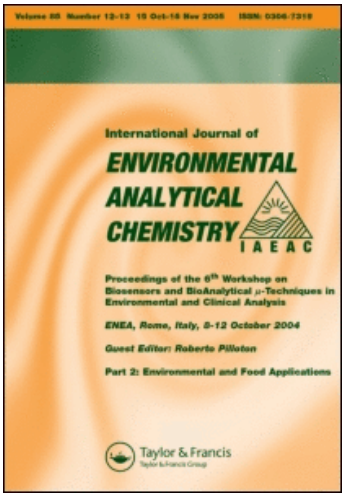

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Polycyclic Aromatic Hydrocarbons (PAHs) in Forest Soils: Critical Evaluation of a New Analytical Procedure

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POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN FOREST SOILS: CRITICAL EVALUATION OF A NEW ANALYTICAL PROCEDURE

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A new method is presented allowing the determination of PAHs in the highly problematic organic humus layers of forest soils. Emphasis was put on the extraction process and on a reliable quantification procedure. Fundamental elements of the developed method are: saponification of samples in an ultrasonic bath, partitioning of PAHs into hexane, clean-up of extracts by means of solid-phase extraction, and quantification by GC-MS using deuterated internal standards.

Saponification was successful, probably due to hydrolysis and expansion effects on the humus matrix, hence sorbed PAHs were more accessible to the solvent and could be extracted more efficiently. The presented extraction procedure is therefore an interesting alternative to conventional solvent extraction methods, especially for sorptive carbon-rich matrices.

The developed method guarantees an accurate and reproducible determination of PAHs in extremely difficult matrices even at trace levels. This was shown by testing the efficiency of the individual steps of the method, and by sample exchange with an external laboratory.

KEY WORDS: Polycyclic aromatic hydrocarbons (PAH), analysis, saponification, solid phase extraction, forest soils.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) attracted scientific interest because of the carcinogenic potential of single compounds in combination with their ubiquitous occurrence. Soil is one of the most important sinks for PAHs, which are deposited in the gaseous state or associated with particles even at sites far from industry. PAH contents in organic humus layers of forest soils may be extraordinarily high[1-6] due to an effective atmospheric deposition on plant surfaces[7] and the great affinity of PAHs to the C-rich organic humus layers.

However, chemical analysis of organic humus layers of forest soils is particularly difficult. The two main problems are extraction efficiency and coextraction of non-target substances. Analytical difficulties are enhanced by the fact that the EPA series of 16 PAHs, which was originally developed for water analysis, is now commonly accepted as the reference for PAH analysis of soils. Because of the great range of physico-chemical properties of these PAHs (Table 1), the individual steps of analysis like extraction or clean-up of extracts cannot be optimally adapted to every single compound.

Table 1 Selected physicochemical properties of PAHs included in the EPA 16 standard[8].

EPA 16:	*Naphthalene*	–	*Benzo(ghi)perylene*
log vapor pressure (Pa)	1.1	–	–6.2
log K_{ow}	3.4	–	7.2
Aqueous solubility (ng/ml)	32000	–	0.2

Problems may arise from the highly different volatility and an increasing hydrophobicity of the 16 PAHs with degree of condensation. Therefore, suitable measures must be taken to keep losses within justifiable limits, or to compensate for them.

In view of the problems combined with PAH analysis of forest soils, the main target of this work was to develop a method which guarantees—especially for the organic humus layers—the accurate, reproducible and economic determination of PAHs even at trace levels.

EXPERIMENTAL

Sampling

Selected samples were taken from the organic and mineral horizons of forest soil profiles. Aluminium bowls, which were heated at 300°C overnight and prerinsed with methanol and toluene served for the transport of samples to the laboratory. After air drying and removal of branches, roots and stones, the humus materials were crumbled and mineral soils were sieved to < 2 mm. Samples were stored in the dark in closed glass containers which were prerinsed with methanol and toluene and heated at 300°C before use.

Instrumentation

A Hewlett-Packard (Waldbronn, FRG) gas chromatograph Model 5890 II was equipped with a 5971A mass-selective detector, an automated splitless and on-column injector and a Macherey-Nagel (Düren, FRG) GC column (25 m × 0.25 mm × 0.24 μm). High-purity helium (99.9996%) was used as carrier gas; run control and evaluation of chromatographic results was carried out with Chemstation software supplied by the manufacturer.

A 320 Watt ultrasonic bath (Bandelin, Berlin, FRG) was used for extraction; solvent separation, partitioning and reduction of organic solvent volumes were performed using a Heraeus centrifuge (Hanau, FRG), a horizontal shaker (Bühler, Bodelshausen, FRG) and a rotary evaporator (Büchi, Göppingen, FRG) connected to a Büchi vaccum/distillation control unit; a Baker SPE 24 G extraction unit (Groß-Gerau, FRG) equipped with Luer taps (brass, nickel) in combination with 8-ml volume glass columns equipped with PTFE frits (Baker, Groß-Gerau, FRG) were used in the clean-up procedure.

Solvents and chemicals

Toluene (Resi analyzed, Baker), methanol and n-hexane (nanograde, Promochem, Wesel, FRG) were of residue analysis purity; ether was p.a. grade (Merck, Darmstadt, FRG). Water was prepared in the laboratory using a Millipore purification system (Eschborn, FRG). KOH and anhydrous Na_2SO_4 were p.a. grade (Merck).

The following column packings were used in the solid-phase extraction procedure: neutral Al_2O_3; pore diameter 60 Å, particle size 50–200 μm (Baker, No. 05.37) and Silica Gel; pore diameter 60 Å, particle size 40–60 μm (Baker, No. 70245).

PAH standards

Quantification by isotope dilution was carried out using a 16 PAH Kit (Restek, Sulzbach, FRG), perylene and benzo(e)pyrene crystalline (Aldrich, Heidenheim, FRG), all with a purity of at least 99%; the standard solution of deuterated PAHs contained d_8-naphthalene, d_{10}-acenaphthene, d_{10}-fluorene, d_{10}-anthracene, d_{10}-pyrene, d_{12}-chrysene, d_{12}-perylene and d_{12}-benzo(ghi)perylene, all with a purity of at least 98% (Promochem).

DEVELOPMENT OF THE ANALYSIS

Complete extraction of PAHs from carbon-rich matrices is difficult because PAHs are strongly bound to the complex structure of the organic matter. The reason for this phenomenon may be found in the prevailing bonding mechanisms; however, the association between PAHs and organic matter is not accessible for methods characterizing bond types, like NMR. Therefore, indications on the bonding mechanisms of PAHs to organic matter were until now mainly obtained indirectly from adsorption/desorption experiments[9,10]. It was deduced from such experiments that organic nonionic compounds are bound to organic matter by hydrophobic interactions, which can be modelled as a partitioning process.

Adsorption experiments are always following two-stage sorption kinetics. It is thus inferred that two different compartments of bonding sites exist in the organic matter: one compartment that interacts fast and unhinderedly with the chemical environment and a second compartment whose accessibility is controlled by diffusion processes[11,12].

It is generally assumed that the three-dimensional porous structure of organic matter impedes equilibration. This structure results from a complex network of a vast variety of plant, animal and microbial residues at different stages of decomposition and of humic substances. Finally, diffusion processes are triggered by the attempt of the PAHs to reach those hydrophobic regions representing the lowest possible free-energy level.

Another model suggests that hydrophobic regions in the organic matter build up structures similar to membranes or micelles[13]. At the same time the hydrophilic regions turn towards the aqueous environment, thereby isolating the hydrophobic regions. Diffusion processes are then the result of the restricted passage through these membrane-like structures.

It can be supposed that the structure or the diffusion processes in the organic matter are also responsible for the difficulties in extracting PAHs, because soils which were contaminated artificially with PAHs showed a time-dependent extraction efficiency as well. Several researchers described[14–16,32] that the efficiency of extraction decreased with increasing incubation time. Often, only a small fraction of the amount initially spiked is still extractable after a few days. Therefore the term 'bound residues' originally in use only for pesticides is meanwhile common also for hydrophobic contaminants. This

characteristic feature has to be taken into account when an extraction method for PAHs from organic matter is chosen.

Extraction with highly apolar solvents leads to insufficient moistening of hydrophilic regions in the organic matter; thus extraction efficiency is often low. This was confirmed by comparative extraction experiments with different polar solvents or solvent mixtures from complex matrices. Methanol, toluene/methanol and dichloromethane/water mixtures yielded best extraction efficiencies for PAHs from waste or waste compost, fly ash and soils[15,17,18].

It is an interesting attempt to use digestion methods which are able to break down the network of organic compounds, e.g. alkaline saponification. Extraction efficiency may be influenced positively in two ways. First, organic matter is hydrolyzed because of the splitting of labile ester bonds, which facilitates a limited breakdown of the network and therefore a better accessibility to the solvent. This point seems especially interesting for PAH extraction because the most important receptor substances (waxes) are embedded in a framework of polyesters (the cutin)[19].

A second effect may be more important for organic materials in an advanced stage of humification: alkaline conditions cause a microscale extension of the humus matrix due to the mutual repulsion of negatively charged carboxyl groups. The better accessibility to solvents results in a higher extraction efficiency of saponification compared to common solvent extraction[20,21].

Saponification is most effective at high temperature and in the presence of an excess of water. Although methanol-water ratios below 9:1 were considered promising, the standard ratio for saponification used by another group of researchers[22,23], our own experiments with ratios below 9:1 failed because of the hydrophobic character of the organic humus layers.

Moreover special extraction vessels were developed in order to avoid losses of volatile, low-molecular-weight PAHs, featuring a screw cap and PTFE seal to close standard glass centrifuge tubes. Besides good extraction efficiency, an additional advantage of the procedure turned to be that shaking the KOH solution with hexane gave a clear, well separated hexane phase.

Sample preparation

2.5–40 g air-dried soil/organic humus matter were weighed into the centrifuge tubes; the actual sample intake depends on expected concentration levels, 40–60 ml 2 M KOH in methanol-water (10:1) were added and the sample was spiked with the internal standard solution and capped. After 1 h of ultrasonication at 65°C the sample was kept at 65°C overnight. 25 ml 2 M KOH were added and, after a further hour of sonication, the sample was centrifuged at 3000 rpm. The supernatant was transferred into a 250 ml separatory funnel. The residue was extracted a second time with 20–40 ml methanol. The combined methanol-water and methanol solutions were shaken with three 50-ml and one 30-ml portions of hexane on a horizontal shaker for 10 min. After drying of the hexane over anhydrous Na_2SO_4, 100 µl toluene were added as a keeper and the volume was reduced to 200–300 µl in a rotary evaporator at 30°C and 260–275 hPa.

Clean-up

Out of a multitude of methods available, column chromatography on polar adsorbents is being used most frequently. Great efforts have been made during the past years to

develop a miniaturized version. The resulting solid-phase extraction system has a solvent-saving effect and several samples can be treated simultaneously in a short period of time.

A method for clean-up of soil extracts using a coupling of the solid-phase materials C_{18}/CN has already been presented[24]. However, preliminary experiments revealed that this method was unsuitable for the clean-up of organic humus layer extracts. Capacity and specificity of the coupled C_{18}/CN materials for compounds coextracted from the complex matrix were insufficient to ensure a measurement free of interferences.

Therefore a solid-phase extraction system was developed using polar adsorbents. 1 g Al_2O_3 (9% water content) and 750 mg active silica gel were used routinely, poured into 8-ml glass columns and sealed with PTFE frits on both sides. Columns were connected to the extraction unit with Luer taps.

The concentrated extract was transferred onto prepared Al_2O_3/silica gel columns (preparation: dry silica gel weighed into the columns, swirled with hexane and selaed with a PTFE frit, dry Al_2O_3 weighed onto the PTFE frit of the silica gel swirled with hexane and sealed with a further PTFE frit, finally the column packing was washed with 2 column volumes of hexane) with a total of 1 ml hexane and drained until the whole extract was just below the upper PTFE frit.

The 18 PAHs (the EPA series was supplemented with perylene and benzo(e)pyrene) were eluted with 3 ml hexane and 10 ml hexane-ether (20:1) without suction. The eluate was reduced to 20–300 µl, the actual volume depending on the expected PAH concentrations.

Gas chromatography

A GC-MS system was preferred for measurement because of expected problems arising from the complex matrix. Difficulties to identify compounds should be minimized by the additional identification of a compound through mass spectra. Furthermore, compensation for analytical losses and hence a good reproducibility is only possible if internal standards are used. This necessitates quantification by means of GC-MS.

Methods using on-column injection and splitless injection for routine analysis were developed. A glass wool packed liner (Hewlett Packard, 4-mm i.d.) at splitless operation helped to avoid irreproducible substance losses and distorted peaks which were due to repeated injections from the bottom of the vaporizing chamber. These problems are well known from the evaporation in splitless injectors of substances with a high boiling point[25]. Likewise, problems resulting from the addition of non-target substances to the system with each injection were abolished, which normally necessitates a routine shortening of the GC column and frequent cleaning of the MS. The acceptable lifetime of the packed liners was about 4–6 weeks. For the reconditioning of the liners and the preparation of the glass wool, a routine procedure was developed as well. Used liners were treated for 24 h with saturated chromosulphuric acid in order to oxidize adhering carbonized particles. Afterwards the glass surface was etched with saturated methanolic KOH, and deactivated by treatment with a silylating agent in toluene. The plug consists of silylated glass wool which was washed with different solvents before use.

The injection port temperature was held at 320°C, the interface at 280°C. The column oven temperature programme was: 85°C for 4 min; at 15°C/min to 160°C, held for 1.5 min; at 5°C/min to 300°C, held for 5 min. The initial carrier gas velocity was 40 cm/s. Figure 1 shows the standard chromatogram of the 18 PAHs investigated.

For on-column injection, the injection port temperature was kept 3°C above the oven temperature; the carrier gas velocity was kept constant at 40 cm/s by means of an

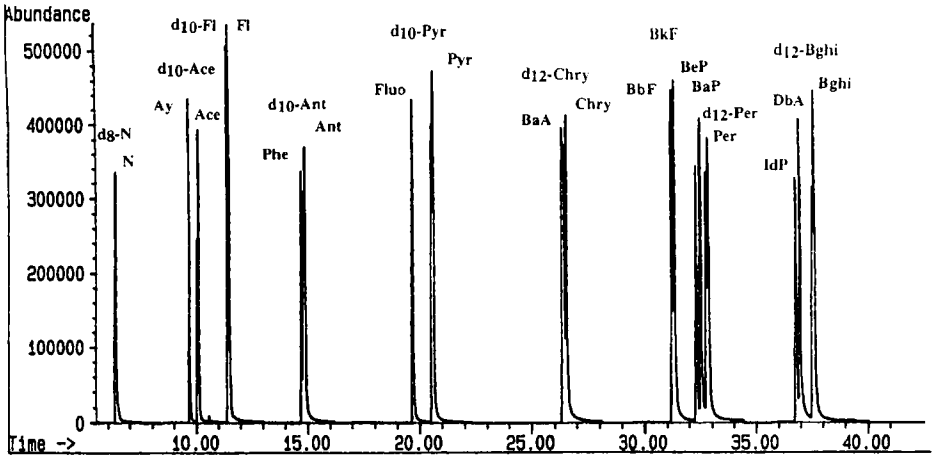

Figure 1 Standard GC-MS chromatogram of the 18 PAHs plus 8 internal standards (PAH/d-PAH 0.8:1). Abbrevations: N (Naphthalene), Ay (Acenaphthylene), Ace (Acenaphthene), Fl (Fluorene), Phe (Phenanthrene), Ant (Anthracene), Fluo (Fluoranthene), Pyr (Pyrene), BaA (Benzo(a)anthracene), Chry (Chrysene), BbF (Benzo(b)fluoranthene), BkF (Benzo(k)fluoranthene), BeP (Benzo(e)pyrene), BaP (Benzo(a)pyrene), Per (Perylene), IdP (Indeno(1,2,3-c,d)pyrene), DbA (Dibenzo(a,h)anthracene), Bghi (Benzo(ghi)perylene).

electronically controlled pressure programme. A protective phenyl-sil-inactivated precolumn (5 m × 0.53 mm) was coupled to the capillary column by means of a universal glass connector.

MSD operating conditions and detection limit

PAH analysis was performed by SIM (Single Ion Monitoring) runs. The tuning operation for the MSD was adapted to user-defined parameters which optimize ion focus, repeller voltage, entrance lens offset and the mass range to maximum sensitivity of the MSD for PAH analysis. The specific detection limit for the instrument used was determined to be 20–30 pg (PAH) for on-column operation and 150–400 pg (PAH) for splitless operation.

Quantification

A calibration curve was prepared using standard solutions of PAH in combination having a constant concentration of deuterated PAH (PAH/d-PAH: 4:1, 1.6:1, 0.8:1, 0.2:1). Figure 1 shows the resulting standard chromatogram. Relative response (PAH/d-PAH) is plotted versus concentration of PAH and used with the analysis of unknown samples.

Aliquots of the d-PAH stock solution were added to samples before extraction. The spike level was adapted to the expected PAH concentration of the respective samples with a range of minimum 0.8 µg/kg to maximum 640 µg/kg.

All extracts from organic humus layers and mineral soils could be analyzed by the present method. Figure 2 gives an impression of the reliable basis for indentification and quantification which the SIM runs represent. One should take into consideration that Oi

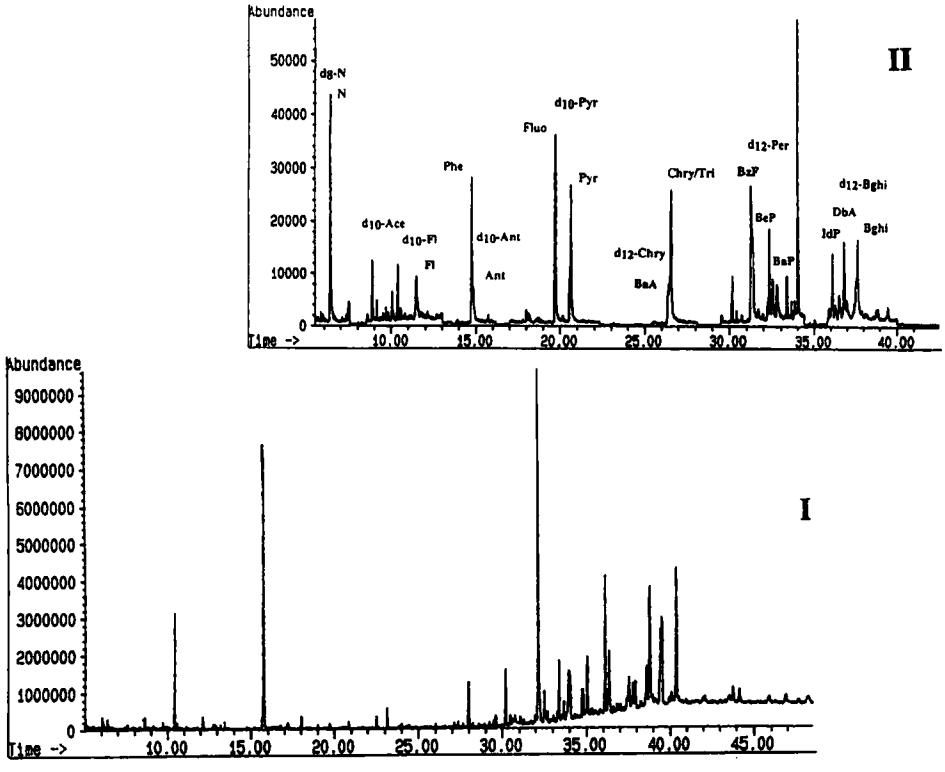

Figure 2 Scan run (I) of an organic humus layer extract (Oi horizon) and the corresponding SIM run (II) which was included in the sample exchange (see *Method comparison*). Abbrevations: Chry/Tri (Chrysene/Triphenylene), BzF (Benzofluoranthenes).

horizons (about 50% organic carbon) are very difficult samples because of generally only low PAH contents but great amounts of non-target substances.

RESULTS

Every step of the procedure susceptible to losses was tested for efficiency with standard compounds.

Partitioning

The first important partitioning process of the method is the transition of the PAHs from the KOH solution to hexane. Because of the salting-out effect[26,27] the recovery of PAHs is already quantitative (> 90%) after a single extraction with hexane. This was examined by recovery experiments with simple standard solutions. Humus-rich samples caused a dark black methanolic-aqueous solution, so the partitioning process with hexane was repeated several times for safety reasons.

Column chromatography

The column chromatography was examined with standard recovery experiments (6 parallels with 40 ng of each PAH). Quantification was performed using eicosane (200 ng) as injection standard.

The results for the PAHs show that column chromatography can be performed without considerable losses (Table 2). Slightly lower recoveries for high-molecular-weight PAHs reflect the strong adsorption capacity of the silica gel which was necessary in view of the great amount of non-target substances due to the complex matrix of the samples studied. Moreover, there are indications that active sites in polar adsorbents are rapidly covered by non-target substances investigating real samples[28], hence it is assumed that no significant losses occurred during sample preparation.

PAH recovery

For method evaluation PAH recoveries were determined for ten samples (Table 3). For this purpose the losses of the initially spiked amounts of internal standards were quantified. Quantification was carried out using d_{10}-fluoranthene as injection standard. Samples from organic horizons and mineral horizons were involved in the recovery experiment. Spiked amounts of d_{10}-fluoranthene were adapted to the expected PAH concentrations and ranged from 30 to 500 ng. Recoveries are independent of the sample matrix and concentration levels.

Table 2 Results of the standard recovery experiment for the Al_2O_3/Silica gel chromatography.

	Recovery [%]	*Standard deviation*
d_8-Naphthalene	95	± 4
Naphthalene	97	± 4
Acenaphthylene	97	± 2
d_{10}-Acenaphthene	97	± 3
Acenaphthene	96	± 2
d_{10}-Fluorene	96	± 2
Fluorene	94	± 3
Phenanthrene	98	± 2
d_{10}-Anthracene	96	± 2
Anthracene	95	± 3
Fluoranthene	97	± 3
d_{10}-Pyrene	96	± 3
Pyrene	96	± 4
Benzo(a)anthracene	92	± 3
d_{12}-Chrysene	92	± 4
Chrysene	92	± 4
Benzo(b,k)fluoranthene	92	± 5
Benzo(e)pyrene	94	± 4
Benzo(a)pyrene	90	± 5
d_{12}-Perylene	91	± 5
Perylene	91	± 5
Indeno(1,2,3-c,d)pyrene	89	± 8
Dibenzo(a,h)anthracene	81	± 11
d_{12}-Benzo(ghi)perylene	88	± 5
Benzo(ghi)perylene	86	± 5

Table 3 Mean recoveries and standard deviation of the standards spiked.

	Recovery [%]	*Standard deviation*
d_8-Naphthalene	76	± 11
d_{10}-Acenaphthene	82	± 6
d_{10}-Fluorene	82	± 11
d_{10}-Anthracene	94	± 6
d_{10}-Pyrene	88	± 7
d_{12}-Chrysene	88	± 8
d_{12}-Perylene	90	± 7
d_{12}-Benzo(ghi)perylene	89	± 7

In view of the complex matrix, losses of about 6–24% are surprisingly low. Similar values were determined by an international standard method in a sandy soil and a garden soil[29].

Internal standards

Internal standards were a special prerequisite for the quality of the developed method. This can be concluded from the categorized relative standard deviations of altogether 393 values determined with the method presented (Figure 3). Internal standards guarantee an excellent correspondence between parallel samples.

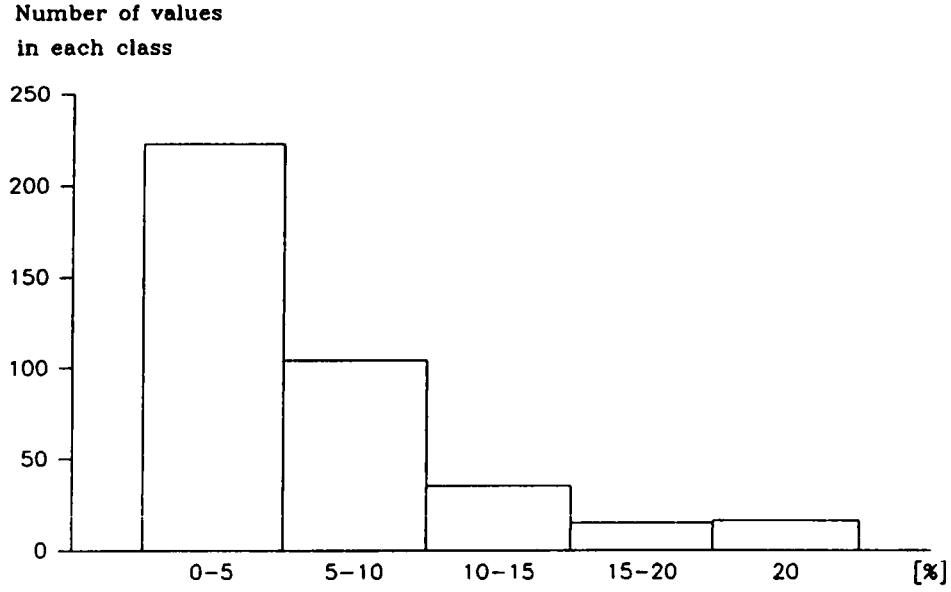

Figure 3 Standard deviations of duplicate samples categorized in the classes 0–5, 5–10, 10–15, 15–20 and > 20%.

Method comparison

In order to check the reliability of values produced with the saponification protocol a sample exchange programme was carried out with the *Staatliches Forschungsinstitut für Geochemie* in Bamberg (branch of the *Bayerisches Geologisches Landesamt*, Munich). Standard material was taken from an organic humus layer (Oe horizon). Eight replications of this material were extracted and analyzed in duplicate to evaluate the reproducibility of the method and measured with on-column injection as well as with splitless injection.

The research institute used two different extraction methods for this comparison: an extraction by shaking with acetone (A)[30], and an extraction method by shaking with petroleum ether/acetone/water (P/A/W)[31]. Extracts were cleaned-up on a silica gel column and measured with a cold on-column injection system by GC-MS. Internal quantification was performed by the respective deuterated analogue of each PAH.

The degree of correspondence between different methods was good (Table 4), especially for shaking with petroleum ether/acetone/water and saponification.

Figure 4 reveals that values determined with extraction method A are lower for almost all compounds compared with saponification.

Saponification was also examined with different soil horizons to test the influence of different matrices on extraction efficiency. Extraction methods P/A/W and A (see above)

Table 4 Comparison of the results (µg/kg) for the three extraction methods (A, P/A/W and S; S = extraction combined with saponification).

	Extraction method											
	A				*P/A/W*				*S*			
Analyte	*n*	*µg/kg*		σ_{n-1}	*n*	*µg/kg*		σ_{n-1}	*n*	*µg/kg*		σ_{n-1}
Naphthalene	2	35	±	4	2	38	±	2	8	44	±	1
Acenaphthylene	1	10		–	–	n.q.*		–	8	17	±	3
Acenaphthene	–	n.q.*		–	–	n.q.*		–	8	1	±	0
Fluorene	4	7	±	1	5	11	±	2	8	17	±	1
Phenanthrene	4	102	±	8	5	149	±	10	8	186	±	10
Anthracene	4	15	±	2	5	24	±	3	8	27	±	3
Fluoranthene	4	232	±	21	5	337	±	39	8	393	±	15
Pyrene	4	159	±	19	5	250	±	17	8	231	±	9
Benzo(a)anthracene	4	78	±	10	5	107	±	7	8	89	±	3
Chrysene/Triphenylene	4	219	±	16	5	529	±	40	8	499	±	16
Benzo(b,j,)fluoranthene**	4	314	±	25	5	577	±	21	8	519	±	45
Benzo(k)fluoranthene**	4	–		–	5	–		–	8	257	±	35
Benzo(e)pyrene	4	198	±	23	5	339	±	49	8	324	±	30
Benzo(a)pyrene	4	97	±	7	5	123	±	4	8	132	±	14
Perylene	4	26	±	3	5	23	±	3	8	26	±	3
Indeno(1,2,3-c,d)pyrene	4	109	±	19	5	172	±	24	8	200	±	8
Dibenzo(a,h)anthracene	4	35	±	6	5	45	±	4	8	35	±	3
Benzo(ghi)perylene	4	102	±	9	5	160	±	25	8	157	±	8

*(n.q.)Acenaphthene could not be quantified using extraction methods P/A/W and A; acenaphthylene could not be quantified using extraction method P/A/W due to chromatographic interferences from non-target compounds of similar molecular weights which mask the trace amounts of PAH.

**only the sum benzofluoranthene (b,j,k) concentrations was determined in the research institute.

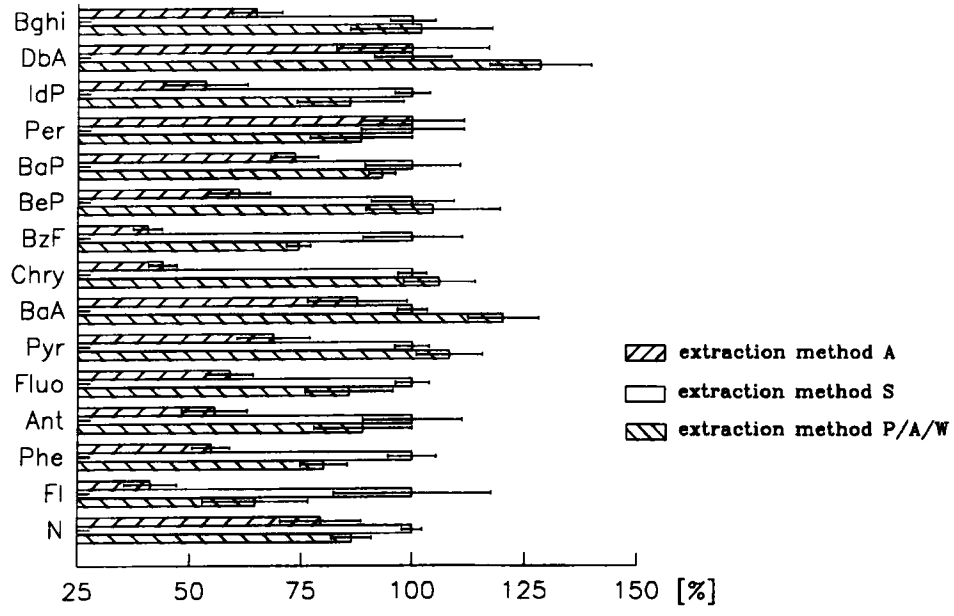

Figure 4 Comparison of extraction methods A, S, and P/A/W, with S value as 100% Symbol ⊢ marks the standard deviation.

served again for comparison. For this purpose samples were taken (Oi, Oi/Oe, Ah, Bt) from a soil profile which had been investigated as a reference location by a third researcher[6]. Four replicates were investigated of each sample for the saponification method, one extraction of each sample was carried out for the A and P/A/W method. The data are shown in Table 5.

Generally the correspondence of the values determined with the saponification, and the P/A/W and A methods was good again, even at very low concentrations (only few micrograms per kg in the Bt horizon). However there are also distinct differences of a rather unsystematic nature. It is not clear whether the reason for this phenomenon is the influence of the matrix or analytical artefacts. At any rate the comparison clearly demonstrates the difficult task of analyzing carbon-rich matrices like organic humus layers.

In view of these results a definite recommendation cannot be given which extraction method is preferable for the complex matrix represented by organic humus layers.

Uncomplicated clean-up and measurement and the high inherent reliability of values due to an excellent reproducibility are in favour of saponification, but the disadvantage is a relatively high requirement of time.

The development of an extraction method which copes with the various difficulties described under *Extraction*, whether via saponification or with solvent mixtures like P/A/W, seems to follow the right way. This is also stressed by a recently published paper[32] which present results of a combination of an organic solvent extraction and subsequent saponification.

Table 5 Comparison of the results (µg/kg) for the three extraction methods P/A/W, A and S at different soil horizons.

Analyte	*Extraction method*											
	A				*P/A/W*				*S*			
Soil horizons:	*Oi*	*Oi/Oe*	*Ah*	*Bt*	*Oi*	*Oi/Ot*	*Ah*	*Bt*	*Oi*	*Oi/Oe*	*Ah*	*Bt*
Naphthalene	38	35	17	7	48	46	13	14	6	16	19	7
Acenaphthylene	n.q.	n.q.	n.q.	n.q.	1	n.q.	n.q.	n.q.	n.q.	2	10	0
Acenaphthene	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.
Fluorene	7	17	4	1	17	14	5	3	5	4	2	0
Phenanthrene	16	25	53	3	22	27	53	3	33	41	71	5
Anthracene	2	0	7	0	2	1	4	2	n.q.	3	16	1
Fluoranthene	34	76	199	4	34	67	176	6	31	80	157	5
Pyrene	25	40	109	3	50	38	95	3	20	47	101	4
Benzo(a)anthracene	7	18	43	1	17	17	36	2	3	17	47	2
Chrysene/Triphenylene	29	67	165	4	53	64	157	5	17	87	234	9
Benzo(b,j,k)fluoranthene	52	124	317	9	87	123	250	15	32	172	945	26
Benzo(e)pyrene	75	101	218	7	86	91	204	6	12	66	241	8
Benzo(a)pyrene	14	24	72	2	18	30	57	5	5	23	76	2
Perylene	2	0	18	n.q.	15	n.q.	16	n.q.	n.q.	4	16	0
Indeno(1,2,3-c,d)pyrene	26	51	109	4	45	55	91	4	13	59	124	5
Dibenzo(a,h)anthracene	n.q.	9	23	1	8	n.q.	19	2	3	10	27	1
Benzo(ghi)perylene	12	33	64	2	23	33	57	3	8	41	93	4
Σ 20 PAH (µg/kg)	339	620	1418	48	526	605	1233	73	188	672	2179	79

CONCLUSIONS

The method developed permits the correct determination of PAHs in extremely difficult matrices like organic humus layers, and also in mineral soils; the time consumed in the analytical procedure is acceptable. Internal standards guarantee an excellent repeatability of results.

The development of an extraction method that is able to release satisfactorily hydrophobic contaminants from a complex matrix like soil organic matter has to orientate itself to the nature of the substrate analysed. Extraction combined with saponification seems very promising for this purpose.

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