiked peat, 0.0087 in the moderately spiked

peat and 0.0098 in the spiked sand. The F3a p-values were 0.0032 in the highly spiked peat, 0.0004 in the moderately spiked peat and 0.0687 in the spiked sand. The F3b p-values were 0.0001 in the highly spiked peat, 0.0012 in the moderately spiked peat and 0.0061 in the spiked sand. The F4 p-values were 0.0007 in the highly spiked peat, 0.0027 in the moderately spiked peat and 0.00001 in the spiked sand. The BOC-adjusted p-values were 0.013 in the highly spiked peat and 0.0001 in the moderately spiked peat. In contrast, all of the p-values were non-significant in the clean processed peat. The p-values were 0.5481 F3a, 0.0772 F3b, 0.6333 F4 and 0.1330 BOC-adjusted PHC F3. The clean natural peat had non-significant F3b (0.2916), F4 (0.1529) and BOC-adjusted PHC F3 (0.2810) p-values, with a slightly significant F3a (0.0184) p-value.

Table 2.2: Analysis of variance between Day 0 and Day 300 carbon range concentrations
F test p values, 95% confidence, triplicate data, logcentred transform compositional analysis

Sample Days/ Analytes	**P1** Clean Processed Peat	**P2** Clean Natural Peat	**sP1**[a] Highly Spiked Processed Peat	**sP2**[b] Moderately Spiked Natural Peat	**sS**[b] Moderately Spiked Sand plus Bacteria and Nutrients
F2[c]	--	--	0.0003*	0.0087*	0.0098*
F3a[c]	0.5481	0.0184*	0.0032*	0.0004*	0.0687
F3b[c]	0.0772	0.2916	0.0001*	0.0012*	0.0061*
F4[c]	0.6333	0.1529	0.0007*	0.0027*	0.00001*
BOC F3[d]	0.133	0.281	0.013*	0.0001*	NC

[a]sP1 - whole crude oil nominal spike concentration: F2-F4 = 19,608 mg/kg; F2 = 6,608 mg/kg; F3 = 10,000 mg/kg; F4 3,000 mg/kg.
[b]sP2 and sS - whole crude oil nominal spike concentration: F2-F4 = 2,942 mg/kg; F2 = 1,000 mg/kg; F3 = 1,500 mg/kg; F4 450mg/kg.
[c]Compositional data analysis included: F2 (C10-C16), F3 (C16-C34), F3a (C16-C22), F3b (C22-C34), F4 (>C34).
dCompositional data analysis included: F2 (C10-C16), BOC-adjusted PHC F3 (see Formula 2.1) and F4 (>C34).
NC – Non-detectable therefore not calculated
*Values less than 0.05 indicate significant differences between Day 0 and Day 300 concentrations

CONCLUSIONS

The microcosm results demonstrated that the measured total F3 concentrations (by CWS methods) in the clean peat soils falsely exceeded the CWS PHC F3 coarse soil guideline, whereas only one of the BOC-adjusted PHC F3 concentrations did not exceed

the guideline. In contrast, all of the Day 0, Day 150 and Day 300 total measured F3 and BOC-adjusted PHC F3 concentrations in the highly spiked peat and the moderately spiked peat exceeded the F3 soil guideline. In this study, the BOC-adjusted PHC F3 calculation provided a useful Tier 2 tool for conservatively estimating authentic PHC F3 concentrations in peat soils.

The F2:F3b ratios in the clean peat were one to two orders of magnitude lower than the ratios in the spiked peat and sand. The ratios in the clean peat were also one to two orders of magnitude lower than the 14 light to heavy crude oils. In this study, the F2:F3b ratio provided a useful Tier 2 tool for indicating PHC absence in clean peat versus PHC presence in crude oil contaminated peat and sand.

These microcosm results support potential field applications of BOC-adjusted PHC F3 calculation combined with the F2:F3b ratio to resolve false PHC F3 detections in clean peat soils. However, this new Tier 2 approach requires field validation studies to identify differences that may exist between F2, F3 and F4 data generated from this controlled microcosm study versus large-scale natural environments. Potential standardization of this new approach could minimize unnecessary ecological disruptions of thousands of peatland sites throughout Canada, while also eliminating unnecessary site remediation costs.

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CHAPTER 3

Diesel drilling waste contaminated manure compost PHC chemistry analysis: A new mathematical GC-FID approach to resolving false detections of petroleum hydrocarbons in manure compost

OVERVIEW

Canada is a global leader in crude oil and natural gas production, with a total of 518,758 wells drilled since 1955. Drilling wastes represent the largest waste stream generated by oil and gas exploration and production activities. Diesel oil based drilling wastes contain toxic petroleum hydrocarbons (PHCs), which must be remediated and/or disposed of in accordance with regulatory environmental protection requirements. Land farming is a common bioremediation technique. Drilling waste is mixed with nutrients and/or composted manure, and spreadonto open fields. Natural biodegradation occurs as soil microorganisms convert PHCs into non-toxic compounds such as water and carbon dioxide. Bioremediation projects require soil chemistry monitoring conducted in accordance with the Canadian Council of Ministers of the Environment (CCME) *Reference Method for the Canada-Wide Standard (CWS) for Petroleum Hydrocarbons in Soil* and Tier 1 PHC soil quality guidelines. However, the CWS methods co-extract natural background biogenic organic compounds (BOCs), such as waxes, fatty acids, sterols, etc., which originate from plant, animal and microbial matter. Elevated BOC concentrations can cause false exceedances of CWS PHC soil guidelines. This study presents a new approach to resolving this problem, through the results of a 300-day diesel drilling waste bioremediation microcosm experiment. Gas Chromatography Flame Ionization Detector (GC-FID) chromatogram patterns were monitored for CWS PHC carbon range fractions F2 (C10-C16), F3 (C16-C34), F4 (>C34) and sub-fractions F3a (C16-C22) and F3b (C22-C34) in clean manure compost and sand, compared to diesel drilling waste spiked manure compost and sand. The experiment results demonstrated two important trends: i) The total F3 range was strongly dominated by sub-fraction F3b (77%-84%) in the clean compost,

while the F3b distribution was only 11% in the diesel drilling waste; and ii) F2 concentrations were non-detectable in the compost, but they composed 25% of the entire F2-F4 range in the diesel drilling waste. This study used F3a and F3b percentages of the total F3 range to calculate BOC-adjusted authentic PHC F3 concentrations. This study also showed that F2:F3b threshold ratio of 0.10 indicated PHC presence ($\leq$0.10) versus PHC absence (>0.10) in compost and soil. These two complimentary mathematical approaches adhered to the existing CWS PHC soil extraction and analysis standards, while resolving false F3 PHC soil guideline exceedances at a Tier 2 level. The results indicated that only moderate bioremediation of diesel drilling waste was achieved, which was likely due to the sorption properties of the organophilic clay, which is a common component of oil-based drilling mud (OBM). PHCs become strongly sorbed within organophilic clay interstial layers, which significantly reduces bioaccessibility and therefore biodegradation by most bacteria species.

INTRODUCTION

Canada is the 3rd largest natural gas producer (3.9 million m^3/day or 24.5 million barrels/day) and the 6th largest crude oil producer (288,000 m^3/day or 1.8 million barrels/day) in the world (NEB 2011; CAPP 2012). Crude oil and natural gas production relies on drilling activities to locate natural deposits buried beneath land and sea. A total of 518,206 onshore wells and 552 offshore wells have been drilled in Canada since 1955 (Håvard 2010; CAPP 2011). The majority of drilled wells are located in the province of Alberta, which currently produces 83% and 70% of all Canadian natural gas and crude oil, respectively (CAPP 2011).

The drilling process requires drilling fluid, also commonly referred to as drilling mud. Drilling mud is primarily used to: stabilize boreholes; continuously suspend and return cuttings for removal from boreholes; and to clean, cool and lubricate drill bits. Used drilling mud holds large amounts of rock cuttings generated by the grinding action of the drill bit. Vibrating screens are used to separate the liquid mixture from the rock cuttings. Recovered fluids are recycled back into the borehole, while contaminated rock cuttings are stored in tanks or waste pits for disposal and/or remediation. Drilling wastes are the most significant waste sources produced by conventional oil and gas exploration and production activities. On average, one drilled well generates approximately 1,350 m^3 (or 8,505 barrels) of drilling waste, which is equal to 130 standard dump truck loads (Veil 1998; Dunbar 2009; Devold 2010).

Oil-based mud (OBM), as opposed to water-based mud (WBM), is the preferred type of drilling fluid used for horizontal drilling and for resolving difficult drilling conditions (NPC 2011; ERCB 2012). OBM is comprised of oil (commonly diesel fuel), emulsifiers, modified bentonite organophilic clay, a dispersed aqueous phase and either barite or hematite. This mixture creates an effective water-in-oil emulsion for ease of drilling. However, the diesel component is difficult to bioremediate because of low water solubility and strong PHC sorption capabilities of the organoclay interstial layers (Crocker et al. 1995; Ball et al. 2012). Diesel fuel has a carbon range of approximately C8-C24, which is produced from the fractional distillation of crude oil at temperatures between 200 $^{\circ}$C and 350 $^{\circ}$C under atmospheric pressure. Diesel fuel consists of complex PHC mixtures primarily including 30% normal and branched alkanes, 45% cyclic alkanes and 4% polycyclic aromatic hydrocarbons (PAHs) (Coles et al. 2009).

Diesel contains toxic PHCs, which can be highly volatile, persistent and mobile in water and in soils. Diesel drilling waste risks to environmental and human health are managed in accordance with Canadian regulatory guidelines (Department of Justice, Canada 2012). The Alberta Energy Resources Conservation Board recently released *Directive 050: Drilling Waste Management* (ERCB 2012), which sets requirements for the treatment and disposal of drilling wastes generated within the province of Alberta. PHC contaminated drilling waste management options include: landfill disposal, abiotic thermal treatments and biodegradation treatments. Ball et al. (2012) provides a detailed cost-benefit review of these drilling waste treatments.

Ex-situ bioremediation methods are required to meet *Directive 050*, which states that drilling waste biodegradation site closure approvals can be obtained if the land spread PHC concentrations do not exceed the equivalent land use capability soil quality endpoints, as listed in Table 1.1 (Chapter 1). Soil quality endpoints listed in *Directive 050* are aligned with the Alberta Tier 1 Soil and Groundwater Remediation Guidelines (Alberta Environment 2010) and the Canadian Council of Ministers of Environment Tier 1 Soil PHC guidelines (CCME 2008a).

The Canadian Council of Ministers of the Environment (CCME) *Reference Method for the Canada-Wide Standard (CWS) for Petroleum Hydrocarbons in Soil* provides standardized PHC soil chemistry analysis methods for evaluating clean and contaminated soil, compost and wastes (CCME 2001a). Introduction Chapter 1 includes detailed descriptions of these methods. Briefly, the CWS PHC Tier 1 analytical standards use hexane, acetone and dichloromethane (DCM) solvents to extract polar and non-polar PHCs, which are classified by four carbon number ranges/fractions, including F1 (C6-C10), F2

(C10-C16), F3 (C16-C34) and F4 (>C34). However, the CWS PHC solvents inadvertently co-extract polar and non-polar biogenic organic compounds (BOCs) originating from fresh and decayed plant, animal and microbial matter. This co-extraction problem is recognized as an issue by the CCME (CCME 2001a; CCME 2008c). False PHC detections are a significant issue for bioremediation methods that mix diesel drilling waste with BOC-enriched organic material in the form of soils or compost materials (ie. manure, straw, wood chips, garden litter, etc.). As discussed in Chapter 2, high BOC concentrations in clean peat microcosms caused false exceedances of the CWS PHC F3 soil quality guidelines. In that study, Gas Chromatography Flame Ionization Detector (GC-FID) chromatogram patterns were used to visually and quantitatively distinguish BOC patterns in peat soil from PHC patterns in crude oil. These signature patterns provide the basis for the F2:F3b ratio threshold of 0.10, which indicated PHC absence (<0.10) in clean peat, while indicating PHC presence ($\geq$0.10) in spiked peat and sand. F3a and F3b percentages of the total F3 concentrations provided the basis for the BOC-adjusted PHC F3 approach, which calculated authentic PHC F3 concentrations.

The primary objectives of this study were to: i) document the standard CWS PHC F2-F4 concentrations of fresh and aged (Day 0, Day 150 and Day 300) compost in the presence and absence of diesel drilling waste; ii) calculate BOC-adjusted PHC F3 concentrations in clean compost and in diesel drilling waste spiked compost; and iii) utilize the F2:F3b ratio to indicate PHC presence versus absence in clean compost and diesel drilling waste contaminated compost.

MATERIALS AND METHODS

Microcosm design

The indoor microcosm study was conducted at the ALS Environmental Laboratory, located in Waterloo, Ontario. Clean and diesel drilling waste spiked manure compost and sand treatments were housed in 70 L rectangular (30 cm x 35 cm x 66 cm) glass tanks, at depths of approximately 15 cm. Test conditions were maintained to promote microbial degradation. Deionized water was routinely added (every 3 or 4 days) to maintain a moisture content of approximately 70% in the compost. Due to the low water holding capacity of the sand, moisture content varied from the surface to the bottom layer. Average moisture levels of 20% were monitored by collecting and homogenizing full depth samples prior to analysis. Aerobic conditions were maintained by placing 5 cm long aeration stones under the substrate in each tank. The air temperature of the laboratory was maintained at 22 $^{\circ}$C. The temperature within each tank was increased by approximately 2 $^{\circ}$C by the placement of a lamp, lit by a 60-Watt incandescent light bulb, under the lid of each tank. The manure and sand was loosely covered with aluminum foil to promote heating, while allowing volatilization to occur. This was done for the purpose of promoting diesel drilling waste PHC biodegradation and volatilization. The microcosm environmental conditions were maintained 24 hours per day for the entire 300-day study period.

Material Sources: Diesel Drilling Waste, Manure Compost and Silica Sand

Diesel drilling waste, comprised of rock cuttings coated with drilling fluid, was supplied by Imperial Oil, Alberta. The drilling waste was shipped in 20 L plastic containers

and stored at 4 °C. The waste contained approximately 12% diesel oil with F2, F3 and F4 concentrations of 26,200 mg/kg, 114,000 mg/kg and 414 mg/kg, respectively. The pH level was 8.2 and the sodium adsorption ratio (SAR) was below the method detection limit of 2.0 meq/L. Nitrate and nitrite, were less than the 1.0 mg/kg detection limits. Total phosphorus and potassium concentrations were 230 mg/kg and 1,320 mg/kg, respectively. Barium was the only trace metal to exceed the CCME and Alberta Tier 1 surface soil agricultural limit of 750 mg/kg, with a concentration of 3,040 mg/kg (CCME 2001b; ERCB 2012). However, the diesel drilling waste spike concentrations used in this microcosm experiment diluted the barium concentration to an approximate range of 30 mg/kg to 350 mg/kg, which did not exceed the 750 mg/kg limit. The heterotrophic plate count in the diesel drilling waste was less than the method detection limit of 10,000 colony forming units (CFU)/g.

Manure compost was provided by the University of Guelph Ridgetown Agricultural Research Group, located in Ridgetown, Ontario. The compost was prepared by mechanically mixing solid beef cattle manure and straw at a ratio of 10:1 for aeration purposes. The mixture was set into one static pile in an indoor composting facility. Natural microbial processes increased the temperature of the mixture to 40 °C within 3 days, with an average temperature of 50 °C maintained during the two-week composting period. The matured compost had a total organic carbon content (TOC) of 38% and an average particle size of 1.5 cm. The pH value of 8.0 was within the neutral range of 6.5-8.5, which is compatible with PHC biodegradation requirements (Das and Preethy 2011). The sodium adsorption ratio (SAR) of 1 meq/L was within the ecologically acceptable limit of 5 meq/L (CCME 2001b). The phosphorus, nitrate and potassium concentrations were 6,060 mg/kg,

376 mg/kg and 5,295 mg/kg, respectively. Nitrite was less than the 1.0 mg/kg detection limit. The bacteria concentration was approximately $4.0x10^{11}$ CFU/g.

Silica sand, purchased from Anachemia Science (Richmond, British Columbia), was used as an inorganic control soil for the purpose of monitoring diesel drilling waste PHC degradation in the absence of manure BOC interferences. The sand had been pre-washed with deionized water and dried. It had a neutral pH of 7.5, 0.0 % TOC by weight and non-detectable bacteria levels (<10,000 CFU/g). Nutrient levels were non-detectable for phosphorus (<50 mg/kg), nitrate (<1.0 mg/kg), nitrite (<1.0 mg/kg) and potassium (<100 mg/kg). The sand treatments were nutrient amended and inoculated with bacteria on Day 0 for the purpose of promoting similar biodegradation processes that were present in the manure compost. Ammonium nitrate and potassium phosphate were added to the sand at concentrations that were similar to the nitrate, potassium and phosphorus concentrations in the composted manure. The following three gram-negative aerobic bacteria were identified as the most dominant species in the compost: *Sphingomonas multivorum, Sphingomonas paucimobilis* and *Brevundimonas vesicularis*. Members of the genera *Sphingomonas* and *Brevundimonas* are included among many different microbes known to degrade hydrocarbons (Chaîneau et al. 1999; Kobayashi et al. 2009). *Sphingomonas* spp. have been shown to degrade PAHs (Kryachko et al. 2012) and *Brevundimonas* spp. have been shown to degrade aliphatic hydrocarbons (Llado et al. 2012). The manure bacteria species were identified and cultured by GAP Laboratories, located in London, Ontario. Although inorganic agar broth was previously used in the Chapter 2 peat microcosms, the manure bacteria could not be cultured at sufficiently high concentrations. Consequently, basal enrichment broth was used to culture the manure bacteria instead. The bacteria broth

was added to both the clean sand and diesel drilling waste contaminated sand at approximate concentrations of $6.0x10^8$ CFU/g, which was the highest possible concentration that could be cultured. The previous bacteria studies indicated that this concentration was high enough to effectively biodegrade PHCs in soil.

Preparation of clean and diesel drilling waste spiked treatments

Different quantities of diesel drilling waste, based on PHC concentration, were used to prepare the highly spiked manure compost (sM1), moderately spiked manure compost (sM2) and the moderately spiked sand (sS). Diesel drilling waste was analyzed for F2-F4 concentrations. The moisture content of the compost and sand was measured in order to calculate the desired spike concentrations as mg of diesel oil per kg of dry weight soil. Spiked compost and sand were prepared in small batches to aid homogeneous mixing of the diesel drilling waste. Compost or sand was evenly spread onto a 60 cm x 30 cm aluminum pan and the weight was recorded on a digital scale. A pre-determined quantity of drilling waste was evenly deposited across the surface of the compost or sand. The spiked material was then transferred to an aluminum bowl and homogenized with an electric mixer. All batches of either spiked or clean compost and sand were mixed and stored separately in food grade plastic bags at -20 $^{\circ}$C. On the day of the experiment, all clean and contaminated batches were thawed and placed into the appropriate microcosm tanks.

The microcosm experiment consisted of 5 treatments conducted in triplicate. The treatments are described as follows: S – clean silica sand amended with bacteria and nutrients; **sS** - silica sand amended with bacteria and nutrients, spiked with a moderate nominal concentration of 2,613 mg/kg F2-F4 (2,000 mg/kg F3) diesel drilling waste; **M** –

clean manure compost; **sM1** – manure compost spiked with a high nominal concentration of 13,400 mg/kg F2-F4 (10,000 mg/kg F3) diesel drilling waste; **sM2** – manure compost spiked with a moderate nominal concentration of 2,613 mg/kg F2-F4 (2,000 mg/kg F3) diesel drilling waste. The sand treatments were used to monitor PHC levels in the absence of BOCs. Manure compost was spiked with moderate and high concentrations of diesel drilling waste to document false and authentic PHC F3 soil guideline exceedances on Day 0, Day 150 and Day 300.

Microcosm Experiment Monitoring and Sampling Procedures

Soil samples were collected from the microcosms on a monthly basis. Approximately 300 ml of compost or sand (full depth) was scooped from the centre of each microcosm and the remaining compost or sand was manually homogenized and left undisturbed until the next sampling period. All samples were homogenized using an electric mixer and placed into amber glass jars with Teflon lined lids. The samples were immediately stored at -20 $^{\circ}$C until PHC analysis was performed.

All samples were analyzed for conductivity, pH and redox levels. Samples were prepared by mixing a slurry of compost or sand and deionized water at a 1:2 ratio. Conductivity ranged from 0.127 to 0.814 dS/m; pH levels ranged from 6.5 to 9.3 and redox aerobic levels ranged from +28mV to +134 mV. These values were within acceptable microbial degradation requirements and were maintained during the entire 300-day study period (Alberta Agriculture and Food 2011; Das and Preethy 2011).

F2, F3, F4 PHC Soil Extraction and GC-FID Analysis

Detailed descriptions of extraction materials and methods used in this experiment are described in Chapter 2. Briefly, ALS Environmental conducted the F2-F4 PHC soil extractions and GC-FID analyses in compliance with the CCME Reference Method for the *Canada-Wide Standard (CWS) for Petroleum Hydrocarbons in Soil – Tier 1 Method* (CCME 2001a). The author of this study conducted all of the GC-FID chromatogram integrations. Only the F2, F3 and F4 carbon ranges were analyzed for the reason that the CCME user guidance document identifies biogenic interferences as occurring in those three ranges (CCME 2001b).

While this study used the same analytical surrogate o-terphenyl for the Day 0 samples, octacosane was used for the Day 150 and Day 300 samples. Both surrogates provided the same ability to evaluate the extraction recovery rate of 60-120%. The second difference is that the Day 0 samples in this study were spiked with the internal standard alpha-androstane for method development purposes. The same external standards described in Chapter 2 were used to determine F2, F3 and F4 concentrations in all of the Day 0, Day 150 and Day 300 samples. All samples were Soxtec heat extracted in a mixture of 50:50 acetone and hexane solvent. Polar compound removal in the extracts utilized a 50:50 mixture of hexane and DCM (dichloromethane) followed by an in-situ silica gel treatment. Sample extracts were analyzed by the Agilent 6890Ns GC-FID using an on-column injector and a 0.32 mm x 0.1 µm x 30 m capillary 100% poly(dimethylsiloxane) column. CWS PHC external standards included a calibration mix of C10, C16 and C34, ATSM D5442 C12-C60 linearity standard, and an Accustandard FTRPH Calibration/Window Defining Standard. A pentacontane (nC50) solution was used to determine a retention time and

response factor standard for C10 to C50 hydrocarbons. An external standard was used to identify the C22 peak for distinguishing the F3a and F3b sub-fraction ranges. All concentrations were reported on a dry weight basis.

Statistical analysis

This study utilized the same statistical analysis techniques and software that are described in Chapter 2. A compositional data approach was followed, with each variable representing a proportion of the total composition. The F2, F3a, F3b and F4 p-values were calculated as components of the same group. The BOC-adjusted F3 p-values were calculated as components of the F2, F4, BOC-adjusted PHC F3 group. This compositional approach required modifications according to standard statistical methods (Aitchison 1986), which transformed the data on a logratio basis (Ecozcue and Pawlowsky-Glahn 2011). The experiment data were transformed by the logcentred transform expressed as:

$$z_i = \log(x_i/g(x_D)) \quad (i = 1, \ldots, D), \qquad \text{(Eqn. 3.1)}$$

where $g(x_D)$ is the geometric mean of the composition

The R statistical software package (RDCT 2011) was used to generate balanced two-way analysis of variance (ANOVA) F-test *p*-values. *p*-values for significant differences between the Day 0 to Day 300 for each of the F2, F3a, F3b and F4 concentrations. Mean significant differences between the triplicate soil samples were not generated due to their insufficient sample sizes. The control (C) and clean sand (S) data were not included in the statistical analysis because all of the F2, F3 and F4 concentrations

were less than their respective method detection limits of 10 mg/kg, 50 mg/kg and 50 mg/kg. The F2 concentrations in the clean compost (M) were below the 10 mg/kg method detection limit and were therefore calculated as half the limit (5 mg/kg). The F3 concentrations in the diesel waste spiked sand (sS) were less than the 50 mg/kg detection limit and were also calculated as half the detection limit (25 mg/kg).

RESULTS AND DISCUSSION

The diesel drilling waste spiked manure compost and sand were monitored for changes in F2, F3, F4, F3a, F3b and total F2-F4 concentrations, percentage distributions and GC-FID chromatogram patterns on Day 0, Day 150 and Day 300. Mean data for the triplicate samples are presented in summary Table 3.1. Individual sample data are presented in appendix Table B-1.

F2, F3, F4 and F2-F4 concentration changes over time

Clean sand and diesel drilling waste spiked sand

Diesel drilling waste spiked sand and clean sand mixed with nutrients and bacteria were monitored over a 300-day period to document PHC degradation in the absence of detectable BOCs. Clean sand (S) had non-detectable F2, F3 and F4 concentrations for all three sample periods (data not shown). All of the moderately spiked sand (sS) samples had higher F3 than F2 concentrations, while F4 concentrations were less than the 50 mg/kg detection limit (Figure 3.1). The Day 0 F2 concentration of 823 mg/kg was 59% lower on Day 150 and 73% lower on Day 300. The Day 0 F3 concentration of 2,653 mg/kg remained

Table 3.1: Days 0, 150, Day 300 mean results for clean manure and diesel drilling waste spiked manure, sand. F2, F3, F4, F3a, F3b concentrations, F3a and F3b percentages, F2:F3b ratios, BOC-adjusted PHC F3

Sample Days/ Analytes	**M** Clean Manure	**sM1**[a] Highly Spiked Manure	**sM2**[b] Moderately Spiked Manure	**sS**[b] Moderately Spiked Sand + Bacteria & Nutrients
Measured Day 0				
F2-F4 (mg/kg)	1,068 ±304	12,473 ±560	3,727 ±352	3,501 ±671
F2 (mg/kg)[c]	<10±0	2,467 ±118**	613±46**	823±30**
F3 (mg/kg)[c]	667±140*	9,653 ±429**	2,817 ±262**	2,653 ±665**
F4 (mg/kg)[c]	397±170	353±23	297±45	<50±0
F3a (mg/kg)[c]	87±27	7,915±379**	2,028±191**	2,282±584**
F3b (mg/kg)[c]	580±115*	1,738±74**	788±73*	371±82*
F3a (% of F3)[c]	13%±2	82%±1	72%±1	86%±0
F3b (% of F3)[c]	87%±2	18%±1	28%±1	14%±0
F2/F3b ratio	0.01±0.00	1.45±0.06	0.79±0.01	2.37±0.63
PHC F3 (mg/kg)[d,e]	104±32	9,242 ±441**	2,369 ±222**	NC
Bacteria (CFU/g)	4E+11±6.0E+09	3E+11±3E+10	2E+11±3E+10	5E+07±4E+07
Measured Day 150				
F2-F4 (mg/kg)	438±114	5,104 ±1,935	1,468 ±508	3,025 ±593
F2 (mg/kg)	<10±0	590 ±208**	130±39	336±60**
F3 (mg/kg)	336 ±96*	4,353 ±1,634**	1,222 ±402*	2,663 ±534**
F4 (mg/kg)	97±20	161±98	116±68	<50±0
F3a (mg/kg)	74±26	3,395±1,229**	770±233*	2,237±460**
F3b (mg/kg)	262±70	958±406*	452±170*	426±74*
F3a (% of F3)	22%±2	78%±2	63%±2	84%±0
F3b (% of F3)	78%±2	22%±2	37%±2	16%±0
F2/F3b ratio	0.01 ±0.00	0.64±0.09	0.29±0.03	0.77±0.03
PHC F3 (mg/kg)[f]	86±30	3,938 ±1418**	899±271*	NC
Bacteria (CFU/g)	5E+10±7E+09	5E+10±1E+10	4E+10±8E+09	4E+08±2E+07
Measured Day 300				
F2-F4 (mg/kg)	481 ±267	4,580 ±1,178	1,612 ±58	2,302 ±74
F2 (mg/kg)	<10±0	526±140**	133±21	224±26**
F3 (mg/kg)	364±193*	3,897 ±991**	1,293 ±110*	2,053 ±99**
F4 (mg/kg)	111±75	157±49	185 ±99	<50±0
F3a (mg/kg)	84±49	3,000±748**	815±61*	1,704±71**
F3b (mg/kg)	280±144	896±243*	478±59*	349±28*
F3a (% of F3)	23%±2	77%±1	63%±2	83%±2
F3b (% of F3)	77%±2	23%±1	37%±2	17%±0
F2/F3b ratio	0.02 ±0.02	0.59±0.03	0.28±0.04	0.66±0.12
PHC F3 (mg/kg)[g]	100±57	3,497 ±869**	946 ±72*	NC
Bacteria (CFU/g)	5E+11±8E+11	7E+10±2E+10	4E+10±3E+10	2E+08±2E+07

[a]sM1 – diesel drilling waste nominal spike: F2-F4 = 13,400 mg/kg; F2 = 3,400 mg/kg; F3 = 10,000 mg/kg; F4 <50 mg/kg.
[b]sM2 and sS - whole crude oil nominal spike: F2-F4 = 2,613 mg/kg; F2 = 613 mg/kg; F3 = 2,000 mg/kg; F4 <50 mg/kg.
[c]F2 (C10-C16), F3 (C16-C34), F3a (C16-C22), F3b (C22-C34), F4 (>C34).
[d]BOC-adjusted PHC F3 concentrations; Formula 3.1.
[e]86%F3a:14%F3b ratio in Spiked Sand (sS1) used as diesel drilling waste source for Day 0, Formula 3.1 calculations
[f]84%F3a:16%F3b ratio in Spiked Sand (sS1) used as diesel drilling waste source for Day 150, Formula 3.1 calculations
[g]83%F3a:17%F3b ratio in Spiked Sand (sS1) used as diesel drilling waste source for Day 300, Formula 3.1 calculations
*Single asterisk indicates the F2 and/or F3 concentration(s) exceeded the CWS PHC coarse soil guidelines
**Double asterisk indicates the F2 and/or F3 concentration(s) exceeded the CWS PHC coarse and fine soil guidelines
Mean ± standard deviation (n=3) ; NC – PHC F3 not calculated for spiked sand; Values reported on dry weight basis.

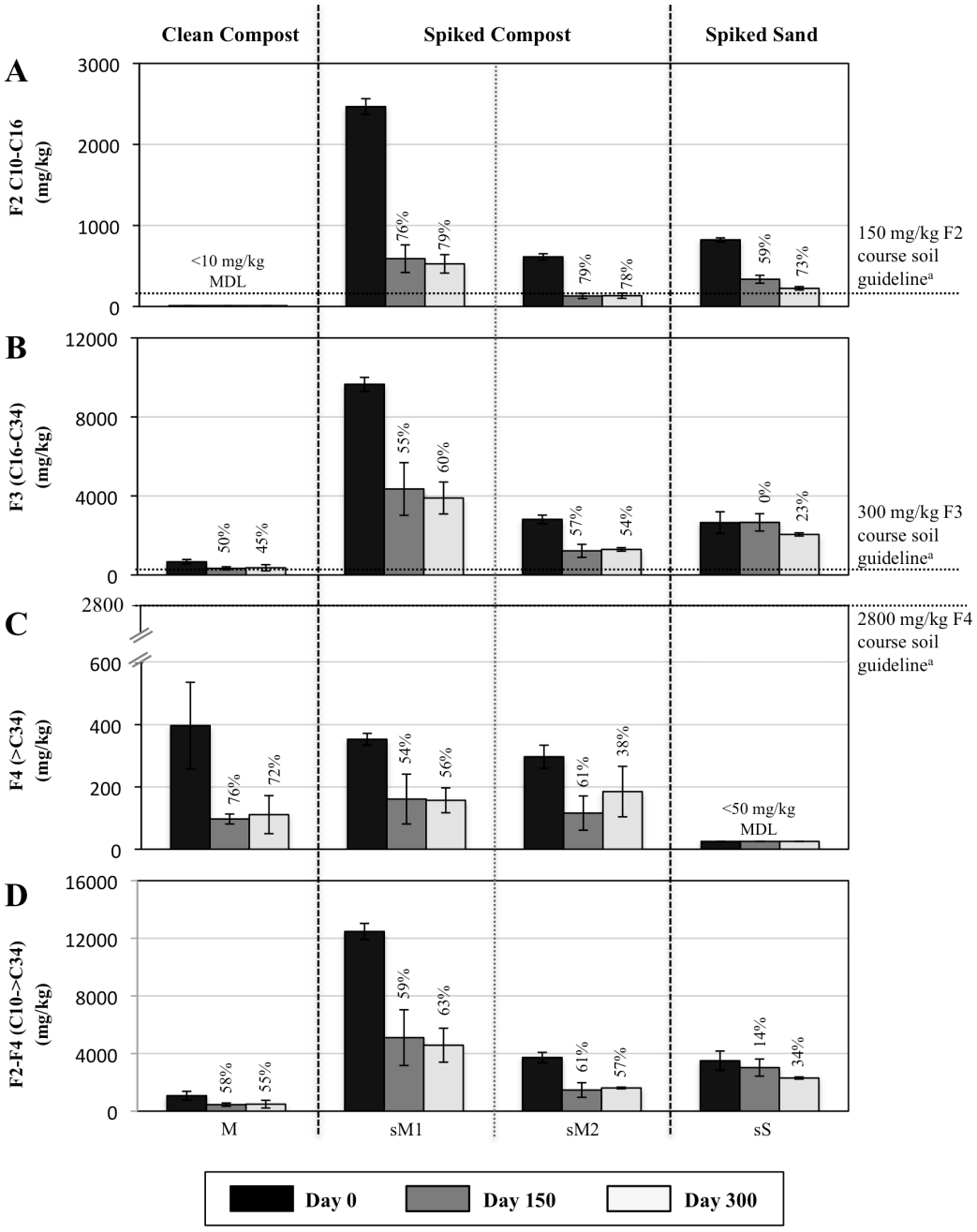

Figure 3.1. CWS PHC concentrations for F2 **(A)**; F3 **(B)**; F4 **(C);** and F2-F4 **(D)** in clean and diesel drilling waste spiked manure and sand on Day 0, Day 150 and Day 300. Results are expressed as the mean of three replicate sample concentrations with standard deviation bars. Numbers above bars represent percentage decreases of mean concentrations on Day 150 and Day 300. [a]Tier 1 PHC course surface soil guideline for agricultural/residential/parkland land uses in potable groundwater conditions (CCME 2008a). MDL – method detection limit. M-clean manure. sM1-manure spiked with nominal 13,400 mg/kg F2-F4 diesel drilling waste. sM2 – manure spiked with nominal 2,613 mg/kg F2-F4 diesel drilling waste. sS - sand spiked with nominal 2,613 mg/kg F2-F4 diesel drilling waste.

unchanged on Day 150, but decreased by 23% on Day 300. The Day 0 total F2-F4 concentration of 3,501 mg/kg was14% lower on Day 150 and 34% lower on Day 300.

The 73% F2 and 23% F3 decreases on Day 300 in the drilling waste spiked sand were much lower than the 93% F2 and 82% F3 decreases in the Chapter 2 crude oil spiked sand treatments. Given that diesel PHCs are comprised of a lighter carbon range (C8 to C24) than crude oil PHCs (C6 to >C34), greater reductions in PHCs due to volatilization and biodegradation may have been expected for diesel drilling waste after 300 days. Sanscartier et al. (2010) observed greater F2 (79%) and F3 (82%) reductions in sand contaminated with diesel fuel (indigenous microbes present) after only 110 days. The F2 and F3 concentration reductions were primarily attributed to abiotic volatilization processes.

The relatively low F3 reduction rates observed in this study suggest that diesel associated with the drilling mud may not have been bioavailable for microbial decomposition. This may be due to PHC adsorption onto and absorption within drilling mud organoclay particles (Ball et al. 2012), which would have effectively limited volatilization and biodegradation processes. After a 150-day lag period, some reduction in F3 PHCs occurred on Day 300. This may be due to possible changes in the microenvironment and/or acclimation of the cultured manure compost microbial community to the diesel drilling waste and sand substrate (Mariano et al. 2007; Moliterni et al. 2012). The mean 73% reduction of F2 concentrations combined with the presence of bacteria (Table 3.1) suggests the utilization of at least some of the F2 PHCs by microbes. Natural production of microbial metabolites (biosurfactants) could alter both the surficial and interstitial absorption of PHCs bound to organoclay in drilling muds, promoting increased bioavailability of F3 PHCs and the subsequent loss of F3 concentrations by

abiotic and biotic processes. Further discussion of the bacterial consortium cultured from manure compost is provided in the following section.

Clean manure compost and diesel drilling waste spiked manure compost

The clean manure compost (M) had non-detectable F2 concentrations of less than 10 mg/kg (Table 3.1, Figure 3.1). Day 0 The F3 (667 mg/kg) and F4 (397 mg/kg) mean concentrations decreased by 50% and 76%, respectively, on Day 150. There was little change on Day 300, with mean F3 and F4 decreases of 45% and 72%, respectively. The Day 0 mean F2-F4 concentration of 1,074 mg/kg decreased by 58% on Day 150, with a similar value of 55% on Day 300. These decreases may be attributed to natural degradation of BOCs, which can occur under optimal conditions of neutral pH, aeration and 22°C ambient air temperature (Kroetsch et al. 2011).

The F3 concentrations were comparatively higher than either the the F2 or F4 concentrations in the highly spiked manure compost (sM1) and in the moderately spiked manure compost (sM2) (Table 3.1, Figure 3.1). All of the F2, F3 and F4 concentrations decreased considerably on Day 150, but there were minimal changes on Day 300. The Day 0 mean F2 concentration of 2,467 mg/kg in the highly spiked compost decreased by 76% and 79% on Day 150 and Day 300, respectively. The Day 0 mean F3 concentration of 9,653 mg/kg decreased by smaller percentages of 55% and 60% on Day 150 and Day 300, respectively, The Day 0 mean F4 concentration of 353 mg/kg decreased by 54% and 56% on Day 150 and Day 300, respectively. The Day 0 mean F2-F4 concentration of 12,473 mg/kg decreased by 59% and 63% on Day 150 and Day 300, respectively.

The Day 0 mean F2 concentration of 613 mg/kg in the moderately spiked compost, decreased by 79% on Day 150 and 78% on Day 300. The Day 0 mean F3 concentration of

2,817 mg/kg decreased by 57% and 54% on Day 150 and Day 300, respectively. The Day 0 mean F4 concentration of 297 mg/kg decreased by 61% on Day 150 and 38% on Day 300. The Day 0 mean F2-F4 concentration of 3,727 mg/kg decreased by 61% on Day 150 with a similar decrease of 57% on Day 300.

The author is not aware of any studies that are similar to this diesel drilling waste spiked manure compost microcosm experiment. However, the Day 150 mean F2-F4 percentage reductions of 59% in the highly spiked compost and 61% in the moderately spiked compost were similar to the TPH (F2-F4) reductions observed in the following two bioremediation experiments of diesel drilling waste contaminated loam soils. Chaîneau et al. (1995) observed a mean 54% TPH reduction in 150 days and Steliga et al. (2009) observed a mean 64% TPH reduction in 130 days. Further TPH reductions (81%, Day 260) were observed in the Steliga et al. (2009) study when bacteria cultured from the drilling waste contaminated soils were inoculated into the bioremediated soils after 130 days. In comparison, other bioremediation studies using diesel oil contaminated soils mixed with manure observed rapid TPH reductions. Coles et al. (2009) reported a 78% mean reduction of 10,000 mg/kg TPH after 45 days and Wellmann et al. (2001) reported 81% mean reduction of 5,000 mg/kg TPH within 41 days. These studies suggest that diesel alone degrades more efficiently than diesel mixed with drilling mud.

There are several factors that could account for the moderate reduction rates of diesel drilling waste observed in the current study and in other published studies as well (Chaîneau et al. 1995; Steliga et al. 2009). The most probable limiting factor influencing biodegradation of diesel drilling waste is the high absorption of PHCs within small orthoclay micropores, which restricts the bioaccessibility of PHCs to microbes. For

example, in this study, the three dominant bacterial species (*S. multivorum, S. paucimobilis* and *B. vesicularis*) in the composted manure have cell diameters that are more than two orders of magnitude wider than the interlayer spaces of the organoclay (Zakharavo et al. 2010; Theng et al. 2012). PHC uptake in these three species occurs by passive intracellular absorption across the cell membrane (Lu et al. 2000; Uyttebroek et al. 2007). Consequently, these bacteria are only able to degrade PHCs that are adsorbed to the clay surface but they cannot physically access the PHCs that are absorbed between the clay microlayers. Theng et al., (2012) observed insignificant biodegradation of phenanthrene in bentonite organoclay that was inoculated by *Sphingomonas sp*. (Theng et al., 2012). Crocker et al. (1995) compared the bioavailability of naphthalene in spiked treatments of organoclay to two types of bacteria with extracellular (*Pseudomonas putida*) versus intracellular (*Alcaligenes sp*.) uptake mechanisms. That study determined that *P. putida* directly degraded the surficially adsorbed naphthalene via extracellular secretions of enzymes, in addition to promoting naphthalene desorption from the interior of the clay particle layers. In contrast, the *Alcaligenes sp.* was only able to utilize the aqueous naphthalene phase. The bacterial consortium in the current study could explain the relatively low F2-F4 reduction rate in the moderately spiked sand (34%) and the higher reduction rates in the highly spiked compost (63%) and in the moderately spiked compost (57%).

PHC sorption strength is defined by cation exchange coefficients (CECs). CEC could have also contributed to the lower diesel biodegradation rates in the spiked sand versus the spiked manure treatments. CEC estimates the number of exchange sites in a soil sample that are capable of adsorbing positively charged cations by electrical attraction. Cations are held by negatively charged particles of clay and humus called “colloids”. Sand has a very

low CEC of 1-5 cmol/kg, while negatively charged clay has a high CEC of >30 cmole/kg and humus has the highest CEC of 100-300 cmole/kg (Alberta Agriculture and Food 2011). Diesel PHCs adsorb strongly to drilling mud organoclay particles, which reduces its bioavailability. The CEC in the spiked sand would be too low to desorb the PHCs from the clay. It is however possible that the spiked manure, with the highest CEC, would be capable of desorbing at least some of the PHCs from the organoclay particle surfaces. The resulting combined effects of PHC adsorption to humic matter and solubilization into the aqueous phase would improve bioavailability for microbial biodegradation (Kobayashi et al. 2009; Das and Preethy 2011). Differences in diesel desorption from organoclay, particularly for PHC F3, could partially explain the 0% mean F3 reduction in the spiked sand, relative to the 57% mean F3 reduction in spiked compost on Day 150 (Figure 3.1). Although the bacteria inoculum used for the sand treatment was cultured from the manure compost, bacterial numbers were consistently lower in the sand versus the compost treatments (Table 3.1), which could also affect biodegradation rates. Further, studies suggest that bacteria derived from composted manure had reduced biodegradation activities once removed from their indigenous substrate (Kästner and Mahro 1996).

Compliance with PHC F2, F3 and F4 soil quality guidelines

F2 concentrations were non-detectable in the clean compost (Figure 3.1A), which indicated the absence of BOC interferences in the C10-C16 carbon range. Correspondingly, the presence of F2 in the spiked compost and sand indicated the presence of diesel PHCs. On Day 0, all of the spiked compost and sand F2 concentrations exceeded the CWS PHC F2 coarse soil guideline of 150 mg/kg. The F2 concentrations in the highly spiked compost

(sM1) and the moderately spiked sand (sS) remained above the F2 guideline on Day 150 and Day 300. In contrast, the F2 concentrations in the moderately spiked compost (sM2) were reduced to below the F2 guideline on Day 150 and Day 300. These data demonstrate that elevated F2 concentrations in the compost and sand indicated the presence of diesel PHCs.

The F3 concentrations in the clean compost, spiked compost and spiked sand remained above the CWS PHC F3 coarse soil guideline of 300 mg/kg on Day 0, Day 150 and Day 300 (Figure 3.1B). In contrast, the clean compost, spiked compost and spiked sand had F4 concentrations that were well below the CWS PHC F4 coarse soil guideline of 2,800 mg/kg on Day 0, Day 150 and Day 300 (Figure 3.1C). These data demonstrate that the false exceedances of the CWS PHC soil guideline by clean compost occurred only in the F3 carbon range.

F2, F3, F4, F3a and F3b GC-FID Chromatogram Patterns

GC-FID chromatograms were used to quantify the F2, F3 and F4 concentrations in accordance with CWS PHC soil analysis methods (CCME 2001a). The current study also used GC-FID chromatograms to monitor PHC degradation patterns, in addition to visually distinguishing clean compost from spiked compost and sand. Figure 3.2 illustrates Day 0 and Day 300 GC-FID chromatograms for clean compost (M), highly spiked compost (sM1), moderately spiked compost (sM2), moderately spiked sand (sS) and the diesel drilling waste spike source. The F2-F4 carbon range chromatogram patterns in the diesel drilling waste spiked blank (Figure 3.2A) and the Day 0 diesel drilling waste spiked sand (sS) (Figure 3.2C) were virtually identical, with uniformly shaped PHC UCMs and

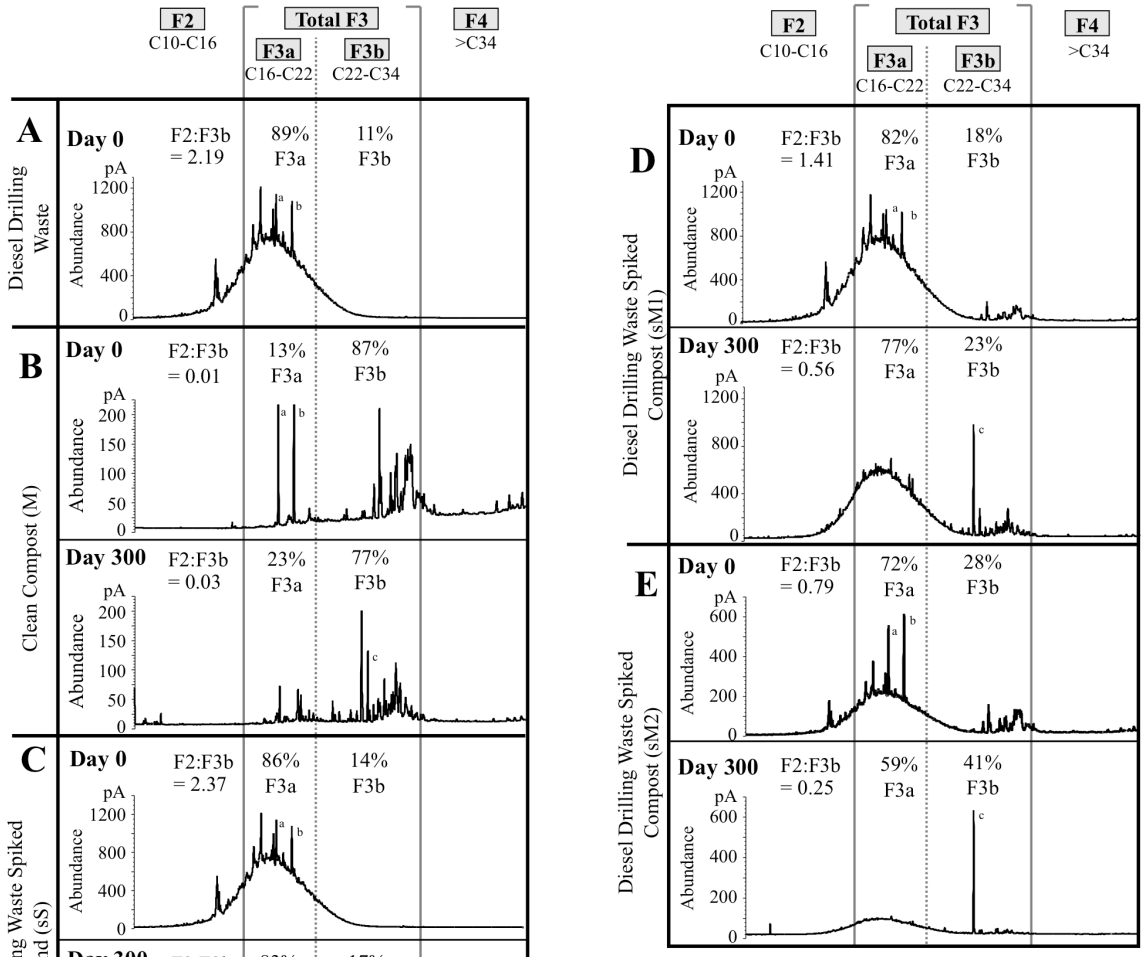

Figure 3.2. GC-FID chromatogram examples for Day 0 and Day 300. **(A)** diesel drilling waste spiked blank. **(B)** M - clean manure compost. **(C)** sS - sand spiked with nominal 2,613 mg/kg F2-F4 diesel drilling waste. **(D)** sM1 – manure compost spiked with nominal 13,400 mg/kg F2-F4 diesel drilling waste. **(E)** sM2 – manure compost spiked with nominal 2,613 mg/kg F2-F4 diesel drilling waste. Panels B to E represent one of three replicate samples analyzed for each treatment group. F2:F3b ratios and F3a and F3b percentages of total F3 calculated as mean values (Table 3.1). S – clean sand treated with bacteria broth not included in figure due to non-detectable F2-F4 concentrations. All chromatograms were produced by CWS PHC Soil extraction and analysis method (CCME 2001a). Diesel drilling waste spikes were administered on Day 0 only. [a]o-terphenyl surrogate. [b]alpha-androstane internal standard. [c]octacosane surrogate.

identical resolved peaks. The Day 300 spiked sand chromatogram had fewer resolved peaks and a smaller UCM. The F3a sub-fraction range dominated all of the Day 0, Day 150 and Day 300 spiked sand chromatograms.

The clean compost chromatogram patterns (Figure 3.2B) were predominantly located within the F3b sub-fraction range, which differed from the above noted diesel drilling waste and spiked sand patterns. The Day 0 and Day 300 clean compost chromatograms had many resolved peaks and less prominent, irregularly shaped BOC UCMs.

The highly spiked compost and moderately spiked compost chromatograms combined the distinctive diesel drilling waste PHC pattern and the clean compost BOC pattern. The PHC pattern in the Day 0 highly spiked compost (Figure 3.2D) was virtually identical to the diesel drilling waste and spiked sand patterns. Although the compost BOC pattern was present, it was less obvious due to the larger scale of this chromatogram. The same PHC and BOC patterns were present in the Day 0 moderately spiked compost as well (Figure 3.2E). The Day 300 PHC patterns were relatively less pronounced in the highly spiked compost and in the moderately spiked compost.

Changes in F3a and F3b percentages over time

Changes in the Day 0, Day 150 and Day 300 F3a and F3b percentages in the clean compost, spiked compost and spiked sand are illustrated in Figure 3.3. All of the clean compost samples were dominated by the F3b range, while all of the spiked manure and sand samples were dominated by the F3a range. To varying degrees, the F3a percentages decreased in all of the spiked compost and sand treatments. This is attributed to the

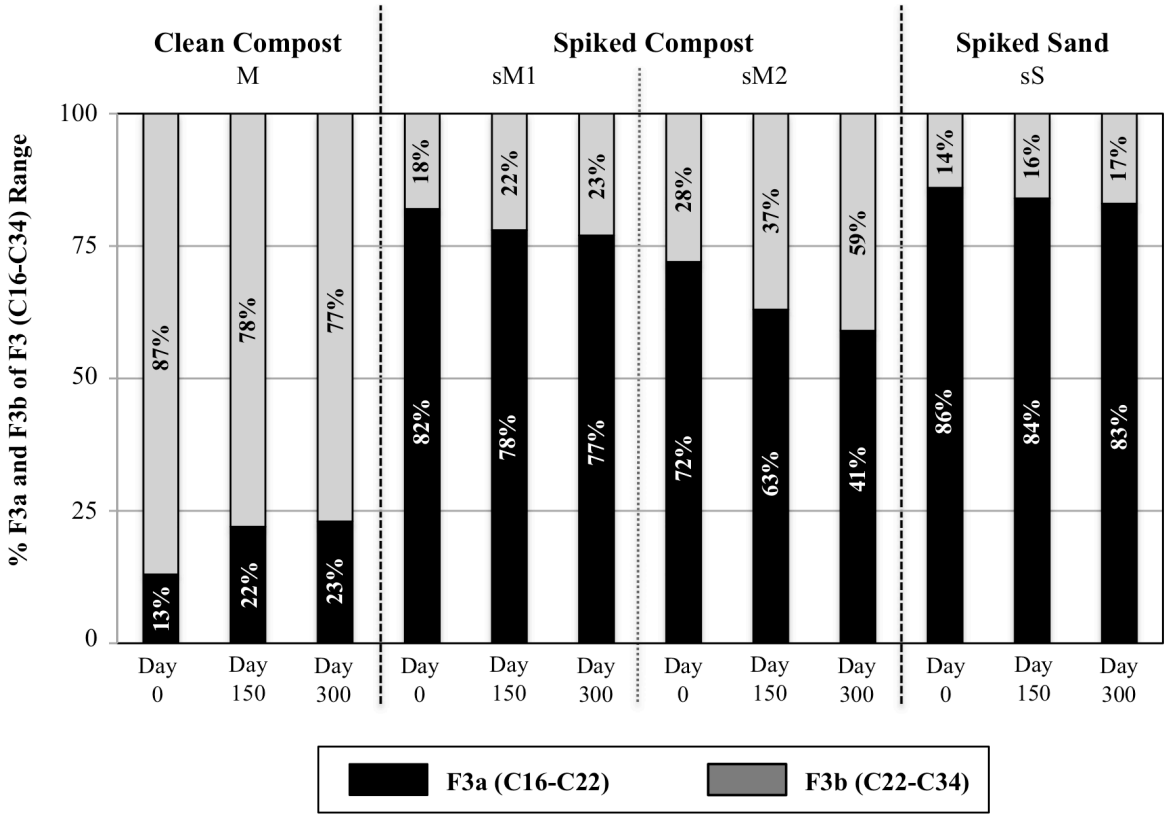

Figure 3.3. F3a (C16-C22) and F3b (C22-C34) percentage distributions of F3 (C16-C34) range on Day 0, Day 150 and Day 300. Results expressed as the mean of three replicate sample values. M - clean manure. sM1 – manure spiked with nominal 13,400 mg/kg F2-F4 diesel drilling waste. sM2 – manure spiked with nominal 2,613 mg/kg F2-F4 diesel drilling waste. sS - sand spiked with nominal 2,613 mg/kg F2-F4 diesel drilling waste.

volatilization of PHC compounds in the C16-C22 range (Killops and Al-Juboori 1990; Wang et al. 2011) and also to short-term and long-term PHC biodegradation processes (Dutta and Harayam 2000; Prince et al. 2003; Wang et al. 2011).

There were minimal changes in the spiked sand, with mean F3a percentages of 86%, 84% and 83% on Day 0, Day 150 and Day 300, respectively. The greatest changes occurred in the moderately spiked compost, with mean F3a percentages of 72%, 63% and 41% on Day 0, Day 150 and Day 300, respectively. There were relatively smaller F3a percentage

decreases in the highly spiked compost, with mean values of 82%, 78% and 77% on Day 0, Day 150 and Day 300, respectively. In contrast, the F3a percentages increased in the clean compost, with mean values of 13%, 22% and 23% on Day 0, Day 150 and Day 300, respectively.

Comparison of the diesel drilling waste spiked compost data to the crude oil spiked peat data (Chapter 2) indicated that F3a percentages decreased in all of the spiked treatments for both studies. However, the mean F3a percentages on Day 300 had decreased by only 3% in the diesel drilling waste spiked sand, as compared to 16% in the crude oil spiked sand. The mean F3a percentages decreased by only 5% in the highly spiked compost, but decreased by 13% in the highly spiked peat. The moderately spiked compost had a higher mean F3a decrease of 31% as compared to 14% in the moderately spiked peat. While the clean compost had a mean F3a increase of 10%, the clean natural peat had 0% change and the clean processed peat decreased by 2%.

BOC-adjusted PHC F3 Calculation Description and Rationale

As described in the Chapter 2 crude oil and peat microcosm study, sub-fractions F3a (C16-C22) and F3b (C22-C34) were used to calculate BOC-adjusted PHC F3 concentrations in clean and contaminated peat samples. The BOC-adjusted PHC F3 calculation (Formula 3.1) was applied to the Day 0, Day 150 and Day 300 clean and diesel drilling waste spiked compost samples as well. The BOC-adjusted PHC F3 concentrations were generated as the sum of the measured PHC F3a concentration and the calculated PHC F3b concentration.

BOC-adjusted PHC F3 (mg/kg) (Formula 3.1)

= measured total F3a (mg/kg) + calculated PHC F3b (mg/kg)

= a + (b/c x a)

a = Measured F3a concentration in compost sample

b = Measured %F3b of total F3 in diesel drilling waste spike source

c = Measured %F3a of total F3 in diesel drilling waste spike source

Figure 3.4A illustrates that the diesel drilling waste was dominated by the F3a range (89%), while the clean compost (Figure 3.4C) was dominated by the F3b range (85%). The spiked compost (Figure 3.4B) had intermediate distributions of 73% F3a and 27% F3b. The following example provides a BOC-adjusted PHC F3 calculation for diesel drilling waste spiked compost (Figure 3.4B) at a nominal F3 concentration of 2,000 mg/kg. The 89% F3a and 11% F3b percentages in the diesel drilling waste (Figure 3.4A) were used as the spike source in the calculation. While the measured F3 concentration was 2,540 mg/kg, the BOC-adjusted PHC F3 calculation determined that the authentic PHC F3 concentration from the diesel spike was 2,083 mg/kg.

BOC-adjusted PHC F3 (mg/kg)

= measured F3a (mg/kg) + calculated PHC F3b (mg/kg)

= 1,854 + (0.11/0.89 x 1,854)

= 2,083 mg/kg

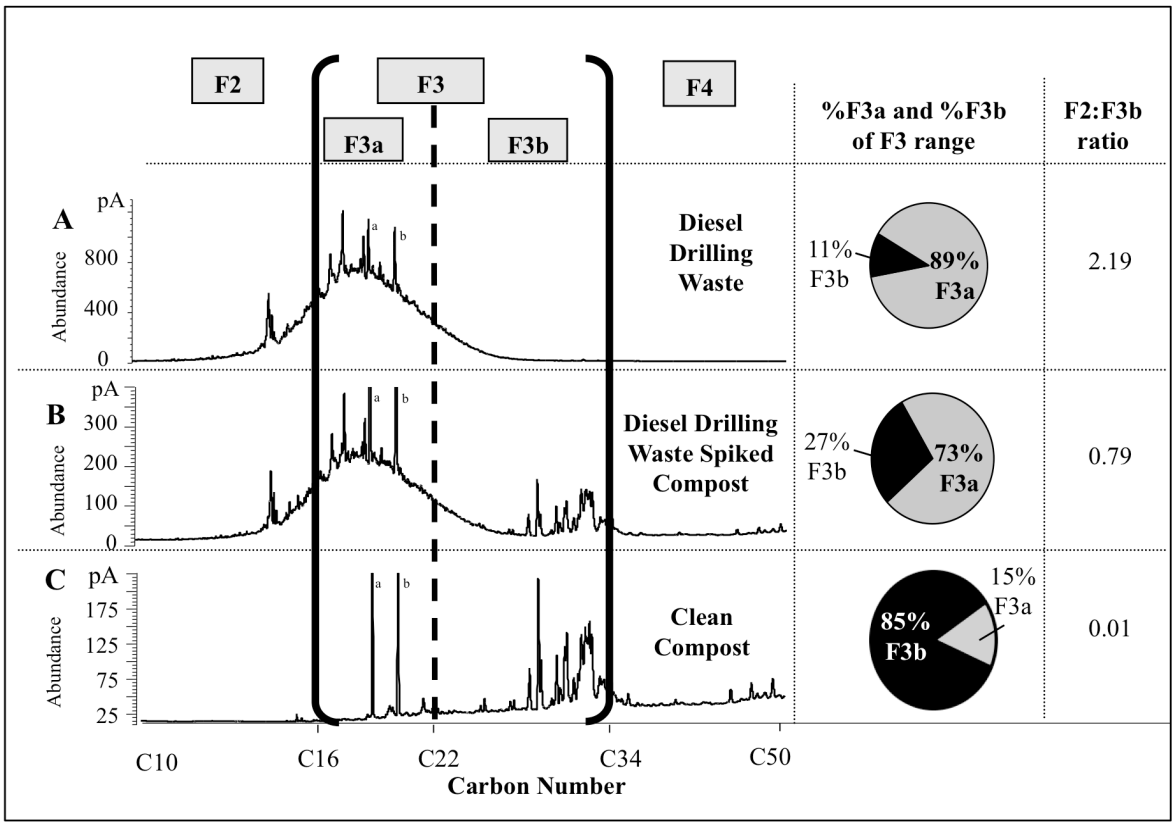

Figure 3.4. Example GC-FID chromatograms of PHC F2 (C10-C16), F3 (C16-C34), F4 (>C34), sub-fractions F3a (C16-C22) and F3b (C22-C34); F3a and F3b percentage pie charts; and F2:F3b ratios. **(A)** Diesel drilling waste. **(B)** Manure spiked with diesel drilling waste – nominal F3 concentration of 2,000 mg/kg, measured F2 = 610mg/kg, measured F3a = 2,081 mg/kg, measured F3b = 769. **(C)** Clean manure – measured F2 = <10 mg/kg, measured F3a = 120 mg/kg, measured F3b = 680 mg/kg. The clean manure F2:F3b ratio calculation used an F2 concentration of 5 mg/kg, which was half of the F2 detection limit. [a]o-terphenyl surrogate. [b]alpha-androstane internal standard.

Rationale for using the spiked sand F3a and F3b percentages to calculate the BOC-adjusted PHC F3 concentrations in the clean and diesel drilling waste spiked manure compost

As discussed in Chapter 2, the spiked sand provided a means of measuring authentic PHC F3a and F3b percentages without detectable BOC interference. The spiked sand and spiked compost treatments were prepared by the same procedures and they were exposed to the same environmental conditions and extraction methods. The F3a and F3b percentages in the spiked sand were therefore used as the representative diesel drilling waste source for

calculating the Day 0, Day 150 and Day 300 BOC-adjusted PHC F3 concentrations in the clean and spiked compost samples.

BOC-adjusted PHC F3 concentrations

All of the Day 0, Day 150 and Day 300 BOC-adjusted PHC F3 concentrations were less than the measured total F3 concentrations that were generated by the CWS PHC method (Figure 3.5). All of the total F3 and BOC-adjusted PHC F3 concentrations in the highly spiked compost (sM1) and moderately spiked compost (sM2) exceeded the 300 mg/kg CWS PHC F3 guideline. The total measured F3 concentrations in all of the spiked compost samples had therefore authentically exceeded the PHC F3 soil guideline. In contrast, all of the total measured F3 concentrations in the clean compost (M) exceeded the guideline, while the BOC-adjusted PHC F3 concentrations were below the guideline. The total F3 concentrations in these clean compost samples had therefore falsely exceeded the PHC F3 soil guideline.

F2:F3b ratio indicator of PHC presence versus absence in manure compost

In this microcosm experiment, F2:F3b threshold ratio of 0.10 was used to indicate PHC absence (<0.10) in clean compost versus PHC presence ($\geq$0.10) in diesel drilling waste spiked compost and sand. As discussed in Chapter 2, the F2:F3b ratio was calculated as the measured F2 concentration divided by the measured F3b concentration. The F2:F3b threshold ratio is based on observations made during each phase of this study, including: i) The total F3 range in clean compost was strongly dominated by the F3b range, while diesel

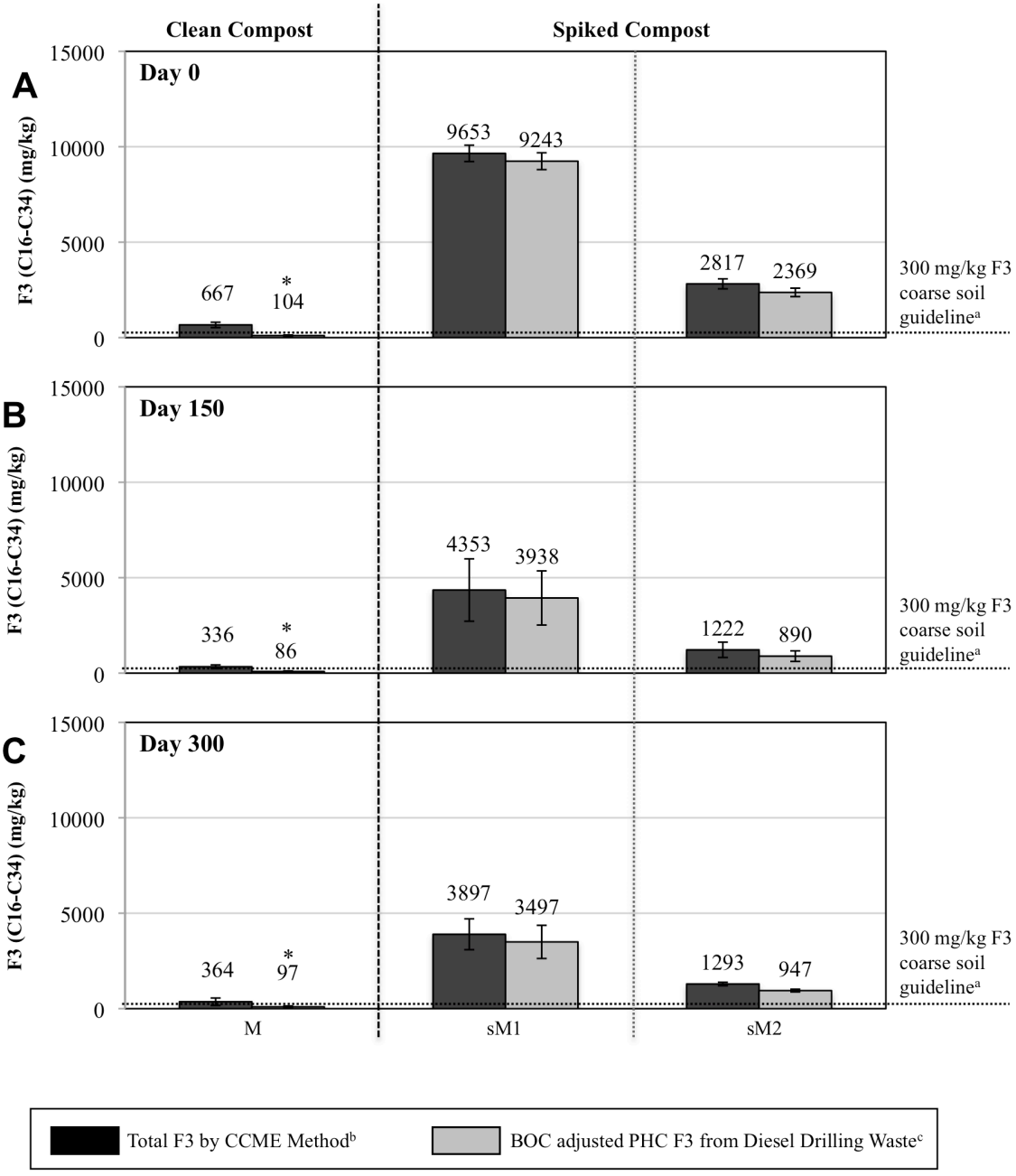

Figure 3.5. Measured F3 and calculated BOC-adjusted PHC F3 concentrations in clean and diesel invert spiked manure on: **(A)** Day 0; **(B)** Day 150; and **(C)** Day 300. [a]CWS PHC Tier 1 F3 coarse surface soil guideline for agricultural/residential/parkland land uses in potable groundwater conditions (CCME 2008a). [b]Total F3 measured by CWS PHC method (CCME 2001a). [c]BOC-adjusted PHC F3 concentration from diesel drilling waste, calculated by Formula 1. *Asterisk indicates concentrations that did not exceed the F3 guideline. M – clean manure; sM1 – manure spiked with nominal 13,400 mg/kg F2-F4 diesel drilling waste; sM2 – manure spiked with nominal 2,613 mg/kg F2-F4 diesel drilling waste.

drilling waste was strongly dominated by the F3a range; and ii) F2 concentrations were non-detectable in the clean compost, but they were strongly present in the diesel drilling waste.

F2:F3b ratios

The F2:F3b ratios in all of the spiked compost and spiked sand samples were at least one order of magnitude higher than the ratios in the clean compost samples (Figure 3.6). The F2:F3b ratios were greater than the 0.10 in all spiked compost and sand samples, regardless of F3 concentrations, weathering stages or TOC levels. The F2:F3a ratios in the highly spiked compost (sM1) ranged from 0.54 to 1.52 with a mean of 1.45. The moderately spiked compost (sM2) had relatively lower ratios ranging from 0.25 to 0.80. The moderately spiked sand (sS) ratios were similar to the highly spiked compost, with a range of 0.56 to 3.10. The F2:F3b ratios in all of the clean compost (M) samples were less than 0.10, with a range of 0.01 to 0.04.

Figure 3.6 A illustrates that seven of the nine clean compost samples falsely exceeded the 300 mg/kg CWS PHC F3 soil guideline. In contrast, all nine samples had BOC-adjusted PHC F3 concentrations that did not exceed the guideline. These data demonstrated that combining the F2:F3b ratios with the BOC-adjusted PHC F3 concentrations provided a stronger indication of false F3 guideline exceedances by the clean compost samples. Perhaps more importantly, the diesel drilling waste spiked compost F3 concentrations and the BOC-adjusted PHC F3 concentrations were very similar, with the same guideline exceedances occurring in all of the samples.

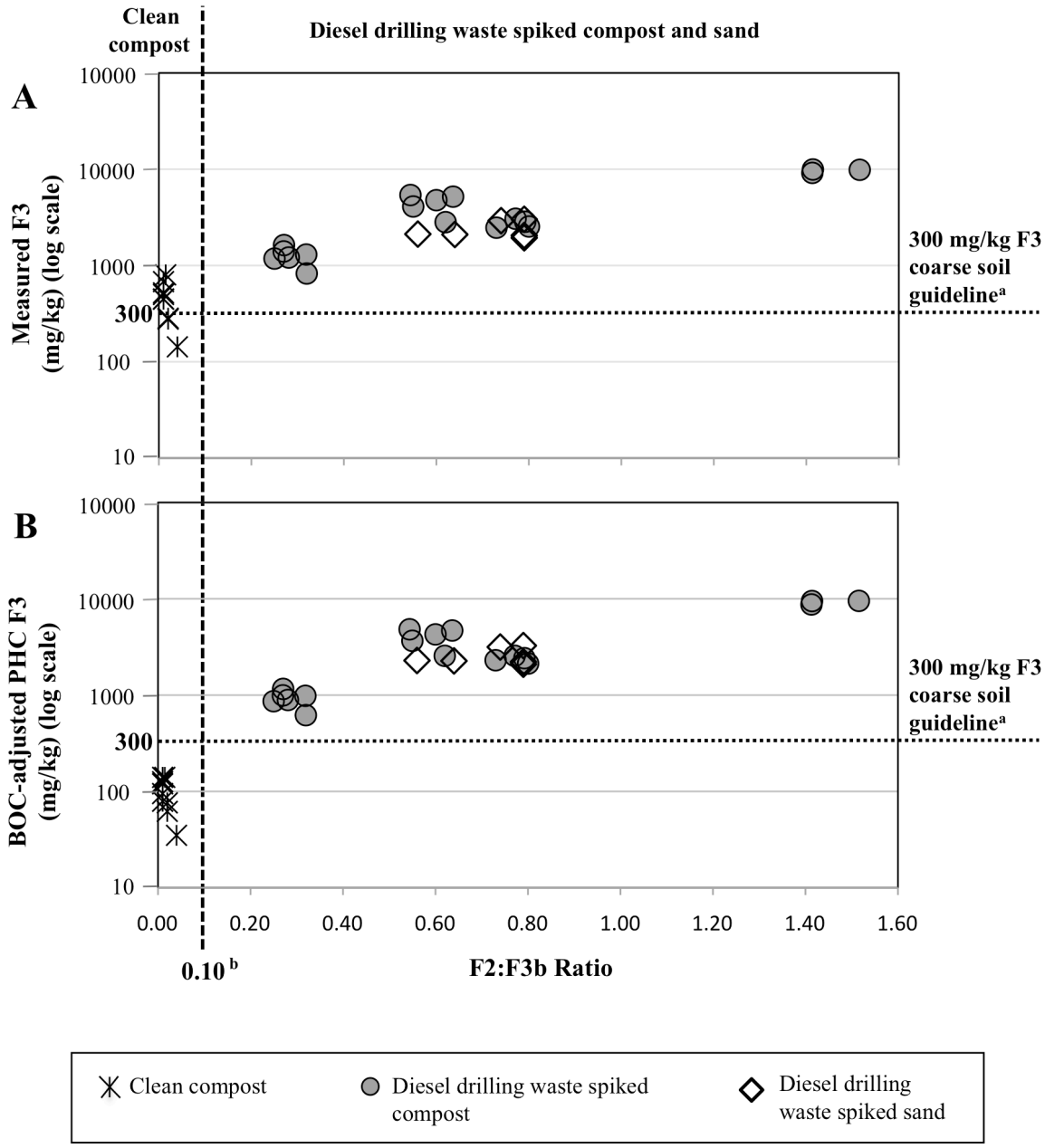

Figure 3.6. Comparison of Day 0, Day 150 and Day 300 clean manure compost and diesel drilling waste spiked manure compost and sand. **(A)** Measured F3 concentrations and measured F2:F3b ratios; and **(B)** Calculated BOC-adjusted PHC F3 concentrations and measured F2:F3b ratios. [a]CWS PHC Tier 1 F3 coarse surface soil guideline for agricultural/residential/parkland land uses in potable groundwater conditions (CCME 2008a). [b]All clean manure compost had F2:F3b ratios less than or equal to 0.10, while all diesel drilling waste spiked manure compost and sand samples had F2:F3b ratios greater than 0.10.

The F2, F3, F4, F3a and F3b data were used to develop a decision process for determining if a soil sample should be included or excluded from a soil remediation zone at a diesel contaminated site (Chapter 5, Figure 5.3).

Question 1 (CWS PHC Tier 1): Do the F2 and/or F4 concentrations exceed soil quality guidelines?

- "Yes" – Sample location is contaminated and would require remediation.

  The F2 concentrations in all of the Day 0 diesel drilling waste spiked compost and sand samples were several times higher than the soil guidelines. These contaminated samples would therefore be included within the PHC contaminated soil remediation zone.

  "No" – Proceed to Question 2. (All of the clean compost samples had non-detectable F2 concentrations. However, some of the Day 150 and Day 300 moderately spiked compost samples had detectable F2 concentrations that did not exceed the F2 guideline. Both scenarios would lead to Question 2. All of the F4 concentrations were well below the F4 guideline, which was due to the absence of diesel PHCs in the F4 range.).

Question 2 (CWS PHC Tier 1): Does the F3 concentration exceed the CWS PHC 300 mg/kg F3 soil guideline?

- "No" – Sample location would not require remediation.
- "Yes" – proceed to Question 3.

Question 3: Does the diesel drilling waste contamination source have an F2:F3b ratio greater than or equal to the 0.10 PHC presence threshold value?

- *"Yes"* – Proceed to Question 4 (The diesel drilling waste used in this experiment did have an F2:F3b ratio of greater than or equal to 0.10).
- *"No"* – Do not proceed because diesel drilling waste contamination source does not meet carbon range requirements necessary for the F2:F3b evaluation and/or BOC-adjusted PHC F3 soil evaluation.

Question 4: Does the GC-FID chromatogram pattern match the diesel invert and/or clean compost GC-FID pattern(s)

- "Yes" – Proceed to Question 5 (All of the sample GC-FIDs matched the diesel drilling waste spike source and/or the clean compost/sand).
- "No" – The sample may be contaminated by a non-diesel PHC product and must therefore be excluded from the decision tree evaluation

Question 5 (CWS PHC Tier 2): Is the F2:F3b ratio greater than or equal to the 0.10 PHC presence threshold value?

- "No" – the sample location would not require remediation. (The false F3 guideline exceedances in the clean manure compost were resolved by the F2:F3b ratio. All of the clean manure samples with greater than 300 mg/kg F3 concentrations had F2:F3b ratios of less than 0.10, while all of the spiked manure and sand had ratios that were greater than 0.10.).
- "Yes" – proceed to Question 6

Question 6 (CWS PHC Tier 2): Is the BOC-adjusted F3 concentration greater than the CWS PHC 300 mg/kg F3 soil guideline?

- *"No" – the sample location would not require* remediation. (Most of the clean manure samples had F3 concentrations that exceeded the F3 soil guideline, but all of the BOC-adjusted F3 concentrations were less than the guideline).
- "Yes" –Proceed to Question 7 (None of the clean compost samples had BOC-adjusted F3 concentrations that exceeded the F3 soil guideline).

Question 7 (CWS PHC Tier 2): Does the PAH and/or analysis verify the presence of diesel PHCs?

- "No" – The sample location would not require remediation.
- "Yes" – The sample location would require remediation.

Statistical analysis results

Day 0 and Day 300 ANOVA F-test *p*-values were calculated as F2, F3a, F3b,F4 and BOC-adjusted PHC F3 compositional data (Table 3.2). F2, F3a, F3b and F4 *p*-values were calculated as components of one group. In this context, *p*-values for each fraction were relative to the other components as well. *p*-values greater than the critical value of 0.05 were identified as not significantly different, while values less than 0.05 were significantly different. The clean compost F2 *p*-values are not presented due to non-detectable F2 concentrations. The spiked sand F4 *p*-values are not presented due to non-detectable F4 concentrations. As previously discussed, BOC-adjusted PHC F3 concentrations were not calculated for the spiked sand. For this reason, *p*-values are not presented for the spiked sand.

The F2 *p*-values were highly significant in the highly spiked compost (0.0022), moderately spiked compost (0.00001) and the spiked sand (0.00001).. The BOC-adjusted

F3 *p*-values were also highly significant in the highly spiked compost (0.0008) and the moderately spiked compost (0.0008). These results indicate that significant PHC degradation did occur in all of the spiked compost and sand treatments. The clean compost had a significant BOC-adjusted F3 p-value of 0.360. The F3a *p*-values were non-significant in the highly spiked compost (0.0938) and in the spiked sand (0.6198). the F3a p-values were non-significant in the moderately spiked compost (0.0003) and in the clean compost (0.0413). The F3b *p*-values were significant in the highly spiked compost (0.0058) and spiked sand (0.0372). The F3b p-values were non-significant in the moderately spiked compost (0.1158) and clean compost (0.0758). The F4 *p*-values were significant in the clean compost (0.0421) and non-significant in the highly spiked compost (0.6182) and in the moderately spiked compost (0.6246). The diesel drilling waste PHCs did not extend into the F4 carbon range, indicating that the F4 concentration decreases in clean compost and spiked compost were due to degradation of BOCs.

These ANOVA F-test *p*-value trends are comparatively different from those observed in the Chapter 2 crude oil contaminated peat experiments. In the previous study, all of the spiked peat and sand treatments had significant F3a decreases.. In this study however, the F3a concentrations did not demonstrate such clear trends. Studies indicate that diesel degradation rates tend to be faster than crude oil degradation rates (Peressutti et al. 2003; Sanscartier et al. 2010). However, such a comparison is not relevant to this experiment because diesel drilling waste was used rather than pure diesel fuel/oil. Organoclay used in diesel drilling mud is known to reduce PHC biodegradation rates due to adsorption/absorption effects (Ball et al. 2012). PHCs in the lightest F2 carbon range would be free to volatilize. However, organoclay particles would trap the heavier PHC

compounds, which would limit their bioavailability to microbial degradation processes. These factors may explanation the relatively lower PHC degradation rates that were observed in this diesel drilling waste spiked compost experiment.

Table 3.2: Analysis of variance between Day 0 and Day 300 carbon range concentrations
F test p values, 95% confidence, triplicate data, logcentred transform compositional analysis

Sample Days/ Analytes	M1 Clean Manure Compost	sM1[a] Highly Spiked Manure Compost	sM2[a] Moderately Spiked Manure Compost	sS[b] Moderately Spiked Sand +Bacteria +Nutrients
F2[c]	NC	0.0022*	0.00001*	0.00001*
F3a[c]	0.0413*	0.0938	0.0003*	0.6198
F3b[c]	0.0758	0.0058*	0.1158	0.0372*
F4[c]	0.0421*	0.6182	0.6246	NC
PHC F3[d]	0.0360*	*0.0008	*0.0008	NC

[a]sM1 – diesel drilling waste nominal spike concentration: F2-F4 = 13,400 mg/kg; F2 = 3,400 mg/kg; F3 = 10,000 mg/kg; F4 <50 mg/kg.

[b]sM2 and sS - whole crude oil nominal spike concentration: F2-F4 = 2,613 mg/kg; F2 = 613 mg/kg; F3 = 2,000 mg/kg; F4 <50 mg/kg.

[c]Compositional data analysis included: F2 (C10-C16), F3 (C16-C34), F3a (C16-C22), F3b (C22-C34), F4 (>C34).

dCompositional data analysis included: F2 (C10-C16), BOC-adjusted PHC F3 (see Formula 1) and F4 (>C34).

NC – Non-detectable therefore not calculated

*Values less than 0.05 indicate significant differences between Day 0 and Day 300 concentrations

CONCLUSIONS

The results of this study demonstrated that the PHC and BOC GC-FID chromatogram patterns distinguished clean compost from diesel drilling waste spiked compost and sand. The BOC-adjusted PHC F3 calculation used F3a and F3b percentage distributions to calculate the authentic PHC F3 concentrations. The F2:F3b threshold ratio of 0.10 indicated PHC presence (≥0.10) in the diesel spiked compost and sand, while indicating PHC absence (<0.10) in the clean compost. These complimentary approaches were used to resolve false exceedances of PHC F3 soil guidelines in clean samples and in fresh and weathered samples as well. These conclusions are consistent with the Chapter 2

study, which used the same Tier 2 approach to resolve BOC interferences in clean peat and crude oil spiked peat.

The current study also documented moderate PHC F2-F4 reduction rates of diesel in drilling waste using composted manure, which was likely limited by PHC bioavailability in the presence of drilling mud organoclay. These results emphasize the need for enhanced bioremediation techniques for diesel drilling waste, beyond traditional land farming methods, to possibly include inoculations with extracellular enzyme producing bacteria and fungi.

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CHAPTER 4

Petroleum hydrocarbon chemistry field survey of Canadian background soils: Implications for a new mathematical GC-FID approach to resolve false detections of petroleum hydrocarbons in clean soils

OVERVIEW

Petroleum hydrocarbons (PHC) are among the most common soil contaminants throughout the world. PHC chemistry analysis is a critical component of environmental risk assessments and site remediation projects. The *Reference Method for the Canada-Wide Standard (CWS) for Petroleum Hydrocarbons (PHC) in Soil* was developed by the Canadian Council of Ministers of the Environment (CCME) for the purpose of providing laboratories with methods for producing accurate and reproducible PHC soil analysis results. The CWS PHC document includes Tier 1 numerical soil quality guidelines that apply to the following four carbon ranges/fractions: F1 (C6-C10), F2 (C10-C16), F3 (C16-C34) and F4 (>C34). The CWS PHC soil analysis methods and quality guidelines were developed and validated for soils with approximately 5% total organic carbon (TOC). However, organic peat soils in this study have much higher TOC levels of up to 41%. Peat is naturally enriched with biogenic organic compounds (BOCs), such as waxes and fatty acids. Co-extracted BOCs are misidentified as PHCs, which can result in false exceedances of PHC soil guidelines. The primary objective of this CWS PHC background soil field survey was to evaluate false PHC detections in soil samples collected from 34 non-commercial/non-industrial Canadian sites with no histories of contamination. The list of analytes included: soil type, TOC, polycyclic aromatic hydrocarbons (PAHs), F2, F3, F4, F3a (C16-C22) and F3b (C22-C34). The soils include a wide range of mineral and organic soil types, with TOC levels ranging from 0.7% to 41%. PAH concentrations indicative of petroleum sources were non-detectable in all of the soil samples. F2 concentrations were non-detectable in 30 of the 34 samples, with low F2 concentrations of less than 28 mg/kg detected in only four samples. F3 was the only fraction to exceed the CWS PHC 300 mg/kg

F3 coarse soil guideline. The F3 concentrations ranged from less than 50 mg/kg to 1,431 mg/kg, with 15 samples exceeding the guideline. The F3 guideline was often exceeded by soils with TOC percentages ranging from 3% to 41%. The guideline was exceeded by all soils with greater than 26% TOC. The F3b percentages were high, with average and median F3b percentages of 93%. As discussed in Chapter 2 and Chapter 3, the absence of crude oil and diesel PHCs in organic soils and compost was indicated by F2:F3b ratios of less than 0.10. The F2:F3b ratios were all less than 0.10 in the samples that also had high F3 concentrations of greater than the CWS PHC 300 mg/kg guideline.

INTRODUCTION

Petroleum hydrocarbon (PHC) soil contamination is a globally recognized issue. There are an estimated 14,173 PHC contaminated sites located throughout Canada (ELM 2006; Sanscartier et al. 2009, TBS 2011). The most common PHC contamination products include crude oils, heating oils and transportation fuels. By definition, "*A contaminated site is one at which substances occur at concentrations above background levels and pose, or are likely to pose an immediate or long-term hazard to human health or the environment, or exceed the levels specified in policies and regulations"* (Office of the Auditor General of Canada 2012). This definition emphasizes the critical role that "background" concentrations play when evaluating petroleum hydrocarbon (PHC) soil contamination risks. This Canadian background soil survey documented PHC concentrations and Total Organic Carbon content (TOC) in 34 soils collected from non-commercial and non-industrial sites with no histories of contamination. TOC is a gross measure of all forms of organic carbon found in PHCs and in natural organic matter (e.g. decaying plant and animal

matter). TOC in mineral soils can be as low as 0%, while organic peat soils in this and other studies range from 20% to 60% (Chapter 2; Szajdak et al 2007; Li et al 2004; Zhou et al 2005). The following discussion explains why false PHC detections can occur in clean organic soils.

The *Reference Method for the Canada-Wide Standard (CWS) for Petroleum Hydrocarbons (PHC) in Soil*, was developed by the Canadian Council of Ministers of the Environment (CCME) in 2001 (CCME 2001a). The standards provide analytical methods for laboratories to generate accurate and reproducible PHC soil chemistry results. CWS PHCs are reported according to the following carbon ranges/fractions: F1 (C6-C10), F2 (C10-C16), F3 (C16-C34) and F4 (>C34). The CWS PHC Tier 1 standards include generic soil quality guidelines for each of the four fractions, as shown in Table 1.1. Exceedances of these generic guidelines may require site-specific evaluations conducted at the Tier 2 and/or Tier 3 levels. The PHC soil guidelines are based on a risk assessment approach that considers site-specific factors such as land use, groundwater potability and fine/coarse soil texture. It is important to note that the soil texture categories apply to mineral soils (silt, clay, sand, gravel), which do not apply to organic peat soils. Selection of the most stringent or less stringent soil texture category may be based on site-specific risks of contaminants migrating to off-site locations.

Although the CWS PHC soil extraction solvents are intended to target PHC compounds, they inadvertently co-extract biogenic organic compounds (BOCs) as well. The term "BOC" refers to natural organic compounds, such as waxes and fatty acids, which are produced by soil organisms such as microbes, plants, insects, etc. Extracted BOCs are misidentified as PHCs, which can cause false exceedances of PHC soil guidelines. The

Canada-wide Standard for Petroleum Hydrocarbons (PHC) in Soil, User Guidance document, recommends the following solution to the problem of falsely elevated PHC concentrations, *"If there is reason to suspect that soils may have a high organic carbon content, it may be beneficial to collect samples for organic carbon content analysis from background soils, and to analyze background soils for PHC concentrations*." (CCME 2008b). The next step in this process is to subtract background F2, F3 and F4 concentrations from the contaminated soil F2, F3 and F4 concentrations. However, this background subtraction approach can be highly problematic when natural variability in parent material, depth and hydrologic regimes produce widely variable results (Alberta Environment 2010). Distinguishing clean organic background soils from PHC contaminated soils can present significant problems for the following scenarios:

i) Delineating PHC contamination boundary zones at spill sites;
ii) Determining if PHC contaminated soils have achieved bioremediation targets; and
iii) Determining if abandoned, vacant and/or unfamiliar sites are PHC contaminated at all.

Chapter 2 and Chapter 3 demonstrated that CWS PHC Gas Chromatography Flame Ionization Detector (GC-FID) chromatogram patterns could be used to distinguish clean peat from crude oil and clean manure compost from diesel drilling waste. The key indicators of clean peat and manure compost were as follows:

i) The total F3 concentrations were dominated by the F3b sub-fraction range (C22-C34); and
ii) The F2:F3b ratios were less than 0.10.

The primary objective of this study was to determine if these same indicators could be applied to soils with a range of mineral to organic compositions. The second objective was to determine if there was a correlation between increasing F3 concentrations and increasing TOC percentages.

MATERIALS AND METHODS

Soil Sample Site Selections

Soil samples were collected by the Geological Survey of Canada and Natural Resources Canada as part of the *North American Soil Geochemical Landscapes Project* to generate systematic continent-wide data on background variations in soil chemistry and physical characteristics (Government of Canada 2008). Soil sample site selections were based on a low-density 40 km x 40 km grid, with one sample collected every 1,600 km^2. All sample sites had no known contamination histories. A total of 34 soil samples were collected from the provinces of Alberta, British Columbia and Newfoundland for analysis in this study.

Soil Sampling Methods

Soil samples were collected from depths of 5 cm below the root zone and within an area of 100 cm^2 using clean shovels. The samples were placed into food grade freezer bags and couriered to ALS Laboratories located in Waterloo, Ontario, Canada. The 34 soil samples were kept frozen at -20 $^{\circ}C$ until they could all be analyzed as one group.

Forensic Chemistry Analysis

Eight of the 34 soil samples were selected for PHC presence versus absence forensic analysis conducted by the Environment Canada Oil Spill Research Laboratory (Ottawa, Ontario, Canada). The eight samples were selected to represent the full range of high to low TOC percentages within the larger group. Detailed descriptions of the forensic analysis methods and list of analytes are included in Wang et al. 2008 and Wang et al. 2010. The list of analytes are briefly described as follows: i) Polycyclic Aromatic Hydrocarbons (PAHs) are multiple aromatic benzene rings consisting of carbon and hydrogen. Alkylated and non-alkylated PAHs were used to indicate the absence versus presence of liquid and partially combusted PHCs from unrefined (i.e. crude oil) and/or refined (i.e. diesel) products. Although crude oil is dominated by substituted forms of PAHs, several non-substituted forms may be detected as well (i.e. acenaphthene, fluorine, naphthalene, phenanthrene). Refined PHCs are also dominated by substituted PAHs, with relatively higher proportions of non-substituted PAHs as well. While both PAH forms can identify liquid PHC sources, they can also identify pyrogenic PHCs originating from point or non-point atmospheric deposition sources as well. ii) PHC biomarkers (i.e. steranes, hopanes, etc.) were used in this study to indicate the presence versus absence of crude oil. PHC biomarker chemical structures originate from ancient biological sources such as plants, algae and/or microorganisms from which the crude oil was formed. PHC biomarkers are valued forensic tools because of their PHC specific structures and environmental persistence. iii) Carbon Preference Index (CPI) refers to the ratio of odd to even carbon numbered *n*-alkanes in the C21-C34 range. Most PHC sources have virtually equal distributions of odd to even *n*-alkane numbers with CPI values that are close to the number one (unity). In contrast,

biogenic organic matter (i.e. plant/animal tissues, fecal waste, etc.) is dominated by odd *n*-alkanes, which may have much higher CPI values ranging from 2 to 12. Mixtures of PHCs and BOCs tend to have intermediate CPI values; iv) Unresolved Complex Mixtures (UCM) can be visually identified as a distinctive "hump" which appears on GC-FID chromatograms between the resolved peaks baseline and the solvent baseline. GC-FID analysis cannot resolve the thousands of compounds that are present in PHC products. As a result, PHC complex mixtures appear as a consolidated UCM hump on GC-FID chromatograms. PHC UCMs are regularly shaped as compared to irregularly shaped BOC UCMs, which appear in clean organic soils.

F2, F3 and F4 PHC Soil Extraction and GC-FID Analysis

Detailed descriptions of extraction materials and methods used in this study are described in Chapter 2. Briefly, the PHC soil extractions and GC-FID runs were conducted by ALS Environmental. All methods were based on the CCME Reference Method for the *Canada-Wide Standard (CWS) for Petroleum Hydrocarbons in Soil – Tier 1 Method* (CCME 2001a). The author of this study conducted all of the GC-FID chromatogram integrations. F1 was not analyzed because the CCME user guidance document refers to biogenic interference occurring only in the F2, F3 and F4 carbon ranges (CCME 2008b).

Trace/organic/pesticide grade solvents and acids were purchased from Caledon Laboratories located in Georgetown, Ontario, Canada. Soxtec heat extraction in a 50:50 mixture of acetone and hexane solvents was used to extract all soil samples. Polar compounds were removed with a 50:50 mixture of hexane and DCM solvents followed by an in-situ silica gel treatment. The extracts were analyzed by an Agilent 6890N® GC-FID

on-column injector fitted with a 0.32 mm x 0.1 um x 30 m capillary column 100% poly(dimethylsiloxane) and a flame ionization detector. External standards included a CWS PHC calibration mix of C10, C16 and C34, ATSM D5442 C12-C60 linearity standard and an Accustandard FTRPH Calibration/Window Defining Standard. A retention time and response factor standard for C10-C50 hydrocarbons was determined by a pentacontane (nC50) solution. An external standard was used to identify the C22 peak for distinguishing the F3a and F3b carbon ranges. All concentrations were reported on a dry weight basis.

PAH Extraction and Analysis

ALS Environmental analyzed all soil samples for nineteen non-alkylated PAHs identified in the CCME PAH soil guidelines for protection of environmental and human health (CCME 2010a; CCME 2010b), including: acenaphthene, acenaphthylene, acridine, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(g,h,i)perylene, benzo(k)fluoranthene, benzo(g,h,i)perylene, chrysene, dibenzo(a,h)anthracene, fluoranthene, fluorene, indeno(1,2,3-cd)pyrene, naphthalene, phenanthrene, pyrene, quinoline. 1-, 2- methylnapthalene substituted PAHs were analyzed as well. All materials and methods were in compliance with EPA SW846 8270. The standards and reagents used in the method, including their grade and suppliers, are listed as follows. Trace organic/pesticide grade methanol and toluene, distilled in glass, were purchased from Caledon Laboratories. Semi-volatile internal standard mix, 2000 μg/mL in DCM, was purchased from Supelco, located in Bellefonte, Pennsylvania, United States. 8270 surrogate standard mix, 4000 μg/mL in DCM, was purchased from Supelco. PAH custom mix, 2000 μg/mL in DCM:Benzene, was purchased from Supelco. PAH Custom

Mix, 2000 μg/mL in DCM, was purchased from CPI International, located in Toronto, Ontario, Canada. Quality assurance measures included one method blank and one duplicate sample for each group of twenty samples or less. The method detection limits were as follows: <0.8 mg/kg for Acridine, <0.05 mg/kg for 1-methylnaphthalene, 2-methylnapththalene, acenaphthene, acenaphthylene, antracene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(g,h,i)perylene, chrysene, dinbenzo(a,h)anthracene, fluoranthene, fluorene, indeno(1,2,3-cd)pyrene, pyrene and quinoline, <0.03 mg/kg for phenanthrene, <0.02 for mg/kg benzo(a)pyrene and benzo(k)fluoranthene and <0.01 for mg/kg naphthalene. The duplicate low and high tolerance levels were ± 50% relative percent difference. All samples were spiked with 2-fluorobiphenyl and p-d14-terphenyl and evaluated for a recovery tolerance rate of 60%-120%. All non-disposable glassware was washed with soap and hot water, rinsed with 25% hydrochloric acid solution, then rinsed with de-ionized tap water followed by oven drying at approximately 110 °C ± for 1-2 hours.

The PAH extraction procedure is described as follows. A 5 g soil sample was spiked with 2-fluorobiphenyl and p-d14-terphenyl surrogates and extracted into toluene. 1 mL of the resulting extract was then transferred to a 2 mL auto sampler vial and the internal standard mix was added. The extract was analyzed for PAHs using a Gas Chromatography Mass Spectrometer (GC/MS), specifically a 7890 GC/5975 MS using a splitless inlet mode with a 30m x 0.53mm x 0.5um capillary poly (5% diphenyl/95% dimethyl siloxane) phase and a Selective Ion Monitoring (SIM) mode.

Total Carbon, Total Organic Carbon and Total Inorganic Carbon Analysis

Total carbon (TC), total organic carbon (TOC) and total inorganic carbon (TIC) soil analysis was conducted by the Geologic Survey of Canada. A Leco CR-412 Carbon Analyser® method involved soil sample combustion and measurement of the released CO_2 by infrared detection. Each soil sample was split into two sub-samples. Total carbon content was determined on one sub-sample, and inorganic carbon was determined on the second sub-sample after ashing to remove the organic carbon. The organic carbon content was determined by subtracting the inorganic carbon from the total carbon content.

Statistical analysis

The statistical program StatPlus® was used to plot lines of best fit and to calculate R^2 values. Correlations between TOC percentages and individual F2, F3, F4 concentrations and F3a, F3b percentages were evaluated.

RESULTS AND DISCUSSION

Background soil survey data including: F2, F3, F4, F3a, F3b, TOC and soil type is presented in Table 4.1. The table also identifies the eight samples that were submitted for PHC presence versus absence forensic analysis.

PHC presence versus absence forensic analysis results

Forensic analysis, conducted by the Environment Canada Oil Spill Research Laboratory, determined that crude oil PHCs were absent in all eight soil samples. Trace

amounts of pyrogenic PAHs were detected, which likely originated from atmospheric deposition sources (Wang et al. 2008 and Wang et al. 2010).

Standard PAH Analysis - EPA SW846 8270

EPA PAH concentrations were non-detectable in all 34 soil samples.

F2, F3 and F4 compliance with CWS PHC soil quality guidelines

The CWS PHC F2 guideline is 150 mg/kg for both coarse and fine soils. The CWS PHC F3 guidelines for coarse and fine soils are 300 mg/kg and 1,300 mg/kg, respectively. The CWS PHC F4 guidelines for coarse and fine soils are 2,800 mg/kg and 5,600 mg/kg, respectively. As shown in Table 4.1, the F3 concentrations in 15 (44%) samples exceeded the coarse soil guideline (300 mg/kg) and two samples (6%) exceeded the fine soil guideline (1,300 mg/kg). The F3 concentrations in five samples (15%) were less than the 50 mg/kg detection limit. The F3 concentrations ranged from a minimum of <50 mg/kg to a maximum of 1,431 mg/kg. The average and median values were 309 mg/kg and 235 mg/kg, respectively. The F2 and F4 concentrations in all 34 samples were less than the most stringent coarse soil quality guidelines. The F2 concentrations were detectable in four (12%) of the 34 soil samples. The concentrations ranged from <10 to 28 mg/kg, with average and median values of <10 mg/kg. The F4 concentrations were non-detectable in seven (21%) samples. The F4 concentrations ranged from a minimum of <50 mg/kg to a maximum of 1,580 mg/kg. The average and median F4 values were 407 mg/kg and 260 mg/kg, respectively.

Table 4.1: Background PHC soil field survey results

Site Number	Soil Type[a]	%TOC[d]	Fractions (mg/kg)[b]			% of F3[c]		F2:F3b Ratio
			F2	F3	F4	%F3a	%F3b	
1	Sa/L	0.7%	<10	<50	<50	NA	NA	NA
2[e]	Si	1.6%	<10	<50	<50	NA	NA	NA
3	Si/L	1.6%	<10	233	340	10%	90%	0.02
4	Si/L	1.7%	<10	<50	<50	NA	NA	NA
5	Sa/L	2.0%	<10	108	130	9%	91%	0.05
6	Si/L	2.6%	<10	88	120	19%	81%	0.07
7	Sa/L	2.8%	<10	*357	340	8%	92%	0.02
8	Si/L	2.9%	<10	198	250	9%	91%	0.03
9[e]	Si/L	3.0%	<10	55	<50	5%	95%	0.10
10[e]	Sa/L	3.0%	<10	264	270	7%	93%	0.02
11	Si/L	3.1%	<10	<50	<50	NA	NA	NA
12	Si/L	3.5%	<10	84	130	10%	90%	0.07
13	L	3.8%	<10	<50	<50	NA	NA	NA
14	Si/L	4.7%	26	237	290	8%	92%	0.12
15	L	6.2%	<10	*492	220	1%	99%	0.01
16[e]	L	6.9%	<10	185	110	5%	95%	0.03
17	O/L	7.2%	<10	72	<50	7%	93%	0.07
18	Si/L	7.7%	<10	84	53	4%	96%	0.06
19	O/L	8.3%	<10	*386	240	3%	97%	0.01
20	O/L	10.0%	<10	147	140	6%	94%	0.04
21	O/L	15.5%	<10	*378	360	4%	96%	0.01
22	O/L	17.7%	<10	51	330	8%	92%	0.11
23	O/P	19.5%	11	*1,004	1,230	8%	92%	0.01
24[e]	O/P	19.7%	<10	*482	570	12%	88%	0.01
25	P	20.1%	28	*561	690	9%	91%	0.05
26	O	20.2%	<10	*469	410	4%	96%	0.01
27	P	21.2%	<10	61	140	10%	90%	0.09
28	P	26.5%	<10	*778	670	5%	95%	0.01
29	P	28.3%	<10	*507	580	5%	95%	0.01
30[e]	O/P	28.9%	<10	**1,335	1,580	4%	96%	0.00
31[e]	P	35.9%	23	*1,236	1,290	7%	93%	0.02
32	P	38.8%	<10	*815	680	10%	90%	0.01
33[e]	P	39.0%	<10	*1,210	1,040	3%	97%	0.00
34	P	41.4%	<10	**1,431	1,443	2%	98%	0.00

Table 4.1: Background PHC soil field survey results

Site Number	Soil Type[a]	%TOC[d]	Fractions (mg/kg)[b]			% of F3[c]		F2:F3b Ratio
			F2	F3	F4	%F3a	%F3b	
Minimum	NA	0.7%	<10	<50	<50	1%	81%	0.00
Maximum	NA	41.4%	28	1,431	1,580	19%	99%	0.12
Average	NA	13.4%	<10	309	407	5%	93%	0.04
Median	NA	7.5%	<10	235	260	4%	93%	0.02
STDV	NA	12.7%	6	419	438	3%	4%	0.04

[a]Soil type abbreviations: Si - silt, Sa - sand, L - loam, O - organic, P - peat
[b]CWS PHC Fractions: F2 (C10-C16), F3(C16-C34), F4 (>C34)
[c]F3 Sub-fractions: F3a - (C16-C22), F3b (C22-C34)
[d]TOC - Total Organic Carbon
[e]One of eight samples submitted for PHC forensic analysis. PHC indicators from liquid/non-pyrogenic sources were not detected in any of these samples.
NA - Not analyzed
STDV - Standard Deviation.
*One asterisk indicates that F3 concentration exceeded the 300 mg/kg coarse soil guideline (CCME 2008a).
**Two asterisks indicate that F3 concentration exceeded the 300 mg/kg coarse soil guideline and the 1,300 mg/kg fine soil guideline (CCME 2008a).
Soil type and TOC data provided by Geological Survey of Canada.

To the author's knowledge, there are no published studies that focus specifically on false detections of CWS PHC F2, F3 and F4 concentrations in background soils. In 2011 however, the Ontario Ministry of Environment issued F2, F3 and F4 PHC background soil standards, as detailed in *Soil, ground water and sediment standards for use under Part XV.1 of the Environmental Protection Act - Table 1: Full Depth Background Soil* (MOE 2011). The MOE background standards for coarse and fine soils, for all land use types are as follows: 15 mg/kg F2; 75 mg/kg F3; and 75 mg/kg F4. The 10 mg/kg F2 method detection limit used in the current study was less than the 15 mg/kg MOE background standard. The F2 concentrations in three (9%) of the 34 background soil survey samples were slightly above the MOE standard with values of 26 mg/kg (Site 14), 28 mg/kg (Site 25) and 23 mg/kg (Site 31). Twenty-five (74%) of the samples exceeded the MOE background F3 standard and 26 (77%) exceeded the F4 background standard. Comparative

differences between MOE background standards and the F2, F3 and F4 concentrations observed in the study may be possibly attributed to soil types and TOC, which are highly variable throughout Canada and within each province as well. Differences between the MOE F2, F3 and F4 background standards and the data generated by this study may be at least partially attributed to the different geographical soil sample locations. Another important factor relates to possible difference in extraction and polar cleanup methods, which are permitted by the CWS PHC soil standards document: "*Soxhlet* (heat) *extraction apparatus is the benchmark method for the C10 to C50 hydrocarbons, but other suitable extraction methods can be substituted provided that validation data demonstrate that the substitute method provides data comparable to the benchmark method."* (CCME 2001). As an example, laboratories may choose to use either Soxhlet/Soxtec heat extractions or mechanical cold extraction methods. Siddique et al. (2006) and Kelly-Hooper (unpublished data) observed acceptable PHC recovery correlations between hot and cold extraction methods. However, clean peat extraction studies conducted by Kelly-Hooper (unpublished data) determined that Soxtec heat extractions recovered higher BOC concentrations than were recovered by cold extraction methods. Another important factor to be considered is the use of various silica gel polar material cleanup methods, which can produce a wide range of BOC recoveries as well (Wang et al. 2012). Standard PHC analysis methods always include some form of silica gel treatment. The CWS PHC standards allow up to two silica gel treatments for highly organic materials. Various silica gel treatment methods are permitted. For example, insitu treatments pour sample extracts through funnels containing 5 grams of silica gel. On-column treatments pack long tubes with silica gel and gradually pour sample extracts through the tubes. Insitu treatments are time efficient and cost

efficient but achieve less efficient BOC removals. On-column treatments are less time efficient and less time efficient but have greater BOC removal efficiencies.

Total Organic Carbon Percentages and Correlations to F2, F3, and F4 data

The carbon percentages in all of the soils were primarily composed of organic carbon, with very low amounts of inorganic carbon. The Total Organic Carbon (TOC) percentages ranged from 0.7% to 41%, with an average of 13% and a median of 8% (Table 4.1). There was no correlation between the TOC and F2 concentrations, with a low R^2 value of 0.03 (Figure 4.1). In contrast, there was a positive correlation between increased F3 concentrations and increased TOC percentages, with a high R^2 value of 0.74. There was also a positive correlation between increased F4 concentrations and increased TOC percentages, with a high R^2 value of 0.68. The 300 mg/kg F3 coarse soil guideline was exceeded by soil samples with TOC percentages ranging from 3% to 41%. The only two samples to exceed the 1,300 mg/kg F3 fine soil guidelines had high TOC percentages of 29% and 41%.

Correlations between TOC percentages and F3a (C16-C22) and F3b (C22-C34) percentages

As discussed in Chapter 2, the total F3 range in the clean peat soils was dominated by the F3b range. The background soils in the current study were also dominated by F3b, with a range of 81% to 99% and average and median values of 93%. Figure 4.2 provides example GC-FID chromatograms, which illustrate F3b dominance in organic and inorganic

soils. There was a very low correlation between TOC and F3a and F3b percentages, with an

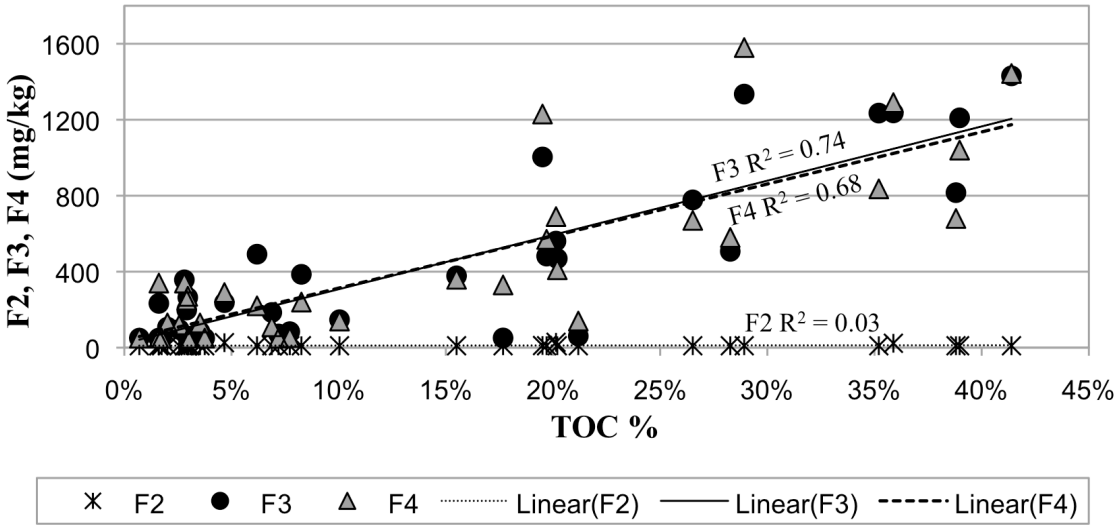

Fig. 4.1. Correlations between TOC percentages and F2, F3, F4 concentrations in soil survey samples

R^2 value of 0.06 (Figure 4.3). These data indicate that the F3b percentages were much higher than the F3a percentages in all of the inorganic and organic soils, regardless of TOC percentages.

F2:F3b ratios

For the purposes of the current study, "low" F3 and F3b refers to concentrations that did not exceed the CWS PHC 300 mg/kg F3 coarse soil guideline. "Low" F2:F3b refers to ratios that are less than the 0.10 threshold value, which is based upon the results of the crude oil contaminated peat experiment (Chapter 2) and the diesel drilling waste contaminated manure compost experiment (Chapter 3). In both experiments, all of the PHC contaminated peat and manure compost samples had F2:F3b ratios of greater than 0.10, while all of the clean peat and manure compost samples had ratios of less than 0.10.

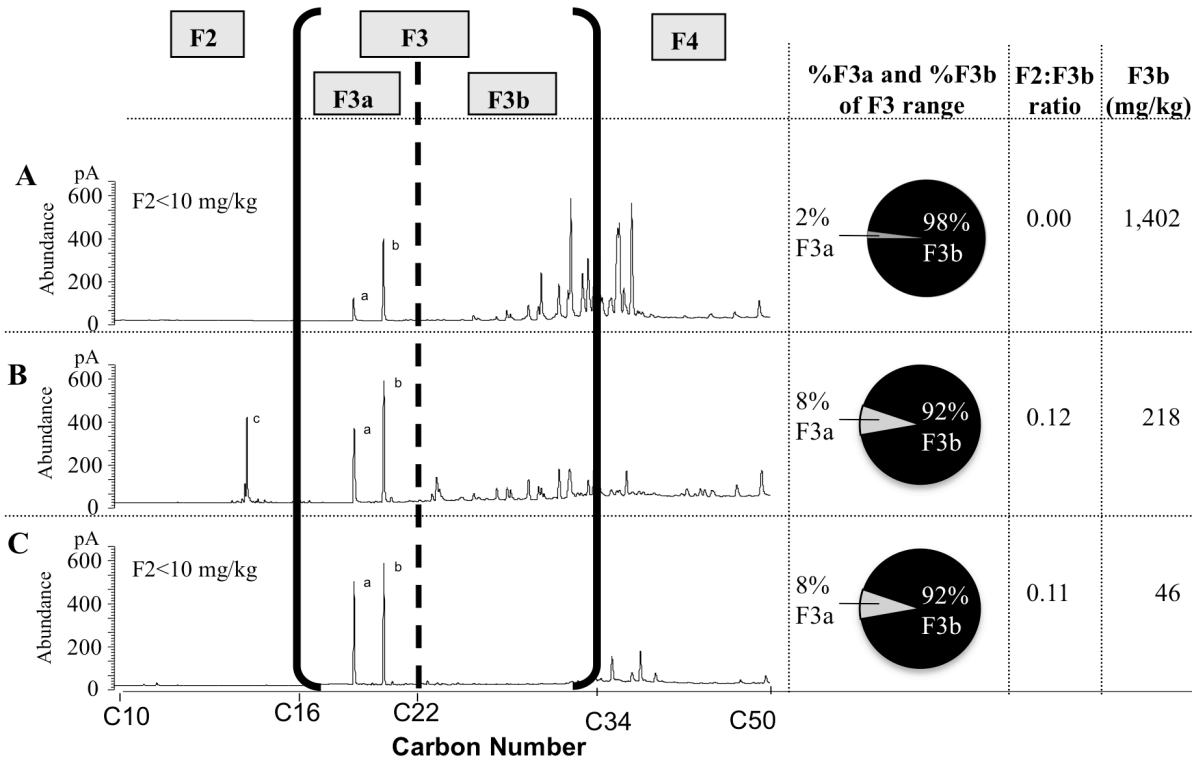

Fig. 4.2. Example GC-FID chromatograms of PHC F2 (C10-C16), F3 (C16-C34), F4 (>C34), sub-fractions F3a (C16-C22) and F3b (C22-C34); F3a and F3b percentage pie charts; and F2:F3b ratios. **(A)** peat site #34 - measured F2 = <10 mg/kg, measured F3a = 29 mg/kg, measured F3b = 1,402 mg/kg. **(B)** Silt loam site #14 - measured F2 = 26 mg/kg, F3a = 19, F3b = 218. **(C)** Silt loam site #22 - measured F2 = <10 mg/kg, measured F3a = 5 mg/kg, measured F3b = 46 mg/kg. F2 concentrations in Samples A and C were less than the 10 mg/kg detection limit. The F2:F3b ratio calculations used 5 mg/kg F2 concentrations, which was half of the F2 detection limit. [a]o-terphenyl surrogate. [b]alpha-androstane internal standard. [c]Unusual biogenic peak in F2 range, 26 mg/kg.

The background PHC soil survey results determined that the lowest F2:F3b ratio of 0.00 was measured only in peat with at least 29% TOC, while the highest F2:F3b ratio of 0.12 was measured in silt loam with 5% TOC. The average and median F2:F3b ratios were 0.04 and 0.02, respectively, for all 34 soils. Figure 4.2 illustrates example chromatograms for soils with a wide range of F3 concentrations and F2:F3b ratios. The peat sample collected from site number 34 (Figure 4.2A), had the highest F3 concentration of 1,431 mg/kg. The non-detectable F2 concentration and the high F3b concentration in this peat sample produced the lowest F2:F3b ratio of 0.00. In contrast, the silt loam sample collected

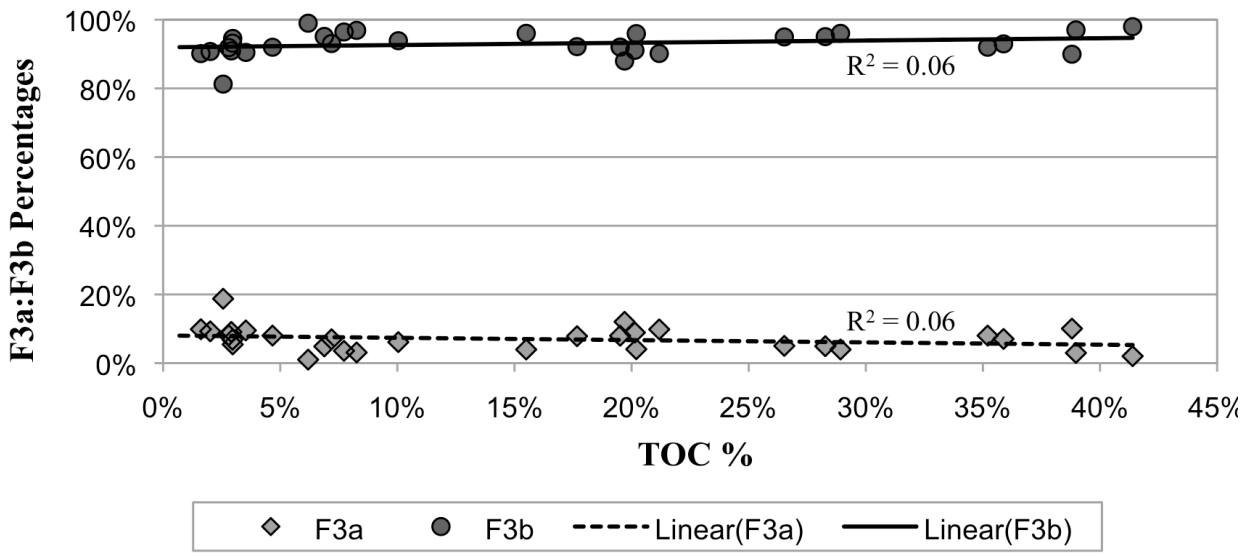

Figure 4.3. Correlations between TOC percentages and F3a and F3b percentages in soil survey samples

from site number 14 (Figure 4.2B) was one of only four samples with detectable F2 concentrations. This chromatogram includes an unusual biogenic peak in the F2 range, with a concentration of 26 mg/kg. The detectable F2 concentration combined with the low F3b concentration produced the highest F2:F3b ratio of 0.12. The silt loam sample collected from site number 22 (Figure 4.2C) had non-detectable F2 and a low F3b concentration of 46 mg/kg, which produced a low F2:F3b ratio of 0.11.

The Figure 4.4 scatter plot includes the F2:F3b ratios (x-axis) and F3 concentrations (y-axis) for 30 of the 34 samples with detectable F2, F3 and/or F4 concentrations. The scatter plot illustrates that all of the samples with greater than 300 mg/kg F3 concentrations had F2:F3b ratios of less than 0.06. These data indicate that F2:F3b threshold value of 0.10 did indicate false exceedances of the 300 mg/kg F3 coarse soil guideline for these background soil samples.

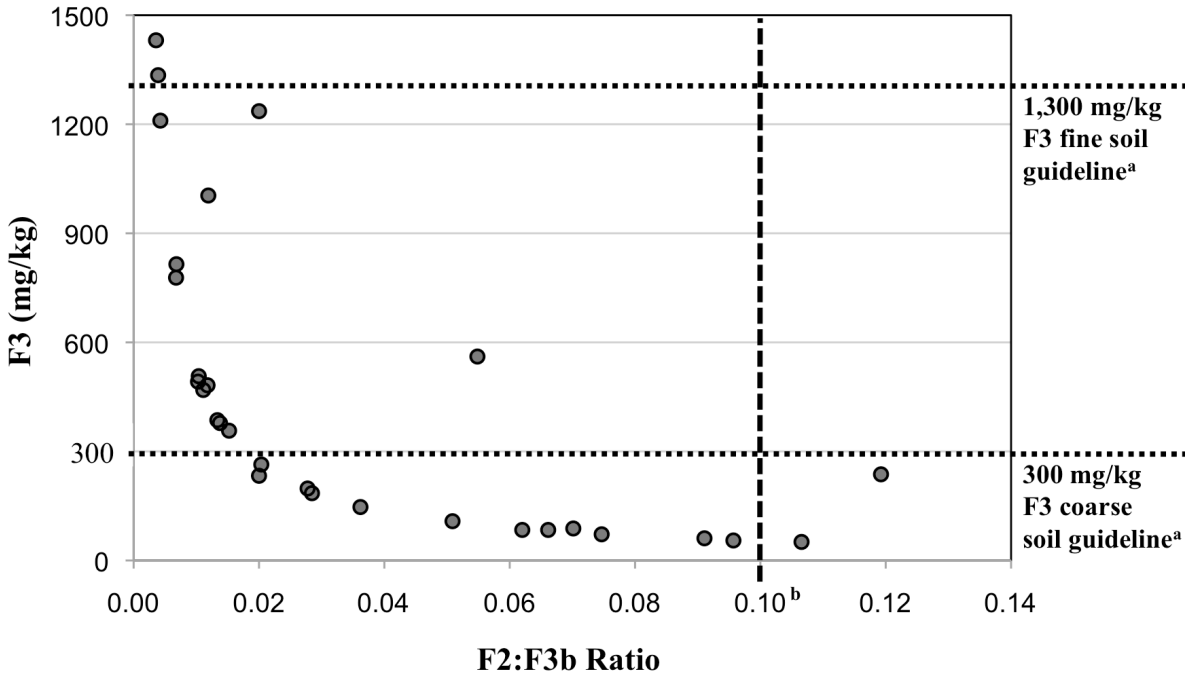

Figure 4.4. F3 concentrations and F2:F3b ratios in background soils. [a]Tier 1 PHC fine and coarse surface soil guidelines for agricultural/residential/parkland land uses in potable groundwater conditions (CCME 2008a). [b]F2:F3b ratios less than 0.10 indicate PHC absence. F2:F3b ratios greater than or equal to 0.10 indicate PHC presence. All of the soil samples with greater than 300 mg/kg F3 concentrations had F2:F3b ratios of less than 0.06.

CONCLUSIONS

Approximately half of the 34 background soil samples falsely exceeded the CWS PHC F3 300 mg/kg F3 coarse soil guideline and two samples falsely exceeded the 1,300 mg/kg F3 fine soil guideline. These false exceedances were identified by the following analytical results:

- All alkylated and non-alkylated PAHs were either non-detectable or present in trace concentrations that were indicative of atmospheric deposition sources;
- Absence of petroleum biomarkers;
- Absence of PHC signature UCMs in GC-FID chromatograms;
- F3b percentages were all greater than 80%;

- Most of the F2 concentrations were less than the 10 mg/kg method detection limit, with four of the 34 samples slightly above; and
- All of the samples with greater than 300 mg/kg F3 concentrations had F2:F3b ratios of less than 0.10, which was also the case for the clean peat and clean manure compost data presented in Chapter 2 and Chapter 3, respectively.

The TOC results identified a positive linear correlation between increasing TOC percentages and increasing F3 concentrations. Although the F3 concentrations ranged widely between the mineral and organic soils, the F3b percentages were consistently higher than the F3a percentages regardless of TOC percentages. There was no correlation between TOC and the F3a and F3b percentages, meaning that the F2:F3b ratio and the BOC-adjusted PHC F3 calculation used in Chapter 2 and Chapter 3, could be applied to all soil types. The F2:F3b ratio is most applicable to defining the line that separates contamination zones from surrounding clean background soils. The BOC-adjusted PHC F3 calculation is applicable to determining if marginally contaminated soils have authentically exceeded the CWS PHC F3 soil guidelines. It should be emphasized that the use of the F2:F3b ratio and the BOC-adjusted PHC F3 calculation is limited to evaluations of lighter PHC products (i.e. crude oils, diesel, jet fuel, kerosene, etc.) which extend into the F2 and F3a carbon ranges. This approach cannot be applied to sites that are contaminated by heavier PHC products, (i.e. motor oil, asphalt, tar, etc.) which only extend into the F3b and F4 carbon ranges.

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CHAPTER 5

Summary Discussion and Conclusions

Carbon Distribution Patterns in Clean Soils and Compost

The results of this study contribute to a better understanding of F2, F3 and F4 GC-FID chromatogram patterns in clean soils and compost. The key observations presented in Chapters 2, 3 and 4 are described as follows:

- F2 (C10-C16) concentrations were very low with few samples exceeding10 mg/kg.
- F3 (C16-C34) and F4 (>C34) concentrations in mineral soils were less than the 50 mg/kg detection limits.
- F3 and F4 concentrations in clean peat and compost were elevated. All of the F3 concentrations exceeded the CWS PHC 300 mg/kg course soil guideline. All of the F4 concentrations were less than the CWS PHC 2,800 mg/kg course soil guideline.
- The F3 concentrations in the 34 background soils were highly variable. The F3 concentration ranged from <50 mg/kg to 1,431 mg/kg, with a standard deviation of 419 mg/kg. These ranges are primarily attributed to the large variety of soil types included in the survey, which had a wide total organic carbon (TOC) range of 0.7% to 41.4%.

The study results also provided a new understanding of sub-fractions F3a (C16-C22) and F3b (C22-C34) patterns in clean soils and compost. Chapters 2, 3 and 4 demonstrated that the F3 carbon range in all of the compost samples and in the clean organic and mineral soils was strongly dominated by the F3b carbon range. As discussed in Chapter 2, at least 90% of the F3 concentrations in all of the clean peat samples were composed of the F3b range. Chapter 3, demonstrated that at least 76% of the F3 concentrations in all of the clean compost samples were composed of the F3b range. Chapter 4 showed that all of the background soils had at least 81% F3b, with an average of 93% F3b.

Approaches to Resolving False Exceedances of CWS PHC F3 Soil Guidelines

The study results presented in Chapters 2, 3 and 4 demonstrated that the Tier 1 CWS PHC GC-FID chromatogram patterns could be used to mathematically resolve false exceedances of F3 soil quality guidelines due to BOCs on a Tier 2 basis. This conclusion is based on the following observations:

- Most clean soil samples and manure compost samples had non-detectable F2 (C10-C16) concentrations, with only a few samples having slightly detectable F2 concentrations. The F2 concentrations were strongly prevalent in all of the crude oils and in the diesel drilling waste.
- The F3 concentrations (C16-C34) in all of the clean soils and manure compost samples were strongly dominated by the F3b sub-fraction range (C22-C34). The crude oils had relatively equal distributions of the F3a (C16-C22) and F3b sub-fraction ranges. The diesel drilling waste was strongly dominated by the F3a sub-fraction range.

These consistent trends were used to develop two complimentary calculations: i) the F2:F3b ratio for indicating crude oil and/or diesel PHC presence versus absence; and ii) the BOC-adjusted PHC F3 calculation for estimating possible crude oil and/or diesel PHC F3 concentrations in soil and compost. Figure 5.1 combines the F2:F3b ratios and F3 concentrations for 108 clean and PHC contaminated samples that were analyzed in the crude oil spiked peat microcosm experiment (Chapter 2), the diesel drilling waste spiked manure compost experiment (Chapter 3) and the background PHC soil field survey (Chapter 4). Figure 5.1 demonstrates that the F2:F3b ratios in all of the contaminated

samples were greater than the 0.10 PHC presence threshold value. All of the clean samples with greater than 300 mg/kg F3 concentrations had F2:F3b ratios that were less than the 0.10 PHC absence threshold value. These data confirmed that the F2:F3b ratio was able to identify false and authentic exceedances of the CWS PHC F3 soil guideline for all of the samples that were analyzed for this study.

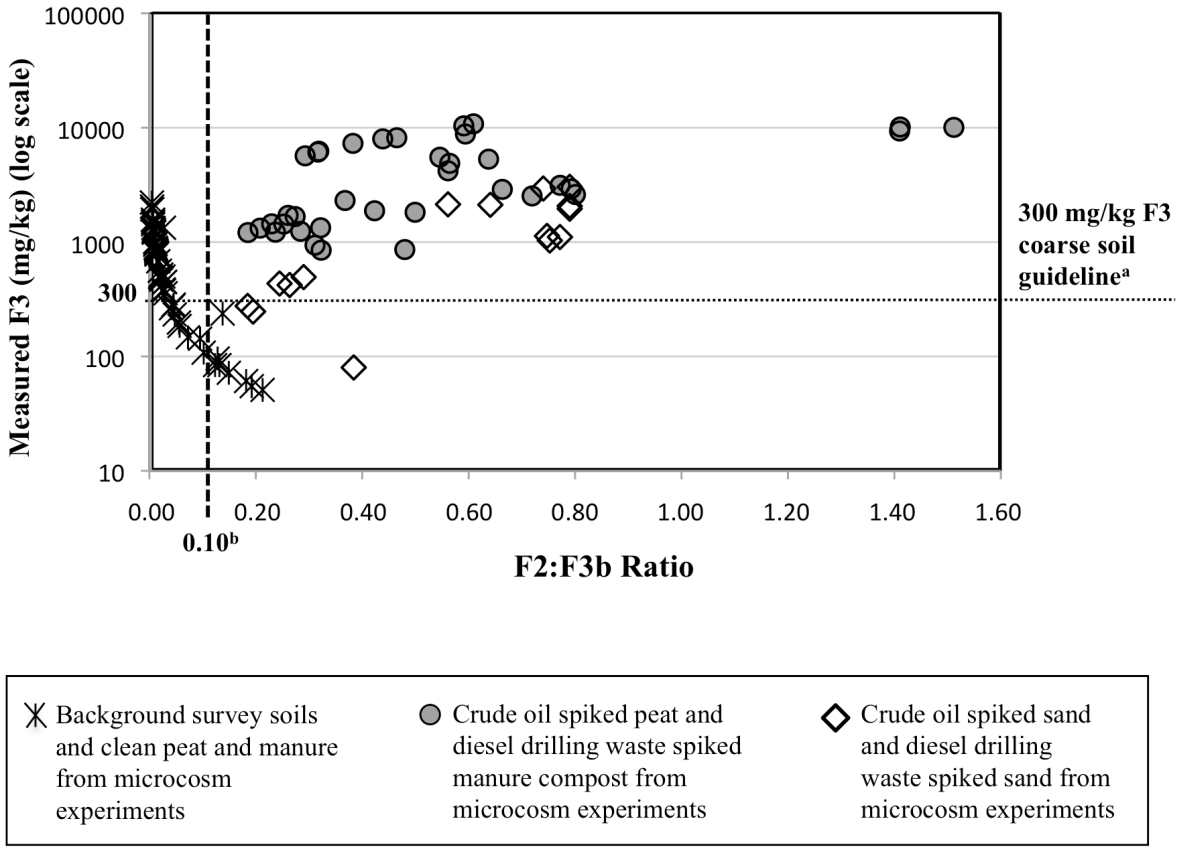

Figure 5.1. Comparison of measured F3 concentrations to F2:F3b ratios. Plot includes 108 samples analyzed for the background soil survey and the Day 0, Day 150 and Day 300 crude oil and diesel drilling waste microcosm experiments. [a]Tier 1 PHC coarse surface soil guideline for agricultural/residential/parkland land uses in potable groundwater conditions (CCME 2008a). [b]All clean samples with greater than 300 mg/kg F3 concentrations had F2:F3b ratios of less than 0.10. All crude oil and diesel drilling waste spiked samples had greater than 0.10 F2:F3b ratios, regardless of high or low F3 concentrations.

Figure 5.2 compares the measured F3 concentrations to the BOC-adjusted PHC F3 concentrations for all of the crude oil spiked peat microcosm experiment samples (Chapter

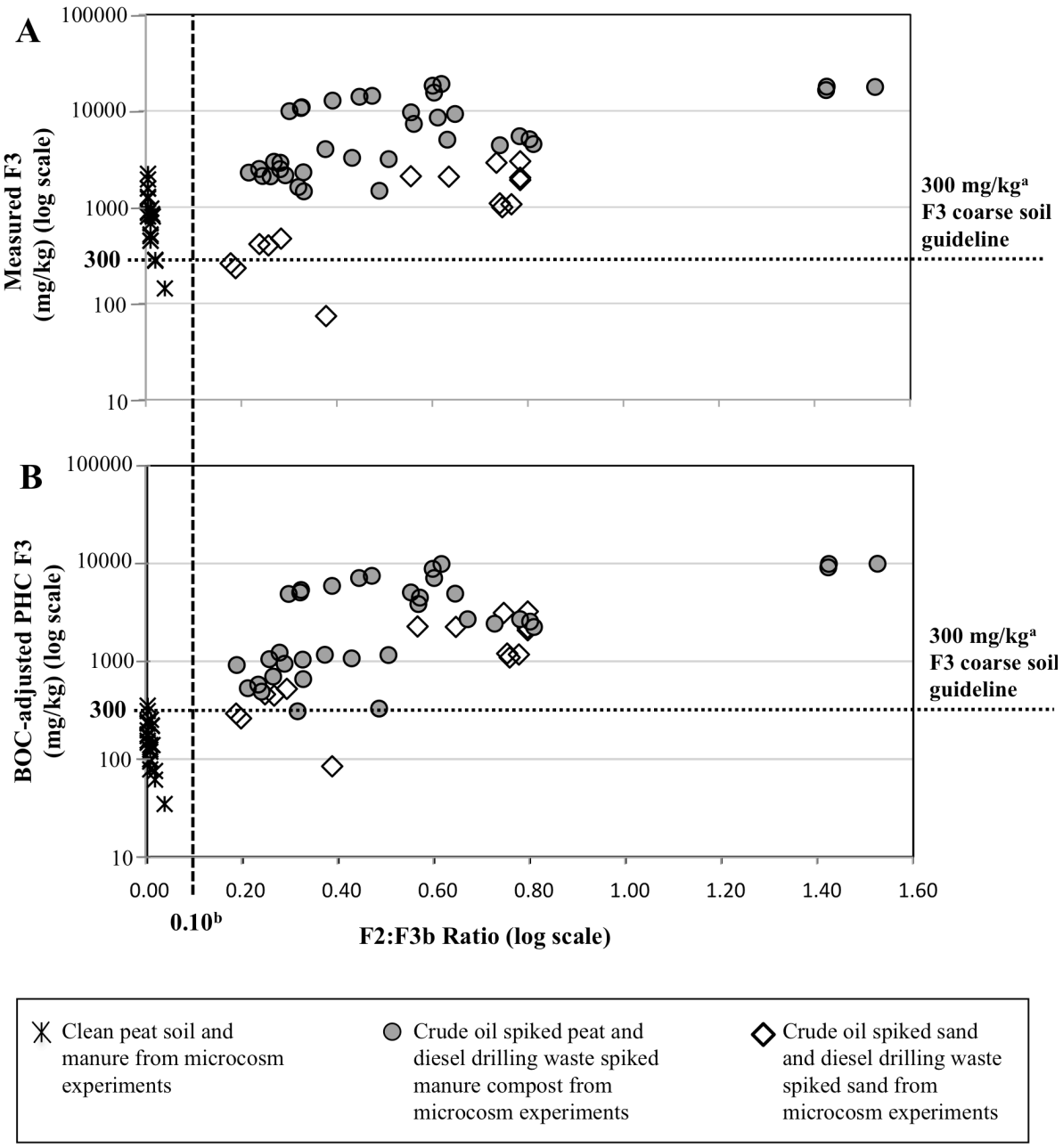

Figure 5.2. Comparison of F2:F3b ratios to F3 concentrations (A) and BOC-adjusted PHC F3 concentrations (B) in clean peat and crude oil spiked peat and sand from microcosm experiment (Chapter 2), clean manure and diesel drilling waste spiked manure and sand from microcosm experiment (Chapter 3). [a]Tier 1 PHC coarse surface soil guideline for agricultural/residential/parkland land uses in potable groundwater conditions (CCME 2008a). [b]Crude oil and diesel presence versus absence threshold value – ratios less than or equal to 0.10 indicate PHC absence while ratios greater than 0.10 indicate PHC presence. Panel B demonstrates that combining the BOC-adjusted PHC F3 concentrations with the F2:F3b ratios provided a stronger indication of false F3 soil guideline exceedances in clean soils and manure compost.

2) and for the diesel drilling waste spiked manure compost experiment samples (Chapter 3). While the measured F3 concentrations (Figure 5.2A) in 25 of the 27 clean peat and manure compost samples falsely exceeded the F3 guideline, the BOC-adjusted PHC F3 concentrations (Figure 5.2B) were below the guideline in all 27 samples. Figure 5.2B also demonstrates that combining the F2:F3b ratios with the BOC-adjusted PHC F3 concentrations was the strongest approach to resolving false F3 guideline exceedances at crude oil and/or diesel contaminated sites.

Comparison of current CCME approach (subtraction of background concentrations) and the new BOC-adjusted approach to address biogenic interferences

The current CCME approach for addressing BOC interferences in organic soils is to subtract the measured F2-F4 concentrations in clean background soils from the F2-F4 concentrations in contaminated soils. Tables 5.1 and 5.2 compare the current CCME background subtraction approach to the new BOC-adjusted PHC F3 approach for the crude oil and diesel drilling waste microcosm experiments.

Table 5.1: Comparison of current CCME approach (subtract background concentration) and BOC-adjusted approach to address biogenic interferences for F3 concentrations in crude oil spiked peat

Crude Oil Spiked Peat Experiment		**Current CCME Approach (Subtract Background) F3 (mg/kg) mean±SD**			**New Approach (BOC-adjusted) F3 (mg/kg) mean±SD**	**Percent difference**
Treatment	**Sample Day**	**Spiked Peat**	**Clean Peat**	**Subtracted Value**	**Calculated Value**	
High crude oil spiked in processed peat						
[a]sP1	**Day 0**	**9,793±1039**	**1,921±378**	**7,872**	**8,229**	**+4%**
sP1	**Day 150**	**7,648±452**	**1,168±198**	**6,480**	**6,524**	**+1%**
sP1	**Day 300**	**5,876±288**	**1,579±355**	**4,297**	**4,864**	**+12%**
Moderate crude oil spiked in natural peat						
[b]sP2	**Day 0**	**1,953±261**	**1,235±344**	**718**	**1070**	**+33%**
sP2	**Day 150**	**1,140±459**	**882±73**	**258**	**418**	**+38%**
sP2	**Day 300**	**1,295±109**	**831±55**	**464**	**501**	**+7%**

[a]sP1 – processed peat spiked with whole crude oil nominal F3 concentration of 10,000 mg/kg
[b]sP2 – natural peat spiked with whole crude oil nominal F3 concentration of 1,500 mg/kg

Table 5.2: Comparison of current CCME approach (subtract background concentration) and BOC-adjusted approach to address biogenic interferences for F3 concentrations in diesel drilling waste spiked manure compost

Diesel Drilling Waste Spiked Manure Compost Experiment		**Current CCME Approach (Subtract Background) F3 (mg/kg) mean±SD**			**New Approach (BOC-adjusted) F3 (mg/kg) mean±SD**	**Percent difference**
Treatment	**Sample Day**	**Spiked Compost**	**Clean Compost**	**Subtracted Value**	**Calculated Value**	
High diesel spiked						
sM1	**Day 0**	9,653 ±429	667±140	**8,968**	9,242 ±441	**+3%**
sM1	**Day 150**	4,353 ±1,634	336 ±96	**4,017**	3,938 ±1418	**-2%**
sM1	**Day 300**	3,897 ±991	364±193	**3,533**	3,497 ±869	**-3%**
Moderate diesel spiked						
sM2	**Day 0**	2,817 ±262	667±140	**2,150**	2,369 ±222	**+9%**
sM2	**Day 150**	1,222 ±402	336 ±96	**886**	899±271	**+1%**
sM2	**Day 300**	1,293 ±110	364±193	**929**	946 ±72	**+2%**

[a]sM1 – composted manure spiked with whole crude oil nominal F3 concentration of 10,000 mg/kg
[b]sM2 – composted manure spiked with whole crude oil nominal F3 concentration of 2,000 mg/kg

In general, 75% of the spiked samples had BOC-adjusted PHC F3 concentrations that were similar (± 10%) to the background subtracted F3 concentrations. All other samples had BOC-adjusted PHC F3 concentrations that were higher (> 10%) than the background subtracted F3 concentrations. The BOC-adjusted PHC F3 calculation accounts for the F3b concentrations that can be attributed to BOC sources. This calculation provides a conservative approach to addressing false exceedances of CWS PHC F3 soil guidelines due to BOC interferences.

There were greater differences between approaches for the crude oil/peat experiment than the diesel drilling waste/manure experiment, particularly for moderate concentrations of crude oil in natural peat. Clean peat material, both natural (831-1235 mg/kg F3) and processed (1168-1921 mg/kg F3) had higher concentrations of F3 than the clean manure compost (336-667 mg/kg F3). Although all material was homogenized, there was evidence of high variability for F3 concentrations for both clean and spiked material due to the heterogenous natural of

the material as well as spiking protocols. This variability will also be evident in field samples. Such variability may be reduced to increasing the sample size used for extraction. Current CCME methods require 5 g of material for analysis, however given the heterogeneity of peat material, larger sample sizes may be beneficial for reducing variability.

The background subtraction approach is based on the assumption that BOC F3 concentrations would be the same in clean and contaminated soils that are collected from the same location and have the same soil texture. However, the peat microcosm data was highly variable even though the peat was homogenized prior to sampling. The background subtraction approach can work well if there is an exact match between the background reference soil and the contaminated soil and if the soil has a homogenous texture. However, this approach cannot be used in situations where background reference soils do not exist and/or the reference soils do not have homogenous textures. For example, it could not be used at remediation facilities that combine contaminated soils from many different locations. As another example, background subtracted concentrations could significantly over-estimated or under-estimated authentic PHC contamination levels in non-homogeneous soils such as peat.

Suitable Applications of F2:F3b ratio and BOC-adjusted PHC F3 concentrations

The F2:F3b ratio and the BOC-adjusted F3 calculation approach has strengths and limitations that must be considered when deciding if it is suitable for applications to specific sites. This approach can only be used to evaluate light carbon range PHC contamination sources that extend into the F3a range. Examples of suitable light PHC

products include: diesel, crude oil, kerosene, jet fuel, etc. Heavy PHC products are unsuitable because they are primarily confined to the F3b portion of the F3 range. Soils contaminated by heavy products would be falsely identified as clean samples. Examples of unsuitable heavy PHC products include: motor oil, tar, asphalt, bitumen, etc.

Verification of PHC contamination sources in soil samples using GC-FID patterns requires training and expertise on the part of the reviewer. The reviewer must be able to visually distinguish PHC UCM patterns originating from different PHC products in addition to distinguishing PHC patterns from BOC patterns. Familiarity with clean soil BOC patterns is especially important if clean background soils for a particular site and/or remediation facility are not available for analysis.

The F2:F3b ratio is a sensitive tool for determining PHC presence versus absence in organic samples. However, the ratio is unsuitable for inorganic soils with low F3 concentrations of less than 300 mg/kg. Mineral soils have low F3b concentrations, which generate high F2:F3b ratios that exceed the $\geq$0.10 PHC presence threshold. For example, Figure 4.4C (Chapter 4) shows a clean background soil sample with a low F3 concentration of 51 mg/kg and a high F2:F3b ratio of 0.11. Although the low F2:F3b ratio would identify clean mineral soils as PHC contaminated, the F3 concentrations would not exceed the CWS PHC 300 mg/kg soil guideline. In this case however, the sample would not be identified as a toxicity risk because the measured F3 concentration of 51 mg/kg did not exceed the CWS PHC 300 mg/kg F3 guideline.

The F2:F3b ratio would also be unsuitable for soils with unusual BOCs in the F2 range. BOCs in the F2 range would elevate F2:F3b ratios to above the $\geq$0.10 PHC presence threshold, resulting in false conclusions that clean samples are PHC contaminated. For

example, Figure 4.4B (Chapter 4) background soil GC-FID chromatogram includes a biogenic peak in the F2 range. The F2 concentration of 26 mg/kg and the F3b concentration of 218 mg/kg, resulted in a high F2:F3b ratio of 0.12. The elevated F2:F3b ratio falsely indicated that this clean sample was contaminated. However, the sample would not trigger risk management issues because the measured F3 concentration of 237 mg/kg did not exceed the CWS PHC 300 mg/kg F3 guideline.

The BOC-adjusted PHC F3 calculation can only be used if the F3a:F3b percentages in the PHC contamination source is known. These percentages can be easily determined for fresh spills where the free product can be readily sampled and analyzed. However, characterizing F3a:F3b percentages in PHC contamination sources at weathered sites requires a different approach. A soil analysis survey would be used to identify the soil sample with the highest F3a concentration. This sample would be selected as most representative of the weathered PHC contamination source. The F3a:F3b percentages in that sample would then be used to calculate the BOC-adjusted PHC F3 concentrations in all of the other soil samples. For example, the Day 300 highly spiked peat sample (Chapter 2, Table 2.1) distributions of 26% F3a and 74% F3b would be used to calculate the BOC-adjusted PHC F3 concentrations in the moderately spiked peat and in the clean peat. This approach provides a cautious estimate of authentic PHC F3 concentrations at weathered sites and where free PHC product analysis is not possible. This approach could however significantly overestimate authentic PHC concentrations at marginally contaminated sites.

The BOC-adjusted PHC F3 calculation cannot be used for samples with unusual BOCs in the F3a range. BOCs in the F3a range would elevate the calculated PHC F3 concentrations to levels that falsely exceed the CWS PHC F3 soil guidelines. Although this

did not occur in any of the 112 samples analyzed for this study, it could potentially occur in other soils and compost materials.

Decision tree process for assessing site remediation requirements

Figure 5.3 illustrates a decision tree for determining if a soil sample requires remediation due to authentic PHC contamination. The decision tree theoretical concept is described as follows.

CWS PHC Tier 1 Analysis – Conduct soil chemistry analysis of F2, F3 and F4 concentrations in accordance with the Tier 1 CWS PHC methods. Samples with concentrations that are below the F2, F3 and F4 soil guidelines would not require remediation and the evaluation would end. Samples with concentrations that do exceed the CWS PHC F2 and/or F4 guidelines would require remediation due to authentic PHC contamination and the evaluation would end. Samples that only exceed the F3 soil guideline would proceed to the next step to determine if they have falsely exceeded the guideline.

Verify PHC Contamination Source – The next step is to verify that the contamination source is a light PHC product such as diesel and/or crude oil. The evaluation would stop if the PHC source has an F2:F3b ratio of <0.10 because it would falsely indicate PHC absence. Further evaluation could be conducted if the PHC source has a ratio of ≥ 0.10, which would indicate PHC presence. The next step in the process would be to evaluate the GC-FID chromatogram patterns for the soil/compost sample. It is essential that the pattern in the sample matches the clean BOC signature and/or the diesel and/or crude oil contamination source. A non-matching pattern would indicate the presence of a different

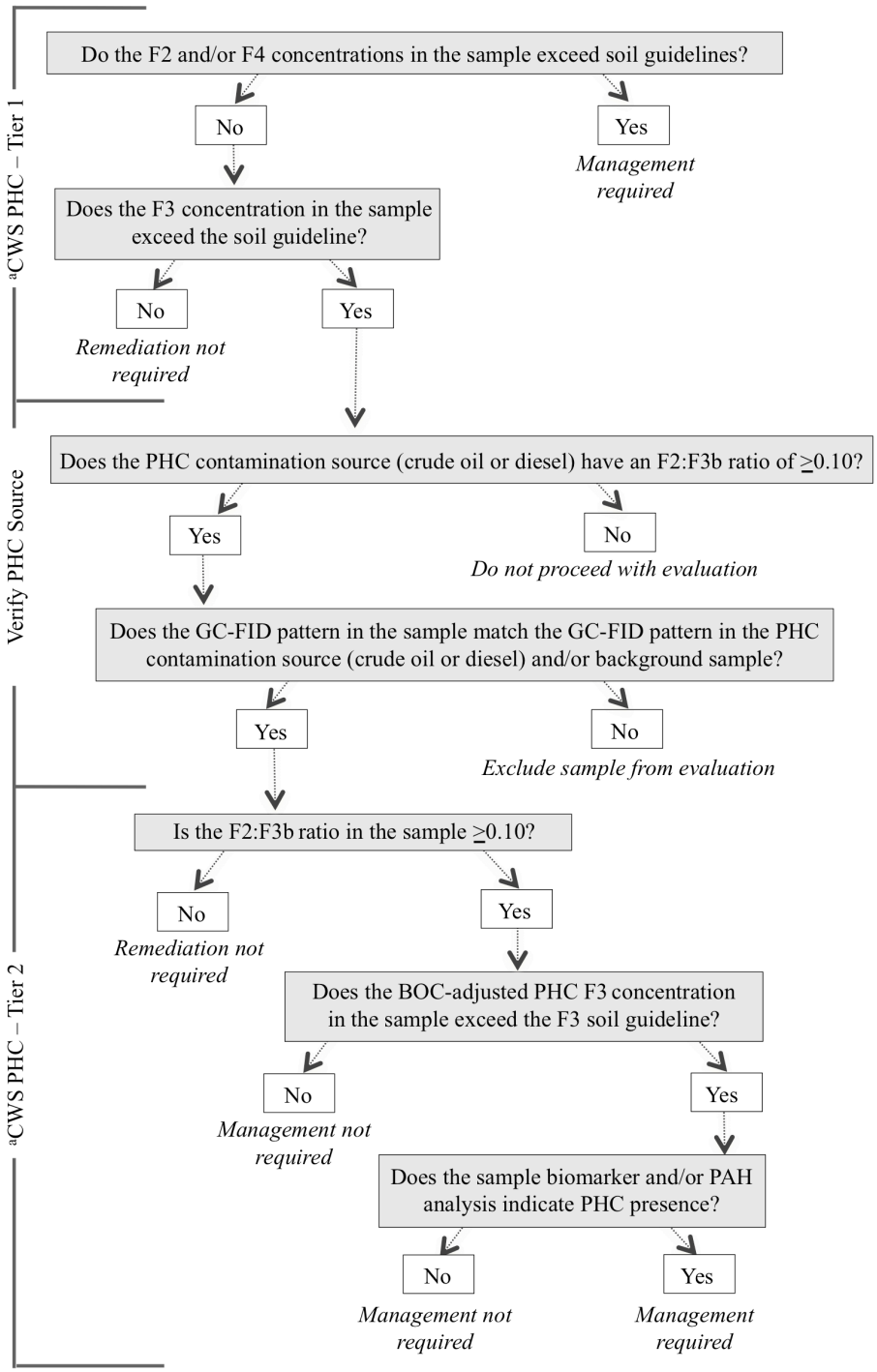

Fig. 5.3. Tier 2 decision process for determining if an organic soil/compost sample location requires remediation due to contamination by crude oil and/or diesel PHCs. [a]Canada Wide Standard (CWS) for Petroleum Hydrocarbons (PHC) in Soil (CCME 2001a). F2 (C10-C16); F3 (C16-C34); and F4 (>C34). F2:Fb calculated as the F2 concentration divided by the sub-fraction F3b (C22-C34) concentration. BOC-adjusted PHC F3 concentration calculated by Formula 1.

PHC product and the evaluation would end. Matching patterns would verify that the sample is either clean or is contaminated by diesel and/or crude oil, which would proceed to the next step in the evaluation.

CWS PHC Tier 2 Analysis – The next step would be to calculate the sample F2:F3b ratio. Samples with ≤0.10 F2:F3b ratios would indicate PHC absence and would not require further evaluation. Samples with ≥0.10 F2:F3b ratios would indicate the potential presence of PHCs, and the evaluation would proceed to the BOC-adjusted PHC F3 calculation. BOC-adjusted PHC F3 concentrations that do not exceed the CWS PHC F3 soil guideline would not require further evaluation. Concentrations that do exceed the guideline may be authentically contaminated and would proceed to the PAH and/or biomarker forensics analysis step. The forensics analysis results would determine if the sample is clean, which would not require remediation or if it contaminated and would require remediation. Example applications of this decision tree are presented in the Chapter 2, crude oil contaminated peat experiment results and in the Chapter 3, diesel drilling waste contaminated compost experiment results.

This decision tree is applicable to several different scenarios diesel and crude oil contamination of soils and/or compost materials.

- May be applied independently or in conjunction with the background subtraction approach at freshly contaminated sites;
- Aged and highly weathered sites;
- Sites with no representative background reference soils; and
- Remediation facilities with multiple sources of contaminated soils.

The practical benefits of these combined Tier 2 approaches are described as follows:

- Under appropriate circumstances, biogenic interference resolutions would not require clean reference soil samples from background reference sites;
- Biogenic interference issues would be resolved through the use of F2, F3, F3a (C16-C22) and F3b (C22-C34) carbon range data generated by the CWS PHC Tier 1 soil analytical standard;
- Only requires standard commercial laboratory GC-FID analytical equipment and a basic level of analytical expertise. Chromatogram integrations and chemistry reporting procedures could be readily incorporated into the CWS PHC Tier 1 standards; and
- The Tier 2 BOC-adjusted F3 PHC approach is time efficient and cost efficient.

Future Research Requirements

It is recommended that contaminated site field studies be conducted to determine if the F2:F3b ratio and the BOC-adjusted F3 calculation data generated in this controlled laboratory study apply to field situations as well. The adoption of this new approach as a CWS PHC Tier 2 method could prevent unnecessary disruptions and remediation of clean soils, which would provide many economic and environment benefits.

REFERENCES

Aitchison J. 1986. *The Statistical Analysis of Compositional Data*, Chapman and Hall, New York, 416 p.

Alberta Agriculture and Food. 2011. Nutrient management planning guide. *http://google.agriculture.alberta.ca/search?q=nutrient+management+planning+guide&site=rtw_collection&client=rtw_frontend&proxystylesheet=rtw_frontend&output=xml_no_dtd&filter=0&proxyreload=1&getfields=*&numgm=5*

Alberta Energy and Utilities Board (EUB). 2006. EUB provincial surveillance and compliance summary 2005. Report #ST99-2006.

Alberta Environment. 2003. Focus on Wetlands. Pub No I/934 0-7785-2477-9 (Printed Edition), 0-7785-2478-7(On-line Edition)

Alberta Environment. 2010. Alberta Tier 1 soil and groundwater remediation guidelines. ISBN: 978-0-7785-9947-0 (On-line Edition).

Association of Petrochemicals Producers in Europe (APPE). 2012. From petrochemicals to everyday products. http://www.petrochemistry.net/from-petrochemicals-to-everyday-products-flowchart.html

Ball AS, Stewart RJ, Schliephake K. 2012. A review of the current options for the treatment and safe disposal of drill cuttings: review article. *Waste Manage Res* 30:457-473.

Brassington KJ, Hough RL. 2007. Weathered hydrocarbon wastes: a risk management primer. Crit Rev Env Sci Technol 37:199-232.

Canadian Association of Petroleum Producers (CAPP). 2009. Crude oil pipeline map. http://www.capp.ca/getdoc.aspx?DocID=191097

Canadian Association of Petroleum Producers (CAPP). 2011. Oil wells completed in Canada. http://www.capp.ca/library/statistics/handbook/ pages/ statisticalTables.aspx?sectionNo=1#r1Fl8EzrLPNQ

Canadian Association of Petroleum Producers (CAPP). 2012. Basic Statistics. http://www.capp.ca/library/statistics/basic/Pages/default.aspx

Canadian Council of Ministers of the Environment (CCME). 2001a. Reference method for the Canada-wide standard (CWS) for petroleum hydrocarbons in soil – Tier 1 method. ISBN 1-896997-01-5, Publication No. 1310.

Canadian Council of Ministers of the Environment (CCME). 2001b. Canadian soil quality guidelines for the protection of environmental and human health. www.ccme.ca/ourwork/soil.html?category_id=44

Canadian Council of Ministers of the Environment (CCME). 2008a. Canada-wide standard for petroleum hydrocarbons in soil 2008 report. www.ccme.ca/assets/pdf/phc_standard_1.0_e.pdf

Canadian Council of Ministers of the Environment (CCME). 2008b. Canada-wide standard for petroleum hydrocarbons (PHC) in soil: scientific rationale – supporting technical document. PN 1399 ISBN 978-1-896997-77-3 PDF.

Canadian Council of Ministers of the Environment (CCME). 2008c. Canada-wide standard for petroleum hydrocarbons (PHC) in soil: user guidance. PN 1398. http://www.ccme.ca/assets/pdf/pn_1398_phc_user_guide_1.1_e.pdf

Canadian Council of Ministers of the Environment (CCME). 2010a. Canadian soil quality guidelines – carcinogenic and other polycyclic aromatic hydrocarbons (PAHs) –

(environmental and human health effects) – scientific guidelines document (revised). PN 1445. ISBN 978-1-896997-94-0 PDF.

Canadian Council of Ministers of the Environment (CCME). 2010b. Canadian soil quality guidelines for the protection of environmental and human health – polycyclic aromatic hydrocarbons 2010. http://www.ccme.ca/publications/ ceqg_rcqe.html

Cermak JH, Stephenson GL, Birkholz D, Wang Z, Dixon DG. 2010. Toxicity of petroleum hydrocarbon distillates to soil organisms. *Environ Toxicol and Chem* 29:268-2694.

Chaîneau CH, Jean-Louis M, Oudot J. 1995. Microbial degradation in soil microcosms of fuel oil hydrocarbons from drilling cuttings. *Environ Sci Technol* 29:1615-1621.

Chaîneau CH, Morel J, Dupont J, Bury E, Oudot J. 1999. Comparison of the fuel oil biodegradation potential of hydrocarbon-assimilating microorganisms isolated from a temperate agricultural soil. *Sci Total Environ* 227:237-247.

Chaîneau CH, Morel JL, Oudot J. 2000. Vertical infiltration of fuel oil hydrocarbons in an agricultural soil. *Toxicol Environ Chem* 74:11-124.Coles C, Patel TR, Akinnola AP, Helleur RJ. 2009. Influence of bulking agents, fertilizers and bacteria on the removal of diesel from a Newfoundland Soil. *Soil & Sediment Contamination,* 18:383-396.

Coles CA, Patel TR, Akinnola A, Helleur RJ. 2009. Influence of bulking agents, fertilizers and bacteria on the removal of diesel from a Newfoundland soil. Soil Sed Contam 18:383-396.

Cooper GM. 2000. The biosynthesis of cell constituents. *The cell: A molecular approach*, 2nd edition. Sinauer Associates, Sunderland, MA, USA. http://www.ncbi.nlm.nih.gov/books/NBK9956

Crocker FH, Guerin WF, Boyd SA. 1995. Bioavailability sorbed to cationic surfactant-modified smectite clay. *Environ Sci Technol* 29:2953-2958.

Das N, Preethy C. 2011. Microbial degradation of petroleum hydrocarbon contaminants: an overview. *Biotechnol Res Int* 2011:1-13.

Department of Justice Canada. 2012. Canada oil and gas operations act. http://laws-lois.justice.gc.ca/Search/Search.aspx?txtS3archA11=spill&txtT1tl3=%22Canada+Oil+and+Gas+Operations+Act%22&h1ts0n1y=0&ddC0nt3ntTyp3=Acts

Devold H. 2010. Oil and gas production handbook, an introduction to oil and gas production. OSLO: ABB AS. ISBN 978-82-997886-2-5. *http://www.abb.com/abblibrary/downloadcenter.*

Dibble JT, Bartha R. 1979. Effect of environmental parameters on the biodegradation of oil sludge. *App Environ Microb* 37:729-739.

Dunbar RB. 2009. Canada's oil sands – a world scale hydrocarbon resource. Strategy West Inc. http://*www.strategywest.com/downloads/StratWest_OilSands.pdf*

Dutta TK, Harayam S. 2000. Fate of crude oil by the combination of photooxidation and biodegradation. *Environ Sci Technol* 34:1500-1505.

Ecozcue JJ, Pawlowsky-Glahn V. 2011. Basic concepts and procedures, *in* *compositional data analysis theory and applications*, edited by V. Palowsky-Glahn and A. Buccianti, Wiley, New York, USA 12-28.

Energy Resources Conservation Board (ERCB), Alberta. 2012. Directive 050: drilling waste management. *www.ercb.ca/directives/Directive050.pdf*

Environmental Labour Market Research (ELM). 2006. Who will do the cleanup? Canadian labour requirements for remediation and reclamation of contaminated sites. http://www.mah.gov.on.ca/AssetFactory.aspx?did=4824

Evans RJ, Felbeck GT Jr. 1983. High temperature simulation of petroleum formation –III effect of organic starting material structure on hydrocarbon formation. *Org. Geochem* 4:153-160.

Fingas M. 1997. Studies on the evaporation of crude oil and petroleum products: I. the relationship between evaporation rate and time. *J Hazard Mater* 56:227-236.

Federation Canadian Municipalities (FCM). 2012. Brownfields sustainability snapshot.

Kettles, I.M., and Lacelle, B. 2000. Peatlands of Canada database. Geological Survey of Canada Open File 3834.

Golebiowski M, Bogus MI, Paszkiewicz M, Stepnowski P. 2011. Cuticular lipids of insects as potential biofungicides: methods of lipid composition analysis. *Anal Bioanal Chem* 399:3177-3191.

González J, Fernández E, Figueiras FG, Varela M. 2013. Subtle effects of the water soluble fraction of oil spills on natural phytoplankton assemblages on mesocosms. *Estuar Coast Shelf S 124:13-23.*

Government of Canada. 2008. North American Soil Geochemical Landscapes Project. *Government of Canada Publications, http://publications.gc.ca site/eng /336292 /publication.html*

Guerin TF. 1999. Bioremediation of phenols and polycyclic aromatic hydrocarbons in creosote contaminated soil using ex-situ land treatment. J Haz Mat B 65:305-315.

Håvard D. 2010. Oil and gas production handbook, an introduction to oil and gas production, Ed 2.3. ISBN 978-82-997886-2-5. http://search.abb.com/library/Download.aspx?DocumentID=9AKK104295D4314&LanguageCode=en&DocumentPartId=&Action=Launch

Hawthorne SB, Grabanski CB, Martin E, Miller DJ. 2000. Comparison of Soxhlet extraction, pressurized liquid extraction, supercritical fluid extraction and subcritical water extraction for environmental solids: recover, selectivity and effects on sample matrix. *J Chrom A* 892:421-433.

Harayama S, Kishira H, Kasai Y, Shutsubo K. 1999. Petroleum biodegradation in marine environments. *J Mole Microbiol Biotechnol* 1:63-70.

Hunt JM. 1979. Petroleum geochemistry and geology. W.H. Freeman and Company, United States of America.

Kettles IM, Lacelle B. 2000. Peatlands of Canada database. Geological Survey of Canada Open File 3834.

Kästner M, Mahro B. 1996. Microbial degradation of polycyclic aromatic hydrocarbons in soils affected by the organic matrix of compost. *Appl Microbial Biotechnol* 44:668-675.

Killops SD, Al-Juboori MAHA. 1990. Characterization of the unresolved complex mixture (UCM) in gas chromatograms of biodegraded petroleums. *Org Geochem* 15:147-160.

Kobayashi T, Murai Y, Tatsumi K, Limura Y. 2009. Biodegradation of polycyclic aromatic hydrocarbons by *Sphingomonas* sp. enhanced by water-extractable organic matter from manure compost. *Sci Tot Env* 407:5805-5810.

Kroetsch DJ, Xiaoyuan G, Chang SX, Saurette DD. 2011. Organic soils of Canada: part 1. wetland organic soils. *Can. J. Soil Sci*. 91:807-822.

Kryachko Y, Dong X, Sensen CW, Voordouw G. 2012. Compositions of microbial communities associated with oil and water in a mesothermic oil field. *Int J Gen Molec Microbiol* 101:493-506.

Kumari D. 1986. Analysis of wax and resin components from a Minnesota peat bog. *Intern J Coal Geol* 8:99-109.

Labud V, Garcia C, Hernandez T. 2007. Effect of hydrocarbon pollution on the microbial properties of a sandy and a clay soil. *Chemosphere* 66:1863-1871.

Li Li, Zhao Z, Huang W, Peng P, Sheng G, Fu J. 2004. Characterization of humic acids fractionated by ultrafiltration. *Org Geochem* 35:1025-1037.

Llado S, Solanas AM, de Lapuente J, Borras M, Vinas M. 2012. A diversified approach to evaluate biostimulation and bioaugmentation strategies for heavy-oil-contaminated soil. *Science of the Total Environment*. 435:262-269.

Li Li, Zhao Z, Huang W, Peng P, Sheng G, Fu J. 2004. Characterization of humic acids fractionated by ultrafiltration. *Org Geochem* 35:1025-1037.

Lu YH, Chai TJ, Hwang DF. 2000. Isolation of bacteria from toxic dinoflagellate Alexandrium minutum and their effects on algae toxicity. *J Nat Toxins* 9:409-417.

Mariano AP, Katoaka APAG, de Agelis DF, Bonotto DM. 2007. Laboratory study on the bioremediation of diesel oil contaminated soil from a petrol station. Brazilian. *J Microbiol* 38:346-353.

Ministry of the Environment, Ontario (MOE). 2011. Soil, ground water and sediment standards for use under Part XV.1 of the Environmental Protection Act. PIBS # 7382e01.

Moliterni E, Rodriguez L, Fernández FJ, Villaseñor J. 2012. Feasibility of different bioremediation strategies for treatment of clayey and silty soils recently polluted with diesel hydrocarbons. *Water Air Soil Poll* 223:2473-2482.

National Energy Board (NEB). 2011. Estimated production of Canadian crude oil and equivalent. http://www.neb.gc.ca/clf-nsi/rnrgynfmtn/sttstc/ crdlndptrlmprdct/ stmtdprdctn-eng.html

National Petroleum Council (NPC). 2011. Waste management, Paper #2-24, working document of the NCP North American Resource Development Study. www.npc.org/Prudent...Papers/2-24_Waste_Management_Paper.pdf

Nelson DR, Hines H, Stay B. 2004. Methyl-branched hydrocarbons, major components of the waxy material coating the embryos of the viviparous cockroach *Diploptera punctata. Comp Biochem Physiol B* 138:265-276.

Office of the Auditor General of Canada. 2012. 2012 spring report of the commissioner of the environment and sustainable development: Chapter 3 – federal contaminated sites and their impacts. http://www.oag-bvg.gc.ca/internet/English/parl_ cesd_ 201205_03_e_36775.html

O'Reilly K, Thorsen W. 2010. Impact of crude oil weathering on the calculated effective solubility of aromatic compounds: evaluation of soils from Ecuadorian oil fields. *Soil Sed Contam* 19:391-404.

Peressutti SR, MA Hector, OH Pucci. 2003. Dynamics of hydrocarbon-degrading bacterioscenosis of an experimental oil pollution in Patagonian soil. *Int Biodeter Biodegr*. 52:21-30.

Prince RC, Garrett RM, Bare RE, Grossman MJ, Townsend T, Suflita JM, Lee K, Owens EH, Sergy GA, Braddock JF, Lindstrom JE, Lessard RR. 2003. The roles of photooxidation and biodegradation in long-term weathering of crude and heavy fuel oils. *Spill Sci Technol B* 8:145-156.

RDCT (R Development Core Team). 2011. R: a language and environment for statistical computing, R foundation for statistical computing. ISBN: 3-9000051-07-0, http://www.R-project.org.

Ruiz-Gutiérrez V, Pérez-Camino M.C. 2000. Update on solid-phase extraction for the analysis of lipid classes and related compounds. J Chrom A 885:321-341.

Samuels L, Kunst L, Jetter R. 2008. Sealing plant surfaces: cuticular wax formation by epidermal cells. *Annu Rev Plant Biol* 59:683-707.

Sanscartier D, Zeeb B, Koch I, Reimer K. 2009. Bioremediation of diesel-contaminated soil by heated and humidified biopile system in cold climates. *Cold Reg Sci Technol* 55:167-173.

Sanscartier D, Kenneth R, Zeeb B, George K. 2010. Management of hydrocarbon-contaminated soil through bioremediation and landfill disposal at a remote location in northern Canada. *Can J Civil Eng* 37:147-155.

Serrano A, Gallego M, González JL. 2006. Assessment of natural attenuation of volatile aromatic hydrocarbons in agricultural soil contaminated with diesel fuel. *Environ Pollut* 144:203-209.

Siddique T, Rutherford PM, Arocena JM, Thring RW. 2006. A proposed method for rapid and economical extraction of petroleum hydrocarbons from contaminated soils. *Can J Soil Sci* 86:725-728.

Steliga T, Kapusta P, Jakubowicz P. 2009. Effectiveness of bioremediation processes of hydrocarbon pollutants in weathered drill wastes. *Water Air Oil Pollut* 202:211-228.

Szajdak L, Brandyk T, Szatylowicz J. 2007. Chemical properties of different peat-moorsh soils from the Biebrza River Valley. *Agron Res* 5:165-174.

Tamocai C. 2006. The effect of climate change on carbon in Canadian peatlands. *Glob Planet Change* 53:222-232.

TBS (Treasury Board of Canada Secretariat). 2011. Federal contaminated sites inventory. http://www.tbs-sct.gc.ca/fcsi-rscf/cm-eng.aspx?clear=1

Theng BKG, Aislabie J, Fraser R. 2001. Bioavailability of phenanthrene intercalculated into an alkylammonium-montmorillonite clay. *Soil Biol Biochem* 33:845-848.

Uyttebroek M, Spoden A, Ortega-Calvo JJ, Wouters K, Wattiau P, Bastiaens L, Springael D. 2007. Differential responses of uebacterial, *Mycobacterium*, and *Sphingomonas* communities in polycyclic aromatic hydrocarbon (PAH)-contaminated soil to artificially induced changes in PAH profile. *J Environ Qual* 36:1403-1411.

Veil JA. 1998. Costs for off-site disposal of nonhazardous oil field wastes: salt caverns versus other disposal methods. Argonne National Laboratory http://*www.ead.anl.gov/pub/doc/saltcost.pdf*

Volkman JK, Holdsworth DG, Neill GP, Bavor HJ Jr. 1992. Identification of natural, anthropogenic and petroleum hydrocarbons in aquatic sediments. *Sci Tot Env* 112:203-219.

Wang XB, Chi CQ, Nie Y, Tang YQ, Tan Y, Wu XL. 2011. Degradation of petroleum hydrocarbons (C6-C40) and crude oil by a novel *Dietzia* strain. *Bioresource Technol* 102:7755-7761.

Wang ZD, Stout SA. 2007. Chemical fingerprinting of spilled or discharged petroleum. *In:* Wang ZD, Stout SA, ed, *Oil spill environmental forensic*. ed. Elsevier, Oxford, UK pp 1-53.

Wang ZD, Yang C, Fingas M, Hollebone B, Un HY, Oh JR. 2007. Petroleum biomarker fingerprinting for oil spill characterization and source identification. In Wang ZD, Stout SA, ed, *Oil spill environmental forensic*. ed. Elsevier, Oxford, UK pp 73-146.

Wang ZD, Yang Z, Yang C, Hollebone B, Brown CE, Landriault, M, Sun J, Mudge SM, Kelly-Hooper F. 2010. Fingerprinting of oil hydrocarbons and biogenic organic compounds in Canadian "Background" soil and plant samples (phase I of the PERD project: "distinguishing and quantifying petrogenic and biogenic hydrocarbons in contaminated and background soils. *Proceedings of the 33rd AMOP Technical Seminar on Environmental Contamination and Response* 1:1-56

Wang Z, Yang C, Yang Z, Hollebone B, Brown CE, Landriault M, Sun J, Mudge SM, Kelly-Hooper F, Dixon DG. 2012. Fingerprinting of petroleum hydrocarbons (PHC)

and other biogenic organic compounds (BOC) in oil-contaminated and background soil samples. *J. Environ. Monit.* Advance Article DOI: 10.1039/C2EM30339F.

Wellman DE, Ulery AL, Barcellona MP, Duerr-Auster S. 2001. Animal waste-enhanced degradation of hydrocarbon-contaminated soil. *Soil Sed Contam* 10:511-523.

Zakharavo YR, Adel'shin RV, Parfenova VV, Bedoshvili YD, Likhashway YV. 2010. Taxonomic characterization of the microorganisms associated with the cultivable diatom *Synedra acus* from Lake Baikal. 2010. *Microbiol* 79:679-687.

Zhou W, Xie S, Meyers PA, Zheng Z. 2005. Reconstruction of late glacial and Holocene climate evolution in southern China from geolipids and pollen in the Dingnan peat sequence. *Org Geochem* 36:1272-1284.

Appendix A

Crude oil spiked peat microcosm experiment:

detailed chemistry results

Table A: Day 0, Day 150, Day 300 raw data for clean peat and whole crude oil spiked peat and sand microcosm samples

Sample Days and Analytes	P1 Clean Processed Peat					P2 Clean Natural Peat					sP1 Highly Spiked Processed Peat				
	A	B	C	Mean	SD	A	B	C	Mean	SD	[5]A	[5]B	[5]C	Mean	[6]SD
DAY 0															
F2,F3,F4 (mg/kg)	4,655	4,219	2,925	3,933	900	2,053	2,633	1,543	2,076	545	15,518	18,790	18,531	17,613	1,819
F2[c]	<10	<10	<10	<10	0	<10	<10	<10	<10	0	3,216**	3,733**	3,656**	3,535**	279
F3[c]	2,221**	2,046**	1,496**	1,921**	378	1,280*	1,555**	871*	1,235*	344	8,618**	10,591**	10,170**	9,793**	1,039
F4[c]	2,429	2,168	1,424	2,007	521	768	1,073	667	836	211	3,684*	4,466*	4,705*	4,285*	534
F3a(% of F3)[c]	5%	4%	5%	5%	1%	9%	7%	8%	8%	1%	37%	42%	39%	39%	3%
F3b(% of F3)[c]	95%	96%	95%	95%	1%	91%	93%	92%	92%	1%	63%	58%	61%	61%	3%
F2:F3b ratio[d]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.59	0.61	0.59	0.60	0.01
PHC F3 (mg/kg)[e,f]	236	174	159	190	41	245	232	148	208	52	6,784**	9,464**	8,438**	8,229**	1,352
DAY 150															
F2,F3,F4 (mg/kg)	2,564	1,962	2,579	2,368	352	1,431	1,569	1,573	1,524	81	13,463	12,378	14,001	13,281	827
F2	<10	<10	<10	<10	0	<10	<10	<10	<10	0	2,283**	1,897**	2,441**	2,207**	280
F3	1,331**	947*	1,225*	1,168*	198	833*	967*	848*	882*	73	7,814**	7,136**	7,994**	7,648**	452
F4	1,228	1,010	1,349	1,196	172	593	597	720	637	72	3,366*	3,345*	3,566*	3,425*	122
F3a(% of F3)	4%	4%	5%	4%	1%	10%	10%	10%	10%	0%	33%	30%	34%	32%	2%
F3b(% of F3)	96%	96%	95%	96%	1%	90%	90%	90%	90%	0%	67%	70%	66%	68%	2%
F2:F3b ratio	0.00	0.01	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.44	0.38	0.46	0.43	0.04
PHC F3 (mg/kg)[g]	140	100	161	134	31	219	254	223	232	19	6,785**	5,633**	7,152**	6,524**	793
DAY 300															
F2,F3,F4 (mg/kg)	2,603	3,159	4,198	3,320	810	1,513	1,443	1,687	1,548	126	9,459	10,542	10,482	10,161	609
F2	<10	<10	<10	<10	0	<10	<10	<10	<10	0	1,189**	1,407**	1,422**	1,339**	130
F3	1,229*	1,569**	1,939**	1,579**	355	802*	798*	895*	831*	55	5,551**	5,983**	6,096**	5,876**	288
F4	1,369	1,585	2,254	1,736	461	706	640	787	711	74	2,719	3,152*	2,964*	2,945*	217
F3a(% of F3)	5%	7%	5%	6%	1%	6%	7%	6%	6%	1%	26%	25%	26%	26%	1%
F3b(% of F3)	95%	93%	95%	94%	1%	94%	93%	94%	94%	1%	74%	75%	74%	74%	1%
F2:F3b ratio	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.29	0.31	0.32	0.31	0.01
PHC F3 (mg/kg)[h]	198	354*	312*	288	81	155	180	173	170	13	4,655**	4,825**	5,112**	4,864**	231

Table A: Day 0, Day 150, Day 300 raw data for clean peat and whole crude oil spiked peat and sand microcosm samples

Sample Days and Analytes	sP2 Moderately Spiked Natural Peat					sS Moderately Spiked Sand Plus Bacteria and Nutrients				
	A	B	C	Mean	SD	A	B	C	Mean	SD
DAY 0										
F2,F3,F4 (mg/kg)	3,073	3,876	3,274	3,408	418	1,745	1,897	1,876	1,839	82
F2[c]	627**	633**	570**	610**	35	407**	443**	456**	435**	25
F3[c]	1,776**	2,253**	1,831**	1,953**	261	1,020*	1,118*	1,094*	1,077*	51
F4[c]	670	990	873	844	162	318	336	326	327	9
F3a(% of F3)[c]	29%	23%	26%	26%	3%	47%	47%	46%	47%	1%
F3b(% of F3)[c]	71%	77%	74%	74%	3%	53%	53%	54%	53%	1%
F2:F3b ratio[d]	0.50	0.36	0.42	0.43	0.07	0.75	0.75	0.77	0.76	0.01
PHC F3 (mg/kg)[e,f]	1,095*	1,102*	1,012*	1,070*	50	--	--	--	--	--
DAY 150										
F2,F3,F4 (mg/kg)	2,871	2,362	1,680	2,304	598	670	759	646	692	60
F2	365**	344**	248**	319**	62	65	85	67	72	11
F3	1,669**	837*	915*	1,140*	459*	427*	486*	414*	442*	38
F4	837	1181	517	845	332	178	188	165	177	12
F3a(% of F3)	15%	14%	12%	14%	2%	37%	39%	38%	38%	1%
F3b(% of F3)	85%	86%	88%	86%	2%	63%	61%	62%	62%	1%
F2:F3b ratio	0.26	0.48	0.31	0.35	0.12	0.24	0.29	0.26	0.26	0.02
PHC F3 (mg/kg)[g]	658*	308*	289	418*	208	--	--	--	--	--
DAY 300										
F2,F3,F4 (mg/kg)	2,184	2,476	2,326	2,328	146	394	149	429	324	153
F2	244**	280**	232**	252**	25	32	21	33	29	7
F3	1,189*	1,407**	1,290*	1,295*	109	242	78	271	197	104
F4	751	789	804	781	27	120	50	125	98	42
F3a(% of F3)	12%	12%	12%	12%	0%	31%	29%	33%	31%	2%
F3b(% of F3)	88%	88%	88%	88%	0%	69%	71%	67%	69%	2%
F2:F3b ratio	0.23	0.23	0.20	0.22	0.02	0.19	0.38	0.18	0.25	0.11
PHC F3 (mg/kg)[h]	460*	544*	499*	501*	42	--	--	--	--	--

Table A: Day 0, Day 150, Day 300 raw data for clean peat and whole crude oil spiked peat and sand microcosm samples

[a]sP1 - whole crude oil nominal spike concentration: F2-F4 = 19,608 mg/kg; F2 = 6,608 mg/kg; F3 = 10,000 mg/kg; F4 3,000 mg/kg.
[b]sP2 and sS - whole crude oil nominal spike concentration: F2-F4 = 2,942 mg/kg; F2 = 1,000 mg/kg; F3 = 1,500 mg/kg; F4 450 mg/kg.
[c]F2 (C10-C16), F3 (C16-C34), F3a (C16-C22), F3b (C22-C34), F4 (>C34).
[d]Non-detectable F2 concentrations calculated as 5 mg/kg (half of the 10 mg/kg detection limit)
[e]Calculated BOC-adjusted PHC F3 concentrations; Equation 2.2.
[f]The 47%F3a:53%F3b ratio in the spiked Sand (sS1) was used as the crude oil source in the Day 0 Equation 2.2 calculations.
[g]The 38%F3a:62%F3b ratio in the spiked Sand (sS1) was used as the crude oil source in the Day 150 Equation 2.2 calculations.
[h]The 31%F3a:69%F3b ratio in the spiked Sand (sS1) was used as the crude oil source in the Day 300 Equation 2.2 calculations.
*Single asterisk indicates exceedences of the CWS PHC coarse soil guideline(s), F2 = 150 mg/kg; F3 = 300 mg/kg; F4 = 2,800 mg/kg (Table 1.1).
**Double asterisk indicates exceedences of the CWS PHC fine soil guideline(s), F2 = 150 mg/kg; F3 = 1,300 mg/kg; F4 = 5,600 mg/kg (Table 1.1).
"SD" standard deviation.
"--" value not calculated.
Values reported on dry weight basis.

Appendix B

Diesel drilling waste spiked manure compost microcosm experiment: detailed chemistry results

Table B: Day 0, Day 150, Day 300 raw data for clean manure and diesel drilling waste spiked manure and sand microcosm samples

Sample Days and Analytes	M Clean Manure Compost					sM1 Highly Spiked Manure Compost					sM2 Moderately Spiked Manure Compost				
	i	ii	iii	Mean	SD	i	ii	iii	Mean	SD	i	ii	iii	Mean	SD
DAY 0															
F2,F3,F4 (mg/kg)	1,395	795	1,015	1,068	304	12,850	11,830	12,740	12,473	560	3,760	3,359	4,061	3,727	352
F2[c]	<10	<10	<10	<10	0	2,530**	2,330**	2,540**	2,466**	118	610**	569**	661**	613**	46
F3[c]	800*	520*	680*	667*	140	9,940**	9,160**	9,860**	9,653**	429*	2,850**	2,540**	3,060**	2,816**	262
F4[c]	590	270	330	397	170	380	340	340	353	23	300	250	340	297	45
F3a(% of F3)[c]	15%	13%	12%	13%	2%	82%	82%	83%	82%	1%	73%	72%	72%	72%	1%
F3b(% of F3)[c]	85%	87%	88%	87%	2%	18%	18%	17%	18%	1%	27%	28%	28%	28%	1%
F2:F3b ratio[d]	0.01	0.01	0.01	0.01	0.00	1.41	1.41	1.52	1.45	0.06	0.79	0.80	0.77	0.79	0.01
PHC F3 (mg/kg)[e,f]	140	79	95	104	32	9,477*	8,733*	9,516*	9,242*	441*	2,419**	2,126**	2,561**	2,368**	222
Bacteria	4.E+11	4.E+11	4.E+11	4.E+11	6.E+09	3.E+11	3.E+11	2.E+11	3.E+11	3.E+10	2.E+11	2.E+11	2.E+11	2.E+11	3.E+10
DAY 150															
F2,F3,F4 (mg/kg)	569	385	360	438	114	6,124	6,316	2,872	5,104	1,935	971	1,447	1,987	1,468	508
F2	<10	<10	<10	<10	0	738**	680**	352**	590**	208**	94	125	171**	130	39
F3	446*	284	277	336*	96	5,190**	5,400**	2,470**	4,353**	,634**	826*	1,210*	1,630**	1,222*	402*
F4	118	96	78	97	20	196	236	50	161	98	51	112	186	116	68
F3a(% of F3)	23%	23%	19%	22%	2%	78%	77%	80%	78%	2%	64%	63%	61%	63%	2%
F3b(% of F3)	77%	77%	81%	78%	2%	22%	23%	20%	22%	2%	36%	37%	39%	37%	2%
F2:F3b ratio	0.01	0.02	0.02	0.01	0.00	0.64	0.54	0.73	0.64	0.09	0.32	0.28	0.27	0.29	0.03
PHC F3 (mg/kg)[g]	120	76	62	86	30	4,686**	4,825**	2,302**	3,937**	1,418**	618*	889*	1,160*	889*	271
Bacteria	5E+10	5E+10	6E+10	5E+10	7E+09	6E+10	3E+10	5E+10	5E+10	1E+10	5E+10	4E+10	3E+10	4E+10	8E+09
DAY 300															
F2,F3,F4 (mg/kg)	680	177	585	481	267	4,799	3,307	5,633	4,580	1,178	268	409	278	1,612	58
F2	<10	<10	<10	<10	0	531**	384**	664**	526**	140	145	109	145	133	21
F3	500*	143	450*	364*	193	4,100**	2,820**	4,770**	3,896**	990*	1,300*	1,180*	1,400**	1,293*	110
F4	175	29	130	111	75	168	103	199	157	49	123	300	133	185	99
F3a(% of F3)	24%	21%	24%	23%	2%	77%	78%	77%	77%	1%	65%	63%	61%	63%	2%
F3b(% of F3)	76%	79%	76%	77%	2%	23%	22%	23%	23%	1%	35%	37%	39%	37%	2%
F2:F3b ratio	0.01	0.04	0.01	0.02	0.02	0.55	0.62	0.60	0.59	0.03	0.32	0.25	0.27	0.28	0.04
PHC F3 (mg/kg)[h]	140	35	126	100	57	3,663**	2,557**	4,270**	3,496**	868*	982*	864*	993*	946*	72
Bacteria	1E+12	6E+10	1E+11	5E+11	8E+11	7E+10	5E+10	1E+11	7E+10	2E+10	7E+10	2E+10	3E+10	4E+10	3E+10

Table B: Day 0, Day 150, Day 300 raw data for clean manure and diesel drilling waste spiked manure and sand microcosm samples

Sample Days and Analytes	sS Moderately Spiked Sand Plus Bacteria and Nutrients					S Sand+Bacteria+Nutrients				
	i	ii	iii	Mean	SD	i	ii	iii	Mean	SD
DAY 0										
F2,F3,F4 (mg/kg)	2,749	3,718	4,037	3,501	671					
F2[c]	824**	793**	852**	823**	30	<10	<10	<10	<10	0
F3[c]	1,900**	2,900**	3,160**	2,653**	665*	<50	<50	<50	<50	0
F4[c]	<50	<50	<50	<50	<50	<50	<50	<50	<50	0
F3a(% of F3)[c]	86%	86%	87%	86%	0%	--	--	--	--	--
F3b(% of F3)[c]	14%	14%	13%	14%	0%	--	--	--	--	--
F2:F3b ratio[d]	3.10	1.95	2.07	2.37	0.63	--	--	--	--	--
PHC F3 (mg/kg)[e,f]	--	--	--	--	--	--	--	--	--	--
Bacteria	2.E+07	9.E+07	3.E+07	5.E+07	4.E+07	6.E+08	9.E+08	7.E+08	7.E+08	2.E+08
DAY 150										
F2,F3,F4 (mg/kg)	2,344	3,303	3,427	3,025	593	<10	<10	<10	<10	0
F2	269**	358**	382**	336**	60	<50	<50	<50	<50	0
F3	2,050**	2,920**	3,020**	2,663**	533*	<50	<50	<50	<50	0
F4	<50	<50	<50	<50	<50	--	--	--	--	--
F3a(% of F3)	83%	84%	84%	84%	0%	--	--	--	--	--
F3b(% of F3)	17%	16%	16%	16%	0%	--	--	--	--	--
F2:F3b ratio	0.79	0.74	0.79	0.77	0.03	--	--	--	--	--
PHC F3 (mg/kg)[g]	--	--	--	--	--	--	--	--	--	--
Bacteria	4E+08	4E+08	3E+08	4E+08	2E+07	2E+08	2E+08	2E+08	2E+08	2E+07
DAY 300										
F2,F3,F4 (mg/kg)	2,216	2,345	2,345	2,302	74	<10	<10	<10	<10	0
F2	251**	200**	220**	223**	26	<50	<50	<50	<50	0
F3	1,940**	2,120**	2,100**	2,053**	99	<50	<50	<50	<50	0
F4	<50	<50	<50	<50	<50	--	--	--	--	--
F3a(% of F3)	84%	83%	83%	83%	0%	--	--	--	--	--
F3b(% of F3)	16%	17%	17%	17%	0%	--	--	--	--	--
F2:F3b ratio	0.79	0.56	0.64	0.66	0.12	--	--	--	--	--
PHC F3 (mg/kg)[h]	--	--	--	--	--	--	--	--	--	--
Bacteria	2E+08	2E+08	2E+08	2E+08	2E+07	1E+08	8E+07	9E+07	9E+07	8E+06

Table B: Day 0, Day 150, Day 300 raw data for clean manure and diesel drilling waste spiked manure and sand microcosm samples

[a]sM1 – diesel drilling waste nominal spike concentration: F2-F4 = 13,400 mg/kg; F2 = 3,400 mg/kg; F3 = 10,000 mg/kg; F4 <50 mg/kg.
[b]sM2 and sS - whole crude oil nominal spike concentration: F2-F4 = 2,613 mg/kg; F2 = 613 mg/kg; F3 = 2,000 mg/kg; F4 <50 mg/kg.
[c]F2 (C10-C16), F3 (C16-C34), F3a (C16-C22), F3b (C22-C34), F4 (>C34).
[d]Non-detectable F2 concentrations calculated as 5 mg/kg (half of the 10 mg/kg detection limit)
[e]Calculated BOC-adjusted PHC F3 concentrations; Equation 2.2.
[f]The 86%F3a:14%F3b ratio in the spiked Sand (sS1) was used as the diesel drilling waste source in the Day 0 Equation 3.2 calculations.
[g]The 84%F3a:16%F3b ratio in the spiked Sand (sS1) was used as the diesel drilling waste source in the Day 150 Equation 3.2 calculations.
[h]The 83%F3a:17%F3b ratio in the spiked Sand (sS1) was used as the diesel drilling waste source in the Day 300 Equation 3.2 calculations.
*Single asterisk indicates exceedences of the CWS PHC coarse soil guideline(s), F2 = 150 mg/kg; F3 = 300 mg/kg (Table 1.1).
**Double asterisk indicates exceedences of the CWS PHC fine soil guideline(s), F2 = 150 mg/kg; F3 = 1,300 mg/kg (Table 1.1).
"SD" standard deviation.
"--" value not calculated.
Values reported on dry weight basis.