

Research Articles

Polycyclic Aromatic Hydrocarbons in Crude Oil-Contaminated Soil:

A Two-Step Method for the Isolation and Characterization of PAHs

Mohammed Shahid Akhlaq

German Petroleum Institute, Walther-Nernst-Str. 7, D-38678 Clausthal-Zellerfeld, Germany

Corresponding address: Dr. Mohammed Shahid Akhlaq, Institut für Erdöl- und Erdgasforschung, Walther-Nernst-Str. 7, D-38678 Clausthal-Zellerfeld, Germany; e-mail: Shahid.Akhlaq@ife-clausthal.de

Abstract

A two-step analytical method is developed for the isolation and characterization of polycyclic aromatic hydrocarbons (PAHs) in crude oil contaminated soil. In the **first step**, those crude oil components were isolated which are easily mobilized with water from the contaminated soil (determination of groundwater pollution potential). In the **second step**, the fraction containing the remaining crude oil compounds was extracted using toluene. After the cleanup of the fractions, both fractions were analyzed using high-performance liquid chromatography (HPLC).

The HPLC of the toluene extracted fraction shows that along with the sixteen priority pollutants from the US-EPA list, many other polycyclic aromatic hydrocarbons (PAHs) are present as well. It is evident from the chromatograms that a significant amount of PAHs are present as is also the case in the fractions eluted by water.

The described method allows the determination of total organic pollutants from crude oil, some of them being potential groundwater contaminants. The major part of the total pollutants could not be mobilized by water and therefore remains in the soil, which was extracted in the second step.

Keywords: Groundwater pollution; high-performance liquid chromatography (HPLC); HPLC; oil contaminated soil; petroleum hydrocarbons; PAHs; polycyclic aromatic hydrocarbons (PAHs); recovery of pollutants

1 Introduction

The polycyclic aromatic hydrocarbons (PAHs) are known components of petroleum and petroleum-derived products. The PAHs are important environmental pollutants because of their carcinogenicity (MISFELD and TIMM, 1978; HARVEY, 1985). These compounds are routinely determined in in-

dustrial wastewater, drinking water and groundwater. Regulations on these toxic chemicals are already in effect in North America and Europe. The PAHs represent a class of compounds with a high toxic potential and consequently belong to the group of priority pollutants.

High-performance liquid chromatography (HPLC) has been widely used for PAH determination. Ultraviolet (UV) absorption and fluorescence spectrometry provide sensitive and selective detection for PAHs by HPLC (ESCRIVA *et al.*, 1991; WISE *et al.*, 1993; DONG and GREENBERG, 1988; GREMM and FRIMMEL, 1990). According to the US-EPA Method 610 (US-EPA, 1982) and the DIN 38407/8 (Deutsche Einheitsverfahren, 1991), 16 and 6 priority PAHs, respectively, must be determined using HPLC in combination with UV and fluorescence detection.

The contamination of soil with crude oils and fuels cause problems for humans and the environment (DEAN *et al.*, 1995). The survey and removal of such hazardous pollutants are demanded. Seepage, aging, mobilization in water and air are some of the important factors which should be investigated. The leaching ability of oil contaminants with water from soil is of particular interest; a method to determine the groundwater pollution (AKHLAQ, 1995).

A two-step analytical method has been developed for the characterization of soil and groundwater pollution, caused by crude oil-contaminated soil. In the **first step**, those crude oil components are isolated which are easily mobilized from the contaminated soil using water. In the **second step**, the fraction containing the remaining crude oil compounds is extracted from the dry soil (DEAN *et al.*, 1995; GEERDINK *et al.*, 1995). After the cleanup of the fractions (NIRMAIER *et al.*, 1996; RIVERA *et al.*, 1996), both fractions were analyzed using the HPLC technique.

2 Experimental

2.1 Standards and Solvents

The sixteen US-EPA PAHs (used as a standard in this study) were purchased from Aldrich, Germany, and were used as received. The water was purified with a Millipore Q-filter system. The quality of this water is equal to tri-distilled water. The "analar" grade toluene and acetonitrile were supplied by Riedel de Haën, Germany. The crude oils investigated were Arabian heavy, Suria, Jakarta Arco, a North Sea crude oil and two North German crude oils. In order to compare the crude oils with fuels; a diesel fuel was also investigated.

2.2 Soil Preparation and Contamination with Crude Oil

The uncontaminated soil samples were collected from Clausthal-Zellerfeld and Goslar (Germany). After transportation to the laboratory, the soil was air-dried. All large stones and extraneous materials were removed by hand and the soil was pulverized in an electric blender for 5 minutes. The powdered soil was sieved and the 63 to 500 μm fraction was used in this study. The prepared soils were stored in air-tight containers. The 150 g of prepared soil was mixed thoroughly with 1.5 g of crude oil or diesel fuel and equilibrated at 30°C for one week in air-tight vials.

2.3 Elution with Water

Deactivated glass wool was fitted at the bottom of a glass column (800 mm x 5 mm i.d.). 150 g portions of contaminated soil were filled into this column and covered with deactivated glass wool. The soil column was eluted with 500 ml water at a flow rate of about 1 ml per minute. The resulting contaminated water was treated in a liquid-liquid-extraction procedure to extract the organic contaminants with toluene. After separation of the organic phase, the toluene solution was dried using anhydrous calcium chloride.

2.4 Soxhlett Extraction Procedure

Soxhlett extraction was performed on 10 g portions of the dry contaminated soil to which 40 g of anhydrous sodium sulphate was added. The mixture was transferred to a cellulose extraction thimble (Schleicher & Schuell, Germany) and inserted into the soxhlett assembly. This apparatus was fitted with a 250 ml flask containing 100 ml of toluene. The assembly was heated and refluxed for 6 hours. The resulting toluene extract was stored in the dark.

2.5 Cleanup of the Extracted Fractions

The toluene extracts were evaporated under vacuum and dissolved in 1 ml acetonitrile. To remove the unwanted soil matrix (humic substances) from the extracts, a C₁₈ SEP-pak cartridge (Millipore, Waters Chromatography) was used. The bonded phase of the cartridge was solvated

with 10 ml of water and 7 ml of acetonitrile. 1 ml acetonitrile solution of extracts were added to the cartridge and eluted with 10 ml acetonitrile. After the cleanup procedure, the resulting solution was analyzed using the HPLC technique.

2.6 Analysis of Extracts by High-Performance Liquid Chromatography (HPLC)

The analysis of all the extracts was carried out on a HPLC apparatus which was equipped with a quaternary pump (1050 Ti-Series), an autosampler (1050 Series) and a Degasser model (1050 Series), all from Hewlett Packard. For the detection and identification of aromatic hydrocarbons, an HP 1100 (Hewlett Packard) diode array detector (DAD) with a 10 mm flow cell was used in this study. DAD allows the identification of PAHs by means of retention times and UV-VIS spectra of the corresponding compounds. Data acquisition and data analysis were performed using HPLC-Chem-Station (DOS Series) software (Hewlett Packard). The gradient system used in this study for the separation of PAHs is shown in Table 1. The column used consisted of Nucleosil 5 C₁₈ PAH (Macherey-Nagel, Germany) 150 mm x 4.0 mm i.d. with 11 mm pre-column (Macherey-Nagel, Germany) having a particle size of 5 μm .

Table 1: HPLC gradient system (linear) for the separation of polycyclic aromatic hydrocarbons, extracted from the contaminated soils

Time (min)	Moble phase		Flow rate (ml/min)
	A (%)	B (%)	
	Water	Acetonitrile	
Start	10	90	0.50
15	10	90	0.50
20	2	98	1.00
35	2	98	1.00
	Re-equilibration		
40	10	90	0.50
50	10	90	0.50

2.7 Quantification of the isolated fractions

Since some of the isolated material was lost during the cleanup of the extracted fractions, we determined the amount of the extracted fractions gravimetrically prior to the cleanup procedure. A blank sample from the uncontaminated soil was prepared with the above mentioned method and subtracted from the contaminated samples to lead to the neat isolated amounts of contaminants in the corresponding fractions.

3 Results and Discussion

The first step in this study was the isolation of such compounds which were easily mobilized with water from the contaminated soil. In the second step, the fraction containing the remaining crude oil compounds was extracted from the dry soil with toluene. The HPLC of the toluene extracted fraction shows that many other polycyclic aromatic hydrocarbons are present as well along with the sixteen priority pollutants from the EPA list (\rightarrow Fig. 1). It is evident from the chromatograms that a significant amount of PAHs are present as well in the fraction eluted by water (\rightarrow Fig. 1, Chromatogram A).

The chromatograms of the extracted fractions in Figure 1 (Chromatograms A and B) are much more complex than the standard PAHs from the US-EPA list (Chromatogram C). The reason for the poor separation is the co-elution of many different substituted aromatics at the same time. The aliphatic compounds do not contribute to the poor resolution in the chromatograms. The aliphatic compounds do not absorb at $\lambda = 254 \text{ nm}$, so they could not be monitored at a UV wavelength of 254 nm . On the other hand, the aromatic sulphur, nitrogen and oxygen heterocyclic compounds are present in the fractions shown in Figure 1. These heterocyclic aromatic compounds have polarity similar to that of aromatic compounds and

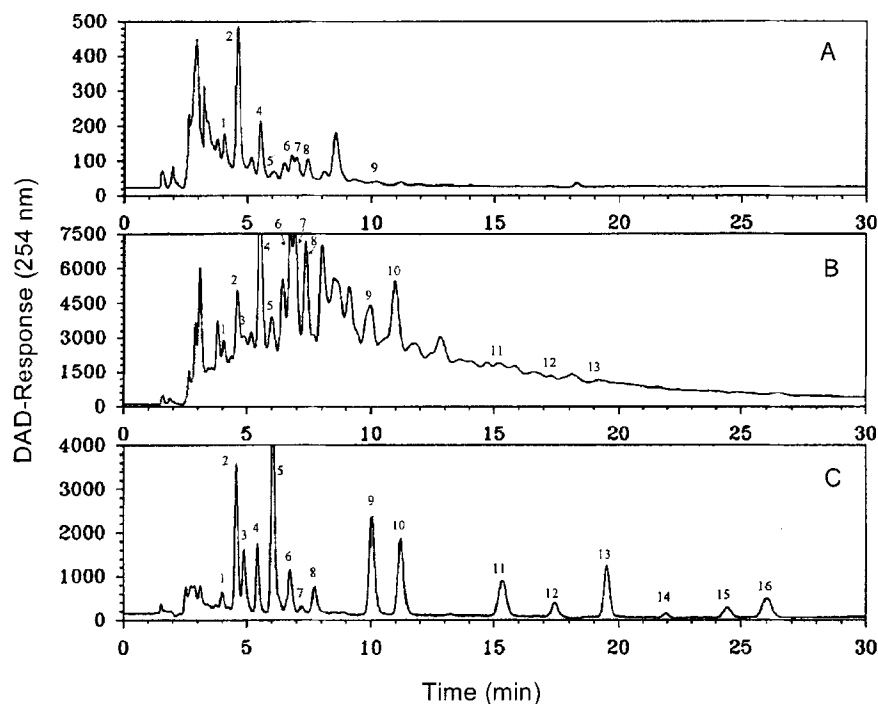


Fig. 1: HPLC of pollutants:

A: Chromatogram of the fraction extracted with distilled water.

B: Chromatogram of the fraction extracted after the water elution from the dry soil with toluene.

C: Chromatogram of the purchased 16 US-EPA PAH₈ standard dissolved in acetonitrile.

Peaks: 1 = Naphthalene; 2 = Acenaphthalene; 3 = Acenaphthene; 4 = Fluorene; 5 = Phenanthrene; 6 = Anthracene; 7 = Fluoranthene; 8 = Pyrene; 9 = Benzo(a)anthracene; 10 = Chrysene; 11 = Benzo(b)fluoranthene; 12 = Benzo(k)fluoranthene; 13 = Benzo(a)pyrene; 14 = Dibenzo(a,h)anthracene; 15 = Benzo(g,h,i)perylene and 16 = Indeno(1,2,3-cd)pyrene.

A 25 μl volume of the solutions was injected. HPLC conditions are provided in the experimental section and Table 1

The crude oils and fuels are complex mixtures containing hundreds of different aliphatic and aromatic components (AKHLAQ, 1993). The aliphatics consist of linear *n*-alkanes, branched-chain alkanes and cycloalkanes (BUNDT *et al.*, 1991), while the major aromatic components are one, two, three and four-ring alkylated derivatives (BUNDT *et al.*, 1991; AKHLAQ and GÖTZE, 1994). In addition, crude oil also contains polar compounds, resins and asphaltenes (AKHLAQ *et al.*, 1996).

Only the aromatic compounds including the 16 US-EPA PAHs were investigated in the present study, the aliphatic compounds extracted with the described method were not

they can be detected at 254 nm (AKHLAQ, 1993). In agreement, the sulphur (1.6%), nitrogen (1.1%) and oxygen (0.8%) is found in the elemental analysis of the collected HPLC fractions.

Since the aromatic compounds are known to be toxic (HARVEY, 1985), all of the aromatic components found in the extracted fractions have to be assumed to be more or less carcinogenic, even if the differences in carcinogenic potential among the PAHs cover several orders of magnitude (KALBERLAH *et al.*, 1995).

In the present study, about 4% of crude oil components were eluted with distilled water. The soxhlett extraction

with toluene of the remaining contaminants of soil resulted in 81% of total yield, e.g. 85% of the crude oil components were isolated with this combined method of water and toluene extraction. It is worth mentioning that the used soil (blank run) does not reveal any aromatic compounds in the HPLC.

To investigate the effect of soil types and the nature of crude oils on the extraction yield, six different crude oil samples and a diesel fuel were included in this study. Moreover, the extraction ability of a sandy soil and a loamy soil was investigated. The results are presented in Table 2. It is evident from Table 2 that more oil components are retained in the sandy soil than in the loamy soil. The total recovery is dependent on the type of crude oil used. The recovery of oils which are rich in colloids and polar compounds (heavy oils) is less than that of the so-called light crude oils. For example, more than 90% of the crude oil components were recovered from a light crude oil (North Sea crude oil). In the case of heavy oils, like Arabian heavy, only 65% of the crude oil substances could be isolated

Table 2: The total recovery of crude oils and diesel fuel compounds as a function of soil type. The experiments were carried out at 20°C

	Sandy Soil [%]	Loamy Soil [%]
Arabian heavy oil	60.3	65.2
Suria oil	72.8	78.6
Jakarta Arco oil	76.3	81.1
North Sea Oil	89.6	90.4
North German oil A	75.4	76.7
North German oil B	81.7	84.9
Diesel fuel	95.8	96.3

from the loamy soils. The contamination of soil with diesel fuels leads to a quantitatively extractable amount of about 96%.

The results indicate that the polar crude oil components, including resins and asphaltenes, are not recovered from the soils by using the method described. These results agree with the wettability experiments of crude oils on quartz sand where the resins and asphaltenes play a dominant role (→ Fig. 2). The adsorbed resins and asphaltenes on quartz sand can only be extracted with a sequence of organic solvents (chloroform, acetone, acetone/chloroform, and methanol/chloroform) from the solid material (AKHLAQ *et al.*, 1996).

3.1 Influence of Temperature on the Extraction Yield

To study the influence of temperature on the extraction yield, the column containing the contaminated soil as well as the elution water was thermostated at 20°C, 40°C and 60°C, respectively (elution model of contaminants for rains on the hot soil during the summer months). The elution and extraction were performed and the yields were determined. Figure 3 (see p.221) shows that the recovery of crude oil components is increased with increasing temperature. For example, about 3.6% of crude oil components were mobilized with water at 20°C. This amount was increased to 6% at 40°C and to 8% of crude oil compounds at 60°C in the fraction eluted with water. The results reflect the better solubility of crude oil components in water with increasing temperature. The experimental results infer that the mobilization of contaminants to groundwater may increase with temperature.

In agreement with the above findings, the soxhlett extraction of the remaining dry soil with toluene resulted in less amounts of contaminants for soils which were eluted previously at high temperature with water (→ Fig. 4, see p. 221), although the total extracted amount of contaminants from the water and toluene fractions only varies between 85 and 87%.

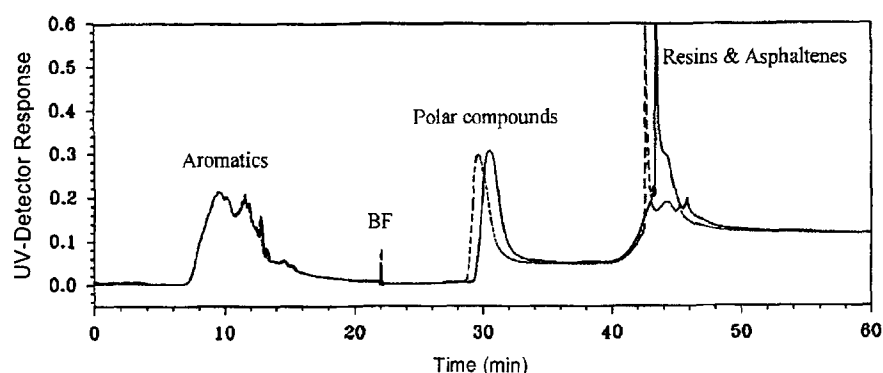


Fig. 2: HPLC of a crude oil solution in cyclohexane before and after wetting with quartz sand. (—) Crude oil solution; (---) Remaining crude oil solution after wetting. HPLC conditions are given in AKHLAQ, 1993

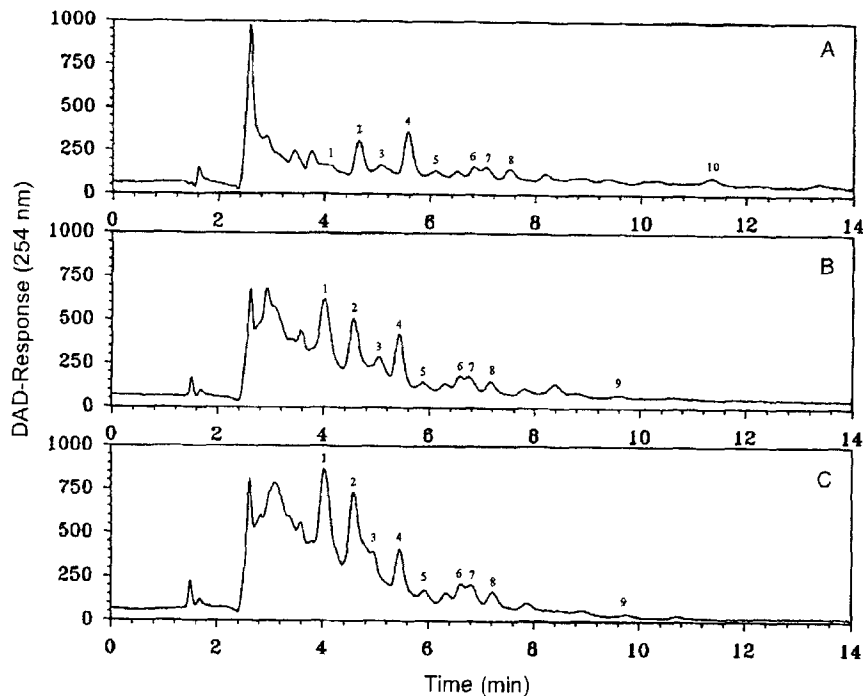


Fig. 3: HPLC of the fractions extracted with water:
 A: Chromatogram of the fraction eluted with water at 20°C.
 B: Chromatogram of the fraction eluted with water at 40°C.
 C: Chromatogram of the fraction eluted at 60°C with water. Peak identification is given in the legend of Fig. 1.
 A 25 µl volume of the solutions was injected. HPLC conditions are provided in the experimental section and in Table 1

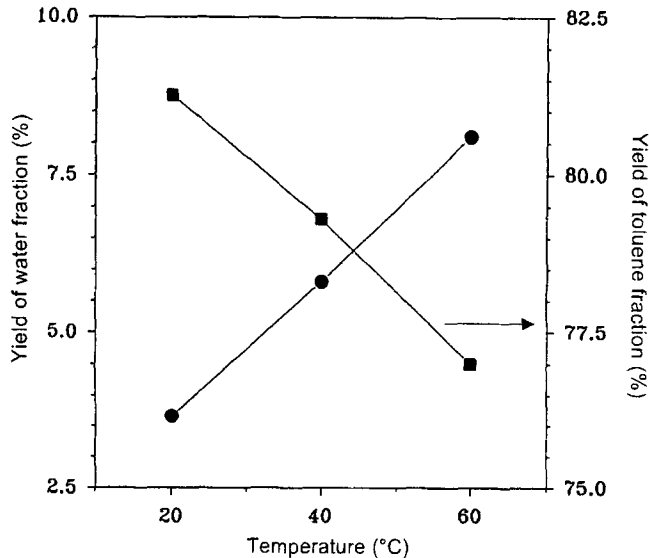


Fig. 4: Comparison of the isolated amounts of crude oil components from the fractions extracted with water and toluene as a function of temperature. (●) Fraction eluted with water; (■) Fraction extracted, after the water elution, from the dry soil with toluene

4 Conclusions

The described method allows the determination of total contaminants in soil. Some of the isolated PAHs as well as other aromatic compounds may potentially contaminate the groundwater. The total recovery of crude oil components from soil is normally better than 85%. The recovery is influenced by the soil type and the composition of crude

oils. The resins and asphaltenes of crude oils cannot be recovered from soil. The wetting behavior of such components plays a important role (AKHLAQ *et al.*, 1997). They can be extracted from the quartz sand with a sequence of organic solvents (AKHLAQ *et al.*, 1996). In any case, the resins and asphaltenes cannot cause a pollution of groundwater. They may biodegrade in the soil.

The high-performance liquid chromatography in combination with a diode array detector (DAD) is a selective and sensitive method for the analysis of polycyclic aromatic hydrocarbons in environmental samples.

The temperature of soil and elution water has a significant effect on the mobility of contaminants towards groundwater. This has to be considered if the oil spill happens during the summer months.

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News & Views: The Shift Programme

Studies on Human Impact on Forests and Floodplains in the Tropics German-Brazilian Program of Scientific Cooperation. Project ENV 52: Soil Fauna & Litter Decomposition in Primary and Secondary Forest and a Mixed Culture System in Amazonia

A research project developed in cooperation between:

Embrapa-CPAA: Empresa Brasileira de Pesquisa Agropecuária - Centro de Pesquisa Agroflorestal da Amazônia Ocidental, Manaus/AM, Brazil. Contact: M. Sc. Marcos Bastos Garcia, e-mail: mgarcia@internext.com.br

SMNK: Staatliches Museum für Naturkunde, Karlsruhe, Germany. Contact: Prof. Dr. L. Beck or Dr. H. Höfer, e-mail: lbeck_smnk@compuserve.com or hhoefer_smnk@compuserve.com

INPA: Instituto Nacional de Pesquisas da Amazônia, Manaus/AM, Brazil. Contact: Dr. C. Martius, e-mail: martius@cr-am.rnp.br

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In the SHIFT programme, several projects are being carried out near the city of Manaus (Central Amazonia, Brazil). Their aim is to study the regeneration and better use of already degraded areas, to diminish the human impact on primary rain forest in Amazonia. "Our basic hypothesis is that soil fauna and microflora communities are extremely important for the maintenance of 'healthy' (functional) nutrient cycles in the systems. We hypothesize, too, that biotic and abiotic factors may be manipulated to optimize the composition of the soil biota, to guarantee the efficient recycling of nutrients and their conservation in the system."

In the present research project, factors like the quantity and quality of the litter produced in the systems, the decomposition

rates, and the abundance, biomass, and respiration of microorganisms and soil animals will be comparatively examined. The studies will be carried out simultaneously in primary and secondary forest and one of the mixed culture systems (a silvicultural plantation of four tree species) in the experimental area established by Embrapa, Manaus, in the project SHIFT ENV-23. Our aim is to obtain data on the specific contribution of the microflora and soil fauna to the decomposition of the organic matter, and on the importance of these processes for the nutrient supply to the plants. In particular, we will focus on studies on the interaction between microflora and fauna of the soil, an aspect that, up to now, has been poorly studied in the tropics.