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IS IT CLEAN OR CONTAMINATED SOIL? USING PETROGENIC VERSUS BIOGENIC GC-FID CHROMATOGRAM PATTERNS TO MATHEMATICALLY RESOLVE FALSE PETROLEUM HYDROCARBON DETECTIONS IN CLEAN ORGANIC SOILS: A CRUDE OIL–SPIKED PEAT MICROCOSM EXPERIMENT

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Abstract: The Canadian Council of Ministers of the Environment (CCME) reference method for the Canada-wide standard (CWS) for petroleum hydrocarbon (PHC) in soil provides chemistry analysis standards and guidelines for the management of contaminated sites. However, these methods can coextract natural biogenic organic compounds (BOCs) from organic soils, causing false exceedences of toxicity guidelines. The present 300-d microcosm experiment used CWS PHC tier 1 soil extraction and gas chromatography–flame ionization detector (GC-FID) analysis to develop a new tier 2 mathematical approach to resolving this problem. Carbon fractions F2 (C10–C16), F3 (C16–C34), and F4 (>C34) as well as subfractions F3a (C16–C22) and F3b (C22–C34) were studied in peat and sand spiked once with Federated crude oil. These carbon ranges were also studied in 14 light to heavy crude oils. The F3 range in the clean peat was dominated by F3b, whereas the crude oils had approximately equal F3a and F3b distributions. The F2 was nondetectable in the clean peat but was a significant component in crude oil. The crude oil–spiked peat had elevated F2 and F3a distributions. The BOC-adjusted PHC F3 calculation estimated the true PHC concentrations in the spiked peat. The F2:F3b ratio of less than 0.10 indicated PHC absence in the clean peat, and the ratio of greater than or equal to 0.10 indicated PHC presence in the spiked peat and sand. Validation studies are required to confirm whether this new tier 2 approach is applicable to real-case scenarios. Potential adoption of this approach could minimize unnecessary ecological disruptions of thousands of peatlands throughout Canada while also saving millions of dollars in management costs. *Environ Toxicol Chem* 2013;32:2197–2206. © 2013 SETAC

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INTRODUCTION

Conventional crude oil is a naturally occurring hydrocarbon-based liquid that is formed over millions of years from buried plant, animal, and microbial remains [1]. Canada is the seventh largest producer of conventional crude oil in the world (http://www.neb.gc.ca/clf-nsi/rnrgynfmtn/sttstc/crdlndptrlmprdct/stmtdprdctn-eng.html). Crude oil is pumped from underground reserves and transported by ships, trucks, and pipelines to oil refineries, where it is converted into products such as heating and transportation fuels, motor oils, and asphalt. Although spill prevention is a key component of crude oil exploration, extraction, and transportation activities, spills can potentially occur during any of these stages. 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 (http://laws-lois.justice.gc.ca/Search/Search.aspx?txtS3archA11=spill&txtT1tl3=%22Canada+Oil+and+Gas+Operations+Act%22&h1ts0n1y=0&ddC0nt3ntTyp3=Act).

The Canadian Council of Ministers of the Environment (CCME) reference method for the Canada-wide standard (CWS) for petroleum hydrocarbon (PHC) in soil [2] is based on a risk assessment approach to managing contaminated sites remediation. The standards can be applied at 3 risk assessment levels or "tiers." Tier 1 is based on generic numerical standards corresponding to 4 land uses (Table 1). Exceeding the tier 1 soil guidelines may lead to detailed site-specific evaluations at the tier 2 or tier 3 levels.

The CWS PHC soil standards are organized into the following 4 PHC carbon-range fractions: F1 (C6–C10), F2 (C10–C16), F3 (C16–C34), and F4 (> C34). The carbon-range fraction F1 consists of nonpolar aliphatic and volatile aromatic PHCs, and F2 consists primarily of nonpolar semivolatile PHCs. Both F2 and F3 contain nonpolar aromatic and aliphatic hydrocarbons. Fraction F4 has low aromaticity and contains small amounts of polar nitrogen, sulfur, and oxygen heteroatoms.

The tier 1 CWS PHC generic soil toxicity guidelines for F1, F2, F3, and F4 carbon ranges are based on risk management of environmental and human health exposures to PHC concentrations for each of the 4 fractions [3]. Tier 1 considers site-specific conditions such as land use, groundwater potability, and mineral soil coarse and fine textures. Highly organic peat soils have low mineral content and do not therefore apply to either the fine or coarse soil categories. Regulatory discretion is used in the selection of the most appropriate fine or coarse soil toxicity guidelines for peat soils, which may depend on site-specific conditions. For example, the most stringent coarse soil guideline might be applied to contaminated sites located in higher risk areas with potable drinking water and/or nearby surface water systems that could carry PHCs to off-site locations.

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Table 1. Tier 1 Canada-wide standard generic guidelines (mg/kg) for petroleum hydrocarbons in surface soils[a]

Land use	Soil texture[b]	Fraction 1	Fraction 2	Fraction 3	Fraction 4
Agricultural/residential/parkland					
	Coarse-grained soil	30	150	300	2800
	Fine-grained soil	170	150	1300	5600
Commercial/industrial					
	Coarse-grained soil	240	260	1700	3300
	Fine-grained soil	170	230	2500	6600

[a]Most stringent Tier 1 soil criteria for potable groundwater protection. Less-stringent criteria may be applied at the discretion of regulatory agencies [3].
[b]Coarse sand and gravel, median grain size of >75 μm; fine silt and clay, median grain size of <75 μm.

The CWS provides analytical methods for generating accurate and reproducible PHC soil chemistry results among Canadian laboratories. The methods use hexane, acetone, and dichloromethane (DCM) solvents for the PHC soil extraction process. However, these solvents coextract biogenic organic compounds (BOCs) originating from fresh and decayed plant, animal, and microbial matter. The CWS PHC methods include a silica gel treatment step, which is used to remove polar BOCs from the final extract. This polar cleanup step was developed and validated on mineral and loam soils [4] with less than 5% total organic content, which is a gross measure of the amount of carbon bound in an organic compound. The 5-g maximum allowable amount of silica gel becomes oversaturated by highly organic soils such as peat, which can have total organic content levels ranging from approximately 35% to 60% [5]. Excess BOCs that cannot be retained by 5 g of silica gel become part of the final PHC extract. Extracted BOCs are misidentified as PHCs, which can cause false exceedences of PHC soil toxicity guidelines. The CCME recommended solution to this problem is to analyze and subtract false PHC concentrations in comparable clean background soils from PHC concentrations in the authentically contaminated soils [2,3]. This approach can, however, be problematic where natural variations in background soil parent material, depth, and hydrologic regimes produce highly varied PHC results [6]. In a worst-case scenario, comparable background soils would not exist at remediation facilities that treat contaminated soil mixtures delivered from many different locations over periods of years.

Alternative forensics methods have been developed over the past 40 yr and continue to be used for petroleum exploration and contaminated soil evaluation purposes [1,7,8]. Forensic analysis tools most relevant to the present study include 1) petroleum biomarkers (e.g., steranes and hopanes), which indicate PHC presence versus absence and PHC sources; 2) polycyclic aromatic hydrocarbons (PAHs; alkylated and nonalkylated), which indicate the absence versus presence of spilled PHCs, in addition to determining if PHCs originate from liquid or pyrogenic (partially combusted) sources; and 3) unresolved complex mixtures (UCM), which can be described as a hump that appears on gas chromatography–flame ionization detector (GC-FID) chromatograms between the solvent baseline and the resolved peaks baseline. The PHC sources have visually pronounced, regularly shaped UCMs, while noncontaminated BOC sources have relatively smaller and irregularly shaped UCMs.

Biomarker and PAH analysis provide excellent tools for determining PHC presence versus absence; however, they require highly specialized expertise and materials that can be too costly and time consuming for large-scale applications. Although UCM analysis is also a valuable tool, it depends on the subjective opinions of chemistry professionals regarding the visual characteristics of UCM patterns in GC-FID chromatograms.

The present study used CWS PHC tier 1 GC-FID chromatograms to mathematically resolve false PHC detections in clean peat and to estimate true PHC concentrations in contaminated soils on a tier 2 basis. This new approach requires only 1 additional integration step to quantify subfractions F3a (C16–C22) and F3b (C22–C34) concentrations and percentages. The F3a and F3b data were used to calculate BOC-adjusted PHC F3 concentrations in peat samples. In addition, F2:F3b ratios were used to indicate PHC absence in clean peat and PHC presence in crude oil–spiked peat. This approach was developed through the results of a 300-d crude oil-spiked peat and sand microcosm experiment. The 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. These data provided the basis for this new tier-2 mathematical approach to resolving false detections of crude oil PHCs and F3 toxicity guideline exceedences in clean peat soils.

MATERIALS AND METHODS

Microcosm experiment design

The microcosm study was conducted indoors at the ALS Environmental laboratory, Waterloo, Ontario, Canada. Each microcosm was housed in a 70-L rectangular (30 cm × 35 cm × 66 cm) glass aquaria fitted with full spectrum lighting to simulate sunlight exposure. Each tank held a soil depth of approximately 15 cm with a 2 cm overlay of deionized water. Aerobic conditions were maintained by placing 5-cm long aeration stones under the peat or sand in each tank. The air temperature of the laboratory facility was maintained at 22 °C. These environmental conditions were maintained for 24 h/d during the entire 300-d experiment.

Microcosm soil types

The soils used in the microcosm experiment included silica sand as a control and 2 sources of peat.The silica sand, purchased from Anachemia Science, had been prewashed with deionized water and dried. Processed peat was purchased from a commercial landscape supplier (collected from a bog located in northern Ontario, Canada), and natural peat was collected from a fen located in Lakeland Provincial Park in northern Alberta, Canada. The natural peat was collected along with the overlying vegetation layer, stored in iced coolers, and shipped to Waterloo, where the vegetation was removed and temporarily held in aquaria until the microcosm experiments had begun.

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 analysis of biomarkers, alkylated and parent PAHs, and UCM

patterns. Wang et al. [9] described the methods and materials used for the forensics analysis. The processed peat and natural peat had high total organic content levels of 45% and 35% by weight, respectively. The natural peat had a neutral pH of 6.7. In contrast, the processed peat had an acidic pH of 4.2, which was below the optimal microbial biodegradation range of 4.5 to 7.5 [10,11]. Calcium carbonate was added to the processed peat for the purpose of increasing the pH to a neutral value of 7.0.

Silica sand was used as an inorganic control to monitor crude oil degradation in the absence of detectable BOCs. The sand had a neutral pH of 7.5, 0.0% total organic content by weight, and nondetectable bacteria levels (<10 000 colony-forming units/g). Nutrient levels were nondetectable 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 all of the sand treatments at similar concentrations that were detected in the 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. Bacteria from the natural peat was cultured in inorganic agar broth (GAP Laboratories), concentrated and added to selected sand microcosms at concentrations of 46×10^6 colony-forming units/g, similar to the total aerobic plate count in the natural peat (3.7×10^5 colony-forming units/g). Gram-negative, aerobic *Burkholderia* sp. was identified as the dominant bacteria in the natural peat, based on a heterotrophic plate count. The purpose of these nutrient and bacteria amendments was to promote similar crude oil degradation processes between the inorganic sand and the organic natural peat.

Federated crude oil description

Whole unweathered Federated crude oil was used in this microcosm study for the reason that it was also used to generate the CWS PHC tier 1 soil toxicity guidelines [4]. Federated crude oil 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.

Microcosm soil treatments

The microcosm experiment consisted of 7 treatments conducted in triplicate. Of these 7 treatments 4 treatments were not spiked with crude oil and 3 treatments were spiked once at the beginning of the experiment to represent a 1-time crude oil spill. The treatments were as follows: (C) control, untreated silica sand; (P1) clean processed peat; (P2) clean natural peat; (sP1) processed peat spiked with a high nominal concentration of 19 608 mg/kg F2 to F4 (9216 mg/kg F3) whole crude oil; (sP2) natural peat spiked with a moderate nominal concentration of 2942 mg/kg F2 to F4 (1383 mg/kg F3) whole crude oil; (S) silica sand amended with bacteria and nutrients; and (sS) silica sand amended with bacteria, nutrients, and spiked with a moderate nominal concentration of 2942 mg/kg F2 to F4 (1383 mg/kg F3) whole crude oil. Overlying moss (*Drepanocladus aduncus*) and herbaceous plants originally harvested with the natural peat were replanted in treatments P2 and sP2. The sand treatments were used to monitor PHC levels in the absence of detectable BOCs. The spiked sand treatment also provided the F3a and F3b percentages as the representative crude oil contamination source for the BOC-adjusted PHC F3 concentration, as described in the *Results and Discussion* section.

Microcosm monitoring and sampling procedures

Microcosm soil samples were collected on day 0, day 150, and day 300. Full-depth 300-mL soil samples were scooped from the center of each microcosm tank and homogenized for 3 min with an electric mixer. Each sample was separated into 3 150-mL aliquots and placed into individual 25-mL amber glass jars with Teflon-lined lids. All of the soil samples were stored at −20 °C prior to PHC analysis. The remaining soils left in each tank were manually homogenized and left undisturbed until the next sampling event.

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, which remained relatively constant during the entire 300-d study period. The pH levels were all within the neutral range of 6.5 to 8.5. Conductivity ranged from 0.293 dS/m to 0.628 dS/m, which is considered to be an ecologically acceptable range [6]. The redox levels ranged from aerobic levels of +88 mV to +154 mV.

F2, F3, and F4 PHC soil extraction and analysis

The F2, F3, and F4 PHC soil extractions and GC-FID runs were conducted by ALS Environmental. The materials and methods used were based on the CCME reference method for the CWS PHC in soil, tier 1 method [2]. The GC-FID chromatogram integrations were conducted by F. Kelly-Hooper. The lowest carbon range, F1 (C6–C10), was not analyzed because the CCME user guidance document identified biogenic interferences as occurring in the F2 to F4 carbon range [3]. Silica-gel 60 and trace/organic/pesticide grade solvents and acids were purchased from Caledon Laboratories. The silica gel was activated by heating at 250 °C (± 25 °C) for 72 h. Soxtec soil extraction quality assurance measures included 1 method blank and 1 duplicate sample for each group of 20 extracted samples or less. The acceptable F2 to F4 method blank concentrations were <10 mg/kg F2, <50 mg/kg F3 and <50 mg/kg F4. The duplicate data quality objectives were <50% relative difference. A 10-g soil sample was mixed with celite as a drying agent and placed into a filter cup. The sample was spiked with the analytical surrogate o-terphenyl (2000 μg/mL) in acetone to evaluate the extraction recovery objective of 60% to 120%. The sample was then packed into an 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 and acetone solvent mixture that was boiled for 2 h. 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 deionized 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. Dichloromethane was added to the decanted hexane at a 50:50 ratio and was mixed with 5 g of silica gel for 5 min 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 (prerinsed with acetone and hexane) to physically separate the silica gel from the solvents. Toluene was added to the beaker containing the solvents and was placed onto a rotary evaporator to remove the remaining DCM from the 10 mL final extract, which was transferred to a glass vial for GC-FID analysis.

F2, F3, and F4 PHC GC-FID analysis procedures

The Agilent 6890Ns GC-FID instrument was equipped with an on-column injector, a 0.32 mm × 0.1 μm × 30 m capillary 100% poly(dimethylsiloxane) column, and a flame ionization detector. The extract injection volume was 1.5 μL. External

calibration standards, Restek CCME 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. 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 5-point calibration curve (10 μg/mL, 50 μg/mL, 100 μg/mL, 250 μg/mL, and 500 μg/mL) was generated at the beginning of each analytical batch. 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

The F2, F3, F4, F3a, and F3b percentages and F2:F3b ratios were analyzed in 14 light to heavy fresh crude oils. The following 8 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 6 fresh crude oils: South Louisiana, Arabian Heavy, Troll, Maya, IFO-180, and Imperial Heavy. The GC-FID analysis methods for these 6 crude oils are described in Wang et al. [12].

Statistical analysis

The F2, F3, F4, F3a, and F3b dry weight concentrations are reported as compositional data, such that each fraction and subfraction represents a proportion of the total composition. When data are expressed in this form, the data must be modified to apply standard statistical methods [13]. The statistical analysis of compositional data requires special treatment by transforming the data based on log ratios [14]. In the present study, the data were transformed using the log-centered transform expressed as

$$z_i = \log(x_i/g(x_D)) \ (i = 1, \ldots, D), \qquad (1)$$

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

The R statistical software package [15] was used to calculate balanced two-way analysis of variance (ANOVA) *F*-test *p* values. The *p* values were calculated for day 0 and day 300 hydrocarbon fractions (F2, F3a, F3b, and F4). The triplicate soil sample data sets were not large enough to estimate mean significant differences. Data for the control and clean sand were not included in the statistical analysis because the F2, F3, and F4 concentrations were less than the following respective method detection limits: 10 mg/kg, 50 mg/kg, and 50 mg/kg. The F2 concentrations in the clean peat (processed and natural) were below the 10-mg/kg detection limit and were therefore calculated as half the detection limit (5 mg/kg). The R software was also used to run quantile-quantile plots and Shapiro-Wilk normal distribution tests on F3a:F3b percentage distributions for the 14 light to heavy crude oils.

RESULTS AND DISCUSSION

Baseline forensics results for the clean natural peat and sand

The biomarker, PAH, and UCM baseline-analysis results determined that liquid and noncombusted hydrocarbons were nondetectable in the clean sand. Only trace pyrogenic PAHs (parent) were detected in the peat samples, likely originating from atmospheric deposition.

F2, F3, F4, and F2 to F4 concentration percentage reductions and PHC soil toxicity guideline exceedences in clean peat and crude oil–spiked sand and peat

The F2 concentrations were less than the 10-mg/kg detection limit in all of the clean peat samples (Table 2). The F2 concentrations progressively decreased from day 0 to day 300 in all of the spiked treatments. The highly spiked peat and moderately spiked peat had similar F2 decreases, while the moderately spiked sand had the greatest decrease. The F2 concentrations in the highly spiked peat and moderately spiked peat exceeded the 150-mg/kg F2 soil guideline on all 3 sample dates. The F2 concentrations in the moderately spiked sand had degraded to below the guideline by day 150 and day 300.

The 300-mg/kg F3 soil guideline was exceeded by all of the clean peat and spiked peat samples during the study period. The F3 concentrations in the moderately spiked sand exceeded the guideline on day 0 and day 150 but decreased to below the guideline by day 300. A steady but relatively smaller decrease was observed in the day 300 highly spiked peat. The F3 concentrations in the moderately spiked peat decreased on day 150, but increased on day 300. The F3 concentrations steadily decreased in the natural peat but fluctuated in the processed peat.

Although F4 concentrations were detected in all of the clean peat, spiked peat, and spiked sand samples, the 2800 mg/kg F4 soil guideline was exceeded only by the highly spiked peat, which steadily decreased by day 300. There was a comparatively greater decrease in the moderately spiked sand, with very little change in the moderately spiked peat. The F4 concentrations fluctuated in the clean processed peat and clean natural peat.

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. [16] reported that sand spiked once with a whole crude oil TPH concentration of 49 200 mg/kg, degraded by 46% at the end of 390 d. This is similar to the 42% F2 to F4 concentration decrease that was observed in the day 300 highly spiked peat in the present study.

The comparatively lower F2, F3, and F4 degradation rates in the crude oil–spiked peat versus the crude oil–spiked sand may be attributed to adsorption and/or partitioning effects, which are known to occur in the presence of organic soil matter [17,18]. The F3 and F4 concentration 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, aeration, and 22 °C ambient air temperature [19,20].

Key factors regarding false detections of PHCs in clean peat were as follows: 1) F2 concentrations were nondetectable only in the clean peat and sand samples but were elevated in all of the spiked peat and sand samples; 2) false exceedences of the CWS PHC soil guideline in clean peat soils occurred only in the F3 range; and 3) F4 concentrations were detectable in all of the peat treatments, but F4 guideline exceedences occurred only in the highly spiked peat.

F2, F3, F4, F3a, and F3b GC-FID chromatogram patterns

The CWS PHC GC-FID chromatograms were used to visually distinguish clean peat from crude oil–spiked peat, in addition to monitoring PHC degradation patterns. Figure 1 illustrates examples of day 0 and day 300 GC-FID

Table 2. Day-0, day-150, and day-300 analysis mean results for clean peat and crude oil spiked peat and sand microcosm samples, F2, F3, F4 concentrations, F3a and F3b percentages, F2:F3b ratios, and BOC-adjusted PHC F3 concentrations[a]

Sample day	Analyte	Clean processed peat	Clean natural peat	Highly spiked processed peat[b]	Moderately spiked natural peat[c]	Moderately spiked sand plus bacteria and nutrients[c]
Day 0						
	Total C10–C34 (mg/kg)	3928 ± 900	2071 ± 545	17 613 ± 1819	3408 ± 418	1839 ± 82
	F2 (mg/kg)	< 10	< 10	3535 ± 279[h]	610 ± 35[h]	435 ± 25[h]
	F3 (mg/kg)	1921 ± 378[h]	1235 ± 344[h]	9793 ± 1039[h]	1953 ± 261[h]	1077 ± 51[h]
	F4 (mg/kg)	2007 ± 521	836 ± 211	4285 ± 534[h]	844 ± 162[h]	327 ± 9[h]
	F3a (% of F3)	5 ± 1%	8 ± 2%	39 ± 3%	26 ± 3%	47 ± 1%[c]
	F3b (% of F3)	95 ± 1%	92 ± 2%	61 ± 3%	74 ± 3%	53 ± 1%[c]
	F2/F3b ratio	0.00 ± 0.00	0.01 ± 0.00	0.60 ± 0.03	0.43 ± 0.05	0.76 ± 0.03
	Calc. PHC F3 (mg/kg)[d,e]	190 ± 41	189 ± 48	8229 ± 1352[h]	107 1 ± 50[h]	NC
Day 150						
	Total C10–C34 (mg/kg)	2373 ± 287	1529 ± 66	13 281 ± 75	2304 ± 88	692 ± 49
	F2 (mg/kg)	< 10	< 10	2207 ± 228[h]	319 ± 51[h]	72 ± 9
	F3 (mg/kg)	1168 ± 162[h]	883 ± 60[h]	7648 ± 369[h]	1140 ± 375[h]	442 ± 31[h]
	F4 (mg/kg)	1196 ± 140	637 ± 59	3426 ± 100[h]	845 ± 271	177 ± 9
	F3a (% of F3)	5 ± 1%	17 ± 2%	32 ± 2%	17 ± 1%	38 ± 1%
	F3b (% of F3)	95 ± 1%	83 ± 2%	68 ± 2%	83 ± 1%	62 ± 1%
	F2/F3b ratio	0.01 ± 0.1	0.01 ± 0.00	0.43 ± 0.04	0.35 ± 0.12	0.26 ± 0.02
	Calc. PHC F3 (mg/kg)[d]	134 ± 31	232 ± 19	6524 ± 793[h]	442 ± 188[h]	NC
Day 300						
	Total C10–C34 (mg/kg)	2991 ± 592	1534 ± 126	10 161 ± 609	2945 ± 217	324 ± 61
	F2 (mg/kg)	< 10	< 10	1339 ± 130[h]	252 ± 25[h]	29 ± 7
	F3 (mg/kg)	1579 ± 355[h]	832 ± 55[h]	5877 ± 288[h]	1295 ± 109[h]	197 ± 104
	F4 (mg/kg)	1736 ± 461	711 ± 74	2945 ± 217[h]	781 ± 27	98 ± 42
	F3a (% of F3)	5 ± 1%	6 ± 1%	26 ± 1%	12 ± 0%	31 ± 2%
	F3b (% of F3)	95 ± 1%	94 ± 1%	74 ± 1%	88 ± 0%	69 ± 2%
	F2/F3b ratio	0.01 ± 0.00	0.01 ± 0.00	0.31 ± 0.02	0.22 ± 0.01	0.25 ± 0.08
	Calc. PHC F3 (mg/kg)[d,g]	239 ± 101	141 ± 48	4865 ± 231[h]	422 ± 145[h]	NC

[a]Values reported on dry weight basis (mean ± standard deviation; $n = 3$).
[b]Whole crude oil nominal spike concentration: F2–F4 = 19 608 mg/kg; F2 = 6078 mg/kg; F3 = 9216 mg/kg; F4 = 4314 mg/kg.
[c]Whole crude oil nominal spike concentration: F2–F4 = 2942 mg/kg; F2 = 912 mg/kg; F3 = 1383 mg/kg; F4 = 647 mg/kg.
[d]Calculated biogenic organic compound-adjusted petroleum hydrocarbon F3 concentrations (Equation 2).
[e]The 47% F3a:53% F3b ratio in the spiked sand was used as the crude oil source in the day 0 Equation 1 calculations.
[f]The 38% F3a:62% F3b ratio in the spiked sand was used as the crude oil source in the day 150 Equation 1 calculations.
[g]The 31% F3a:69% F3b ratio in the spiked sand was used as the crude oil source in the day 300 Equation 1 calculations.
[h]Value exceeds Canada-wide standard petroleum hydrocarbons coarse soil guideline (Table 1).
F2 = carbon fraction C10–C16; F3 = carbon fraction C16–C34; F3a= carbon subfraction C16–C22; F3b = carbon subfraction C22–C34; F4 = carbon fraction >C34; PHC = petroleum hydrocarbon; NC = PHC F3 not calculated for spiked sand.

chromatograms for fresh Federated crude oil, clean peat (processed and natural), crude oil–spiked peat (processed and natural), and crude oil–spiked sand. The directly injected fresh crude oil chromatogram (Figure 1A) illustrates dominance of the F2 range and relatively equal F3a and F3b subfraction distributions. These same PHC patterns were clearly present in the day 0 spiked sand (Figure 1D), 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 (Figures 1B and 1E), with nondetectable F2 and a strong dominance of the F3b subfraction range. The PHC and BOC patterns were present, to varying degrees, in all of the highly spiked peat (Figure 1C) and moderately spiked peat (Figure 1F) 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.

Changes in F3a and F3b percentages over time

The F3b percentages in the processed peat and natural peat were strongly dominant during the entire study (Table 2). The F3b percentages steadily increased only in the spiked peat and sand treatments. This distribution shift toward the F3b range is attributed to the preferential volatilization of PHC compounds in the F3a range [21,22], combined with photo-oxidation and biodegradation [8,22,23]. The F3b percentages became strongly dominant in the degraded moderately spiked peat, indicating that the day 300 total F3 concentrations were predominantly composed of BOCs.

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 (Equation 2). The following section explains the rationale for using the F3a:F3b percentages in the crude oil–spiked sand as the crude oil source in Equation 2.

$$\begin{aligned}&\text{BOC-adjusted PHC F3 concentration (mg/kg)}\\&\quad = \text{measured F3a (mg/kg)} + \text{calculated F3b (mg/kg)}\\&\quad = a + (b/c \times a)\end{aligned} \quad (2)$$

where a represents the measured F3a concentration in peat sample, b represents the measured percentage of F3b of total F3 in crude oil–spiked sand, and c represents the measured percentage of 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:F3b distributions in the crude oil contamination source

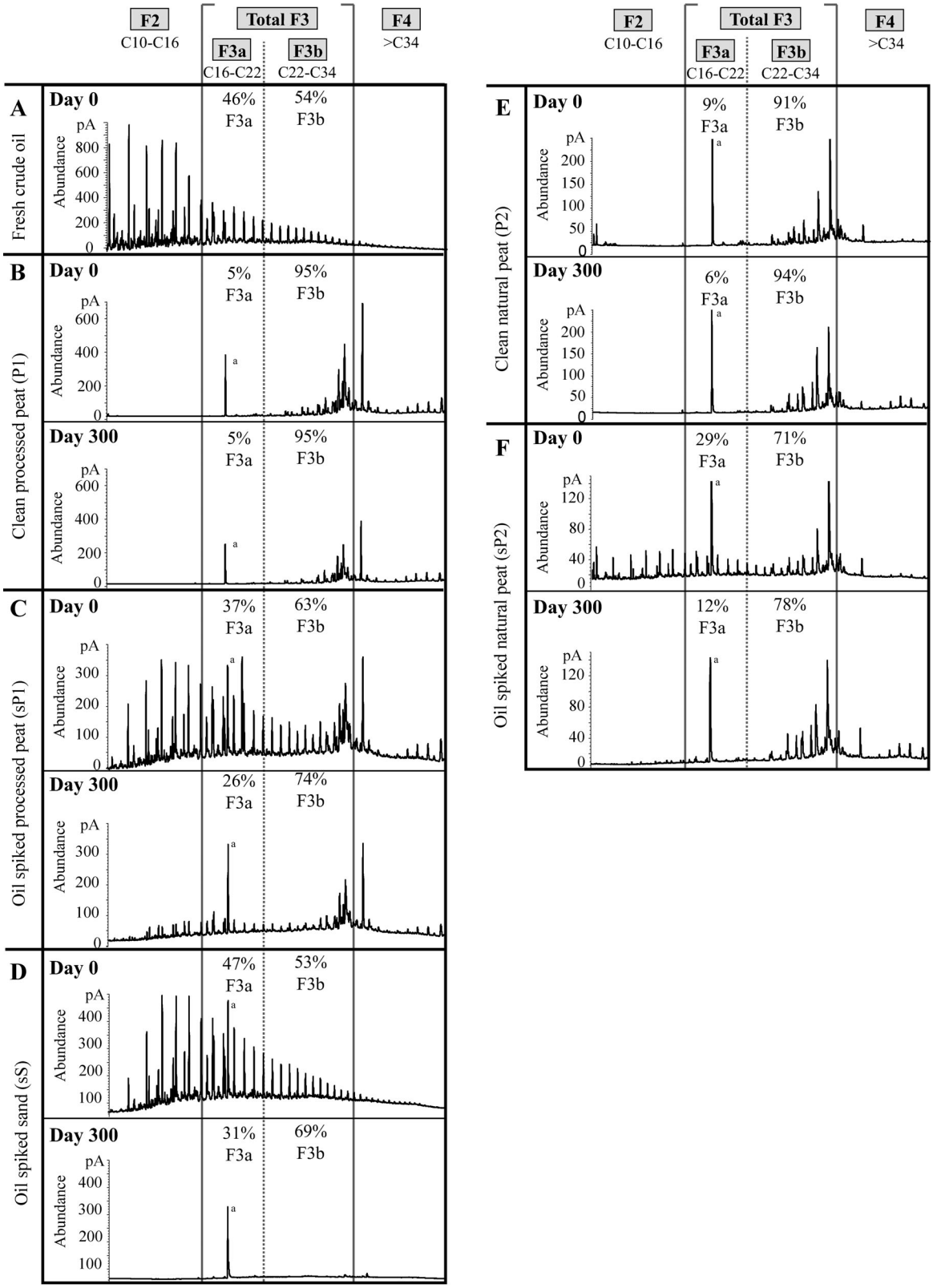

Figure 1. Gas chromatography–flame ionization detector chromatogram examples for day 0 and day 300. (**A**) Fresh whole Federated crude oil; (**B**) clean processed peat (P1); (**C**) processed peat spiked with nominal 19 608 mg/kg F2–F4 crude oil (sP1); (**D**) sand spiked with nominal 2942 mg/kg F2–F4 crude oil (sS); (**E**) clean natural peat (P2); (**F**) natural peat spiked with nominal 2942 mg/kg F2–F4 crude oil (sP2). Panel A chromatogram was produced by direct injection; panels B, C, D, E, and F were produced by the Canada-wide standard for petroleum hydrocarbon (PHC) soil extraction and analysis method [2]. Panels B, C, D, E, and F represent 1 of 3 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). Crude oil spikes were administered on day 0 only. F2 = carbon fraction C10–C16; F3 = carbon fraction C16–C34; F3a= carbon subfraction C16–C22; F3b = carbon subfraction C22–C34; F4 = carbon fraction >C34.
[a]o-terphenyl surrogate.

are known. For example, the chromatogram presented in Figure 2A illustrates that the directly injected fresh crude oil had relatively equal proportions of F3a and F3b (46%:54%). In contrast, however, the clean peat (Figure 2C) was strongly dominated by the F3b range (95%). Peat spiked with Federated crude oil (Figure 2B) had an intermediate F3b percentage of 66%.

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

Comparisons of the GC-FID chromatograms confirmed that there was a loss of the lightest F2 carbon range in the crude oil–spiked sand (Figure 1D), which did not occur in the directly injected fresh crude oil (Figure 1A). The majority of this loss likely occurred by volatilization as the crude oil–spiked sand was vigorously mixed under a fume hood during the spiking procedure. However, the spiked sand and spiked peat treatments were prepared by identical contamination and mixing procedures and 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. For this reason, the F3a and F3b percentages in the day 0, day 150, and day 300 spiked sand were used as the representative crude oil source for calculating the BOC-adjusted PHC F3 concentrations in the clean and spiked peat samples.

BOC-adjusted PHC (petrogenic) F3 concentrations

Formula 1 was used to calculate the day 0, day 150, and day 300 BOC-adjusted PHC F3 concentrations in the clean peat and spiked peat samples (Figure 3; Table 2). All of the BOC-adjusted PHC F3 concentrations in the highly spiked peat (sP1) and moderately spiked peat (sP2) exceeded the CCME PHC F3 300-mg/kg guideline. The total measured F3 concentrations in these spiked samples therefore authentically exceeded the CCME PHC F3 soil guideline. In contrast, the total measured F3 concentrations in the clean processed peat and clean natural peat exceeded the guideline, while the BOC-adjusted PHC F3 concentrations were below the guideline. The total F3 concentrations in the clean peat samples therefore falsely exceeded the CCME PHC F3 soil guideline.

F2:F3b ratio for indicating PHC presence versus absence in soil

In this microcosm experiment, the ratios of measured F2 to measured F3b were used as indicators of PHC absence in clean peat versus PHC presence in crude oil–spiked peat and sand. The F2:F3b ratio is calculated as the measured F2 concentration divided by the measured F3b concentration. All of the clean peat F2:F3a ratios were less than 0.10, while all of the crude oil–spiked peat and sand samples had ratios of greater than 0.10 (Figure 3; Table 2). These data indicate that PHC presence versus absence was identified by an F2:F3b ratio threshold value of 0.10. This mathematical approach identified clean peat soils that had falsely exceeded the F3 toxicity soil guideline and also identified peat and sand soils that were truly contaminated.

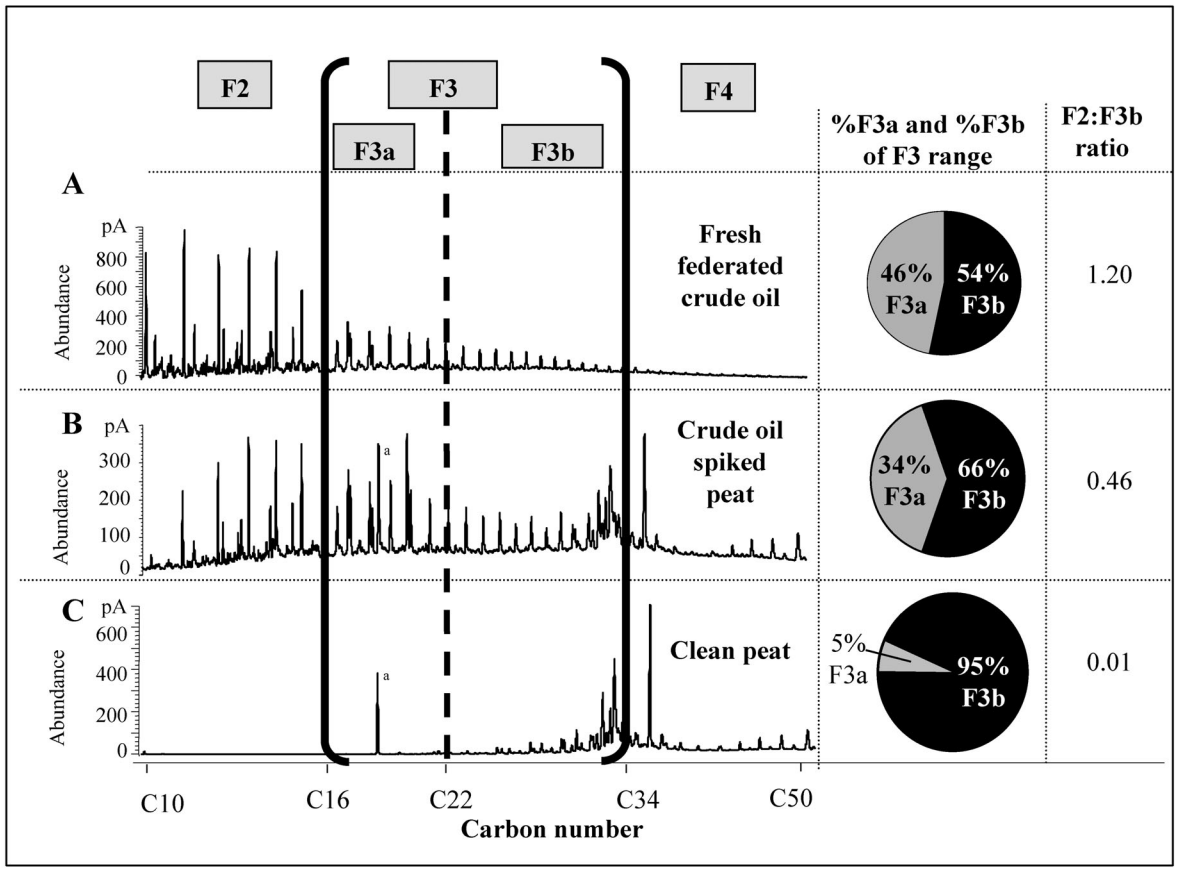

Figure 2. Gas chromatography–flame ionization detector chromatograms of petroleum hydrocarbon (PHC) F2, F3, F4 and subfractions F3a and F3b; pie charts of F3a and F3b percentages; and F2:F3b ratios. (**A**) Fresh crude; (**B**) peat spiked with fresh whole Federated crude oil, nominal F3 = 10 000 mg/kg, measured F3a = 2718 mg/kg, measured F3b = 5276 mg/kg; and (**C**) clean peat, measured F3a = 67 mg/kg, measured F3b = 1264 mg/kg. F2 = carbon fraction C10–C16; F3 = carbon fraction C16–C34; F3a= carbon subfraction C16–C22; F3b = carbon subfraction C22–C34; F4 = carbon fraction >C34.
[a]o-terphenyl surrogate.

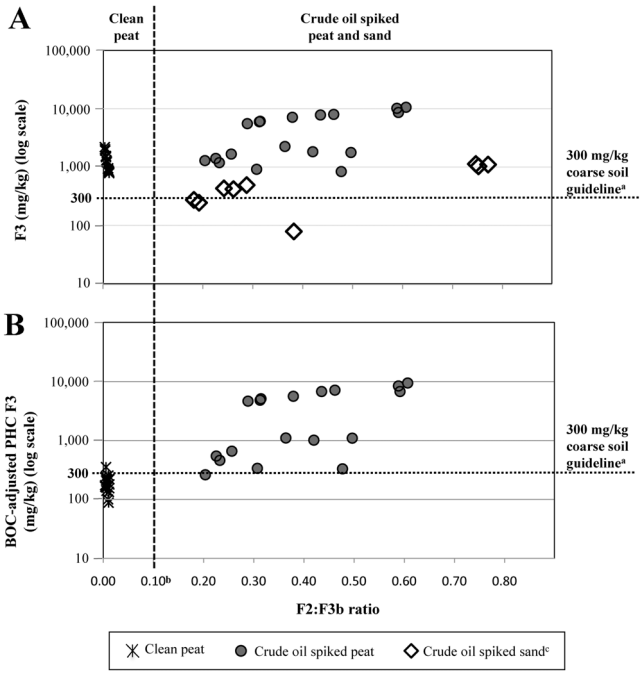

Figure 3. Comparison of day 0, day 150, and day 300 clean peat to crude oil–spiked peat and sand. (**A**) Measured F3 concentrations and measured F2:F3b ratios, and (**B**) calculated biogenic organic compound (BOC)–adjusted petroleum hydrocarbon (PHC) F3 concentrations and measured F2:F3b ratios. F2 = carbon fraction C10–C16; F3 = carbon fraction C16–C34; F3b = carbon subfraction C22–C34;
[a]Tier 1 PHC coarse surface-soil guideline for agricultural/residential/parkland land uses in potable groundwater conditions [3].
[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 calculations

Combination of the F2:F3b ratios with the BOC-adjusted PHC F3 concentrations strengthened this mathematical approach to identifying false PHC F3 exceedences in peat soils. Figure 3A illustrates that the CWS PHC F3 concentrations in all of the clean peat samples exceeded the 300 mg/kg soil toxicity guideline. However, Figure 3B illustrates that BOC-adjusted PHC F3 concentrations for the clean peat samples were below the guideline, with the exception of a slight exceedence by 1 sample. Although this clean sample slightly exceeded the guideline, it was still identified as noncontaminated by the low F2:F3b ratio of less than 0.10. This approach was also useful for evaluating the spiked peat sample with a BOC-adjusted PHC F3 concentration that did not exceed the F3 guideline. Although the low PHC F3 concentrations in this spiked sample did not exceed the guideline, it was still identified as PHC contaminated by the high F2:F3b ratio of greater than 0.10.

Tier 2 decision process for determining if a soil sample location should be excluded or included within a crude oil contaminated peat management zone

The tier 2 decision tree in Figure 4 provides a thought-process framework for determining if a soil sample should be excluded or included within a crude oil contaminated peat management zone. The following example applies specifically to the results of this crude oil spiked peat experiment. This approach required a pre-screening evaluation which confirmed that the GC-FID patterns in each peat sample matched the crude oil contamination source and/or the clean background peat. This approach would have required that any samples with non-matching GC-FID patterns

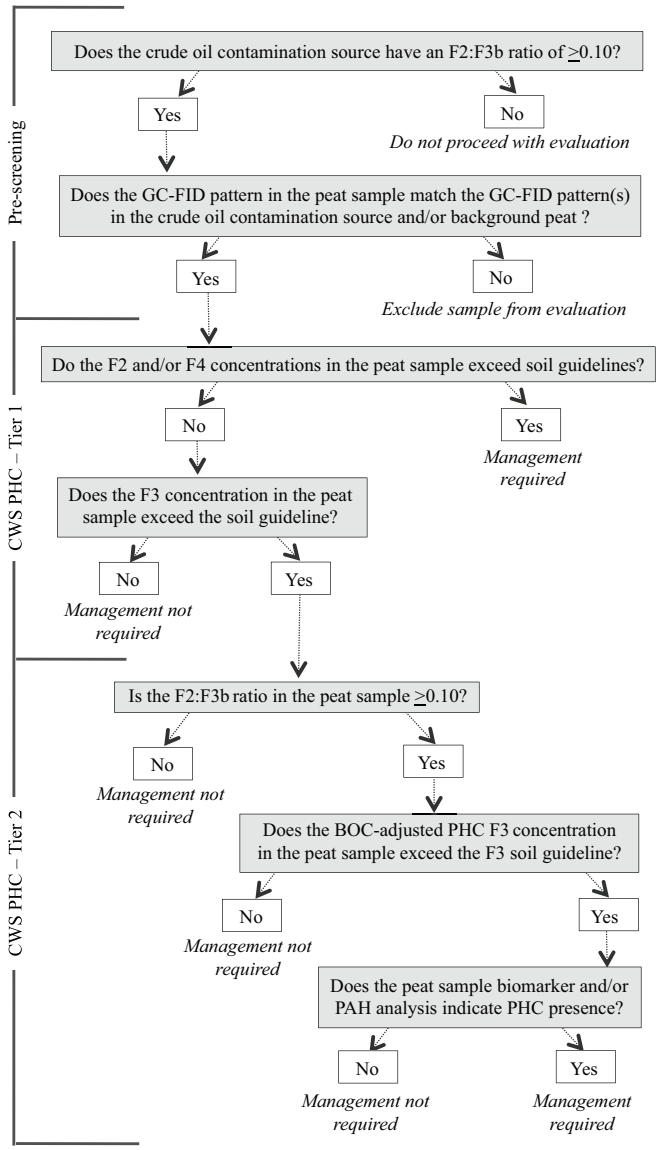

Figure 4. Tier 2 decision process for determining if a peat sample location should be included within a crude oil petroleum hydrocarbon contaminated site-management zone. F2 = carbon fraction C10–C16; F3 = carbon fraction C16–C34; F3b = carbon subfraction C22–C34; F4 = carbon fraction >C34.

be excluded from the tier 2 decision tree process. The tier 2 decision tree concept is described as follows. The first step would be to verify that the F2:F3b ratio in the crude oil contamination source is equal to or greater than the 0.10 PHC presence value. The next step would be to verify that the GC-FID patterns in all of the peat samples match the crude oil and/or the background peat patterns. Samples with non-matching patterns would be excluded from the decision tree process. The next step would be to measure the CWS PHC tier 1 F2, F3 and F4 concentrations in every peat sample. Samples with F2 and/or F4 concentrations that exceed the CWS PHC soil guidelines would be identified as authentically contaminated and would not be further evaluated. Samples that do not exceed the F2 and/or F4 soil guidelines but do exceed the F3 guideline would indicate possible false exceedences due to background BOCs. The next step would be to proceed to the tier 2 F2:F3b ratio PHC presence versus absence evaluation. Samples with less than 0.10 F2:F3b ratios would be identified as clean and would not require further

Table 3. Analysis of variance between day 0 and day 300 carbon range concentrations, *F*-test *p* values, 95% confidence interval, triplicate data, log-centered transform compositional analysis

Analyte	Clean processed peat	Clean natural peat	Highly spiked processed peat[a]	Moderately spiked natural peat[b]	Moderately spiked sand plus bacteria and nutrients[b]
F2	–	–	0.0003	0.0087	0.0098
F3a	0.5481	0.0184	0.0032	0.0004	0.0687
F3b	0.0772	0.2916	0.0001	0.0012	0.0061
F4	0.6333	0.1529	0.0007	0.0027	0.0000

[a]Whole crude oil nominal spike concentration: F2–F4 = 19 608 mg/kg; F2 = 6078 mg/kg; F3 = 9216 mg/kg; F4 = 4314 mg/kg.
[b]Whole crude oil nominal spike concentration: F2–F4 = 2942 mg/kg; F2 = 912 mg/kg; F3 = 1383 mg/kg; F4 = 647 mg/kg.
F2 = carbon fraction C10–C16; F3a= carbon subfraction C16–C22; F3b = carbon subfraction C22–C34; F4 = carbon fraction >C34;

evaluation. Samples with greater than or equal to 0.10 F2:F3b ratios would indicate possible PHC contamination. However, it would be possible for F2:F3b ratios in clean soils to be falsely elevated by unusual BOCs in the F2 carbon range. Evaluation of samples with high F2:F3b ratios would proceed to the tier 2 BOC-adjusted PHC F3 evaluation. BOC-adjusted PHC F3 concentrations that exceed the tier 1 CWS PHC F3 soil guideline may be caused by PHC contamination. However, it would also be possible for BOC-adjusted PHC F3 concentrations in clean peat samples to be falsely elevated by unusual BOCs occurring in the F3a carbon range. Evaluation of samples with high BOC-adjusted PHC F3 concentrations would proceed to the final tier 2 forensics biomarker and/or PAH analysis for verification of PHC presence versus absence.

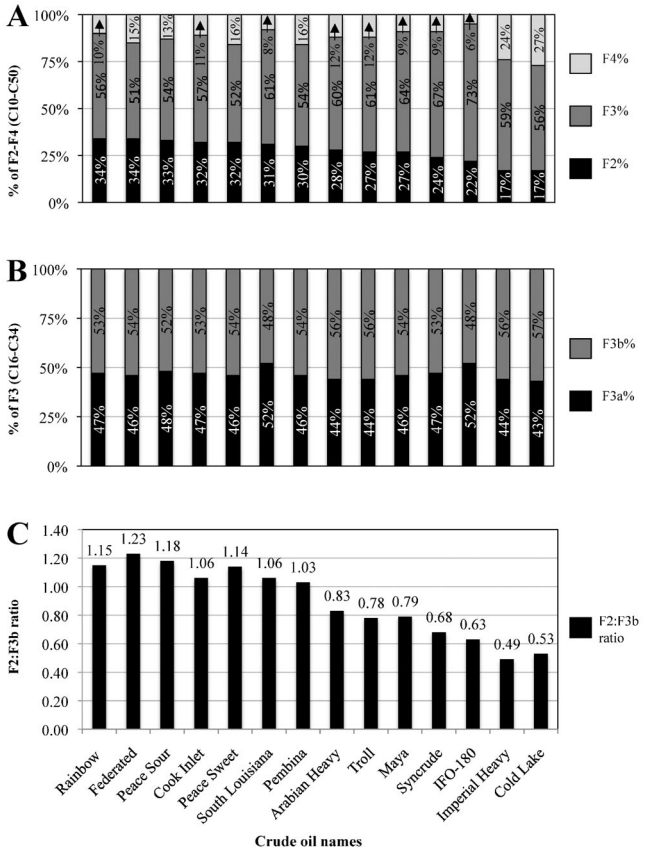

Figure 5. Petroleum hydrocarbon carbon range distributions in light (Rainbow) to heavy (Cold Lake) fresh crude oils. (**A**) Percentages of F2, F3, and F4 within the F2–F4 range (C10–C50); (**B**) percentages of F3a and F3b subfractions within the F3 range; and (**C**) F2:F3b ratios. F2 = carbon fraction C10–C16; F3 = carbon fraction C16–C34; F3a= carbon subfraction C16–C22; F3b = carbon subfraction C22–C34; F4 = carbon fraction >C34.

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

The survey of 14 fresh crude oils included the following analyses: 1) F2, F3, and F4 percentages of the total F2 to F4 carbon range (Figure 5A); 2) F3a and F3b percentages of the total F3 carbon range (Figure 5B); and 3) F2:F3b ratios (Figure 5C). The quantile-quantile and Shapiro-Wilk analysis determined that the F3a: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 among all of the crude oils indicated 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 1 to 2 orders of magnitude higher than the ratios in the clean peat. The high F2:F3b ratios of greater than 0.10 in all of the surveyed crude oils could be used to indicate PHC absence versus presence in the clean and crude oil contaminated microcosm peat soils.

Statistical analysis results

The microcosm day 0 and day 300 ANOVA *F*-test *p* values were calculated as F2, F3a, F3b, and F4 compositional data (Table 3). The compositional-data-analysis approach measured each of the F2, F3a, F3b, and F4 concentrations as part of a whole group, meaning that *p* values for each fraction were relative to the other 3 fractions as well. All of the F2, F3a, F3b and F4 *p* values were strongly significant in the highly spiked peat and moderately spiked peat samples. The spiked sand had strongly significant F2, F3b and F4 *p* values and a slightly non-significant F3a p value. The clean processed peat had strongly non-significant F3a and F4 p values and a slightly significant F3b *p* value. The clean natural peat had strongly significant F3b and F4 *p* values and a slightly non-significant F3a *p* value. These results demonstrate that significant differences primarily occurred in the spiked treatments, while non-significant differences primarily occurred in the non-spiked treatments. These trends are indicative of PHC degradation processes that would have been present in the crude oil spiked peat and sand treatments but would have been absent in the clean peat treatments.

CONCLUSIONS

The new tier 2 approach in the present study is time efficient and cost efficient because it relies on existing CWS PHC soil chemistry analysis methods that would be routinely conducted for typical PHC contaminated soil evaluations. The present study demonstrated that the BOC-adjusted PHC F3 concentrations and the F2:F3b ratios resolved false CWS PHC F3

detections and soil toxicity guideline exceedences in the clean peat. Approximately equal F3a and F3b percentages and high F2:F3b ratios in the 14 surveyed fresh crude oils indicate that this approach could be applied to a range of light to heavy crude oils as well.

Further validation studies are required to determine if this new tier 2 approach could be applied to actual sites located in a wide range of soil types and spill scenarios. The present study demonstrated that this tier 2 approach could potentially resolve false PHC F3 detections at crude oil and diesel contaminated sites. Ongoing field studies are being conducted to evaluate the applicability of this approach to a wider range of PHC spill scenarios.

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