Int. J. Environ. Sci. Technol. (2014) 11:1197–1206
DOI 10.1007/s13762-014-0575-2

ORIGINAL PAPER

Landfarmed oil sludge as a carbon source for *Canavalia ensiformis* during phytoremediation

D. Ramirez · J. Dussan

Received: 25 August 2011 / Revised: 2 February 2013 / Accepted: 15 March 2013 / Published online: 9 April 2014

Abstract Petroleum exploitation in oilfields, especially drilling, generates an oily sludge mixed with hydrocarbons and mineral solids. This oily sludge is sometimes treated by bioremediation and phytoremediation. This investigation established that landfarmed oil sludge provided adequate soil conditions to grow jack beans (*Canavalia ensiformis*) that in turn rhizo- and phytoremediated residual aliphatic and aromatic hydrocarbons in the soil. Landfarming oily sludge adequately reduced jack bean phytotoxicity. Rhizo- and phytodegradation reduced total petroleum hydrocarbons by 57.38 % during 4 months of growing jack beans. Aliphatic hydrocarbons were detected in the roots but not in the aerial parts. Polycyclic aromatic hydrocarbons were translocated to the roots, stems, leaves, and beans, requiring successive cropping to manage all risks associated with some aromatic hydrocarbons found such as: acenaphthylene, anthracene, pyrene, benzo(a)anthracene, benzo(k)fluoranthene, and benzo(a)-pyrene. Landfarming and phytoremediation, perhaps with successive crops, holds the promise of providing inexpensive management of extensive oily wastes when sufficient land is available.

Keywords Bioremediation · Jack beans · Total petroleum hydrocarbons · Polycyclic aromatic hydrocarbons · Rhizodegradation

D. Ramirez (✉) · J. Dussan
Centro de Investigaciones Microbiológicas (CIMIC), Universidad de Los Andes, Cra 1 N. 18 A-10, Bogotá, Colombia
e-mail: diego.ramirez.guerrero@gmail.com

Introduction

Petroleum drilling generates considerable volumes of sludge mixed with hydrocarbons and mineral solids, which is extracted to the surface of the oil field. Once extracted, the sludge has no further use and represents a large waste product in the oil industry (Dibble and Bartha 1979). This waste can be incinerated and buried in secure landfills, but the process is expensive (Vasudevan and Rajaram 2001). Sometimes the waste is dumped directly into the environment causing pollution of the soil and groundwater. A cost-effective and environmentally acceptable alternative is landfarming. After total petroleum hydrocarbons (TPHs) are reduced to tolerable levels to plants by landfarming, phytoremediation can be used to complete the treatment (Kuyukina et al. 2003; Muratova et al. 2008).

Phytoremediation is a low-cost process that consists of the removal or stabilization of contaminants from a site through the use of plants (Schnoor et al. 1995; McCutcheon and Schnoor 2003). The different types of phytoremediation include phytoextraction, phytodegradation, phytostabilization, and phytovolatilization among others (Meagher 2000; McCutcheon and Schnoor 2003; Pulford and Watson 2003; Singh et al. 2007).

Phytoremediation can be considered as a final stage of a sustainable decontamination process (McCutcheon and Schnoor 2003; Muratova et al. 2008). While microorganisms mineralize some organic contaminants, plants tend to act like green livers that transform a broader range of organic contaminants into benign or much less toxic by-products, including basic building blocks of life; or they may enhance rhizosphere biodegradation by rhizosphere microbial communities (McCutcheon and Schnoor 2003). Given the differences

between the stoichiometry of plants and microorganisms, plant transformation may produce fewer by-products that are toxic to animals (Schnoor et al. 1995; McCutcheon and Schnoor 2003).

Aromatic hydrocarbons can be produced in the processes of bioremediation and phytoremediation of petroleum hydrocarbons. Both microorganisms and plants degrade those many times at slower rates. The slower phytotransformation ensures only temporary accumulation of hydrocarbons, for which successive cropping may be necessary to achieve sustainable management. This can ensures that the hydrocarbons can be degraded and phytotransformed (Allard and Neilson 1997; Harms et al. 2003; Hutchinson et al. 2003; McCutcheon and Schnoor 2003).

Legumes are used in phytoremediation because they do not compete with rhizosphere microorganisms for nitrogen due to their ability to fix this chemical element (Gudin and Syratt 1975). The nodulation could also be an important source of hydrocarbon-degrading bacteria (Radwan et al. 2007). Plant uptake and metabolism are influenced and controlled by molecular configuration and size of the chemical compound; the capability of the plant species is also a strong influence in the uptake process (Harms et al. 2003). Jack beans have been used in the phytoremediation of cadmium-contaminated sites (Andrade et al. 2005). Consequently, the current study investigated whether soil treated with landfarmed oil sludge provided an extra supplement of a carbon source for jack beans during the phytoremediation of the residual aliphatic and aromatic hydrocarbons from the sludge. This study was conducted from October 2009 to March 2010. The study was conducted in the "Centro de Investigaciones Microbiológicas (CIMIC)" of the Universidad of Los Andes. The field study was located in the town of Anapoima, Cundinamarca, Colombia.

Materials and methods

Plants and oil sludge

The jack beans seeds (*Canavalia ensiformis*) were sown in a greenhouse. The mixture of soil for germination contained one kilogram of Canadian Sphagnum peat moss per ten kilograms of soil. Plants were transplanted 2 weeks after germination to the field site. At this time, the jack beans had only their characteristic heart-shaped cotyledons. The plants were watered in the early morning every day in the field site.

The oil sludge used in this study came from a western Colombian oilfield. The oil sludge used for the study was landfarmed in 2 months.

Preparation of the site of study

The in-field study was carried out in Anapoima, Cundinamarca, Colombia (GPS coordinates: 4°33′13″N and 74°32′22″W). Anapoima has a warm, dry climate with a temperature between 22 °C and 28 °C. The annual precipitation is 1,300 mm. It is 710 m above sea level. Two plots were prepared at the site, each one with 2.16 m × 1.30 m of dimensions. One plot was oil sludge treated (150 kg of landfarmed oil sludge was poured on the plot), and the other plot, the control, was humus amended with 8.42 kg m^2 in the entire plot (3 kg of humus per m^2). Jack beans were transplanted into both plots; each plot had 80 plants. The period of the study from the transplantation to the final analysis of chromatographies was 4 months.

Initial physicochemical analysis of treatment soils

The physicochemical analysis of the soil before the transplantation included: pH determination, boron-level determination using colorimetry; calcium, copper, total iron, magnesium, manganese, molybdenum, potassium, and zinc contents were determined by atomic absorption spectroscopy; phosphorus and total nitrogen levels were determined by spectrophotometry and the Kjeldahl methods, respectively. The soil samples were stored in aluminum bags at 4 °C until analysis was carried out.

Morphological analysis of plants

Twenty plants were selected randomly in situ for height measurement to the tendrils tips (first measurement: after transplantation; second measurement: 53 days after transplantation). No measurements were made at the end of the study due to excessively long tendrils. Five leaves corresponding to each measureable branch level were collected and pressed using the method described by Barbosa (1984). Measurements were taken three times, at 15-day intervals. The leaf area was calculated with ImageJ (Rasband 1997). Leaves were scanned using a printer-scanner (EPSON© Stylus CX4700) at a resolution of 400 dots per inch. Brightness and contrast were 66 and −19, respectively. The equivalent pixels measured per centimeter were standardized using 70 repetitions. The most frequent measure of 154.76 pixels per centimeter was used to calculate the leaf area in cm^2. Statistical analysis of the treatment effects were evaluated using Statistix 8.0 (Analytical Software, 1998–2003). Student's t test was used in order to compare the treatment methods.

Determination of hydrocarbons in soil and in plants from the oil sludge treatment plot

Total petroleum hydrocarbons in the soil were determined at the beginning and at the end of the study. Ten subsamples of 10 cm of depth, each of 100 g, were taken in each plot following a zigzag pattern. The subsamples were mixed in order to have a representative sample (1,000 g) of the soil. The petroleum hydrocarbons were extracted from soil according to Schwab et al. (1999). The sample weights for the chromatographic analysis were 1.1 and 0.93 g for the initial and final soil sampling, respectively. The soil sample of the beginning of the on-field study was diluted 1:10. The soil sample corresponding to the end of the study was diluted 1:5 because fewer TPHs were expected. Analysis was carried out using a gas chromatography–flame ionization detector (GC–FID). The plants were stored in aluminum bags at 4 °C prior to analysis.

One hundred grams of roots, stems, leaves, and bean pods were taken from the study field at the end of the experiment and stored in aluminum bags at 4 °C prior to analysis. Plant samples were sectioned and processed. The respective weights used for the chromatographies of roots, stems, leaves, and bean pods were 10.57, 10.34, 10.23, and 13.72 g, respectively. The seeds and the bean pods were crushed and mixed. TPHs and polycyclic aromatic hydrocarbons (PAHs) were identified separately using a gas chromatography–mass spectrometry–flame ionization detector (GC–MS–FID) as described by Cofield et al. (2007). Plant samples were previously washed gently with distilled water to clean their surfaces and to remove, as far as possible, compounds that could interfere with the analysis. Approximately 10–13 g of each plant part (wet weight) was weighed. The plant samples were macerated in distilled water using a mortar. A silica column with 100 ml of hexane and 100 ml of dichloromethane was used to separate TPHs and PAHs from plant secondary metabolites. Chlorophyll and other plant metabolites remained in the superior part of the column. PAHs were located in the middle of the column with the dichloromethane phase, and the TPHs were eluted with the hexane. The peaks of the chromatograms were integrated with specialized software to identify their areas and intensities (μV). In order to identify the peaks properly and to differentiate them from the secondary metabolites, a diesel range organics and PAHs range standard was constructed for the chromatography of TPHs and PAHs, respectively. The reduction percentage for each hydrocarbon molecule designated by atoms from the oil sludge was

$$[(\text{Oil sludge treatment}_{\text{initial}} - \text{Oil sludge treatment}_{\text{final}}) * 100]/\text{Oil sludge treatment}_{\text{initial}} \quad (1)$$

where $\text{Oil sludge treatment}_{\text{initial}}$ is the initial concentration of TPHs in ppm and $\text{Oil sludge treatment}_{\text{final}}$ corresponds to the final concentration of TPHs in ppm.

Results and discussion

Crop monitoring

After the third branching, the plants grew tendrils. No visual evidence of phytotoxicity or nutritional deficiencies was observed in either treatment (data not shown). At the end of the study, most of the tendrils had reached immeasurably large heights in both the oil sludge and humus treatments. When the roots were removed from the soil for chromatographic analysis, some nodules were found for both treatments.

Plant height and leaf area comparison of oil sludge and humus treatments

The initial plant height after transplantation (first measurement) and the height after 53 days (second measurement) were measured. The average height of the oil sludge treatment plants was different from the average height of the humus treatment plants ($p < 0.05$) in the first measurement. There were no significant differences between the plant height average of both treatments in the second measurement ($\alpha = 0.05$, $p = 0.4940$).

The leaf area was measured three times during the experiment. Initially, no differences existed between the mean of leaf area of the first branch of both treatments ($\alpha = 0.05$, $p = 0.4499$). During the second measurement 2 weeks later, the mean leaf area at the first and the third branch levels was not significantly different ($\alpha = 0.05$; $p = 0.3626$, $p = 0.1534$, respectively). However, significant differences for the second branch ($\alpha = 0.05$, $p = 0.0098$) were measured. The mean of leaf area for the oil sludge treatment (84.03 cm^2) was greater than the mean leaf area for the humus treatment (92.98 cm^2). No significant differences in the leaf area of the first three branches ($\alpha = 0.05$; $p = 0.2765$, $p = 0.4595$, $p = 0.2304$) were observed during the third measurement made 2 weeks after the second. These lack of differences in height and leaf area between the plants of both treatments implied that the landfarmed oil sludge does not affect the jack beans. This implication was supported by the

previously mentioned lack of phytotoxicity from the soil amended with oil sludge.

Oil sludge and humus treatment soils: initial physicochemical analysis

The initial physicochemical analysis for both treatment soils is shown in Table 1. Macroelements such as nitrogen, potassium, phosphorus, calcium, and magnesium were higher in the humus-treated soil, because of the higher organic matter in this treatment. Table 1 shows that none of the microelements were toxic for plants in either treatment; none of the plants showed symptoms of nutritional deficiency or phytotoxicity. The soil pH in both treatments was close to neutral (6.3 in humus-amended soil and 6.9 in soil treated with oil sludge), so most of the nutrients were not limited by pH. In fact, the greatest availability for most nutrients ranges between pH 6 and 7. However, most minerals are more soluble in soils with lower pH than in soils with neutral or alkaline pH (USDA-NRCS 1999). Despite the higher proportions of elements in the humus-treated soil, the sludge-treated soil had acceptable levels of minerals and nutrients.

Total petroleum hydrocarbons chemical analysis for oil sludge-treated soils and plants

The initial TPHs in the oil sludge-treated soil were 27,647 mg kg^{-1}, and the final level was 11,781.9 mg kg^{-1}.

Fig. 1 Differential comparison of TPHs GC–MS chromatograms of peak areas and intensity for oil sludge-amended initial soil condition (**a**), oil sludge-amended final soil condition (**b**), and oil sludge-amended final plant roots condition (**c**). The peaks of saturated hydrocarbons are indicated. The peaks of unsaturated hydrocarbons (<C-*n*, where *n* is the number of carbon atoms and C states for carbon) are indicated ▶

According to these values, the percentage reduction in TPHs was 57.38 %. The chromatograms of initial and final oil sludge-treated soil are shown in Fig. 1a, b, respectively. The initial oil sludge-amended soil characterization after the oil sludge was landfarmed (Fig. 1a) showed the presence of unsaturated hydrocarbons represented by peaks of greater intensities than almost all of the saturated hydrocarbons. Peaks from molecules with 10–28 carbon (C) atoms are presented in the chromatogram in Fig. 1a. Figure 1a presents an important peak with a high intensity of saturated hydrocarbons with 17 carbon atoms (C-17). Several unsaturated hydrocarbons are also shown in the chromatogram. These unsaturated hydrocarbons have the highest intensities and area. Few representative saturated linear hydrocarbons were present (Fig. 1a). There were also several peaks between C-19 and C-20.

The peaks for the TPHs chromatogram from the oil sludge-treated final soil condition decreased notably compared with the initial characterization (See the *y*-axis scale in Fig. 1b compared to Fig. 1a). The first peaks (Fig. 1b) from C-10 appeared in less retention time than was the case for the chromatogram in Fig. 1a. All the high unsaturated

Table 1 Characterization of oil sludge- and humus-amended soil

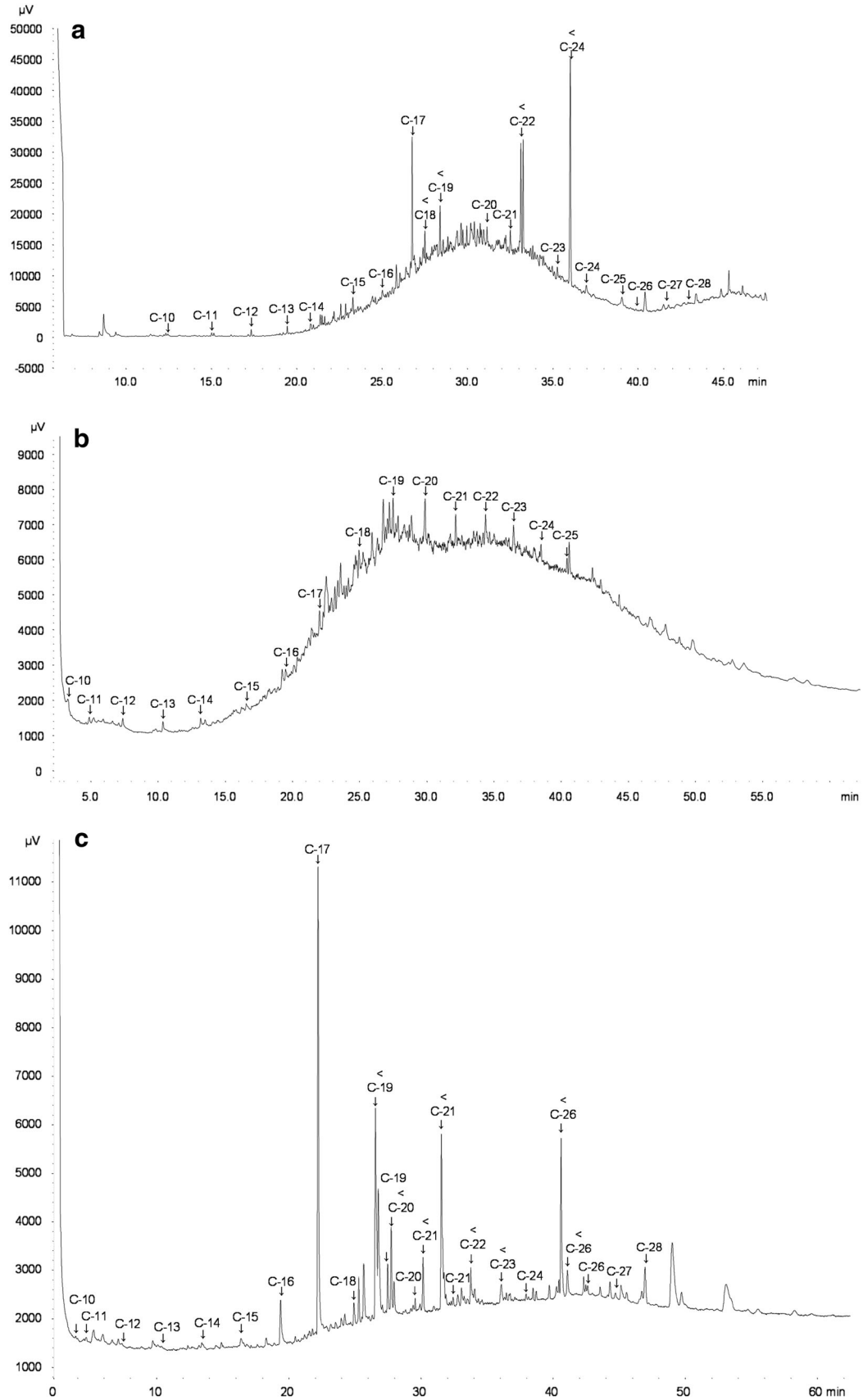
Physicochemical variable[a]	Soil		Plant nutrient deficiency[b]	Phytotoxicity of micronutrients[c]
	Oil sludge-amended	Humus-amended		
Boron	0.19 mg kg^{-1}	0.38 mg kg^{-1}	<20[d]	50–200 (M)
Copper	0.50 mg kg^{-1}	0.60 mg kg^{-1}	<4	20–100 (MH)
Phosphorus	0.87 mg l^{-1}	1.42 mg l^{-1}	<0.2	Not applicable
Molybdenum	< 4.90 mg l^{-1}	< 4.90 mg l^{-1}	<0.5	10–50 (M)
Manganese	6.00 mg kg^{-1}	10.00 mg kg^{-1}	<20[d]	250–500 (LM)
Total iron	6.10 mg kg^{-1}	86.90 mg kg^{-1}	<10–80	>1,000 (L)
Zinc	29.70 mg kg^{-1}	70.00 mg kg^{-1}	<20	100–400 (LM)
Magnesium	93.20 mg kg^{-1}	247.80 mg kg^{-1}	<20	Not applicable
Potassium	155.30 mg kg^{-1}	415.00 mg kg^{-1}	Not available	Not applicable
Total nitrogen	412.83 mg l^{-1}	691.70 mg l^{-1}	Not available	Not applicable
Calcium	1,317.00 mg kg^{-1}	3,959.00 mg kg^{-1}	Not available	Not applicable

[a] Units are in mg kg^{-1} (ppm). Except for molybdenum, phosphorus, and total nitrogen, which are presented in mg l^{-1} (ppm)

[b] From Hodges (2010). The units are in mg kg^{-1} (ppm)

[c] The letters in parentheses indicate the level of phytotoxicity of microelements: H (high), M (moderate), and L (low). The units are in μg g^{-1} leaves dry weight (ppm). From Kabata-Pendias and Pendias (1992). Adapted from Huheey (1972) and Hodges (2010)

[d] Value for soybeans (Fabaceae). Value for boron from Kelling (1999)


a
μV
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
-5000
C-10
C-11
C-12
C-13
C-14
C-15
C-16
C-17
C-18
C-19
C-20
C-21
C-22
C-23
C-24
C-25
C-26
C-27
C-28
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
min
b
μV
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
C-10
C-11
C-12
C-13
C-14
C-15
C-16
C-17
C-18
C-19
C-20
C-21
C-22
C-23
C-24
C-25
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0
55.0
min
c
μV
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
C-10
C-11
C-12
C-13
C-14
C-15
C-16
C-17
C-18
C-19
C-20
C-21
C-22
C-23
C-24
C-26
C-27
C-28
0
10
20
30
40
50
60 min

peaks that occurred in the initial chromatogram (Fig. 1a) were reduced in intensity in the final chromatogram (Fig. 1b). These reductions suggest that the unsaturated hydrocarbons were partially degraded and converted to saturated hydrocarbons as shown in Fig. 1b. The peak of the highest intensity between the C-17 saturated hydrocarbons in Fig. 1a decreased from 32,500 (Fig. 1a) to 4,500 μV (Fig. 1b). Saturated hydrocarbons can be converted into unsaturated compounds, and it sometimes can be done by anaerobic biodegradation (Widdel and Rabus 2001). On the other hand, unsaturated aliphatic and aromatic hydrocarbons can be degraded aerobically and anaerobically (Widdel and Rabus 2001). Low molecular weight petroleum compounds are easier to degrade than higher molecular weight petroleum hydrocarbons (Maila and Cloete 2004). The unsaturated peaks in the initial sample of oil sludge-amended soil were degraded into shortened chain hydrocarbons.

Table 2 shows the percentage of reduction in the concentration of each saturated hydrocarbon including the respective less unsaturated hydrocarbons from the initial to final measurement in the oil sludge-amended soil. It also shows the reduction in the concentration of unsaturated hydrocarbons higher than C-28. Despite the different initial concentrations, greater reductions occurred for saturated hydrocarbons and their respective less unsaturated hydrocarbons carbon atoms (C-) C-10, C-14, and C-15 hydrocarbons, with 94.56, 90.22, and 86.44 %, respectively (Table 2). The higher initial concentrations corresponded to hydrocarbons ranging from C-15 saturated hydrocarbons and their respective less unsaturated hydrocarbons carbon atoms to the C-25 saturated hydrocarbons and their respective less unsaturated hydrocarbons carbon atoms, the percentage of concentration reduction tending to be more than 50 %. An increase in the percentage of saturated hydrocarbons and their respective less unsaturated hydrocarbons carbons atoms C-26, C-27, and C-28 occurred with concentration increases of 482.40, 425.98, and 149.49 %, respectively. The dramatic increase in the percentage of saturated hydrocarbons and their respective less unsaturated hydrocarbons carbons atoms C-26, C-27, and C-28 could be explained by the degradation of molecules with more than 28 carbon atoms as these molecules can be degraded microbially, chemically, or by green plants (Harms et al. 2003). The degradation of unsaturated hydrocarbons is probably difficult due to the presence of double and triple bonds. Some reduction in hydrocarbons could be attributed to degradation processes like volatilization and photo-oxidation by solar radiation (Kuyukina et al. 2003; Lee 2003).

Table 2 Reduction in the concentration of each range of saturated hydrocarbons and their respective less unsaturated hydrocarbons from oil sludge-amended soil

Number of carbon atoms[a]	Concentration (mg kg^{-1})		Reduction (%)
	Initial	Final	
≤10	140.5	7.6	94.6
≤11	14.4	7.6	47.1
≤12	27.8	20.2	27.5
≤13	35.3	11.7	66.8
≤14	212.7	20.8	90.2
≤15	800.7	108.5	86.4
≤16	1,301.4	791.5	39.2
≤17	2,085.1	581.4	72.1
≤18	3,360.0	1,038.2	69.1
≤19	3,619.3	1,254.6	65.3
≤20	3,482.1	1,247.6	64.2
≤21	3,157.9	1,124.3	64.4
≤22	2,886.4	1,048.2	63.7
≤23	2,330.2	1,002.6	57.0
≤24	1,971.2	891.6	54.8
≤25	918.4	758.3	17.4
≤26	128.8	750.1	−482.4
≤27	114.3	601.3	−426.0
≤28	180.7	450.9	−149.5
>28	1,041.7	691.6	33.6

Initial concentrations to final concentrations

[a] Each number includes the values for the saturated number of carbon atoms mentioned, and the symbol (≤) indicates the values of the corresponding unsaturated hydrocarbons

The level of TPHs in plant roots was 64.59 mg kg^{-1}. This quantity was much lower than the presented in the oil sludge-amended final soil condition (11,781.9 mg kg^{-1}). The chromatogram is shown in Fig. 1c. The peak with the greatest intensity (11,500 μV) corresponded to the saturated C-17 found in Fig. 1a, b. Except for the C-17 peak, the unsaturated peaks were more intense than the saturated ones. Much of the higher intensity peaks found in the soil initially (Fig. 1a) were found in the plant roots, indicating at least temporary accumulation of these hydrocarbons from the oil sludge during the 4 months of the study. It is important to mention that the root exudates are rich in biodegradable organic molecules that can stimulate the growth of soil microorganisms and enhance the degradation in the rhizosphere. These exudates can also improve the bioavailability of hydrophobic organic compounds (Muratova et al. 2008). This concept is known as phytoremediation *ex planta* (Alkorta and Garbisu 2001). Some mathematical models that simulate phytoremediation of oil-con-

taminated soils are based on-field data which established that the roots and the rhizosphere are key locations for contaminant transformation. The greater degradation rate of the contaminant adjacent to plant roots occurs because of the increased multiplication of soil microorganisms feeding on root exudates (Thoma et al. 2003). It could take time for the plant to degrade these contaminants; thus, phytoremediation assays showed that this is a long-term process (Carman et al. 1998). The fact that the plants accumulate TPHs in the roots is important because it avoids two important problems in phytoremediation: the biomagnification of contaminants in the food chain and the transpiration (volatilization through leaves, stems, and roots due to leaking membranes) of these organic compounds into the atmosphere.

The patterns of the chromatograms found in the plant stems (data not shown) were rather different from the chromatograms in Fig. 1a–c. No TPHs were found in the aerial parts of the plants with the exception of some polyaromatic hydrocarbons. The chromatograms of the aerial parts of the jack beans were characteristic of secondary metabolites compared with the chromatograms from Oh et al. (1995), Guillén and Manzanos (1998), Ruther (2000), and Canini et al. (2007).

Hydrocarbon translocation to the roots and not to the stems, leaves, and beans established that sorption to root cells was taking place in this study. Inside the roots at least three processes can occur: phytostabilization, phytodegradation, and phytoaccumulation (Schnoor et al. 1995; McCutcheon and Schnoor 2003; Singh et al. 2007). The initial and final soil chromatograms established that rhizodegradation and perhaps phytodegradation occurred. Cofield et al. (2007) indicated most of the phytoremediation of polyaromatic hydrocarbons may have occurred in the roots of fescue (*Festuca arundinacea*), switchgrass (*Panicum virgatum*), and zucchini (*Curcubita pepo*) due to the presence of greater levels of aliphatic and PAHs found in the roots.

Polycyclic aromatic hydrocarbons levels in the plant roots, stems, leaves, and bean pods exposed to oil sludge-treated soil

The detectable PAHs in the plants were the same as those identified in Cofield et al. (2007): Anthracene, pyrene, and benzo(a)anthracene found in roots and leaves of the jack beans. The following were also found: acenaphthylene (roots), benzo(k)fluoranthene (stems and beans), and benzo(a)pyrene (stems). The chromatograms of PAHs found in roots, stems, leaves, and bean pods are shown in Fig. 2a–d, respectively.

The chromatogram corresponding to jack bean roots (Fig. 2a) showed the presence of four PAHs compounds: acenaphthylene (0.627 mg kg^{-1}), anthracene (0.879 mg kg^{-1}), pyrene (1.975 mg kg^{-1}), and benzo(a)anthracene (1.567 mg kg^{-1}). The largest peaks ranged from 8,000 μV to 11,500 μV corresponded to unidentified compounds (Fig. 2a). Rhizodegradation of PAHs has been successfully demonstrated in the rhizosphere, where the levels of recalcitrant aromatic hydrocarbons diminished (Olson et al. 2003). The chromatogram of the plant stems (Fig. 2b) had two PAHs compounds: benzo(k)fluoranthene with one representative peak (0.933 mg kg^{-1}) and benzo(a)pyrene with three peaks (1.614 mg kg^{-1}). The chromatogram of the leaves also showed large unidentified peaks (Fig. 2c). The largest was 225,000 μV. Pyrene was defined by four peaks (1.975 mg kg^{-1}), larger than those of anthracene (0.879 mg kg^{-1}) and the derivative benzo(a)anthracene (1.567 mg kg^{-1}). The chromatogram of the bean pods (Fig. 2d) showed one aromatic compound, benzo(k)fluoranthene (0.757 mg kg^{-1}), with a representative peak of 10,000 μV. The chromatogram also showed an unidentified peak (72,500 μV).

Evidently, anthracene, pyrene, and benzo(a)anthracene were not degraded, because these hydrocarbons were detected in comparable quantities in the roots and in the leaves. As acenaphthylene was not found in the stems, leaves or beans, this compound may have been degraded in the roots or was not transported from the roots to the aerial parts. Yet highly complex benzo(k)fluoranthene and benzo(a)pyrene were detected in stems and beans. Only benzo(k)fluoranthene decreased (by 19 %) along the path from the roots to the beans.

Anthracene, pyrene, and benzo(a)anthracene were the same in roots and leaves. The most complex compound, benzo(k)fluoranthene, with four aromatic rings, was less present in the beans than the stems. The persistence of high molecular weight PAHs (pyrene, benzo(a)anthracene, benzo(k)fluoranthene, and benzo(a)pyrene) in the aerial parts are due to the very slow rate of aerobic biodegradation. Therefore, these compounds are recalcitrant in the environment (Olson et al. 2003). The plant uptake of PAHs is limited due to their low water solubility and high lipophilicity (Hutchinson et al. 2003). It is likely that the organic compounds taken by the plant undergo oxidation or biodegradation in vivo or conjugation and sequestration within the plant (Olson et al. 2003). The PAHs are attached to bacteria as a result of liposolubility. These

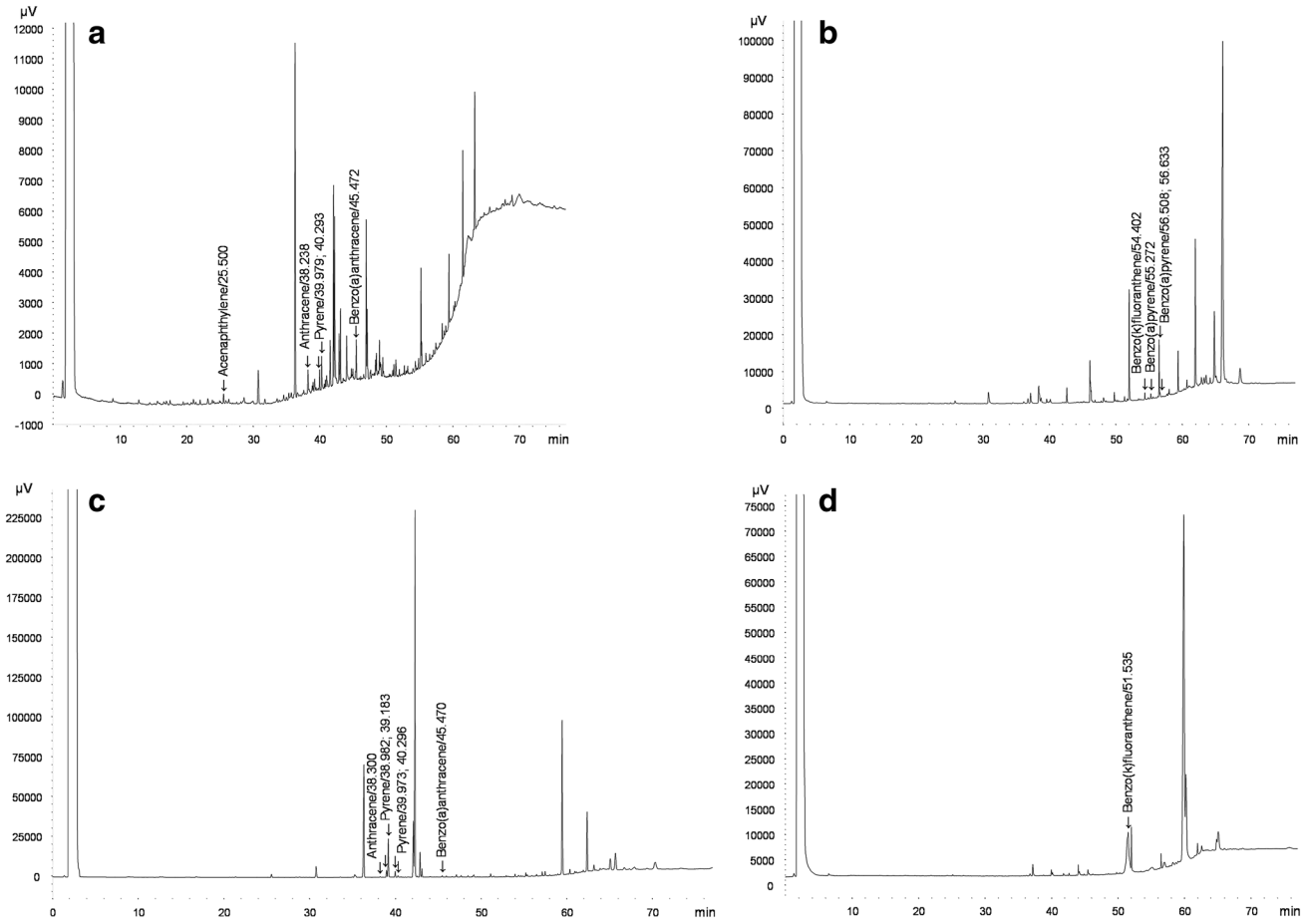


Fig. 2 Chromatograms of oil sludge-exposed plants at the end of 2 months: (a) roots, (b) stems, (c) leaves, and (d) bean pods. The peaks of the PAHs detected are indicated with the name of the compound and the retention time in minutes

aromatics compounds also undergo adsorption, volatilization, photolysis, hydrolysis, and biological transformation as in the case of the petroleum hydrocarbons (Haritash and Kaushik 2009). Benzo(a)pyrene, the most carcinogenic and toxic PAH (Harms et al. 2003), was found in the stem with one of the highest concentrations observed in this study.

Further considerations in the phytoremediation of landfarmed oil sludge

It is important to clarify that some oil levels remnant in the soil and plants will diminish with the application of crop rotation with jack beans or other plants such as grasses and legumes. Previous crop plants should be used as organic soil matter for subsequent plantings to manage hydrocarbon residuals in previous crops, while crop rotations continue to remediate the soil. In each crop rotation, it could be expected that the TPHs levels will be lower than in the previous cycle. Furthermore, it is not recommended to burn the plants because of the potential air-polluting effects. Olson and Fletcher (2000) suggested the application of plant succession in phytoremediation studies.

Conclusion

This study established that landfarmed oil sludge can be used as a source of carbon for *C. ensiformis*, at the same time as remnant petroleum is reduced by phytoremediation. In fact, it is important to make a plant uptake model in order to understand and evidence this process specifically. Aliphatic hydrocarbons remained in the roots and were not taken to the aerial parts; but aromatic hydrocarbons were presented in the stem, leaves, and bean pods. Since the jack beans did not present differences in growth between treatments and there was not phytotoxicity, jack beans can be used as phytoremediators of the landfarmed oil sludge.

Acknowledgments This study was funded by the Research Fund of the Faculty of Sciences of the Universidad de Los Andes (Funding Call 2009-2 for postgraduate research projects) and the "Centro de Investigaciones Microbiológicas" (CIMIC). We wish to thank Vladimir Ramírez (Biointech) for providing the landfarmed oil sludge and Oscar Ariza for his support in the analysis of chromatograms.

References

Alkorta I, Garbisu C (2001) Phytoremediation of organic contaminants in soils. Bioresour Technol 79(3):273–276

Allard AS, Neilson AH (1997) Bioremediation of organic waste sites: a critical review of microbiological aspects. Int Biodeterior Biodegrad 39(4):253–285

Andrade SAL, Jorge RA, Silveira APD (2005) Cadmium effect on the association of jackbean (*Canavalia ensiformis*) and arbuscular mycorrhizal fungi. Sci Agric 62(4):389–394

Barbosa CE (1984) Manual de Técnicas para Colecciones Botánicas. Instituto Nacional de los Recursos Naturales Renovables y del Ambiente (INDERENA), Bogotá, Colombia, pp 4–6

Canini A, Alesiani D, D'Arcangelo G, Tagliatesta P (2007) Gas chromatography–mass spectrometry analysis of phenolic compounds from *Carica papaya* L. leaf. J Food Compos Anal 20(7):584–590

Carman EP, Crossman TL, Gatliff EG (1998) Phytoremediation of No. 2 fuel oil-contaminated soil. J Soil Contam 7(4):455–466

Cofield N, Schwab AP, Banks MK (2007) Phytoremediation of polycyclic aromatic hydrocarbons in soil: part I, dissipation of target contaminants. Int J Phytorem 9(5):355–370

Dibble JT, Bartha R (1979) Effect of environmental parameters on the biodegradation of oil sludge. Appl Environ Microbiol 37(4):729–739

Gudin C, Syratt WJ (1975) Biological aspects of land rehabilitation following hydrocarbon contamination. Environ Pollut 8(2):107–112

Guillén MD, Manzanos MJ (1998) Study of the composition of the different parts of a Spanish *Thymus vulgaris* L. plant. Food Chem 63(3):373–383

Haritash AK, Kaushik CP (2009) Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. J Hazard Mater 169(1–3):1–15

Harms H, Bokern M, Kolb M, Bock C (2003) Transformation of organic contaminants by different plant systems. In: McCutcheon SC, Schnoor JL (eds) Phytoremediation: transformation and control of contaminants. Wiley Interscience, USA

Hodges SC (2010) Soil fertility basics [Internet]. NC Certified Crop Advising Training, North Carolina, USA. Soil science extension [cited 2010 March 2]. http://www.plantstress.com/Articles/min_deficiency_i/soil_fertility.pdf

Huheey JE (1972) Inorganic chemistry. In: McBride MB (ed) Environmental chemistry of soils. Harper and Row, New York

Hutchinson SL, Schwab AP, Banks MK (2003) Biodegradation of petroleum hydrocarbons in the rhizosphere. In: McCutcheon SC, Schnoor JL (eds) Phytoremediation: transformation and control of contaminants. Wiley Interscience, USA

Kabata-Pendias A, Pendias H (1992) Trace elements in soils and plants. In: McBride MB (ed) Environmental chemistry of soils, 2nd edn. CRC Press, Boca Raton

Kelling KA (1999) A2522 understanding plant nutrients: soil and applied boron [Internet]. University of Winsconsin, USA [cited 2010 March 2]. http://www.soils.wisc.edu/extension/pubs/A2522.pdf

Kuyukina MS, Ivshina IB, Ritchkova MI, Philp JC, Cunningham CJ, Christofi N (2003) Bioremediation of crude oil-contaminated soil using slurry-phase biological treatment and land farming techniques. Soil Sediment Contam 12(1):85–99

Lee RF (2003) Photo-oxidation and photo-toxicity of crude and refined oils. Spill Sci Technol Bull 8(2):157–162

Maila MP, Cloete TE (2004) Bioremediation of petroleum hydrocarbons through landfarming: are simplicity and cost-effectiveness the only advantages? Rev Environ Sci Biotechnol 3(4):349–360

McCutcheon SC, Schnoor JL (2003) Phytoremediation: transformation and control of contaminants. Wiley Interscience, USA

Meagher RB (2000) Phytoremediation of toxic elemental and organic pollutants. Curr Opin Plant Biol 3(2):153–162

Muratova AY, Dmitrieva TV, Panchenko LV, Turkovskaya OV (2008) Phytoremediation of oil-sludge-contaminated soil. Int J Phytorem 10(6):486–502

Oh CH, Kim JH, Kim KR, Mabry TJ (1995) Rapid gas chromatographic screening of edible seeds, nuts beans for non-protein and protein amino acids. J Chromatogr A 708(1):131–141

Olson PE, Fletcher JS (2000) Ecological recovery of vegetation at a former industrial sludge basin and its implication to phytoremediation. Environ Sci Pollut Res 7(4):195–204

Olson PE, Reardon KF, Pilon-Smits EAH (2003) Ecology of rhizosphere bioremediation. In: McCutcheon SC, Schnoor JL (eds) Phytoremediation: transformation and control of contaminants. Wiley Interscience, USA

Pulford ID, Watson C (2003) Phytoremediation of heavy metal-contaminated land by trees—a review. Environ Int 29(4):529–540

Radwan SS, Dashti N, El-Nemr I, Khanafer M (2007) Hydrocarbon utilization by nodule bacteria and plant growth-promoting rhizobacteria. Int J Phytorem 9(6):475–486

Rasband WS (1997–2011) ImageJ [Internet]. U. S. National Institutes of Health, Bethesda, Maryland, USA [updated 2011 June 24; cited 2010 Feb 15]. http://rsb.info.nih.gov/ij/

Ruther J (2000) Retention index database for identification of general green leaf volatiles in plants by coupled capillary gas chromatography–mass spectrometry. J Chromatogr A 890(2):313–319

Schnoor JL, Licht LA, McCutcheon SC, Wolfe NL, Carreira LH (1995) Phytoremediation of organic and nutrient contaminants. Environ Sci Technol 29(7):318A–323A

Schwab AP, Su J, Wetzel S, Pekarek S, Banks MK (1999) Extraction of petroleum hydrocarbons from soil by mechanical shaking. Environ Sci Technol 33(11):1940–1945

Singh RP, Dhania G, Sharma A, Jaiwal PK (2007) Biotechnological approaches to improve phytoremediation efficiency for environment contaminants. In: Singh SN, Tripathi RD (eds) Environmental bioremediation techniques. Springer, Berlin, Heidelberg

Thoma GJ, Lam TB, Wolf DC (2003) A mathematical model of phytoremediation for petroleum contaminated soil: model development. Int J Phytorem 5(1):41–55

U. S. Department of Agriculture-Natural Resource Conservation Service (USDA-NRCS) (1999) Soil quality test kit guide. Section 1. Test procedures, and Section 2. Background and interpretive guide for individual tests [Internet]. Soil Quality

Institute, Ames, Iowa [cited 2010 March 2]. http://soils.usda.gov/sqi/assessment/files/test_kit_complete.pdf
Vasudevan N, Rajaram P (2001) Bioremediation of oil sludge-contaminated soil. Environ Int 26(5–6):409–411
Widdel F, Rabus R (2001) Anaerobic biodegradation of saturated and aromatic hydrocarbons. Curr Opin Biotechnol 12(3):259–276