Quantification of Soil Organic Matter as Total Petroleum Hydrocarbons by GC-FID in Non-Contaminated Soils

by

Skanda Vishnu Sundar

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Graduate Supervisory Committee:

Anca G. Delgado, Chair
Paul Dahlen
Natasha Sihota

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ABSTRACT

Soil impacts from crude oil spills in the United States are regulated at the state level using the analytical group *total petroleum hydrocarbons* (TPH) as the primary regulatory metric. TPH concentration in soil is used to enforce and verify compliance with cleanup levels (CULs). While there are significant differences between states concerning TPH CULs based on land use, most states enforce an action level of 100 mg TPH kg^{-1}. The most common standard method for quantification of TPH in soils is EPA Method 8015, which entails extraction of petroleum hydrocarbons by dichloromethane and analysis by gas chromatography with flame ionization detection (GC-FID). Using Method 8015 or similar methods, TPH is defined as the cumulative area of all peaks within a defined analytical range (typically C6-C36). A limitation of TPH standard methods is their lack of specificity for petroleum hydrocarbons (i.e., these methods can also detect and quantify compounds that are an inherent part of natural soil organic matter (SOM)). While the interference of SOM compounds with TPH quantification is known, documentation regarding the extent of this interference is almost absent in the peer-reviewed literature. In this thesis, 15 biogeochemically-diverse soils, uncontaminated by crude oil hydrocarbons, were sampled from geographically diverse locations and investigated in an effort to determine the concentration of SOM that registers as TPH. Solvent extractions using dichloromethane or *n*-pentane in conjunction with GC-FID analysis showed that all soils had detectable concentrations of TPH ranging from 160 to 2700 mg TPH kg^{-1}. Based on the results from this study, it can be concluded that many soils have a higher apparent TPH concentration than most US state-level CULs. In

addition, the data from this study show that soils with a lower pH and/or a higher organic carbon content also have higher concentrations of apparent TPH. Findings from this thesis show that uncontaminated soils have a significant apparent TPH concentration that would be considered part of the TPH originating from contamination and should be accounted for in the regulatory landscape.

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INTRODUCTION

Petroleum exploration, processing, and the management and use of refined petroleum hydrocarbon products can lead to environmental releases with potential impacts to surface water, soil, and groundwater. In the United States, contamination by petroleum hydrocarbons in soil is regulated at the state and/or federal level (Michelsena and Petito Boyce, 1993, Tomlinson and Ruby, 2016). The most common metric for regulation is the analytical group *total petroleum hydrocarbons* (TPH) (United States Environmental Protection Agency, 2020), which defines the action or clean-up levels (CULs) in soil. Action or CULs further involve various analytical expressions of TPH, including total TPH, TPH distinguished as gasoline-range organics (GRO; C_6-C_{10}), diesel-range organics (DRO; C_{10}-C_{28}), and oil-range organics (ORO; C_{28}-C_{36}), and TPH as C_9-C_{36} aliphatic and C_{11}-C_{22} aromatic petroleum fractions (Herzfelder and Golledge, 2004, Tomlinson and Ruby, 2016).

While concentration of TPH for action and CULs can vary significantly between states, sometimes by orders of magnitude(Tomlinson and Ruby, 2016), many states use action and CULs in the order of 100 mg TPH kg^{-1} (Oliver, et al., 1993) (APPENDIX A). The discrepancy in state action and CULs in in part due to various nuances of the regulation. Specifically, eleven (11) states account for the route of exposure when setting action and CULs. Additionally, 21 states consider the end use of the contaminated site when determining allowable TPH concentrations (Tomlinson and Ruby, 2016). As an example, Oklahoma CUL for GRO TPH in a residential-use scenario is 500 mg kg^{-1},

whereas the CUL for GRO TPH in an industrial scenario is 2500 mg kg^{-1} (Oklahoma Department of Environmental Quality, 2020).

Compliance with action and CULs in soil requires quantifying the TPH concentration. The most utilized method for TPH quantification for regulatory purposes is EPA Method 8015 (American Petroleum Institue (API), 2001, Interstate Technology Regulatory Council, 2017), which entails solvent extraction of petroleum hydrocarbons from the soil matrix and analysis of hydrocarbons using gas chromatography (GC) with flame ionization detection (FID) (Becker, et al., 2002). At least 24 US states are currently using Method 8015, 8015B, or 8015D (United States Environmental Protection Agency, 1996, US Environmental Protection Agency, 1996) (APPENDIX B). The recommended solvent in Method 8015 or 8015-based methods (e.g., the Massachusetts Department of Environmental Protection's method for the determination of Extractable Petroleum Hydrocarbons - MADEP-EPH-04) is dichloromethane. Dichloromethane is a slightly polar solvent for extraction of GRO, DRO, and ORO TPH and other semi-volatile or non-volatile analytes (US Environmental Protection Agency, 1996). GC-FID standard methods like Texas Natural Resource Conservation Commission Total Petroleum Hydrocarbons Method 1005 (TNRCC Method 1005) and the Total Petroleum Hydrocarbon Criteria Working Group (TPHCWG) Method (Weisman, 1998) used by 5 states employ *n*-pentane, a non-polar solvent, in the extraction process (Saitas, 2001) (APPENDIX B). Regardless of the extraction solvent, in the GC-FID method, TPH is defined as the cumulative area of all chromatographic peaks eluting within a pre-determined analytical range, typically demarked by the C_6 to C_{36} *n*-alkanes standards

(Tomlinson and Ruby, 2016). For most soils containing petroleum hydrocarbons from crude oil or refined products (e.g., gasoline, diesel, waste oil), the compound peaks are not discrete, they cannot be chromatographically resolved, and are therefore lumped together in an unresolved complex mixture (or hump) (Gough and Rowland, 1990).

While commonly used as a tool in contaminant regulations, an important limitation of the TPH GC-FID analytical method is its lack of specificity for petroleum hydrocarbons (Interstate Technology Regulatory Council, US Environmental Protection Agency, 1996, Schwartz, et al., 2012). The GC-FID method can detect soil compounds that are neither of petroleum origin nor composed of only hydrogen and carbon (US Environmental Protection Agency, 1996, Muijs and Jonker, 2009). As such, various components of soil organic matter (SOM) which are extractable by dichloromethane or *n*-pentane and elute within the C_6-C_{36} unresolved complex mixture are conceivably quantified as TPH (Interstate Technology Regulatory Council). SOM is a complex mixture of compounds from the decomposition and transformation of materials of plant, animal, and microbial origin at various stages and with various degrees of biodegradability (Bot and Benites, 2005, National Resources Conservation Service, 2014). Most agriculturally productive soils contain 30000-60000 mg SOM kg^{-1} (Troeh and Thompson, 2005, Cornell University Cooperative Extension, 2008). Drier, desert soils have around 10000 mg SOM kg^{-1}, whereas soils from wet areas could have up to 90000 mg SOM kg^{-1} (Troeh and Thompson, 2005, Cornell University Cooperative Extension, 2008). Organic compounds within SOM include lipids, proteins, lignin, and unsaturated and condensed hydrocarbons (Tfaily, et al., 2015). An investigation on

extraction of SOM components by solvents with different polarities – water, methanol, *n*-hexane, and acetonitrile – showed that *n*-hexane (a non-polar solvent with similar properties to *n*-pentane (Tables, Murov, 2010, Reichardt and Welton, 2011)) was highly selective towards hydrophobic compounds such as lipids (Tfaily, et al., 2015). Similarly, dichloromethane, the most common solvent in the TPH by GC-FID analytical method, has also been shown to extract lipids and/or other compounds with hydrocarbon-like characteristics (Cequier-Sánchez, et al., 2008). However, the nature of compounds or the magnitude of contribution of SOM to a TPH concentration in soils is neither documented in the peer-reviewed literature, nor is it typically considered in the contamination regulatory landscape.

Commercial laboratories that perform TPH analyses for regulatory compliance acknowledge the issue of non-petroleum organic compounds and their interference with petroleum hydrocarbon quantification in their standard operating procedures for Method 8015B (Eurofins Lancaster Laboratories, 2013). When a petroleum hydrocarbon-contaminate d soil sample is suspected to contain a high concentration of SOM, a recommendation is to use a silica gel column (EPA Method 3630C) on the TPH extract(Eurofins Lancaster Laboratories, 2013). Removal of non-hydrocarbons and other SOM compounds is achieved by adsorbing the relatively polar compounds in the TPH extract on the silica gel (polar substance) and eluting those compounds using a more polar solvent (Council, United States Environmental Protection Agency, 1996). Silica gel columns and EPA Method 3630C are also used to separate TPH from contaminated soils into aliphatic (using *n*-hexane or *n*-pentane) and aromatic (using dichloromethane)

fractions (United States Environmental Protection Agency, 1996, American Petroleum Institue (API), 2001, Herzfelder and Golledge, 2004). However, the use of silica gel columns for removal of SOM compounds in petroleum hydrocarbon-contaminated soil samples presents some challenges. First, for TPH quantification for regulatory purposes, silica gel columns are used only by seven US states and the USEPA (Tomlinson and Ruby, 2016, US EPA, 2017). In all other states, the use of cleanup agents such as silica gel is arbitrary based on the recommendation of the analyst and may not be accepted by the regulatory agency. Second, petroleum hydrocarbons are also removed by the silica gel cleanup process (Zemo and Foote, 2003). Third, according to one investigation, chromatography clean-up agents such as silica gel, aluminum oxide, and Florisil fail to completely remove SOM and other organic material while also obtaining a high recovery of TPH (Muijs and Jonker, 2009).

In this study, we assembled a library of 15 soils without a known history of petroleum hydrocarbon contamination from geographically-diverse locations and with varying biogeochemical properties and subjected them to solvent extraction and analysis by GC-FID according to standard TPH methods (US Environmental Protection Agency, 1996, Herzfelder and Golledge, 2004). Results showed that all soils had detectable concentrations of SOM compounds eluting within the TPH analytical range. The concentrations of "apparent TPH" in soils ranged from 160 to 2700 of mg kg^{-1}. Analysis by GC with mass spectrometry (MS) revealed dichloromethane and *n*-pentane soil extracts used in the GC-FID analysis had only a small fraction of hydrocarbons and a larger fraction of compounds composed carbon, hydrogen, and other elements (non-

hydrocarbons), including lipids, and unidentified compounds, likely of plant and microbial origin.

MATERIALS AND METHODS

Soil library

Topsoil was collected for this study from twelve locations within the US, from one location in Germany, and from one location in Scotland (Table 1). The soils were sampled from areas without a history of petroleum hydrocarbon or other type of contamination. The soils were sampled either manually using a hand trowel or with excavation equipment. Sample depths ranged from 0.2 to 1.5 m. At least one kilogram of soil was collected from each location and was shipped to Arizona State University at ambient temperature. Once received, the soils were stored at 4 °C if they were to be immediately analyzed or at −20 °C if they were to be analyzed at a later time.

Table 1. Description and characteristics of topsoils used in this study. The data are averages with standard deviation of triplicates.

Soil ID	Description	Location	Moisture content (%)	pH	Conductivity (μS cm^{-1})	Organic carbon (mg kg^{-1})
A	Field soil	Gerlach, Nevada, USA	16 ± 0.2	10.1 ± 0.0	37000 ± 50	7200 ± 100
B	Field soil	Michigan, USA	12 ± 0.3	4.9 ± 0.0	200 ± 2	7100 ± 1200
C	Landscaping soil	Tempe, Arizona, USA	2 ± 0.3	8.4 ± 0.0	1400 ± 20	1200 ± 60
D	Field soil in proximity to a crude oil-contaminated site	Undisclosed	9 ± 1.0	8.1 ± 0.1	2400 ± 200	850 ± 60
E	Field soil	Rangeley, Colorado, USA	4.4 ± 0.1	7.7 ± 0.0	3800 ± 60	2900 ± 440
F	Unsaturated soil	Bakersfield, California, USA	7.3 ± 1.6	8.2 ± 0.0	860 ± 10	6800 ± 1400
G	Forest soil	Decatur, Georgia, USA	30 ± 0.2	6.1 ± 0.0	110 ± 10	40000 ± 1300
H	Pasture soil	Oak Harbor, Washington, USA	33 ± 1.5	5.8 ± 0.0	40 ± 3	46000 ± 14000
I	Beach soil	Whidbey Island, Washington, USA	3 ± 0	8.5 ± 0.0	10 ± 1	< 200
J	Mangrove soil	Carolina, Puerto Rico, USA	64 ± 1.0	7.2 ± 0.0	8200 ± 100	65000 ± 6200
K	Field soil in proximity to a river	Andover, New Hampshire, USA	19 ± 2.8	3.8 ± 0.2	90 ± 20	18000 ± 3200
L	Lake soil	Meppen, Germany	3 ± 0.6	4.4 ± 0.0	820 ± 80	13000 ± 4600
M	Field soil in proximity to a lake	Meppen, Germany	7 ± 0.8	3.7 ± 0.0	130 ± 20	61000 ± 5600
N	Forest soil	Salisbury, New Hampshire, USA	59 ± 2.8	4.0 ± 0.1	90 ± 10	97000 ± 23000
O	Field peat soil	Aberdeen, Scotland, UK	86 ± 1.5	5.4 ± 0.1	110 ± 10	80000 ± 1300

Soil chemical characteristics

The pH and conductivity for each soil were measured in slurries of soil and deionized water using an Oakton Multi-Parameter PCSTestr^{TM} 35 probe (Vernon Hills, IL, USA). The ratio of soil to water was 1:1 (w w^{-1}), unless the measured parameter was out of instrument range, in which case a 1:5 soil:deionized water solution was used. The probe was calibrated according to the manufacturer's instructions using Oakton calibration standards.

The concentration of organic carbon in soils was measured using a Shimadzu TOC-V SPH Total Carbon Analyzer with a solid state module (SSM-5000A) as described by Chen et al (Chen, et al., 2016, Chen, et al., 2017). The organic carbon calibration was performed using glucose (Acros organics, NJ, USA) in a range of 0.5-25 mg C.

Solvent extractions from soils and TPH GC-FID analysis

Solvent extracts for TPH analysis by GC-FID were obtained from 5 g of soil air-dried at room temperature for 24 h, and thoroughly mixed with sodium sulfate (Chem Impex Int'l Inc, Wood Dale, IL, USA). Dichloromethane (Fisher Scientific, NJ, USA) was then used for extraction on all soils. A subset of six soils that exhibited the highest apparent TPH concentrations was also extracted with *n*-pentane (Reagents, Charlotte NC, USA). Extraction was completed using EPA Method 3541 (United States Environmental Protection Agency, 1994) (automated Soxhlet with heat) using a Gerhardt Soxtherm instrument (Königswinter, Germany) (Apul, et al., 2016, Apul, et al., 2016). The sulfate-dried soil was placed in a cellulose thimble (Advantec, Dublin, CA, USA) and was secured with a metal frame inside a glass Gerhardt extraction beaker. PTFE boiling chips (2-3 g, Saint-Gobain, Poestenkill, NY, USA) were added to each beaker. Soil was spiked

with 200 µL of a 40 µg mL^{-1} solution of 1-chlorooctadecane and *o*-terphynyl (AccuStandard, New Haven, CT, USA) to monitor the recovery during the extraction process. Then, 125 mL of solvent was carefully poured into the filter inside the extraction beakers and the beakers were placed in the extractor. The Soxtherm extraction parameters were as follows: extraction temperature, 140 °C reduction interval, 4.5 min; and reduction pulse, 3 s. The time for Extraction A (soil sample is completely submerged in solvent) was 30 min while Extraction B (solvent level is below the extraction thimble) was 20 min. The total method time was 1 hr and 14 min. The final extract volume was 10-15 mL. The extracts were then filtered through a 0.2 µm PTFE membrane filter (VWR, Radnor, PA, USA), further concentrated to 1 mL using a gentle stream of N_2 gas, and then transferred into a glass GC vial.

Dichloromethane and *n*-pentane extracts were analyzed using a GC-FID (Shimadzu GC-2010, Columbia, MD, USA) fitted with a Restek Rxi-1HT capillary column (29 m × 0.25 mm × 0.25 µm, Bellefonte, PA, USA). The GC-FID instrument settings were configured as per Method 8015D(US Environmental Protection Agency, 1996) and MADEP EPH-04 (Herzfelder and Golledge, 2004) as follows: injection port temperature, 330 °C; FID temperature, 350 °C; H_2 as carrier gas at 3 mL min^{-1}; air at 400 mL min^{-1}; H_2 as FID feed at 32 mL min^{-1}; and oven temperature programming of 60 °C with a hold time of 1 min, a ramp of 8 °C min^{-1} to 305 °C, and a final hold time of 30 min (total run time of 61.63 min). The extracts were analyzed using collective area integration of peaks and range calibration factors as described in standard methods (Herzfelder and Golledge, 2004) (Saitas, 2001).

A C9-C36 *n*-alkane standard mixture in dichloromethane (AccuStandard, New Haven, CT, USA) was used to create a seven-point calibration curve from 1-200 µg L^{-1}. The calibration for the 1-chlorooctadecane and *o*-terphenyl in the same range was performed using a 1000 µg L^{-1} stock solution in dichloromethane prepared from neat compounds (Sigma-Aldrich, St. Louis, MO, USA). Calibration factors were calculated using Equations 1 and 3, respectively, from Section 9 in the MADEP EPH-04 method (Herzfelder and Golledge, 2004). The average recovery of 1-chlorooctadecane and *o*-terphenyl across all soil extractions (with dichloromethane and *n*-pentane) was 93% ± 13%.

Apparent TPH was defined as the area of all peaks eluting from 0.1 min before *n*-nonane (C9) to 0.1 min after *n*-hexatriacontane (C36), as described in Sections 3 and 9 in the MADEP EPH-04 method (Herzfelder and Golledge, 2004). The distribution of TPH concentration as a function of C range (i.e., GRO, DRO, and ORO) was determined by summing the areas of all peaks within the specific range using the retention times of C_9 to C_{40} *n*-alkanes (Sigma-Aldrich, St. Louis, MO, USA). Triplicate samples were extracted for each soil. The moisture content of the soil was determined, and the apparent TPH concentration was reported as mg TPH per kg of dry soil. The statistical significance of the differences in TPH concentrations extracted using dichloromethane and *n*-pentane was evaluated using a Student's *t* test. A *p* value of 0.05 or less was considered statistically significant.

Solvent extractions from leaves and microbial biomass

Solvent extractions were performed on Arizona ash tree (*Fraxinus velutina*) leaves and microbial biomass. Fresh green leaves were picked from a tree near Arizona State University campus, Tempe, Arizona, USA. The leaves were dried for 6 hours at 105 °C and then were ground with a mortar and pestle. For microbial biomass, a pure culture of *E. coli* DH5α and a mixed culture enriched from soil G (Table 1) were used. The cultures were grown in 2 L flasks containing 1 L of LB broth (Sigma-Aldrich, St. Louis, MO, USA). The *E. coli* culture was inoculated with 0.5 mL of an overnight culture while the mixed microbial culture was inoculated with 0.5 g of soil G (Table 1). The culture flasks were incubated at 30 °C on a platform shaker set at 150 RPM for 48 hr. After 48 hr, 200 mL from each culture were pelleted using 4000 RPM centrifugation for 10 min in an Eppendorf 5810 R centrifuge (Hamburg, Germany). Microbial biomass was then dried overnight at 105 °C. Approximately 0.5 g of leaf material and 0.1 g of microbial biomass were used per extraction. The leaves and microbial biomass were subjected to the same extraction procedure and analysis as the soils including mixing with sodium sulfate, spiking with 1-chlorooctadecane and *o*-terphenyl solution, Soxtherm extraction, and GC-FID analysis as described. The average recovery of 1-chlorooctadecane and *o*-terphenyl for the leaf and microbial biomass extractions was 102% ± 18%. Duplicate leaf and microbial biomass samples were extracted. The moisture content was determined, and apparent TPH concentrations were reported as mg per kg of dry material.

GC-MS compound identification and quantification in soil extracts analyzed by GC-FID

Six dichloromethane and six *n*-pentane soil extracts used in the GC-FID analysis for TPH were subjected to GC-MS analysis for compound identification and quantification. The soil extracts were shipped overnight on dry ice to Eurofins Lancaster Laboratories Environmental, Lancaster, Pennsylvania. The extracts were analyzed using EPA Method 8270D (United States Environmental Protection Agency, 2014) according to the laboratory's standard operating procedure. Soil compounds in either dichloromethane or *n*-pentane extracts were identified using the EPA and/or National Institute of Standards and Technology (NIST) libraries. Total ion chromatograms (TICs) were integrated to produce a listing of all peaks present. The internal standards and all peaks less than 20% of the nearest internal standard peak area were removed from the integration table. The remaining peaks were then searched against the NIST Library for identification. Compounds labelled as "unknown" in Figure 3 did not produce a match within the libraries.

RESULTS

The presence of SOM compounds registering as TPH in a library of 15 biogeochemically-diverse soil (Table 1) from areas without a history of contamination was investigated. The concentrations of apparent TPH are shown in Figures 1 and 2 and the GC-FID chromatograms are included in APPENDIX C. All soils showed detectable concentrations of apparent TPH (Figure 1). The C9-C36-defined concentrations ranged from 160 mg TPH kg^{-1} to 2700 mg TPH kg^{-1} (APPENDIX D). The concentrations of GRO + DRO TPH extracted by dichloromethane were between 60 to 1000 mg kg^{-1} while the ORO concentrations ranged from 90 to 1700 mg TPH kg^{-1} (Figure 1, APPENDIX D). Across soils from this study, more than 91% of the apparent TPH concentration in the dichloromethane extracts and more than 63% in *n*-pentane was detected in the ORO C_{22}-C_{36} range. In five soils, apparent TPH concentrations in the dichloromethane extracts were 13%-54% higher than those in the *n*-pentane extracts (Figure 1). However, the *n*-pentane extracts showed a consistent and significantly higher (Student's paired *t* test, one-tailed distribution, $p < 0.05$) concentration of C_9-C_{12} apparent TPH (Figure 2). Note that all soils had concentrations of TPH in a range C_{36} to C_{40} and > C40 (Figure 2). However, TPH concentrations > C_{40} are not usually accounted for in regulations (US

Environmental Protection Agency, 1996, American Petroleum Institue (API), 2001, Herzfelder and Golledge, 2004, Nelson, et al., 2015).

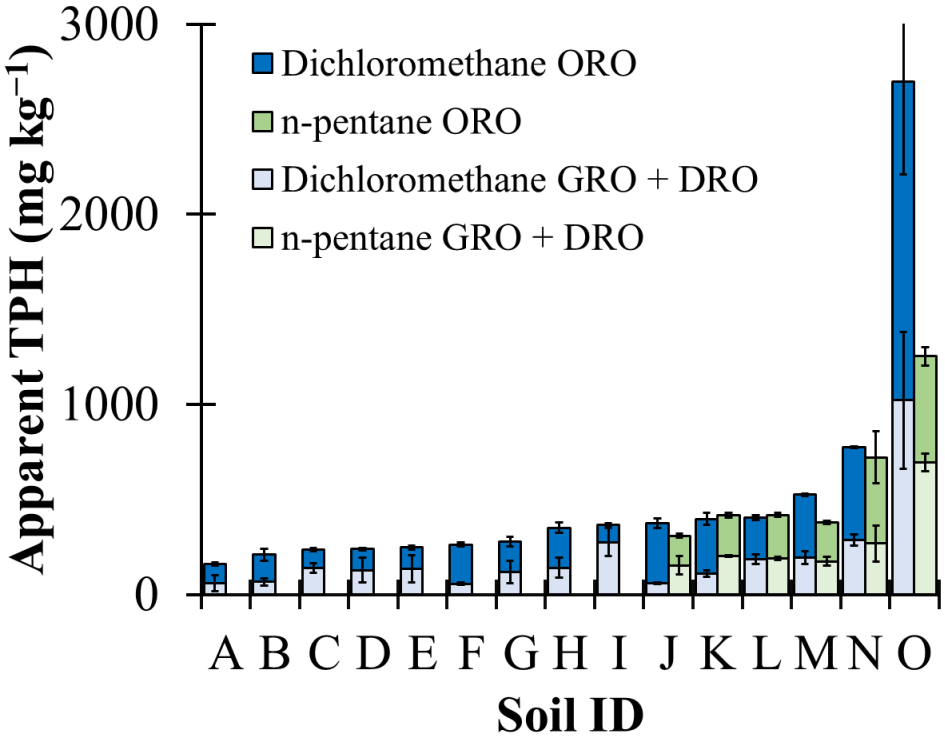

Figure 1. Concentrations of apparent TPH in extracts from uncontaminated topsoils using dichloromethane (15 soils) and *n*-pentane (6 soils). GRO + DRO = C_9 – C_{28} TPH; ORO = C_{28} – C_{36} TPH. The data are averages with standard deviation of triplicate soil extractions.

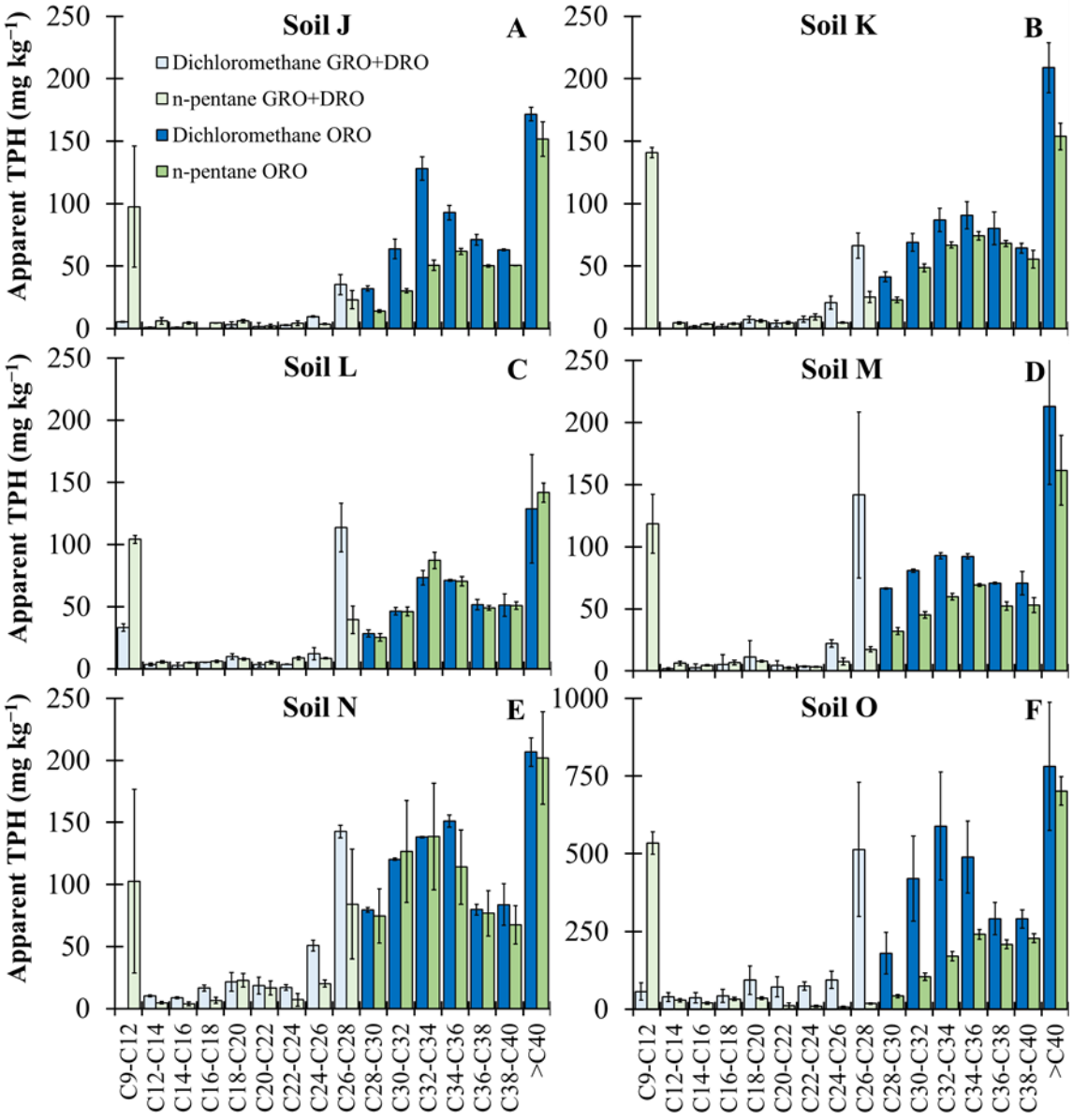

Figure 2. Distribution of apparent TPH as a function of carbon range for soils extracted by dichloromethane and *n*-pentane. Note that soil O has a different y axis scale. The data are averages with standard deviation of triplicate soil extractions.

Given the FID is non-specific and most peaks within a TPH chromatogram are not chromatographically resolved, the standard TPH method cannot give information on compound identity (e.g., by using retention times). We therefore used GC-MS interpretive TICs for the six soils shown in Figure 2 to determine the nature of some of the compounds identified by GC-FID as petroleum hydrocarbons. The identified compounds obtained from the GC-MS analysis in the dichloromethane and *n*-pentane soil extracts are shown in APPENDIX E. Between 11 to 19 compounds were identified in each soil. Some compounds did not produce a known TIC spectrum based on the EPA and NIST libraries and thus were classified as unknown (Figure 3, APPENDIX E).

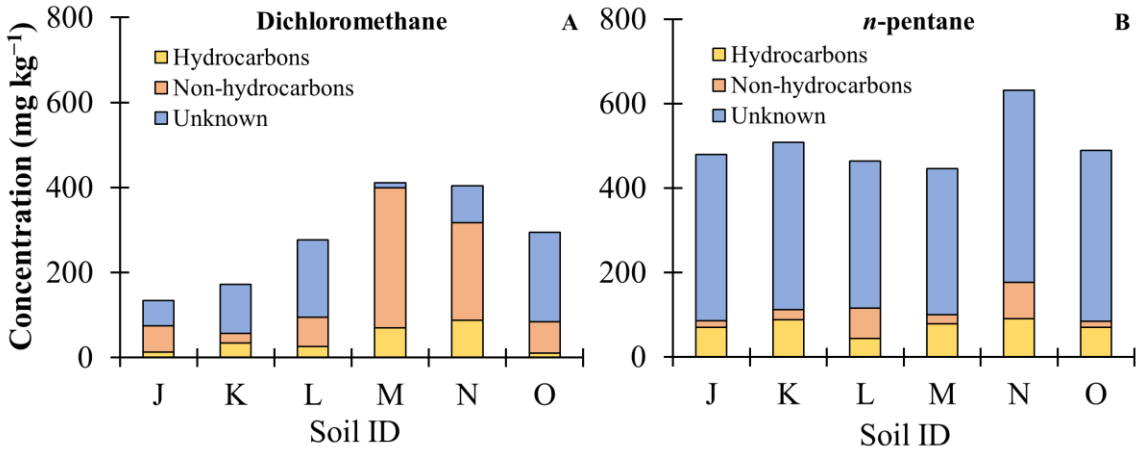

Figure 3. Concentrations of hydrocarbons, non-hydrocarbons, and unknown compounds determined by GC-MS in soils extracted by dichloromethane (A) and *n*-pentane (B) and analyzed by GC-FID in Figure 2.

Based on the outcomes of GC-MS identification, we binned compounds into hydrocarbons, non-hydrocarbons, and unknown compounds and summed their respective concentrations in Figure 3. Hydrocarbons containing at least 7 to 29 carbons were detected in all six soils (APPENDIX E). Common hydrocarbons found in two or more soils included toluene (C_7), mesitylene (C_9), undecane (C_{11}), cyclohexadecane

(C_{16}), tetracosane (C_{24}) (APPENDIX E). However, C6-C8 hydrocarbons such as toluene or xylene (C8) were not quantified by GC-FID as apparent TPH in Figure 2.The combined concentrations of non-hydrocarbons and unknown compounds was overall much higher than hydrocarbons across all soils (Figure 3). For some of the soils, especially in the dichloromethane extracts, the concentration of identified non-hydrocarbons was also much greater than of hydrocarbons (Figure 3). However, it is important to note that the compounds identified and quantified by GC-MS by Eurofins Lancaster Laboratories cannot be exactly cross referenced with the GC-FID analysis for the following reasons: (i) only the first 25 identified compounds were reported (25 is the maximum number of compounds typically reported by this laboratory) and (ii) some peaks were eliminated based on the concentration of the nearest internal standard. Nonetheless, based on the identified hydrocarbons from APPENDIX E and their concentration, it can be inferred that there is overlap between the GC-FID and GC-MS analyses. It can also be inferred that not all compounds identified as apparent TPH in Figure 2 are hydrocarbonsas the concentration of identified hydrocarbons quantified by GC-MS is much lower than the TPH concentration measured by GC-FID in these soils. It is highly likely that non-hydrocarbons quantified by the FID as apparent TPH were lipids, such as stigmasterol, .beta.-Sitosterol, or octadecanal (APPENDIX E). Lipids are primarily composed of hydrogen and carbon, but often have other functional groups such as oxygen, nitrogen, sulfur, and phosphorus (phospholipids) attached (BCcampus).

We quantified apparent TPH by GC-FID in plant and microbial biomass, two types of biological materials present in SOM in their native state and/or in various stages

of decomposition or transformation (National Resources Conservation Service, 2014, Lehmann and Kleber, 2015). The concentrations of apparent TPH extracted from leaves and microbial biomass are reported in Figure 4. The GC-FID chromatograms and the distribution of TPH concentration as a function of carbon range are included in APPENDIX C and F, respectively. The apparent TPH concentration in leaves (9000 mg kg^{-1}) was similar for the extracts with dichloromethane and *n*-pentane (Figure 4, APPENDIX D). The concentrations of apparent TPH for leaves and microbial biomass were similar using dichloromethane as extraction solvent. However, the concentration of TPH was higher when *n*-pentane was used as the extraction solvent, yielding a concentration of 23000 mg kg^{-1} for microbial biomass (Figure 4, APPENDIX D). Similar to our results in soils, *n*-pentane extracted a higher concentration C_9-C_{12} apparent TPH (APPENDIX F). These data highlight that plant and microbial biomass are among the sources of SOM that contributed to an apparent TPH concentration in soils.

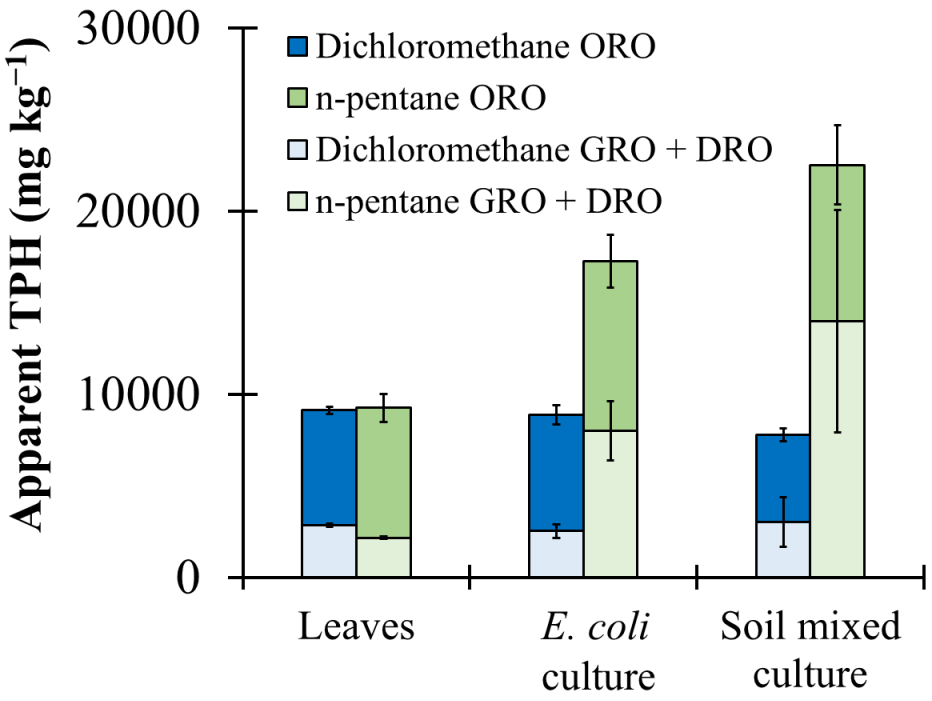

Figure 4. Concentrations of apparent TPH in extracts from leaves and microbial biomass using dichloromethane and *n*-pentane. GRO + DRO = C_9-C_{28} TPH; ORO = C_{28}-C_{36} TPH. The data are averages with standard deviation of duplicate extractions.

The soils used in this study captured a wide range of soil properties including moisture contents of 2% to > 80% (w w^{-1}), pH ranging from 3 to 10, conductivity of 10 µS cm^{-1} to 35 mS cm^{-1}, and concentrations of organic carbon of < 200 to 97000 mg kg^{-1} (Table 1). We performed regression analyses to determine if these soil characteristics could be used as predictors of the magnitude of apparent TPH concentration in soils. Those analyses, with the coefficient of determination R^2 for TPH *vs*. pH, conductivity, salinity, organic carbon, and moisture content, are shown in Figure 5 and APPENDIX C. Typically, an R^2 between 0.3 and 0.5 is considered weakly correlated, while R^2 values between 0.5 and 0.7 are a moderate correlation and values greater than > 0.7 are generally considered to have a strong correlation, respectively (Zikmund and Carr, 2003, Moore

and Kirkland, 2007). Conductivity, salinity, and moisture content of the soils from Table 1 had a weak correlation with the concentration of apparent TPH (Figure 5 and APPENDIX G).

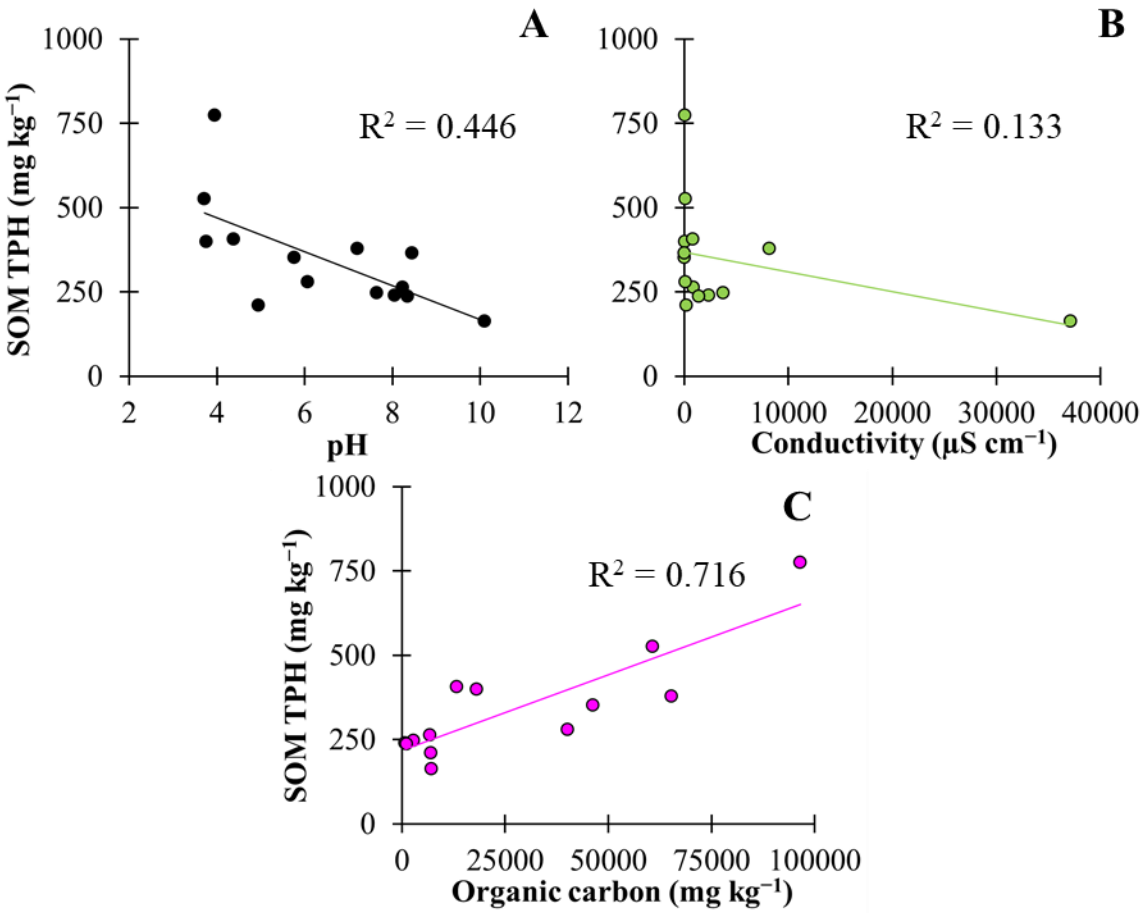

Figure 5. Linear regression analyses between the apparent TPH concentration and select soil properties. The white-filled datum (Soil O) in each panel was not included in the regression analyses. `

Soil pH is a measure of the concentration of hydrogen ions in the soil solution, providing information on the acidity or alkalinity of the soil. pH is an important variable, affected by the type of soil, anion vs. cation concentrations, organic carbon, and

microbial activity, among others (Natural Resources Conservation Service, McCauley, et al., 2017, Neina, 2019). Typical pH in natural soils ranges from 3 to10, with a pH of 5-7 for soils from wet areas and 6.5-9 for soils from drier climates (Queensland Government, 2013). The soils from this study's library captured those ranges (Table 1). Regarding the effect pH has on apparent TPH concentration, results of this study indicated that soil pH has a moderate effect. In general, samples that had a lower pH had a higher apparent TPH concentration than soils with a more alkaline pH. As seen in Figure 5, organic carbon shows a strong correlation with apparent TPH for the soils analyzed in this study. Soils that had higher organic carbon concentrations also displayed higher concentrations of apparent TPH. These data support the use of organic carbon concentration as an indicator of the presence of apparent TPH from SOM.

DISCUSSION

From the results discussed, it is evident that soils without petroleum hydrocarbon contamination have detectable and quantifiable concentrations of apparent TPH due to compounds from SOM native to these soils. This phenomenon warrants further investigation and could potentially have a reaching impact on future TPH regulations. A 2011 survey by the Alaska Department of Environmental Conservation reported that CULs for TPH vary from 50 mg kg^{-1} to 4100 mg kg^{-1} across states (Interstate Technology and Regulatory Council). In a more recent survey capturing state responses from 2012/2014, it was reported that CULs for GRO and DRO range from 30 to 47500 mg TPH kg^{-1} (Tomlinson and Ruby, 2016). The large difference in state CULs are a

consequence of differences in contamination scenarios (residential, industrial, potable groundwater, all other) and the pathways leading to contamination (direct contact, leaching, and "all other" pathways including commercial, non-residential, park user, etc.(Tomlinson and Ruby, 2016). Our study indicated that the concentrations of apparent GRO+DRO TPH in two out of 15 non-contaminated soils were higher than the minimum CULs for a residential site contaminated by direct contact (460 mg TPH kg^{-1}) (APPENDIX D) (Tomlinson and Ruby, 2016) and in all soils (15/15) for "all other" scenarios and pathways (80 mg TPH kg^{-1}) (Tomlinson and Ruby, 2016). The ORO TPH concentration in the soils from Figure 1 and APPENDIX D is also higher than CULs for this fraction in many states (reported range of 99 mg kg^{-1} to 10000 mg kg^{-1}) (Interstate Technology and Regulatory Council). A similar phenomenon was observed in Italy by Vecchiato et al. in 2017 (Vecchiato, et al., 2017). They investigated the contribution of plant residues and effect of different compost practices to TPH concentrations by analyzing background soils from protected woodland areas and agricultural soils in Italy and discovered that the TPH concentrations were higher than the local intervention limit of 50 mg kg^{-1}. They also observed that soils with natural composting had higher TPH concentrations than soils with chemical fertilizers.

An interesting observation in the apparent TPH concentrations was the difference in concentrations and the types of compounds extracted by dichloromethane and *n*-pentane. Our study showed that *n*-pentane extracted a higher concentration of SOM compounds with a lower molecular weight as compared to dichloromethane. Discrepancies in the concentrations of actual TPH and aromatic hydrocarbons in soil

extracted by dichloromethane and *n*-pentane have been documented in previous studies (Hollender, et al., 2003, Kwon and Hwang, 2017). For example, Kwon et al., (2017) (Kwon and Hwang, 2017) documented that dichloromethane extracted a higher TPH concentration from crude oil contaminated soils than *n*-pentane, *n*-hexane, or methanol, which is similar to the results of this study for apparent TPH from SOM. Hollender et al (2002) employed two soils with different biogeochemical characteristics and different concentrations of polycyclic aromatic hydrocarbons (PAH), and performed Soxhlet extractions using dichloromethane and *n*-pentane (Hollender, et al., 2003). That study showed that *n*-pentane extracts yielded a concentrations of C_{10}-C_{22} PAHs that were higher by 117%-146% in both contaminated soils, likely due to a better extraction of non-polar PAHs by *n*-pentane, the non-polar solvent (Hollender, et al., 2003). The concentration of TPH extracted depends on multiple factors such as the type of solvent used (Saari, et al., 2008, Kwon and Hwang, 2017), the physical and chemical characteristics of the soils (Sui, et al., 2014, Devatha, et al., 2019), the interactions of the target compounds with the soil matrix and their concentration (Hollender, et al., 2003) , and the mass ratio of solvent to soil employed in the method (Kwon and Hwang, 2017), among others.

Our study demonstrated that plant and microbial biomass are likely sources of apparent TPH. This contribution was also acknowledged in a study on background soils in Italy by Vecchiato et al. (Vecchiato, et al., 2017). Also, apparent TPH concentrations for grass (14000 mg kg^{-1}), dried oak leaves (18000 mg kg^{-1}), and pine needles (16000 mg kg^{-1}) are listed on the American Petroleum Institute (API) website (American Petroleum

Institue (API), 2001). There is a strong possibility that many of the compounds detected as apparent TPH in leaves and microbial biomass in Figure 4 are in fact lipids. *n*-Hexane and dichloromethane effectively extract lipids from SOM and other organic compounds such as seeds and fish muscle tissue (Cequier-Sánchez, et al., 2008, Tfaily, et al., 2015). Some lipids such as fatty acids are also non-polar (BCcampus). Lipids make up 40% of the bacterial membranes (Todar) and most plants have about 5% of their dry weight as lipids, with leaves containing the greatest amount among plant material(Horwath, 2015). In *E. coli*, one of the sources of biomass investigated for its contribution to an apparent TPH concentration, membranes are comprised of the phospholipid phosphatidylethanolamine (75%), the anionic phospholipid phosphatidylglycerol (20%), and the anionic phospholipid cardiolipin (5%) (Sohlenkamp and Geiger, 2015, Rowlett, et al., 2017). The prevalence of these lipids in plant and microbial biomass with their hydrocarbon-like properties likely explains the high concentrations of apparent TPH associated with these materials.

The GC-MS analysis confirmed that uncontaminated soils contain hydrocarbons extractable by dichloromethane or *n*-pentane. However, sources of hydrocarbons in uncontaminated soils are common and are not a result of crude oil or petroleum hydrocarbon products spill. Liquid and solid alkanes with more than 20 carbon atoms can be present in soils due to the wax covering the leaves of plants and trees and due to the excrements of animals and birds (Gennadiev, et al., 2015). Furthermore, algae, bacteria, and fungi can be sources of odd number alkanes with 15 to 25 carbon atoms. Forest, peat, and steppe fires, volcanic activity, underground rock and mineral activity are

considered to be sources for the presence of PAHs in soils (Gennadiev, et al., 2015). Vecchiato et al., (2017) (Vecchiato, et al., 2017) investigated how different types of soils and different farming and composting practices might affect the hydrocarbons present in soils and concluded that leaf waxes of trees contribute to the hydrocarbon concentration in woodland soils whereas grasses and roots and spruce needles contribute to lower chain alkanes ($\leq C_{20}$) in cultivated soils. The GC-MS analysis also showed that many of the SOM compounds extracted by the solvents used in TPH analysis by GC-FID could not be identified. The lack of identification reflects the complexity of SOM, with organic compounds originating from biomass from plants, animals, microorganisms, and other sources that enter the SOM pool and are microbially degraded or transformed (Natural Resources Conservation Service, 2009, Gennadiev, et al., 2015, Pluske, et al., 2015) . Findings from our study showed that the organic carbon concentration in the soils was strongly and positively correlated to their apparent TPH concentrations. The concentration of organic carbon in soils is influenced by climate, land use, nutrients present, but mostly by type and mass input of organic C and microbial activity (Insam and Domsch, 1988, Natural Resources Conservation Service, 2009, Pluske, et al., 2015, Liao, et al., 2016). The concentration of organic carbon in soils can have a wide range as seen in Table 1.

In a study on crude oil contaminated soil, the organic carbon concentration was tested in approximately 3000 field soil samples from a former oil refinery to determine if organic carbon can be used as a screening tool for high TPH concentrations. Samples which had a high organic carbon concentrations (approximately 421 out of 3000) were

analyzed for TPH using EPA method 418.1 for residual petroleum hydrocarbons or method 8015M for DRO (Schreier, et al., 1999). The authors found that samples having organic carbon concentrations greater than 30000 mg kg^{-1} had TPH concentrations >20000 mg kg^{-1} (Schreier, et al., 1999), concluding that organic carbon can be used as a screening method for high TPH concentrations. The background TPH concentrations in the soils investigated by Vecchiato et al., (2017) (Vecchiato, et al., 2017) were also in accordance with the organic carbon content of the soils ($R^2 = 0.66$). Data from this study's soils confirmed that soils with a high concentration of organic carbon are also likely to have a high concentration of apparent TPH.

In conclusion, this study is the first comprehensive documentation showing that uncontaminated soils from various geographical locations and with various biogeochemical properties contain substantial concentrations of SOM quantified as TPH that are neither "total", nor of "petroleum" origin, nor exclusively composed of "hydrocarbons". The concentrations of apparent TPH in our study soils were higher than many state action or CULs. Data from this work informs on the limitation of the TPH by GC-FID method for quantifying petroleum hydrocarbons released during crude oil or refined product contamination events in soils. Thus, in a scenario where the CUL is below the soil's apparent TPH concentration, remediation would entail partial destruction of SOM to be able to meet the TPH CUL. Findings from this study support establishing action and clean-up levels that account for the concentration of SOM that registers as apparent TPH.

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APPENDIX A

ACTION LEVELS AND CLEANUP LEVELS FOR DIFFERENT FRACTIONS OF TOTAL PETROLEUM HYDROCARBONS

Action level (mg kg^{-1})	Gasoline	Diesel	Oil	Cleanup Level (mg kg^{-1})	Gasoline	Diesel	Oil
≤100	AL, AR, DE, GA, ID, IN, IA, KS, MN, NV, ND, OK, PA, SD, TN, UT, WV, WI	AL, FL, KS, NV, NM, PA, UT, WV, WI	AL, KS, NV, NM, UT, WI, WY	**≤100**	AL, AZ, IN, IL, KS, ME, MT, NV, NH, ND, OK, PA, SD, WA	AZ, KS, ME, MS, NH, NM, OK, PA	AZ, ID, KS, MS, NM, WY
>100	FL	GA, ID, VT	AK, GA	**>100**		WA	
Site specific	AK, AZ, MO, SC	AK, AZ, SC	AZ	**Site specific**	AK, AR, CO, CT, GA, IN, IA, LA, MN, MO, MT, NC, ND, OR, SC, TN, TX, UT, VA, WV, WI	AK, AR, CO, CT, DE, GA, ID, IN, IA, LA, MA, MN, MO, NC, MT, ND, OH, OR, SC, TN, TX, VT, VA, WV, WI	AR, CO, CT, DE, GA, IL, IN, IA, MA, MN, MO, MT, NC, OR, PA, SC, TN, TX, VT, VA, WI

APPENDIX B

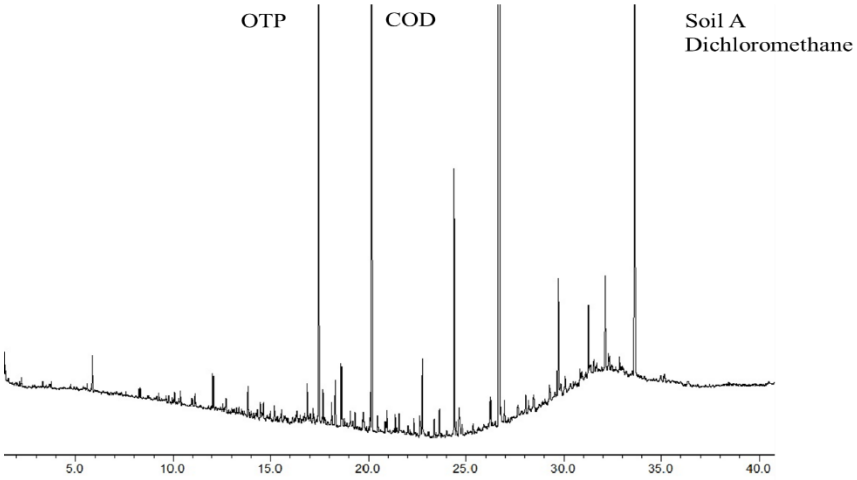
COMMONLY USED ANALYTICAL METHODS TO MEASURE TOTAL PETROLEUM HYDROCARBONS IN UNITED STATES OF AMERICA

Method	States
EPA method 8015 (M)(B)(D)	AL, AZ, AR, CA, CO, FL, HI, IL, LA, ME, MS, NE, NV, NH, NM, NC, ND, OH, OK, UT, VA, WA, WV, WY
MADEP - EPH 04	HI, LA, ME, MA, MS
TNRCC 1005, 1006	AR, LA, OK, RI, TX
Other - EPA 418.1, 9071, 5520, 5035, 8021B, 8260B, 8260, 8020, 8270, 8021, 8040, SW 846, AK 101 - 103, CT ETPH, Delaware DNREC - SIRS, Iowa OA 1,2, Wisonsin WI DNR Modified GRO method, TPHCWG	Varies

APPENDIX C

CHROMATOGRAMS OF SOILS, LEAVES, AND MICROBIAL BIOMASS EXTRACTED WITH DICHLOROMETHANE.

NOTE: COD - 1-CHLOROOCTADECANE AND OTP - O-TERPHENYL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Dichloromethane
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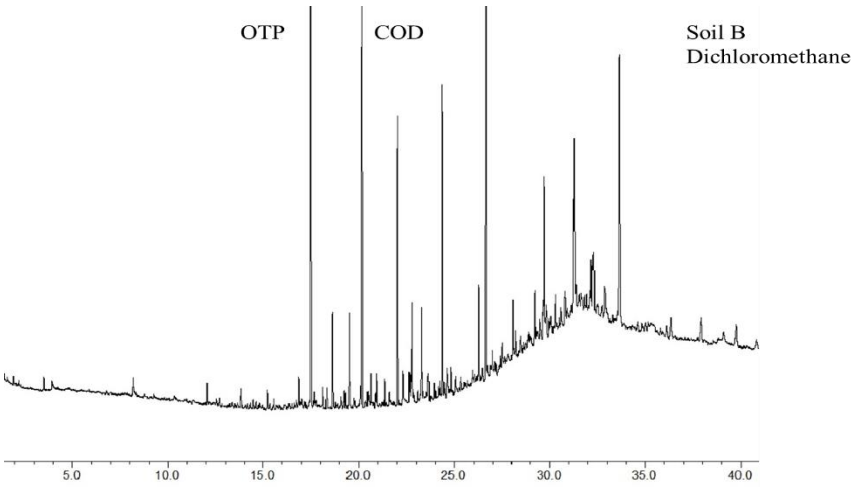
10.0
15.0
20.0
25.0
30.0
35.0
40.0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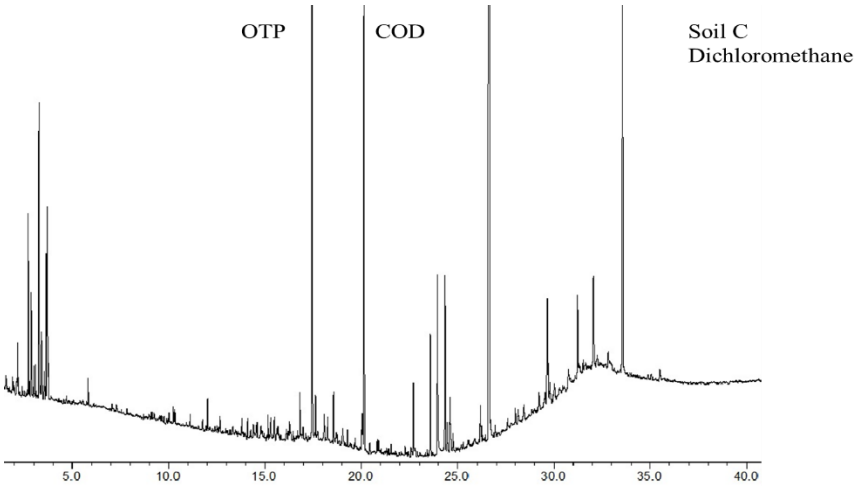
35.0
40.0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COD
Soil C
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20.0
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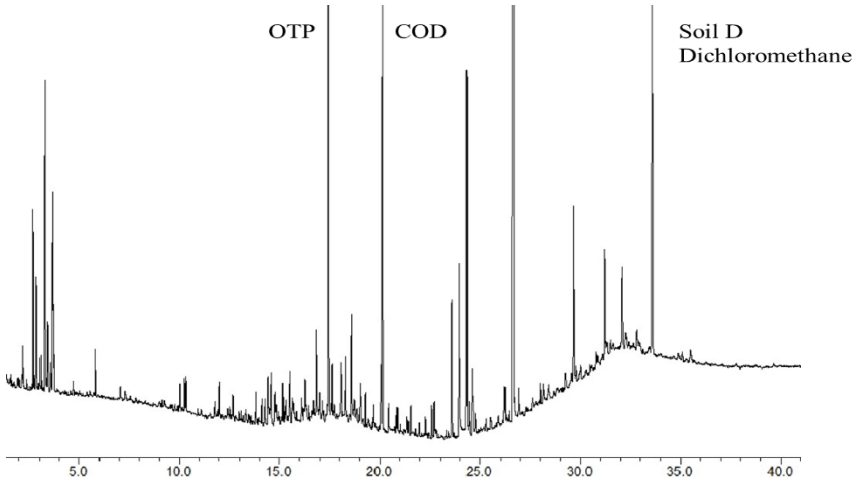
30.0
35.0
40.0


OTP
COD
Soil D
Dichloromethane
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20.0
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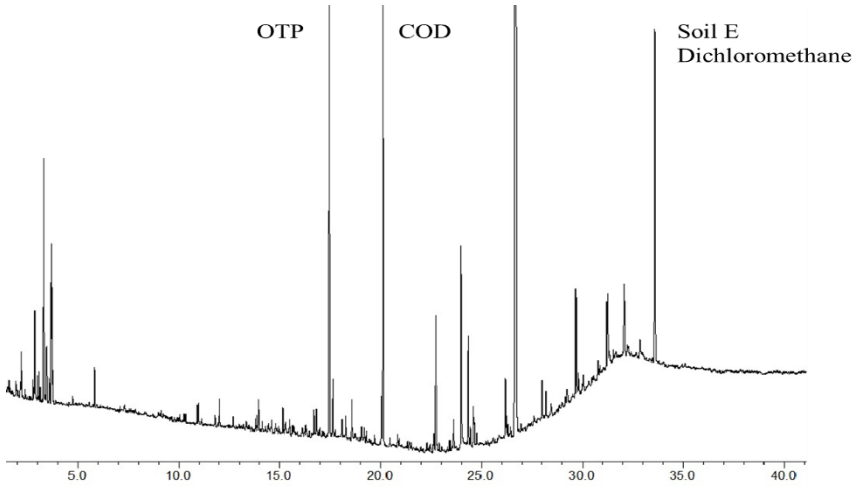
35.0
40.0


OTP
COD
Soil E
Dichloromethane
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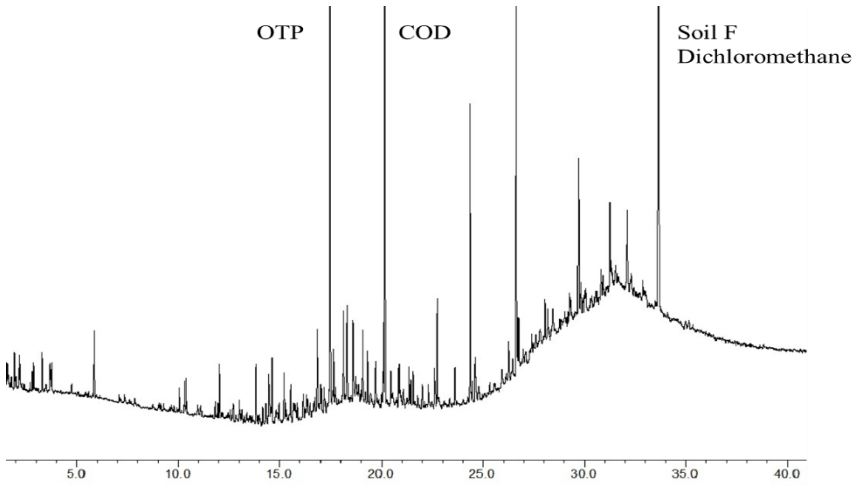
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Soil F
Dichloromethane
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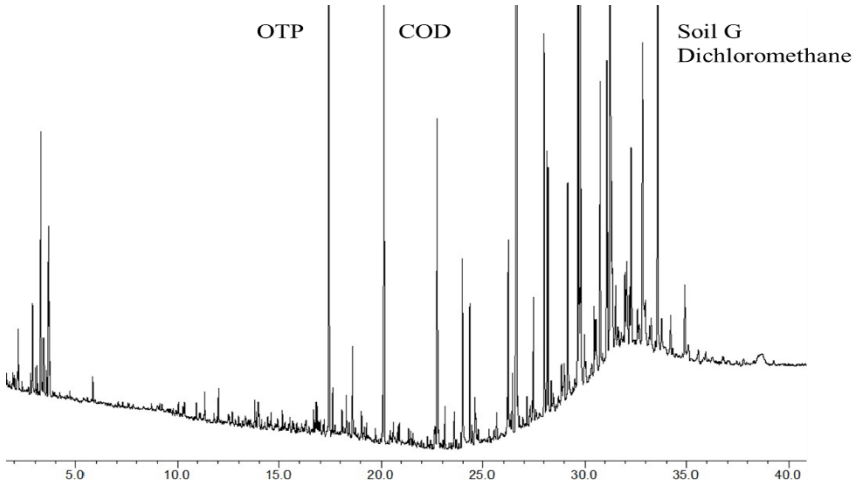
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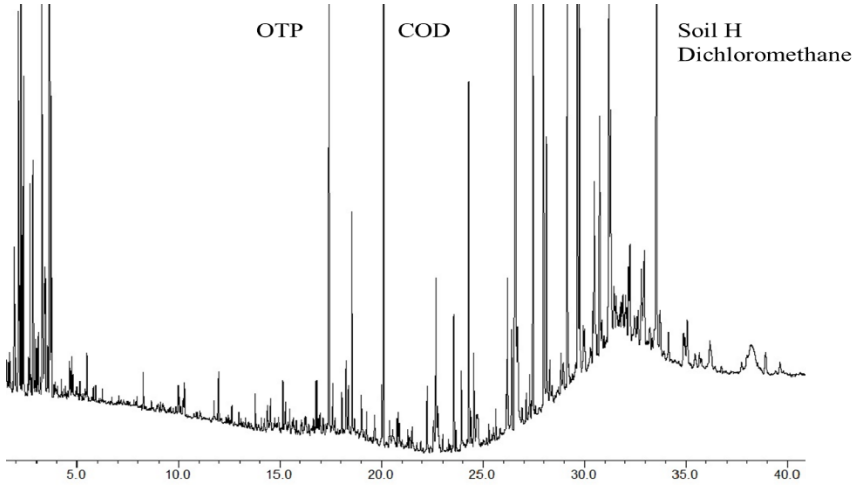
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COD
Soil H
Dichloromethane
5.0
10.0
15.0
20.0
25.0
30.0
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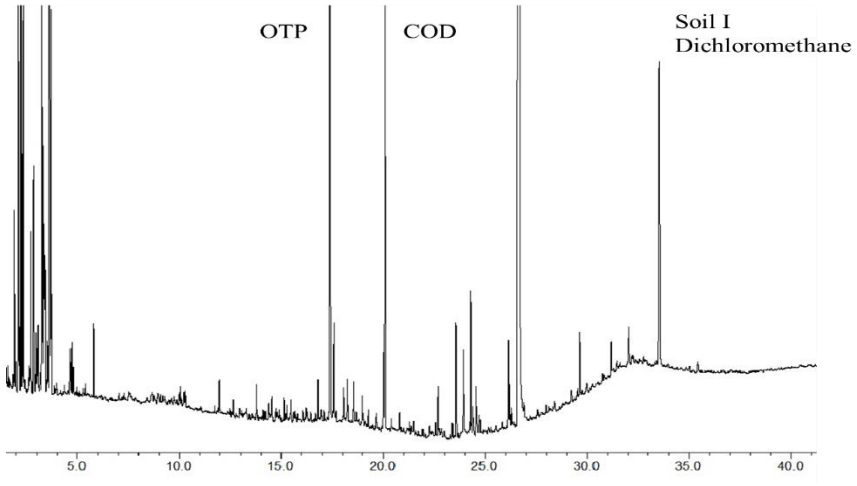
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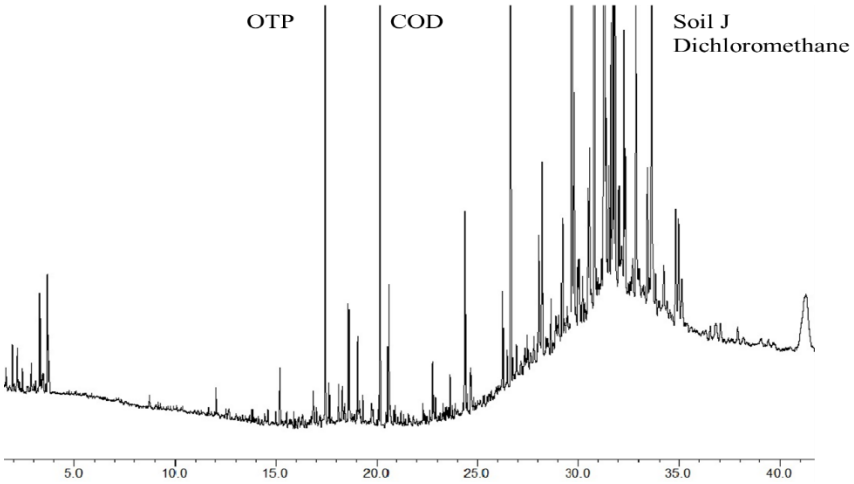
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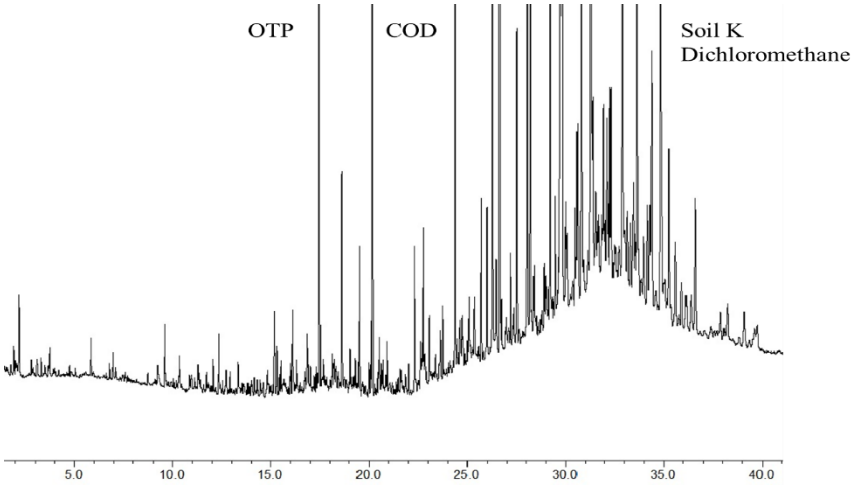
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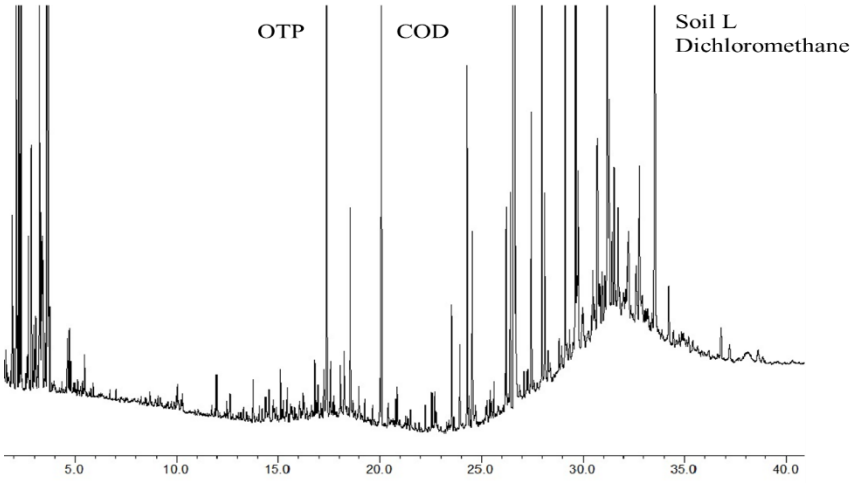
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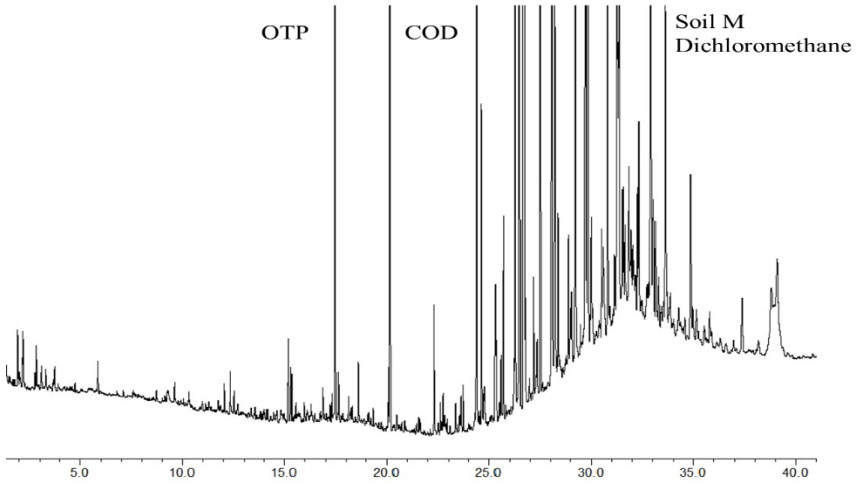
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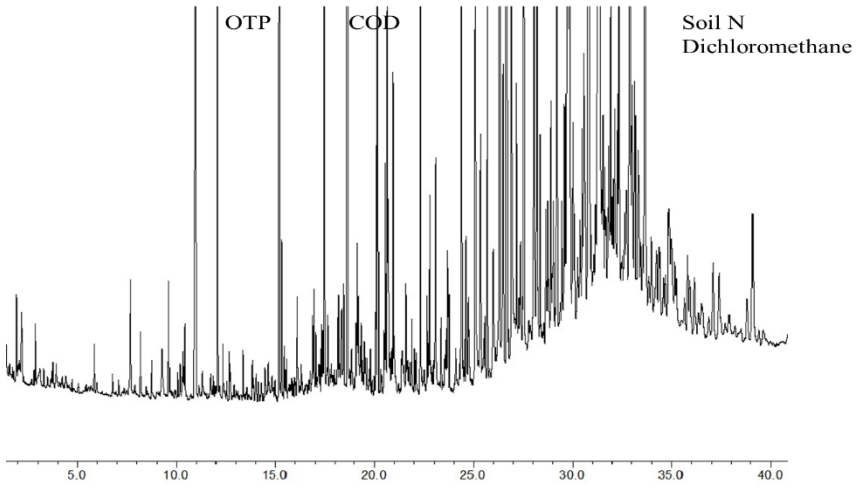
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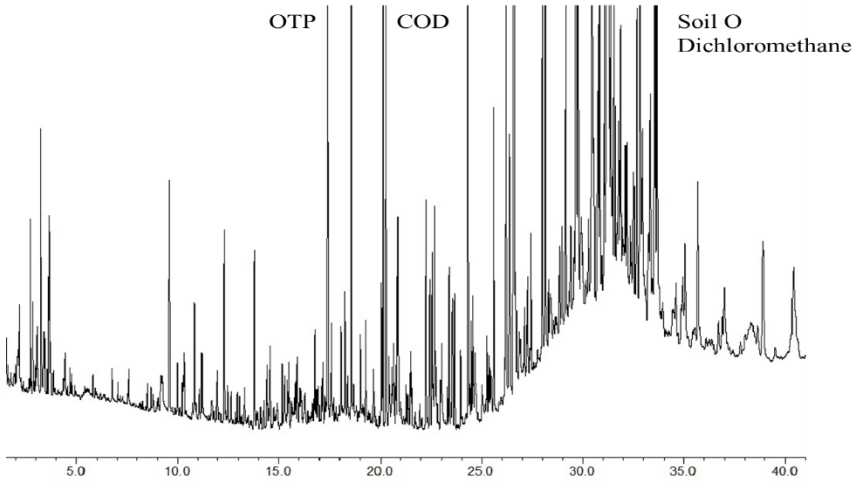
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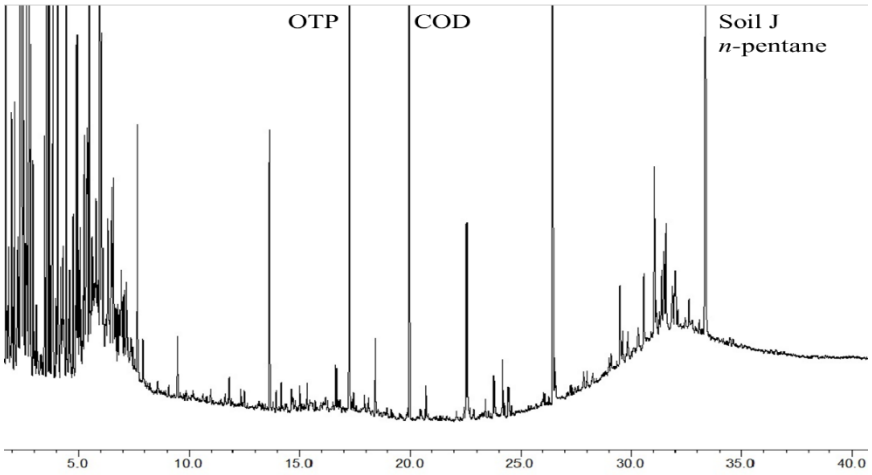
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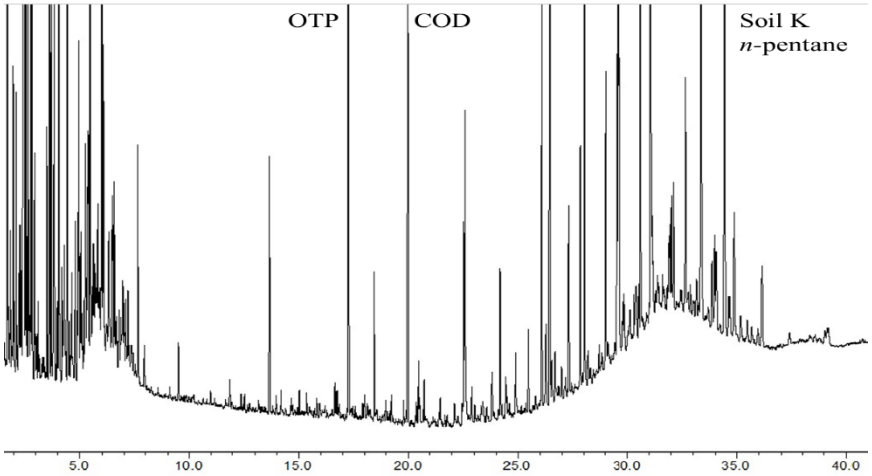
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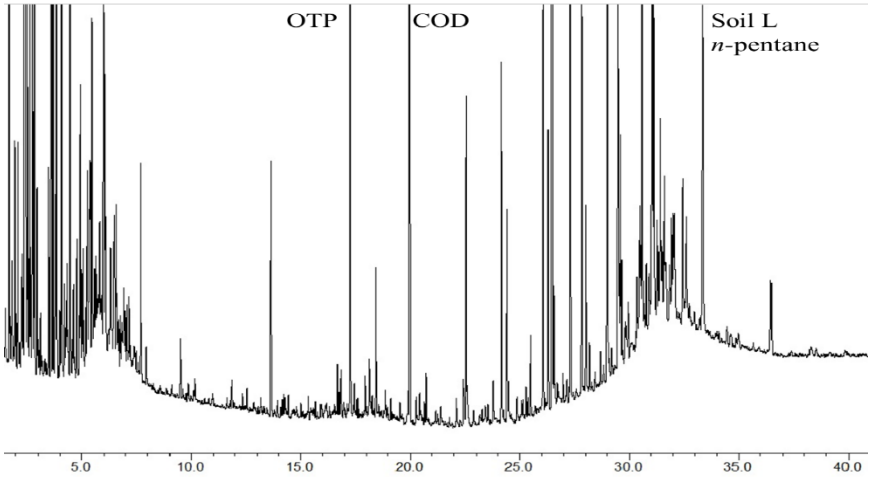
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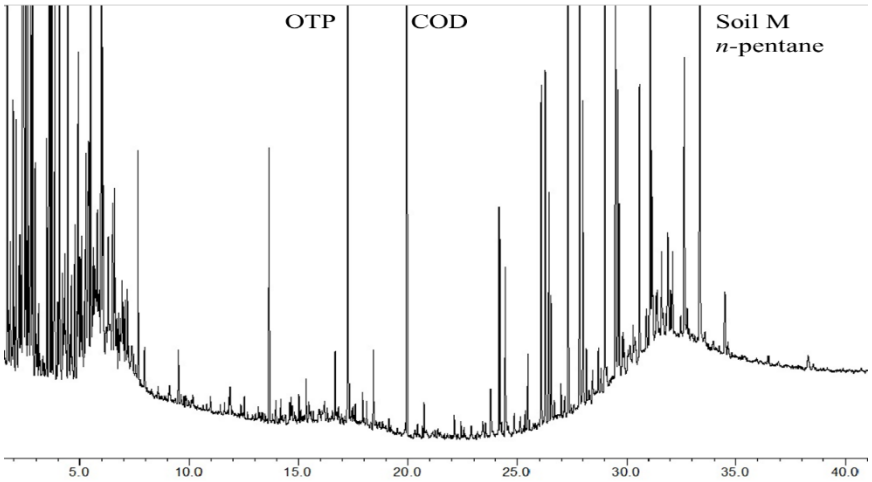
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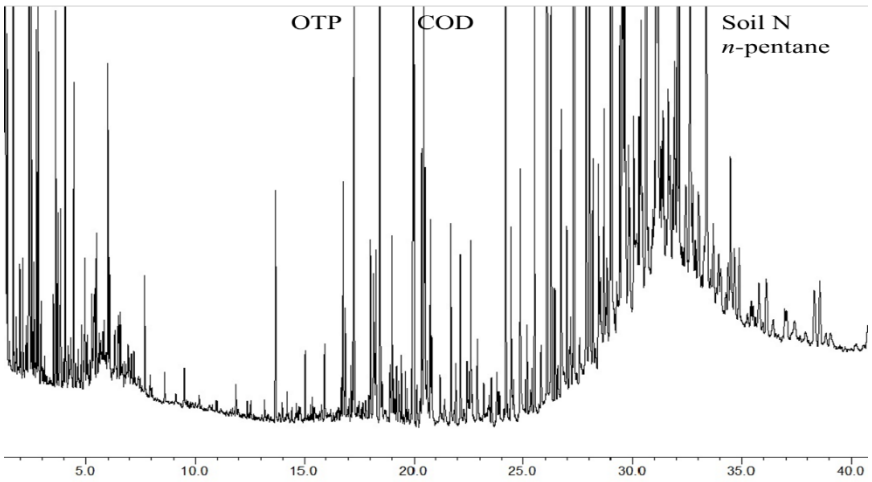
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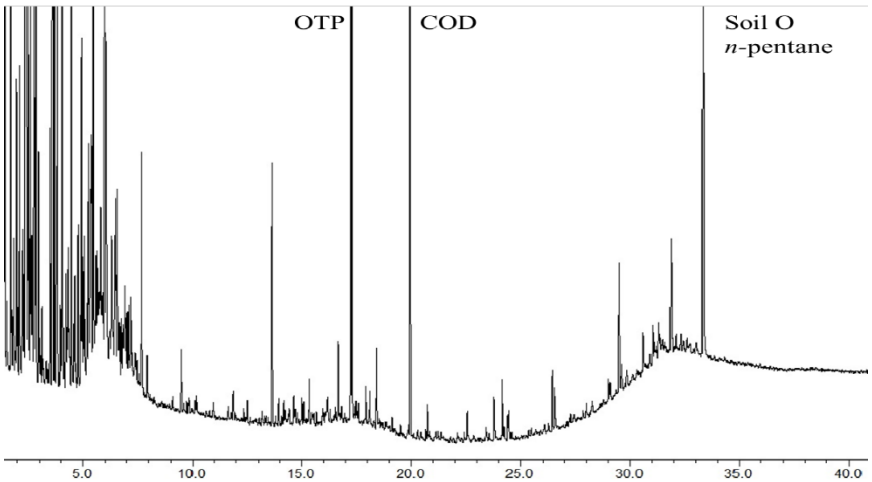
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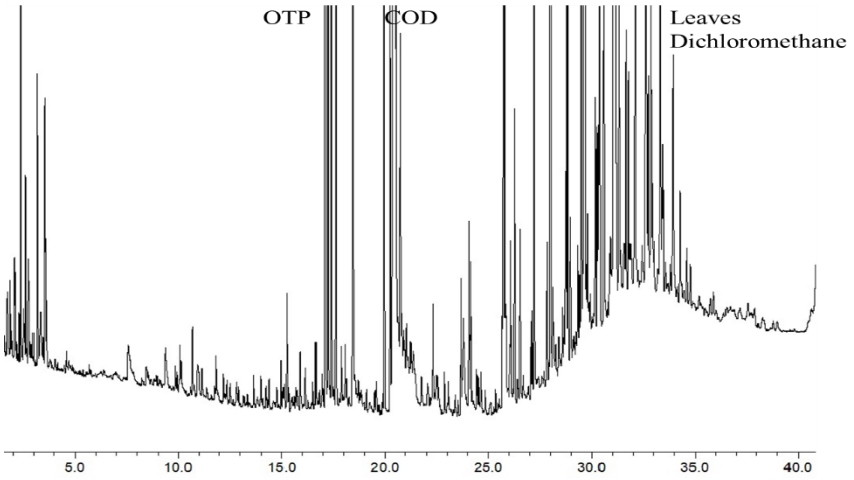
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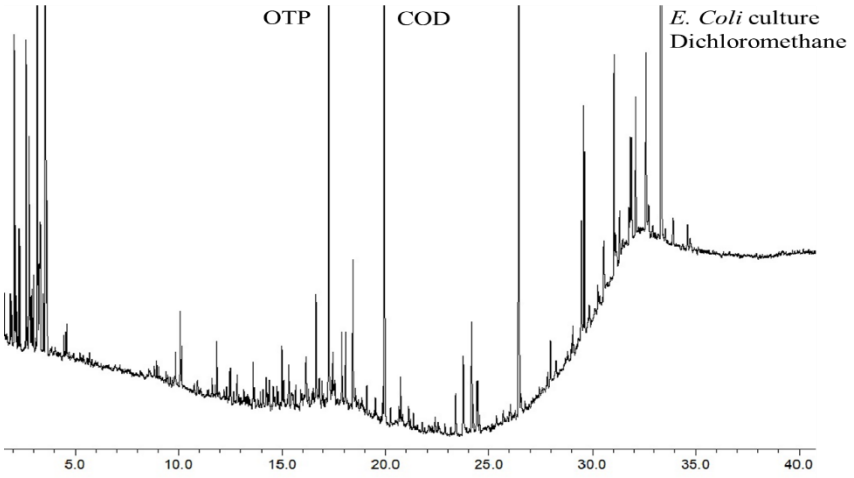
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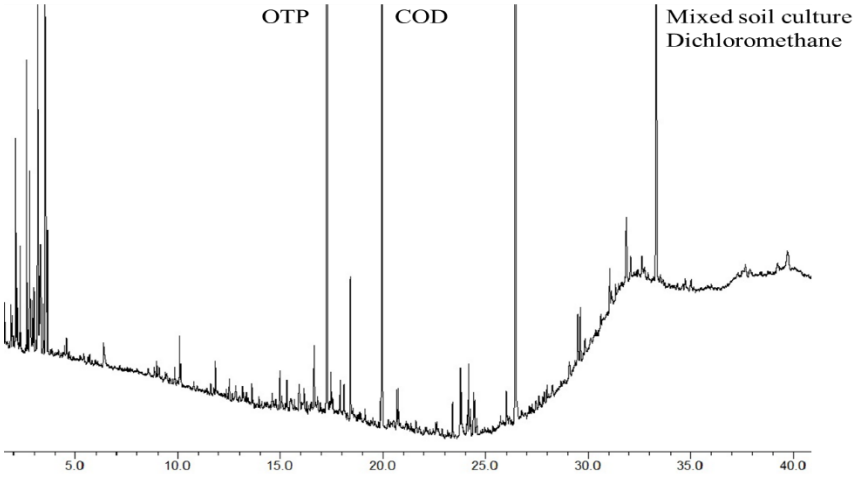
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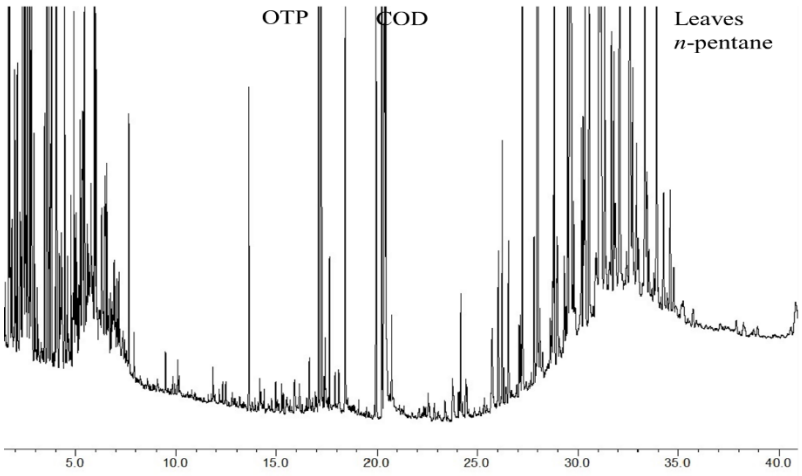
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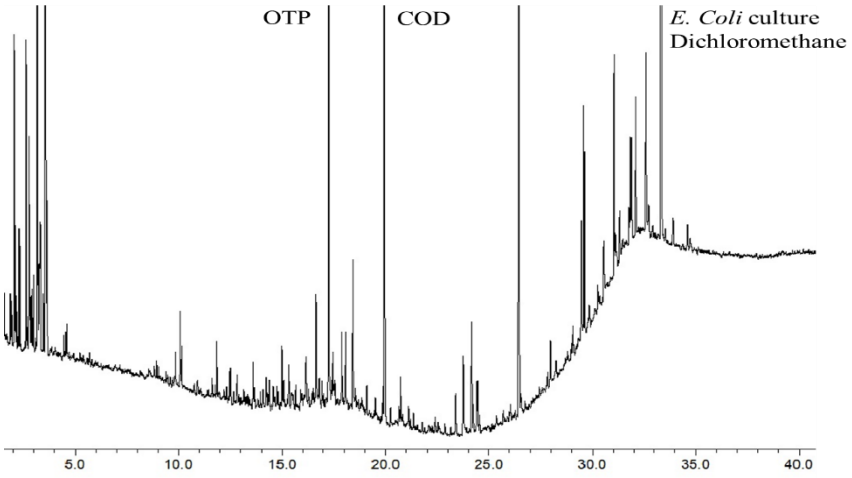
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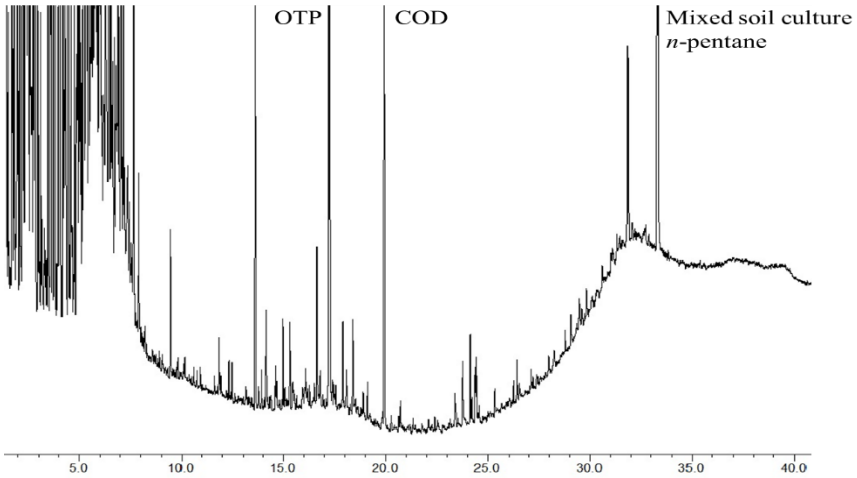
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APPENDIX D

CONCENTRATIONS OF APPARENT TOTAL PETROLEUM HYDROCARBONS IN SOILS AND LEAVES AND MICROBIAL BIOMASS EXTRACTED BY DICHLOROMETHANE AND N-PENTANE

NOTE: GRO – GASOLINE RANGE ORGANICS, DRO – DIESEL RANGE ORGANICS, ORO – OIL RANGE ORGANIC

	Dichloromethane			*n*-pentane		
Sample	**GRO + DRO (mg kg-1)**	**ORO (mg kg-1)**	**Total (mg kg-1)**	**GRO + DRO (mg kg-1)**	**ORO (mg kg-1)**	**Total (mg kg-1)**
Soil A	60	100	160			
Soil B	66	140	210			
Soil C	140	99	240			
Soil D	130	110	240			
Soil E	140	110	250			
Soil F	56	210	270			
Soil G	120	160	280			
Soil H	140	210	350			
Soil I	280	90	370			
Soil J	60	320	380	150	160	310
Soil K	110	290	400	200	210	410
Soil L	190	220	410	190	230	420
Soil M	190	330	520	180	210	390
Soil N	290	490	780	270	450	720
Soil O	1000	1700	2700	700	560	1300
Leaves	2900	6300	9200	2200	7100	9300
E. coli culture	2500	6300	8800	8000	9300	17000
Soil mixed culture	3000	4800	7800	14000	8600	23000

APPENDIX E

COMPOUNDS IDENTIFIED USING GAS CHROMATOGRAPHY/MASS SPECTROMETRY IN SOILS EXTRACTED BY DICHLOROMETHANE AND N-PENTANE

COMPOUNDS IN BOLDFACE ARE IDENTIFIED IN MORE THAN ONE SOIL EXTRACT AND ITALICIZED COMPOUNDS ARE COMPOUNDS IDENTIFIED IN BOTH SOLVENT EXTRACTS OF THE SAME SOI

Dichloromethane					*n*-pentane				
Soil J									
RT (min)	Compound	Molecular Formula	Concentration (mg kg^{-1})	Quality score	RT (min)	Compound	Molecular Formula	Concentration (mg kg^{-1})	Quality score
8.269	Hexathiane	S6	1.9	90	2.328	**Toluene**	C7H8	7.2	95
10.627	**Cyclic octaatomic sulfur**	S8	12	92	3.687	Unknown		340	
11.969	1-Octadecene	C18H36	1.8	96	3.916	o-Xylene	C8H10	5.9	97
12.763	Unknown		16		4.046	**Ethanol, 2-butoxy-**	C6H14O2	7.9	87
13.257	Heptadecane	C17H36	2.6	93	4.587	**Benzene, 1-ethyl-3-methyl-**	C9H12	9.4	95
13.363	Perylene-D12	C20D12	5.9	98	4.875	**Benzene, 1,2,3-trimethyl-**	C9H12	12	97
13.886	Unknown		1.9		5.093	**Mesitylene**	C9H12	10	93
13.916	n-Tetracosanol-1	C24H50O	6	95	5.328	Unknown		4.2	
14.039	26-Nor-5-cholesten-3.beta.-ol-25-one	C26H42O2	8.3	95	5.363	Benzene, 1,3-diethyl-	C10H14	3.9	94
14.086	Unknown		2.3		5.41	Unknown		4.3	
14.18	Unknown		1.4		5.493	Unknown		7.1	
14.292	Nonadecane, 1-chloro-	C19H39Cl	2	91	5.569	Benzene, 1-methyl-3-(1-methylethyl)-	C10H14	6.3	97
14.357	Tetradecanal	C14H28O	4.3	93	5.669	**Undecane**	C11H24	5.6	93
14.392	Unknown		2.8		5.822	Benzene, 1,2,4,5-tetramethyl	C10H14	2.9	95
14.486	Unknown		5.6		5.869	Cyclopentasiloxane, decamethyl-	C10H30O5Si5	3.3	91
14.545	Dotriacontane	C32H66	1.8	91	5.934	Cyclohexane, pentyl-	C11H22	2.5	91

14.592	**Cyclotetracosane**	C24H48	7.3	95	6.034	Benzene, 4-ethyl-1,2-dimethyl-	C10H14	2.6	92
14.639	Unknown		3		6.322	Tetradecane	C14H30	2.4	94
14.715	**.beta.-Sitosterol**	C29H50O	7.8	91	6.892	Cyclohexasiloxane, dodecamethyl-	C12H36O6Si6	2.1	91
14.792	Unknown		6.1		8.439	Unknown		3.7	
14.857	Unknown		2.3		11.533	9-Octadecenamide, (Z)-	C18H35NO	1.9	91
14.927	Unknown		2.5		12.769	Unknown		27	
14.992	5-bromo-4-oxo-4,5,6,7-tetrahydrobenzofurazan	C6H5BrN2O2	8.6	93	14.78	Unknown		3.1	
15.127	Unknown		16		14.986	Unknown		2	
15.262	Stigmast-4-en-3-one	C29H48O	4.6	91	15.133	Unknown		2	
Soil K									
11.968	**Cyclohexadecane**	C16H32	4.8	94	2.328	**Toluene**	C7H8	8.8	95
12.192	Unknown		4.6		3.693	Unknown		360	
12.621	**Cyclotetracosane**	C24H48	9.9	98	3.916	**Benzene, 1,3-dimethyl-**	C8H10	6.6	97
12.768	Unknown		40		4.046	Unknown		8.1	
13.068	***Octadecanal***	C18H36O	2.5	96	4.587	**Benzene, 1-ethyl-2-methyl-**	C9H12	9.8	95
13.257	Unknown		17		4.663	**Mesitylene**	C9H12	4.8	97
13.709	***Oxirane, hexadecyl-***	C18H36O	4.1	90	4.875	**Benzene, 1,2,3-trimethyl-**	C9H12	12	93
13.886	**Tricosane**	C23H48	6	95	5.093	**Benzene, 1,2,4-trimethyl-**	C9H12	10	93
13.915	Unknown		18		5.492	Sulfurous acid, decyl pentyl ester	C15H32O3S	7	90

14.033	Unknown		5.4		5.569	o-Cymene	C10H14	6.3	97
14.18	Unknown		3		5.669	**Undecane**	C11H24	5.5	96
14.221	Unknown		2.4		5.822	**Benzene, 1,2,3,4-tetramethyl**	C10H14	4.9	97
14.274	Unknown		4		6.04	Benzene, 1-ethyl-3,5-dimethy	C10H14	2.6	95
14.362	**1,19-Eicosadiene**	C20H38	8.8	95	6.322	Dodecane	C12H26	2.3	94
14.433	Unknown		4.1		8.439	Unknown		3.2	
14.492	Unknown		2.3		12.621	Cyclopentadecane	C15H30	4.6	92
14.545	*Tetracosane*	C24H50	2.3	87	12.768	Unknown		11	
14.592	13-Tetradecen-1-ol acetate	C16H30O2	4.7	91	13.068	***Octadecanal***	C18H36O	2.3	91
14.639	**2-Pentacosanone**	C25H50O	4.6	86	13.257	*Tetracosane*	C24H50	6.7	97
14.715	**.gamma.-Sitosterol**	C29H50O	6.9	93	13.71	*Oxirane, hexadecyl-*	C18H36O	3.6	91
14.768	Unknown		2.9		13.886	**Hexadecane**	C16H34	3.9	94
14.909	Acenaphtho[1,2-j]fluoranthene	C26H14	2.8	87	13.915	Unknown		11	
15.092	Unknown		3.7		14.363	Oxirane, heptadecyl-	C19H38O	4.6	91
15.168	Unknown		4.9		14.715	**.beta.-Sitosterol**	C29H50O	6.1	96
15.78	Unknown		2.8		15.162	Unknown		2.1	
Soil L									
10.627	***Cyclic octaatomic sulfur***	S8	16	91	2.328	**Toluene**	C7H8	6	95
11.533	*9-Octadecenamide, (Z)-*	C18H35NO	3.9	98	3.687	Unknown		280	
11.969	Cetene	C16H32	8.5	96	4.046	**Ethanol, 2-butoxy-**	C6H14O2	6.3	87
12.621	Hexadecane	C16H34	8.5	95	4.587	**Benzene, 1-ethyl-2-methyl-**	C9H12	7.7	95

12.78	Unknown		140		4.875	**Benzene, 1,2,3-trimethyl-**	C9H12	9.6	97
13.068	**Tetradecanal**	C14H28O	5.9	93	5.093	**Benzene, 1,2,4-trimethyl-**	C9H12	8.3	93
13.257	Docosane, 11-butyl-	C26H54	6.4	95	5.669	**Undecane**	C11H24	4.6	95
13.71	**Oxirane, heptadecyl-**	C19H38O	9	91	8.439	Unknown		3.3	
13.886	*Tetracosane*	C24H50	2.7	90	10.627	*Cyclic octaatomic sulfur*	S8	14	91
13.915	Unknown		4.7		11.533	*9-Octadecenamide, (Z)-*	C18H35NO	3.1	99
13.951	**2-Pentacosanone**	C25H50O	2.4	87	11.98	1-Heptadecene	C17H34	4.9	93
14.192	Unknown		2.8		12.621	Sulfurous acid, 2-propyl tetradecyl ester	C17H36O3S	5.6	91
14.274	Unknown		2.5		12.768	Unknown		40	
14.362	**Octadecanal**	C18H36O	6.6	93	13.068	16-Octadecenal	C18H34O	9.9	94
14.486	**Stigmasterol**	C29H48O	3.9	93	13.257	***Tetracosane***	C24H50	3.2	98
14.592	Unknown		3.6		13.71	**Oxirane, hexadecyl-**	C19H38O	10	98
14.715	***.gamma.-Sitosterol***	C29H50O	10	90	14.357	Tridecanal	C13H26O	4.9	87
14.774	**Stigmastanol**	C29H52O	6.8	93	14.592	Unknown		3.6	
14.862	Unknown		2.6		14.715	***.gamma.-Sitosterol***	C29H50O	14	91
14.921	Unknown		3.7		14.786	Unknown		6.7	
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15.274	Unknown		5.3		14.986	Unknown		2.8	
15.698	Unknown		10		15.139	Unknown		3.9	

15.78	**Friedelan-3-one**	C30H50O	4	95	15.268	Stigmast-4-en-3-one	C29H48O	3.4	90
Soil M									
11.969	**Cyclohexadecane**	C16H32	28	94	2.334	**Toluene**	C7H8	7.3	95
12.298	Sulfurous acid, octadecyl 2- propyl ester	C21H44O3S	1.6	91	3.692	Unknown		320	
12.416	***1,19-Eicosadiene***	C20H38	2.9	95	4.045	**Ethanol, 2-butoxy-**	C6H14O2	7.1	91
12.622	**Docosane, 5-butyl-**	C26H54	23	91	4.587	**Benzene, 1-ethyl-2-methyl-**	C9H12	8.1	95
12.786	1,3-Benzenedicarboxylic acid, bis (2-ethyhexyl) ester	C24H38O4	220	91	4.875	**Benzene, 1,2,3-trimethyl-**	C9H12	9.9	97
12.945	Cyclotetradecane	C14H28	1.8	87	5.092	**Mesitylene**	C9H12	8.4	90
13.069	(Z)-14-Tricosenyl formate	C24H46O2	20	93	5.669	**Undecane**	C11H24	5.8	96
13.269	***1-Heptacosanol***	C27H56O	26	93	5.822	**Benzene, 1,2,4,5-tetramethyl**	C10H14	3.6	96
13.316	2-Heptacosanone	C27H54O	2.3	93	8.439	Unknown		3.4	
13.716	**Oxirane, hexadecyl-**	C19H38O	21	91	11.98	**Hexadecane**	C16H34	4.8	93
13.886	**Tricosane**	C23H48	8.7	95	12.621	1-Heptadecene	C17H34	6.7	93
13.916	1-Docosanol, methyl ether	C23H48O	8.8	87	13.068	13-Docosen-1-ol, (Z)-	C22H44O	4.7	96
13.957	2-Nonacosanone	C29H58O	4.6	91	13.257	**Tetracosane**	C24H50	9	97
14.027	**Oxirane, heptadecyl-**	C19H38O	1.7	94	13.709	*1,19-Eicosadiene*	C20H38	7.6	93
14.363	**Octadecanal**	C18H36O	10	93	13.886	Hentriacontane	C31H64	4.6	95
14.545	Heneicosane, 11-decyl-	C31H64	2.7	95	13.915	*1-Heptacosanol*	C27H56O	2.7	91

14.592	**1-Heptacosanol**	C27H56O	4.3	94	13.951	2-Dotriacontanone	C32H64O	3.3	86
14.639	Unknown		2.5		14.027	Unknown		2.7	
14.715	**.beta.-Sitosterol**	C29H50O	5.5	90	14.356	Unknown		6.1	
14.863	Unknown		2.6		14.539	Eicosane	C20H42	3	
15.157	**1,19-Eicosadiene**	C20H38	2.4	93	14.721	Unknown		4.2	
15.263	Unknown		1.6		14.786	Unknown		2.9	
15.527	Unknown		1.6		14.862	Unknown		3.9	
15.668	Unknown		3.2		14.986	Lup-20(29)-en-3-one	C30H48O	3.1	
15.78	**Friedelan-3-one**	C30H50O	3.9	97	15.127	Unknown		2.8	86
Soil N									
7.869	Cyclododecane	C12H24	7.5	96	2.328	**Toluene**	C7H8	8.1	95
8.916	Dodecyl acrylate	C15H28O2	15	91	3.693	Unknown		340	
10.286	Unknown		7.4		4.045	**Ethanol, 2-butoxy-**	C6H14O2	7.9	91
11.969	**Cyclohexadecane**	C16H32	25	95	4.587	**Benzene, 1-ethyl-3-methyl-**	C9H12	9.4	97
12.621	Nonadecyl pentafluoropropionate	C22H39F5O2	29	91	4.875	**Benzene, 1,2,3-trimethyl-**	C9H12	12	97
12.774	1,3-Benzenedicarboxylic acid, bis (2-ethyhexyl) ester	C24H38O4	79	91	5.092	**Benzene, 1,2,4-trimethyl-**	C9H12	10	93
13.068	**Oxirane, heptadecyl-**	C19H38O	14	94	5.669	**Undecane**	C11H24	5.4	93
13.263	Docosane, 5-butyl-	C26H54	34	93	11.969	Heptafluorobutyric acid, pentadecyl ester	C19H31F7O2	6.3	94
13.715	***Oxirane, hexadecyl-***	C19H38O	28	91	12.621	Nonadecyl trifluoroacetate	C21H39F3O2	13	87

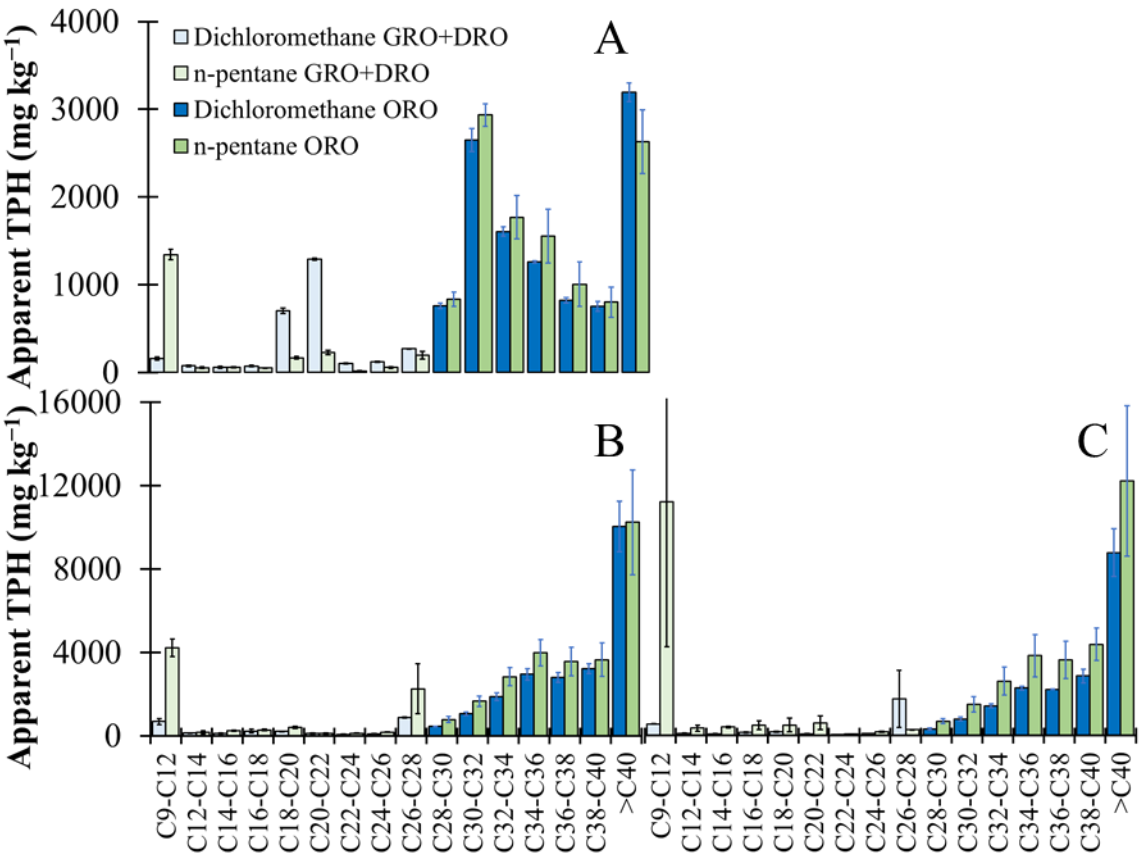
13.886	**Hexadecane, 1-iodo-**	C16H33I	17	91	12.774	Unknown		92	
13.915	Unknown		18		13.068	Heptadecanal	C17H34O	7.2	94
13.957	Unknown		8.1		13.257	Docosane, 11-butyl-	C26H54	24	95
14.033	1-Eicosyne	C20H38	12	93	13.71	***Octadecanal***	C18H36O	18	91
14.18	Unknown		6.1		13.886	Hexadecane	C16H34	15	93
14.362	***Octadecanal***	C18H36O	22	91	13.915	Unknown		9.7	
14.433	Unknown		5.4		13.957	2-Heptacosanone	C27H54O	5.4	90
14.486	***Stigmasterol***	C29H48O	6.7	96	14.033	Unknown		5.5	
14.545	*Heneicosane, 11-pentyl-*	C26H54	9.1	94	14.357	***Oxirane, hexadecyl-***	C19H38O	7.3	87
14.592	1-Docosanol, methyl ether	C23H48O	10	99	14.392	Unknown		4.1	
14.645	Unknown		9.3		14.486	*Stigmasterol*	C29H48O	4	99
14.721	Unknown		13		14.545	*Heneicosane, 11-pentyl-*	C26H54	7.2	93
14.774	Unknown		7.1		14.592	1-Heneicosyl formate	C22H44O2	3.8	87
14.857	Unknown		7.4		14.639	Unknown		4.2	
15.162	Pentadecanal-	C15H30O	8.9	90	14.715	**.beta.-Sitosterol**	C29H50O	8.6	95
15.527	Unknown		5.3		14.768	18.alpha.-Olean-3.beta.-ol, acetate	C32H54O2	3.9	87
Soil O									
11.968	1-Heneicosanol	C21H44O	9.5	95	2.328	**Toluene**	C7H8	8.5	95
12.621	1-Dodecanol, 2-octyl-	C20H42O	5	91	3.693	Unknown		380	
12.674	**2-Pentacosanone**	C25H50O	2.6	91	3.916	**Benzene, 1,3-dimethyl-**	C8H10	6.4	97
12.78	Unknown		130		4.046	**Ethanol, 2-butoxy-**	C6H14O2	8.7	90

13.257	Heptadecane, 9-octyl-	C25H52	4.8	93	4.581	**Benzene, 1-ethyl-2-methyl-**	C9H12	10	95
13.321	2-Heptacosanone	C27H54O	4.1	94	4.657	**Mesitylene**	C9H12	4.8	97
13.709	**Tetradecanal**	C14H28O	4.9	94	4.875	**Benzene, 1,2,3-trimethyl-**	C9H12	12	97
13.745	Unknown		7		5.093	**Benzene, 1,2,4-trimethyl-**	C9H12	10	93
13.886	**Hexadecane, 1-iodo-**	C16H33I	9.4	90	5.493	Unknown		7	
13.915	**1-Heptacosanol**	C27H56O	19	91	5.569	Benzene, 1-ethyl-2,4-dimethy	C10H14	6.3	97
13.957	Unknown		8.9		5.669	**Undecane**	C11H24	5.6	96
14.039	Unknown		4		5.822	**Benzene, 1,2,3,4-tetramethyl**	C10H14	3	96
14.362	Unknown		3.7		6.034	Benzene, 4-ethyl-1,2-dimethy	C10H14	2.6	92
14.392	Campesterol	C28H48O	4.2	95	8.439	Unknown		3.4	
14.486	Unknown		3.8		13.71	Unknown		1.4	
14.539	Unknown		12		13.88	Unknown		1.5	
14.592	**Cyclohexadecane**	C16H32	5.6	94	13.927	Unknown		1.9	
14.639	2-Tetratriacontanone	C34H68O	7	90	14.039	Unknown		2.4	
14.721	Unknown		13		14.198	Unknown		2.2	
14.774	**Stigmastanol**	C29H52O	8.4	91	14.357	Unknown		1.6	
14.921	Unknown		11		14.468	Unknown		2	
15.092	Unknown		4		14.527	Unknown		1.4	
15.527	Unknown		3.8		14.645	Tetratriacontane	C34H70	1.5	91
15.698	Unknown		5		14.721	Cholestan-3-ol, 5-chloro-6-nitro-(3.beta.,5.alpha.,6.beta.)-	C27H46ClNO3	2.5	9

15.78	Unknown		4		14.921	9-O-Pivaloyl-N-acetyl colchinol	C25H31NO6	2.4	37

APPENDIX F

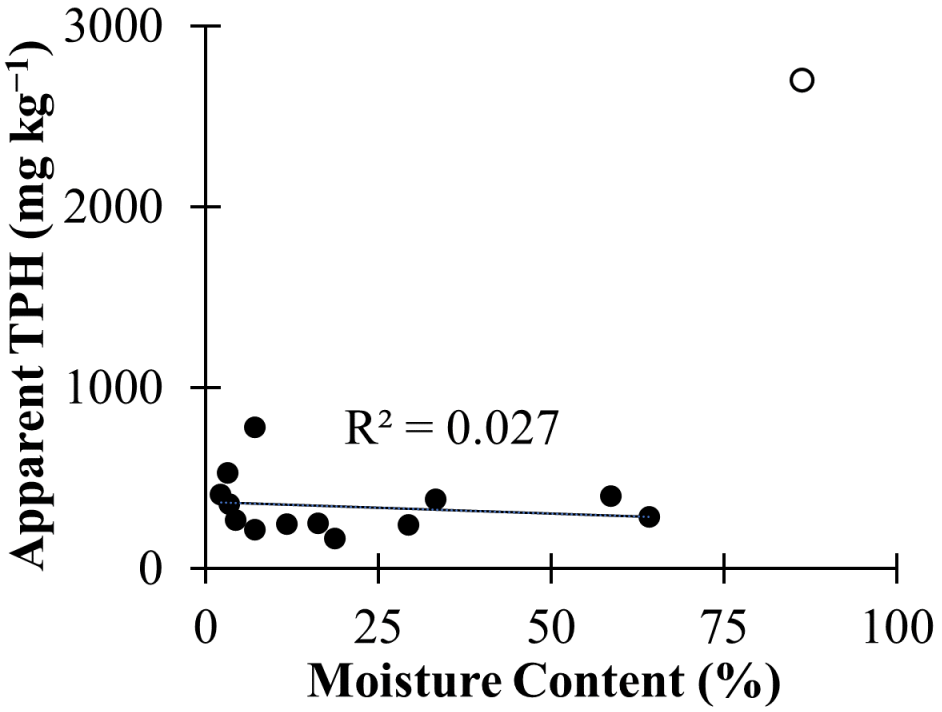
DISTRIBUTION OF NATURAL TPH CONCENTRATIONS AS A FUNCTION OF CARBON RANGE FOR LEAVES AND MICROBIAL BIOMASS



Appendix F. Distribution of total petroleum hydrocarbons (TPH) concentration as a function of carbon ranges in dichloromethane and *n*-pentane extracts from leaves (A), *E. coli* culture (B), and mixed soil culture (C). Note that (A) is on a different y axis scale. The data are averages with standard deviation of triplicate extractions.

APPENDIX G

CORRELATION OF MOISTURE CONTENT OF SOILS WITH NATURAL TPH CONCENTRATIONS



Appendix G. Linear regression analyses between the natural TPH concentration and moisture content. The white-filled datum (Soil O) was not included in the regressio