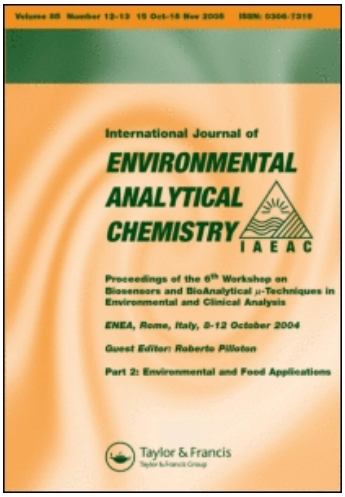

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

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To cite this Article Zerhouni, Peter and Bengtsson, Göran(2001) 'A High Throughput Procedure for Polycyclic Aromatic Hydrocarbons (Pahs) Assayed for Biodegradation in Contaminated Soils', International Journal of Environmental Analytical Chemistry, 79: 1, 1 — 14
To link to this Article: DOI: 10.1080/03067310108034149
URL: http://dx.doi.org/10.1080/03067310108034149

Intern. J. Environ. Anal. Chem., Vol. 79(1), pp. 1-14
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A HIGH THROUGHPUT PROCEDURE FOR POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) ASSAYED FOR BIODEGRADATION IN CONTAMINATED SOILS

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(Received 30 November 1999; In final form 24 May 2000)

This study describes the development of a high throughput procedure for the extraction, purification, and quantification of all 16 polycyclic aromatic hydrocarbons (PAHs) on EPA's priority list, subject to biodegradation assays in a creosote-contaminated soil. We used various combinations of solvents, temperatures, ultrasonication, tumbling, saponification, vacuum centrifugation, adsorption chromatography, gas chromatography, and mass spectrometry to demonstrate that a) individual PAHs were quantified in the soil matrix with coefficient of variations varying between 5 and 28%, b) recovery from a spiked soil was 80% and more for most compounds but dependent on characteristics of the internal standard, c) up to 20 PAH analyses of soil samples can be processed per day by one person, with a reasonably low consumption of solvents, and d) from undetectable levels to >50% of PAHs were biodegraded in 45 days at 23 °C in soil samples that were contaminated with creosote more than 35 years ago.

Keywords: Creosote; compound ageing; ultrasonication; saponification; mass spectrometry

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) form a series of homologous organic pollutants that are produced by air deficient combustion of organic material, like in coal gasification[1]. Creosote, used to impregnate wood, consists to 85% of PAHs[2], and both wood preservation and gas work sites are usually heavily contaminated with PAHs[3]. Other major anthropogenic sources are processing, combustion and disposal of fossil fuels, residential wood heating, and high temperature industrial processes, e.g. smelters[1,4]. The most important natural source is forest fires[1]. The main sink for PAHs is the soil[5]. Microbiological

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degradation is the chief process for natural elimination of PAHs from soils[6], but the *in situ* degradation is a slow process[7].

There is great concern about PAH pollution since compounds with four and more rings are among the most potent carcinogens and mutagens known[8], and low molecular weight PAHs are acutely toxic[9]. PAHs are also known to be immunosuppressive[10]. The detrimental effects of the compounds have urged the United States Environmental Protection Agency (US EPA) to list 16 of them as priority pollutants[11].

The low bioavailability of PAHs in soil is considered to be a main challenge in bioremediation of historically polluted soils. Several studies suggest that only PAHs dissolved in aqueous solution are available for bacterial degradation[12,13], and the low water solubility of the group results in sorption to[14] or other associations[15] with the soil. The longer the PAHs are in contact with the soil, the larger the fraction of PAHs that becomes unavailable, a process referred to as ageing[16]. As a result of ageing, desorption becomes very slow and can limit the rate of degradation[16,17].

The isolation and enrichment of PAHs in environmental samples usually require several liquid/liquid extractions, cleanup and enrichment procedures. Alcoholic saponification may be included in the extraction procedure to release PAHs bound to lipid and humic components[18]. The most common ways of cleanup of the extracts are the application of adsorption and/or size exclusion chromatography. The enrichment procedure may use rotary evaporation, nitrogen evaporation, or different distillation columns, with recovery of individual PAHs varying with the method and solvent used[19]. The identification and quantification of PAHs may use HPLC in combination with selective fluorescence detection, or GC-MS. They have about the same sensitivity but the latter technique has a higher accuracy[20].

A necessary step in the evaluation of the fate of organic pollutants in the field and of the bioremediation potential of a site is the accomplishment of laboratory bioassays. While alleviating the demands for extensive and complicated field sampling, laboratory bioassays of polluted soils and sediments can be designed to include numerous treatments and sufficient samples to get through the criteria for replicability, reproducibility, and the limits for detecting e.g. biodegradation. Those possibilities call for the development of high throughput procedures for the extraction, purification, and quantification of pollutants. While it is praxis to examine only one or a few of the 16 PAHs in degradation studies of polluted soils[e.g. 7,21], our ambition was to analyse all of them and to treat them as a group since a primary aim of bioremediation must be to reduce the total load of PAHs in the soil, even though cleanup criteria for individual compounds

exist[22]. To accomplish this with many samples we developed a high throughput extraction and analysis method for the PAHs.

EXPERIMENTAL

Soil

The soil was taken from an old wood preservation area in Hässleholm, Sweden. At this site railroad sleepers were impregnated with creosote between 1946 and 1965. The soil was sandy, with an organic content of 0.67 % and a pH of 7.2. It was kept at 4°C in tightly sealed plastic containers until sieved through a 2 mm (din 3) mesh, thoroughly mixed, and air dried at room temperature for 65 hours. Subsamples of the soil were frozen and stored at −21°C to determine the starting (T_0) concentration of PAHs. Other subsamples were incubated in autoclaved 100 ml serum bottles in the dark for 45 days at 23°C on a rotating table (70 rpm) to determine the innate capacity for PAH degradation. Each bottle received 15 g of the dried soil and 3 ml of filter sterilized (0.2 μm) groundwater. The water content was maintained by adding 1 ml sterile groundwater every six day. Also dried samples without any addition of groundwater were autoclaved twice at 121°C with three days in between. Both dried and autoclaved soils were incubated to measure abiotic losses. Subsamples of the soil from autoclaved microcosms were frozen at the onset of the experiment and stored at -21°C to determine the starting (T_0) concentration of PAHs. Replicate soil samples from the same wood preservation area were also analysed for PAHs by Hedeselskabet, Viborg, Denmark, using US EPA's method 3540C. Experiments in which the described analytical method was used to determine the influence of carbon enrichment and DOC concentration on biodegradation of PAHs are reported elsewhere (Bengtsson and Zerhouni subm.).

PAH analysis

Extraction of soil

All glassware was burned at 400°C (Carbolite LHT 5/60, England) for 12 hours before use. Microcosm soil was frozen, freeze dried over night and kept in a 10 ml test tube (Kimax, USA) in a freezer until extracted. Fifteen ml of methylene chloride (MeCl) (p.a., Riedel-de Haën, Switzerland) and one ml of internal standard (ISTD), 3,6-dimethylphenanthrene (Sigma-Aldrich, Germany) dissolved in MeCl at a concentration of 50 μg ml^{-1}, was added to 3.0 g of freeze

dried soil in a 45 ml test tube (Kimax, USA) with tight teflon lined screwcap. MeCl is common for PAH extraction[23], but acetone (p.a., Prolabo, France) was also tried since it was suggested to be more efficient than methylene chloride[18], perhaps due to the larger solubility of PAHs in acetone[24]. The test tube was put in an ultrasonic bath (Transsonic 570, Germany) at 40°C or 80°C for 30 min, then left for 17 hours on a vertical rotating wheel (custom made, resembling a wheel of fortune), at 23°C, and centrifuged for 15 min at 1800 rpm. The supernatant was transferred to an 18 ml test tube (Kimax, USA).

The soil residue was again extracted with 15 ml of a methanol (p.a., Prolabo, France) and water mixture (10:1 v/v), saturated with potassium hydroxide (Eka, Sweden). The saponification step was included to increase extraction efficiency[18,25]. Saponification can improve the PAHs' accessibility to solvent in two ways: by partially breaking down the network of organic compounds in the soil, and by expanding the humus matrix through repulsion between negatively charged carboxyl groups. The soil was put in the ultrasonic bath for 30 min, left on the rotating wheel for 17 hours, centrifuged at 1800 rpm for 15 min, and the supernatant was collected into a 45 ml test tube, whereas the extracted soil was discarded.

The alkaline supernatant was extracted with 10 ml of hexane (p.a., Fischer Scientific, England) on the rotating wheel for 30 min at 23°C following Hartman[25]. More than 90% of the PAH content is recovered from the alkaline methanol-water in one hexane extraction according to Hartmann[25], and one extraction was considered enough for this study. To improve separation between the two phases, a few drops of isopropanol (p.a., Merck, Germany) was added before centrifugation for 10 min at 1800 rpm. The hexane phase was transferred to a 10 ml test tube and dried with anhydrous sodium sulphate (Merck, Germany).

The MeCl and hexane extracts were separately evaporated to about 100 µl in a vacuum centrifuge (Savant Speedvac AES 1000, NY, USA) with 50 µl toluene (p.a., Riedel-de Haën, Switzerland) as a keeper. Full vacuum was used for 3 min before cryopumping was started. Low temperature evaporation setting, without radiation, was used. The MeCl took about 75 min to evaporate and the hexane 25 min.

Column clean-up

Aluminium oxide (neutral, 150 mesh, Sigma-Aldrich, Germany) was activated at 170°C for 19 hours and then stored in a desiccator. Four gram was added to 6 ml filtration columns (Supelco, PA, USA) with a PTFE frit in the bottom. The filled column was swirled with hexane, stirred and tamped down with a glass rod. The

two evaporated extracts were dissolved in 2 × 250 µl hexane each and transferred as a pool to the column (1 ml in total).

The aliphatic fraction was first eluted with 10 ml hexane and discarded. The PAHs were eluted with 17 ml MeCl[23] through gravity flow. The PAH containing fraction was collected in an 18 ml test tube. The column was washed with another portion of 10 ml MeCl before the next sample was added to it. The aluminium oxide was discarded and replaced with fresh one after every two samples. The eluate was evaporated without toluene to dryness, or, as in most samples, to a yellow tar of a few µl, in the Speedvac as described above. It took 70 min.

Evaporated soil extracts were dissolved in 100 µl of MeCl and then diluted 5 and 100 times depending on which compounds were to be quantified. All but the three largest PAHs were possible to quantify by gas chromatography-mass spectrometry (GC-MS) at the 1:100 dilution and the three largest were quantified at the 1:5 dilution or undiluted by GC-MS or gas chromatography with flame ionization detection (GC-FID).

GC-MS and GC-FID analysis

A combination of a HP5890 II gas chromatograph and a HP5989B MS Engine (GC-MS) was used to analyse the soil extracts. The extracts contained so many non-target substances that it was impossible to separate the PAH peaks from all other peaks on a GC only, even after column clean-up of the samples. A standard stock solution containing the 16 PAHs, each at a concentration of 2000 µg ml^{-1} in MeCl-benzene (1:1), was purchased from Supelco, PA, USA, and kept at −18°C in a sealed glass ampoul. A dilution of this to 50 µg ml^{-1} of each PAH in MeCl was prepared and spiked with the ISTD, 50 µg ml^{-1}, and kept in the freezer. It was used extensively in method development and for calibration of the instruments. The instruments were recalibrated after every 15 sample, and the MS was autotuned once a day. The MS was used in the SIM aquisition mode when the samples were analysed. One µl sample was injected splitless, split closed 0.8 min, split ratio 1/50. The inlet temperature was 250°C, carrier gas was helium at 1 ml min^{-1}, the capillary column was a HP-5MS, length 30 m, diameter 250 µm, film thickness 0.25 µm. The oven initial temperature was 80°C for 5 min, then increased by 3°C min^{-1} to 130°C, held there for one min, increased by 5°C $min^{-1}$to 200°C, held for one min, increased by 15°C $min^{-1}$to 275°C and kept there for 20 min. The interface temperature was 270°C, the source temperature was 200°C, the quadrupole temperature 100°C, and EI ionization used 70 eV.

To identify the retention times and target ions of the 16 PAHs and the internal standard, a method with the GC-MS in Scan mode (50–550 m/z) was applied.

Some PAHs were difficult to identify and discriminate between because of similar mass spectra and retention times. In those cases the same elution order as in Hartmann[25] was assumed. Benzo(b)fluoranthene (BbF) and Benzo(k)fluoranthene (BkF) were not satisfactorily resolved, which is a common problem[26], so they were treated as one peak throughout the study (Bb/kF).

During method development and for quantification of the three largest PAHs, a HP 6890 gas chromatograph equipped with a flame ionization detector (GC-FID) was used. The injection volume was 5 µl, using pulsed splitless injection at 20 psi for 0.2 min with the same inlet temperature and oven temperature programming as for GC-MS. The detector temperature was 300°C. A 30 m HP-5 capillary column with 320 µm diameter and 0.25 µm film thickness was used with hydrogen as a carrier gas at 1.5 ml min^{-1}.

Evaluation of extraction and analysis methods

The recovery of naphtalene (ICN Biomedicals, OH, USA), anthracene (ditto), phenanthrene (ditto), fluoranthene (Sigma-Aldrich, Germany), pyrene (ditto) and the internal standard (3,6-dimethylphenanthrene) in column clean-up and Speedvac evaporation was investigated using GC-FID as there were no interferring compounds in the samples.

In the column clean-up evaluation, one ml of MeCl with a known PAH content was applied to an aluminium oxide column and the column was washed with 10 ml of hexane. Then the PAHs were eluted with 15 ml MeCl and the eluate analysed. In the Speedvac evaporation test, 15 ml MeCl with a known PAH content were evaporated to dryness with toluene as a keeper, then redissolved in 15 ml MeCl and evaporated once more to dryness, this time without keeper. The residue was redissolved in MeCl and analysed.

To evaluate the entire method, three g (wet weight) of uncontaminated soil (n=3) from the lawn outside the Ecology Building, Lund University, Sweden, were spiked with 50 µg of each PAH, administered in 1 ml MeCl. This soil had higher organic content than the microcosm soil. Samples of the spiked and unspiked soil were left in a vent cupboard for 24 hours at room temperature before frozen and freeze dried.

Data processing and evaluation

The integration of the acquired MS and GC data was done in Chemstation 3.0 and 4.01 (Hewlett Packard), respectively. Chromatograms were integrated using Autointegration for most peaks. Integration results, peak areas and retention times were reported automatically to Microsoft Excel (4.0 and 5.0, respectively). For some peaks with the same target ion and virtually the same retention time,

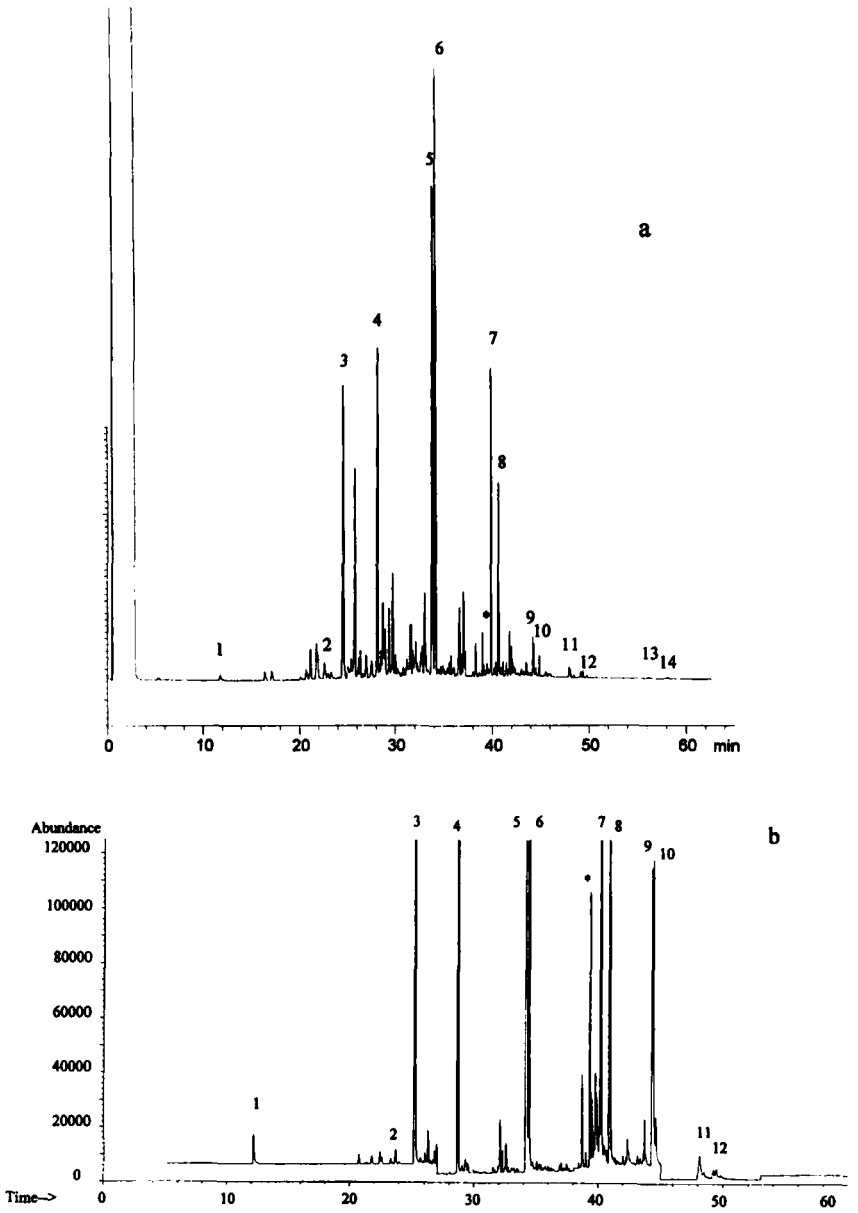

FIGURE 1 PAHs found in a soil extract of a creosote contaminated site after a 45 days laboratory biodegradation assay. The figure shows a gas chromatogram with flame ionization detection (a) and a total ion chromatogram from the mass spectrometer in selected ion monitoring (b). 1 (Nap); 2 (Acy); 3 (Ace); 4 (Flu); 5 (Phe); 6 (Ant); 7 (Flt); 8 (Pyr); 9 (BaA); 10 (Chr); 11 (B(b/k)F); 12 (BaP); 13 (IndP); 14 (BghiP); *(ISTD). The abbreviations are explained in Table I

Chemstation had to be supplied with manual integration instructions to separate the peaks from one another. As the peaks were so close and similar to each other, the slightest peak drift caused problems for the software to report the right peak to Excel. Consequently, peak drift had to be adjusted for quite often when processing the data. The use of qualifier ions could not improve the situation as the PAHs had so similar mass spectra. The response factors ($Rf_i=m_i/A_i$, where m_i is the amount and A_i the area of compound *i*) were updated continuously by the injection of standard solutions. The average peak areas of the 2–3 closest calibration runs were used to calculate response factors for a group of samples.

The sterile controls were included in the study to calculate abiotic losses, i.e. evaporation. A problem was that the four PAHs with the lowest molecular weights were quantitatively lost from the soil during autoclaving, due to evaporation, and it was not obvious that the evaporation during incubation from the autoclaved soil would follow the same pattern as in the non-autoclaved soil. However, we found that the dry and moist sterile samples had the same relative loss of PAHs during the incubation period as the non-autoclaved dry samples. Since the degradation of PAH in dry soil was assumed to be negligible, this finding indicated that the relative evaporation was the same in autoclaved and non-autoclaved samples.

To calculate the evaporation loss correction factor for a compound, the average ($Cs_i(T)/Cs_i(T_0)$), where Cs_i ($\mu g\ g^{-1}$ of soil) is the soil concentration of compound *i* in the sample, from dry, sterile dry and sterile moist treatments at the end of incubation, at time T, were used. Biodegradation of a compound ($\mu g\ g^{-1}$ soil) was calculated as the concentration at the end of incubation subtracted from the initial concentration, corrected for evaporation. The relative degradation, in % of the initial concentration, was also calculated.

RESULTS

The column clean-up recovery efficiency on aluminium oxide was good and similar, 84–88%, for all six compounds tested. The average coefficient of variation (C.V.) was 0,9% (n=3). The substance loss in the Speedvac evaporation test was significant for Nap (all abbreviations of individual PAHs are explained in Table I), but not for the other compounds tested. The recovery of Nap was only 3,4% (S.D.=0,6%, n=3). After a single evaporation without toluene as a keeper, the Nap recovery was 20%. The other five tested compounds had recoveries of 88–95%, increasing with the molecular weight (average S.D.=1,7%, n=3).

TABLE I PAH MS Characteristics, Concentration in Soil, Method Recovery, and Degradation in Bioassay

Compound in order of chromatographic elution	*Abbreviation*	*Target ion(m/z)*	*Conc. at T_0[a] 40 °C(μg g^{-1} soil)*	*C.V.[b] (n=5)*	*Conc. at T_0[a] 80°C(μg g^{-1} soil)*	*Commercial Anal.[c](μg g^{-1} soil)*	*Method Recovery[d] (%)*	*Degradation (%) (n=4)*	*Degradation stand. dev.(%) (n=4)*
Naphtalene	Nap	128,1	60	0,12	76	73	3	28	2
Acenaphtylene	Acy	152,1	18	0,14	44	82	38	24	7
Acenaphtene	Ace	153,1	61	0,09	192	400	49	26	5
Fluorene	Flu	166,1	104	0,09	139	210	70	34	10
Phenanthrene	Phe	178,1	299	0,08	1519	3100	92	54	5
Anthracene	Ant	178,1	36	0,05	142	–	82	25	11
3,6-Dimethylphenanthrene	ISTD	206,1	–	–	–	–	–	–	–
Fluoranthene	Flt	202,1	157	0,07	816	1600	105	n.d.[e]	–
Pyrene	Pyr	202,1	95	0,07	540	1200	105	n.d.	–
Benz(a)anthracene	BaA	228,1	18	0,13	246	440	101	12	2
Chrysene	Chr	228,1	20	0,09	172	400	112	n.d	–
Benzo(b,k)fluoranthene	Bb/kF[f]	252,1	4	0,20	139	290	94	n.d	–
Benzo(a)pyrene	BaP	252,1	4	0,17	58	160	80	20	6
Indeno(1,2,3-c,d)pyrene	IndP	276,1	3	0,28	31	83	70	n.d	–
Dibenzo(a,h)anthracene	dBahA	278,1	1	0,24	31	98	83	n.d	–
Benzo(ghi)perylene	BghiP	276,1	3	0,21	27	–	93	n.d.	–
Total			883		4172	8136			

a. *As measured by the method of this study.*
b. *Coefficient of Variation*
c. *As measured by Hedeselskabet, Viborg, Denmark, using US EPA's method 3540C.*
d. *Recovery from spiked pristine soil using the method of this study (n=3).*
e. Not detected
f. Benzo(b and k)fluoranthene were treated as one compound

Two consecutive analysis of the same sample on the MS had an average C.V. of about 2%, with higher variance for the largest compounds. The MS responded linearly to the injected amount of PAH over the range 2,5–250 ng. All 16 PAHs and the internal standard were tested and most had R^2=0,99. The three largest PAHs had R^2=0,95. The variance of the GC-FID response was small with a C.V. of around 0,8% for three consecutive analyses of the same sample for all compounds tested, except for Nap, that had 1,7%. The GC-FID reported peak area responded linearly (R^2=0,995) to the injected amount of compound over a very wide range, 2,4–616 ng.

Only 3% of the Nap were recovered from the spiked soil (Table I), and the recoveries were also low for Acy (38%), Ace (49%), and Flu and IndP (70%). The other compounds had recoveries of 80–105%. The three PAHs with the lowest molecular weights were also those that had evaporated during the incubation period in autoclaved and non-autoclaved soil, and for which the evaporation losses had to be corrected when the degradation was calculated; Nap by 0.52, Acy by 0.81, and Ace by 0.86. Five replicate measurements made on the PAH concentration of the creosote contaminated soil had a C.V. of less than 15%, except for the five largest compounds, which were present at the lowest concentrations and with C.V. between 20 and 30% (Table I). The recovery of the PAHs from the contaminated soil was low – generally less than 10% – when sonication was made at 40°C but considerably higher – between 32 and 104% – at 80°C compared with the US EPA method used by the commercial laboratory (Table I). This method comparison has some fallacies due to the spatial variability of the creosote contamination and the ageing processes. A single sample randomly taken from the same large batch of soil but sieved and treated separately with our method had between 3 and 5% higher concentrations of individual PAHs than the sample analysed by the commercial laboratory.

The degradation of the compounds followed the same pattern as in many other studies[e.g. 27], with larger relative degradation of low compared with high molecular weight PAHs (Table I). Several of the compounds were degraded by between 20 and 30%, Phe even by 50%, while most of the high molecular compounds were not degraded at all. The standard deviation of those that were degraded was less than or equal to 10% (Table I). If we assume that a degradation bioassay aims at detecting a 10% difference between treatment means of normally distributed samples, with a 80% probability and 0.05% error, we can calculate[28] that 10 replicates would be required if the focus would be on compounds with a standard deviation of 7.3 (Acy) and lower but 19 replicates if also Ant would be considered.

DISCUSSION

The proposed methodology allows one person to process up to 20 PAH analyses of soil samples per day and with a reasonably low consumption of solvents. Since the replicability of the measurements was good for all but the most high molecular weight PAHs, the methodology could be successfully applied in a bioassay of the degradation of PAHs in the soil (Table I).

The method of extraction is crucial in degradation studies. Unless exhaustive, the total PAH content of the soil will be underestimated and biodegradation can be overestimated, since what is interpreted as degradation might just have become non-extractable[18]. The differences in results of the two measurements of the PAH concentration in the contaminated soil was most likely due to a lower extraction efficiency of our method compared with the US EPA's method 3540C, which includes Soxhlet extraction, or a spatial variability of PAH concentrations within the same batch of soil. In addition, the efficiency of extraction methods seems to vary from one soil and sediment matrix to another. Rohrbach and Reed[29]found rotary shaking and Tan *et al.*[30] ultrasonication to be almost as efficient as Soxhlet in extracting PAHs from marine sediments, whereas Griest and Guerin[31] found the Soxhlet procedure to be relatively ineffective in comparison with sonication. Also Noordkamp et al.[32] found rotary tumbling for 40 min to be as efficient as Soxhlet extraction for 7 hr at 50°C.

The tendency of PAHs to stick to the soil as irreversibly sorbed residues as they age[33] tends to protect a fraction of them from extraction. Thus, acetone extraction efficiency decreased by up to 40% during incubation of soil for two months[18], a problem that can be overcome to some extent by alkaline methanol extraction that cleaves the ester bonds that form the macromolecular humic entity and then release the trapped PAH molecules. Even a few hours of ageing can affect the extraction efficiency negatively[15,16], and more lipophilic high molecular weight PAHs age faster than less lipophilic ones[34]. This would also explain why the recovery of the relatively more lipophilic Ant was 10% lower than that of Phe despite equal molecular weight.

The efficiency of solvents also seems to vary from one sample matrix to another. There was no significant difference in extraction efficiency between methylene chloride and acetone, which seems to be the most commonly used solvents for PAH extraction. Although the alkaline saponification was not fully evaluated, we noticed from preliminary tests that it increased the recovery by 30–40%. This agrees with the findings by Eschenbach et al.[18] with fluoranthene and benzo(a)pyrene added to soils. Hartmann[25] found methanol-KOH extraction at 65°C of carbon rich forest soils to be as efficient as shaking with acetone or petroleum ether/acetone/water, whereas Noordkamp et al.[32] showed that

saponification of an industrial soil at 20°C gave about 30% lower yield than rotary tumbling with acetone. A high temperature increases the solubility of PAHs in the solvent and may explain these different outcomes of saponification as well as the increased recovery at 80°C sonication compared with 40°C in our study (Table I). The temperature effect on PAH recovery was also evident in the microwave extraction comparison made by Lopez-Avila et al.[35].

Recoveries in extraction and analysis of spiked pristine soil (Table I) clearly reflects which PAHs were used in the method development. These five PAHs, and the internal standard, had similar molecular weights, except for Nap, and they behaved similarly in column clean-up and Speedvac evaporation tests and had recoveries close to 100%. This was not true for PAHs with other physico-chemical properties that were included in the sample analyses. In the case of Nap, and other small PAHs that evaporated more easily than the internal standard, the method used in this study will underestimate the true amount of compound extracted, and their recoveries were hence low with high standard deviations. Vice versa, compounds with higher molecular weight that evaporated less than the internal standard showed recoveries of more than 100%. The highest molecular weight compounds showed again recoveries less than 100%, probably due to losses during the column clean up. The ideal internal standard would be deuterated analogues of all the analysed compounds[25]. They behave close to their normal counterparts, but they are expensive. Overall, the recoveries in our method of Ant and higher molecular weight compounds were competitive with those of Gonzalez-Vila *et al.*[23] and Hartmann[25].

The varying degrees of ageing, hydrophilicity, and volatility of PAHs call attention to the need for a carefully evaluated, yet reasonably rapid chemical analysis of sorbed PAHs when the bioremediation potential of a contaminated soil is assessed. Because of the great variability in the physico-chemical properties of the PAHs and the complex interaction of each of them with the soil matrix at aged pollution sites, a high throughput method cannot ensure the maximum yield of every single compound. The present method has a high degree of confidence in screening large numbers of samples that have been used in degradation bioassays by the sacrifice of a lower total recovery of the compounds from an old creosote site with aged PAHs. The comparison of extraction methods made in this work and in others[29–32] suggests that extraction yields are site and matrix specific and that more than one extraction method should be evaluated when exhaustive yields are required, especially when the contamination is significant and aged.

Acknowledgements

The soil samples were kindly provided by Erik Andersson at the Department of Biotechnology, Lund University, Sweden. Christer Bergwall at our department gave helpful hints throughout the laboratory work. Financial support from the Swedish Environment Protection Board and Crafoordska Stiftelsen is acknowledged. Skandinaviska Enskilda Banken's Foundation for economic and technical research provided the grant that made the purchase of the mass spectrometer possible.

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