Comparison of various extraction and clean-up methods for the determination of polycyclic aromatic hydrocarbons in sewage sludge-amended soils ☆

G. Codina, M.T. Vaquero, L. Comellas*, F. Broto-Puig

Secció de Cromatografia, CETS Institut Químic de Sarrià (Universitat Ramon Llull), Institut Químic de Sarrià s/n, 08017-Barcelona, Spain

Abstract

Different extraction and clean-up methods for the determination of polycyclic aromatic hydrocarbons (PAHs) in sewage sludge-amended soil were investigated. Soxhlet plus saponification extraction and silica gel clean-up provided the best results. HPLC with a programmable fluorescence detector performed accurate identification and quantification of PAHs. The initial PAH concentrations in sewage sludge-amended soil ranged from 8 ng/g for benzo[a]fluoranthene to 93 ng/g for phenanthrene, with a total PAH concentration of 417 ng/g. The weathering of PAHs in sludged soil was monitored for a 141-day period. The results indicated that low-molecular-mass PAHs were susceptible to abiotic and biotic loss processes.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds that are principally formed during the incomplete combustion of organic material such as coal, petrol, oil and wood. Wastewater catchments receive PAHs from two main sources: industrial and domestic fossil fuel spillages and urban runoff inputs that flush the hydrocarbons deposited on the ground surface from vehicles or heating systems. As a result of their very low aqueous solubility, PAHs are efficiently removed from the water during sedimentation in the wastewater treatment. This results in the formation of sewage sludges that typically contain between 1 and 10 mg/kg of each individual PAH [1–4].

A significant proportion of the generated sewage sludge is applied to land as an organic fertilizer or amendment. Because some PAHs are known or suspected carcinogens, the fate of these compounds in the soil environment is critical in assessing their potential hazard risk [5,6]. However, there is little information about the persistence or loss of PAHs in sewage sludge-amended soil and the mechanism of their biodegradation [7].

A prerequisite to determining the evolution of PAHs in sludged soils is to develop sensitive and effective analytical methods to measure their concentration. Their extraction and isolation from complex samples, where PAHs are only a minor fraction of the organics present, require

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* Corresponding author.
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accurate protocols. Several procedures have been described [8].

This paper discusses the use of Soxhlet or Soxhlet plus saponification extractions, and compares silica gel clean-up according to the EPA 3630B method with the specific PAH clean-up with XAD-2 [9, 10]. An HPLC method with both UV and fluorescence detection was also developed. Using the method developed, the weathering of PAHs from sewage sludge-amended soil was also studied for a 141-day period in a laboratory experiment.

2. Experimental

2.1. Experiment design and details

Anaerobically digested sewage sludge was obtained from DARGISA, a sewage aerobic treatment plant in Girona (Spain). The sludge was air-dried and ground to less than 0.4 mm before soil addition. Limey soil from Bellaterra (Barcelona, Spain) was ground and sieved to less than 2 mm.

High doses of sewage sludge (15%) were mixed with soil and were placed in 6-kg polyethylene containers. Three different treatments were adopted in duplicate: (a) control sewage sludged soil amended with 15% of sewage sludge (CON A, CON B), (b) sludge-amended soil to which hydrocarbons were added at twice the control concentration (PAH 2A, PAH 2B) and (c) sludged soil to which hydrocarbons were added at ten times the control concentration (PAH 10A, PAH 10B). Experimental soils were watered when necessary to maintain a moisture content of 20% in order to ensure half the water-holding capacity, which was appropriate to obtain the best microbiological activity [11]. Soils were sampled after 0, 4, 8, 15, 22, 32, 44, 60, 81 and 141 days. Homogeneous samples were guaranteed by sampling and mixing through all the container depth. The samples were preserved with formaldehyde (0.4%) and were stored in the dark at 4°C. Simultaneously two other experiments were applied: (a) sludge-amended soil with twice the hydrocarbon concentration of the control to which mercury(II) chloride (HgCl$_2$)

(2% by mass) was added to ensure complete sterilization (PAH 2Hg) and (b) sludged soil with ten times the control concentration with the addition of 2% of HgCl$_2$ for sterilization (PAH 10Hg). These soils were sampled after 0 and 20 days.

2.2. Reagents and materials

Dichloromethane, hexane and pentane (Pestipur) were obtained from SDS (Peypin, France). Methanol and acetonitrile of HPLC grade and isooctane for residue analysis were supplied by Merck (Darmstadt, Germany). Toluene (purissimum grade) was purchased from Panreac (Barcelona, Spain) and was glass distilled prior to use. Deionized water was produced with a Millipore Milli-Q system from Waters (Milford, MA, USA). Anhydrous sodium sulphate (analytical-reagent grade) was supplied by Merck and was treated at 300°C for 12 h before analysis. Potassium hydroxide (analytical-reagent grade) and formaldehyde (35–40%) (purissimun grade) were obtained from Panreac. Silica gel 60 (63–200 μm) was supplied by Merck. XAD-2 from Alltech (Deerfield, IL, USA) was used.

Naphthalene (Na), fluorene (Fl), phenanthrene (Pa), anthracene (An), fluoranthene (Fa), pyrene (Pyr), chrysene (Chry), benzo[b]fluoranthene (BbFa), benzo[k]fluoranthene (BkFa) and benzo[a]pyrene (BaPyr) were obtained in pure form from Supelco (Bellefonte, PA, USA). Solid PAHs were dissolved in toluene in order to spike sludged soils. A 610-M commercial PAH mixture from Supelco, containing the previous PAHs and also acenaphthylene (Aci), acenaphthene (Ace), benz[a]anthracene (BaAn), dibenzo[a,h]anthracene (diBahAn), indenopyrene (I-P) and benzo[ghi]perylene (BghiPer), was used in appropriate dilutions with acetonitrile to study the chromatographic system and used as an external standard.

2.3. Soxhlet extraction

About 1 g of sewage sludge or 10 g of sludge-amended soil, thoroughly mixed with 10 g of anhydrous sodium sulphate, was Soxhlet extracted with 200 ml of dichloromethane for 6 h at

a rate of 4–6 cycles/h. The extract was then dried over 0.5 g of anhydrous sodium sulphate. The decanted extract was evaporated at 40°C in a rotary evaporator under reduced pressure to near dryness, dissolved in 1 ml of isooctane or acetonitrile for silica gel or XAD-2 clean-up, respectively, and re-evaporated to less than 1 ml.

2.4. Soxhlet and saponification extraction

Soxhlet extraction was carried out with 200 ml of dichloromethane for 6 h. The solvent was concentrated to 5 ml in a rotary evaporator under reduced pressure. A 100-ml volume of 0.5 M potassium hydroxide in methanol was added and the mixture was refluxed for 4 h in a water-bath at 80°C. After cooling 20 ml of Milli-Q-purified water were added and extraction was performed with hexane (3 × 50 ml). The combined organic extracts were dried over 0.5 g of anhydrous sodium sulphate. The decanted extract was evaporated at 40°C in a rotary evaporator under reduced pressure to near dryness, dissolved in 1 ml of isooctane or acetonitrile for silica gel or XAD-2 clean-up, respectively, and re-evaporated to less than 1 ml.

2.5. Silica gel clean-up

Silica gel was activated at 130°C for 16 h. The glass column (1.2 cm I.D.) was slurry packed in dichloromethane with silica gel (10 g) and a top layer of anhydrous sodium sulphate (0.5 g). The column was rinsed with 40 ml of hexane before use. The extract was transferred on to the column and sequentially eluted with 25 ml of hexane and 30 ml of hexane–dichloromethane (60:40) to give fractions enriched in alkanes and PAHs, respectively. The second eluate was evaporated under reduced pressure to near dryness and replaced with exactly 1 ml of acetonitrile.

2.6. XAD-2 clean-up

The glass column (10.4 × 1.2 cm I.D.) was slurry packed in methanol with XAD-2. The column was rinsed with 15 ml of pentane, 32 ml of toluene and 25 ml of methanol before use.

The extract was transferred on to the column and sequentially eluted with 25 ml of methanol, 15 ml of pentane and 32 ml of toluene to give fractions enriched in polar compounds, alkanes and PAHs, respectively. The third eluate was evaporated under reduced pressure to near dryness and replaced with exactly 1 ml of acetonitrile.

2.7. Chromatographic instruments and conditions

A Tracer-Spherisorb ODS analytical column (5 μm, 243 mm × 4 mm I.D.) and a Spherisorb ODS guard column (4 mm I.D. cartridge), both from Teknokroma (Sant Cugat, Spain), were used. The chromatographic system consisted of a Waters Model 600E pump with a Rheodyne (Cotati, CA, USA) 20-μl loop injector. A Model 991 photodiode-array detector with integration software from Waters, a Model 470 fluorescence detector from Waters and a Model D-2000 integrator from Merck–Hitachi (Tokyo, Japan) were also used.

Acetonitrile and water were used as eluent components at a flow-rate of 1 ml/min. Linear gradient elution from 50% acetonitrile at 0 min to 100% acetonitrile at 25 min was applied, followed by isocratic elution with acetonitrile for 17 min.

The UV detector was set at 254 nm in combination with the diode-array detector to identify PAHs in the samples. The fluorescence excitation and emission wavelengths were changed during the chromatographic separation in order to obtain better sensitivity. The excitation/emission wavelengths were set as follows: 280/340 nm at 0 min, 250/376 nm at 16 min, 286/460 nm at 18 min, 305/430 nm at 19.5 min and 296/425 or 305/425 nm at 28 min to detect BghiPer or I-P, respectively.

3. Results and discussion

3.1. High-performance chromatography

The precision of the retention time within one day ranged from 0.2 to 0.8%; analyses from

day-to-day produced only a slight decrease in precision. The peak-height precision within one day ranged from 2.5 to 6.7% for the fluorescence detector and from 1.7 to 4.0% for the UV detector. The peak area precision was poorer, from 2.7 to 8.7% and from 2.3 to 3.7% for the fluorescence and UV detectors, respectively. The reproducibility from day-to-day of peak heights produced only a slight decrease in precision. Therefore, PAH determinations were performed by comparison of the peak heights with those of an external standard injected daily.

HPLC with fluorescence detection provided a linear response from 64 to 0.002 g/ml for each PAH and detection limits between 9 ng/ml for Na and 0.1 ng/ml for BkFa. HPLC with UV detection (254 nm) was also linear from 64 to 0.002 g/ml, but had higher detection limits between 62 ng/ml for Aci and 1 ng/ml for An. The detection limits calculated with a signal-to-noise ratio of 3 (IUPAC criteria) were decreased between 2- and 15-fold using fluorescence detection compared with UV detection.

3.2. Analytical procedure

A sample of sewage sludge-amended soil was submitted to the above-described extraction and clean-up methods. The subsequent PAH fractions were analysed by HPLC with photodiode-array and programmable fluorescence detection. The chromatograms are displayed in Fig. 1.

According to their retention times and UV spectra, several PAHs could be identified in the sludged soil: Pa, An, Pyr, Chry/BaAn, BbFa and BaPyr. Interferences from most other compounds in the extract were greatly reduced with fluorescence detection, resulting in the additional identification by retention times of Fl/Ace, Fa, BkFa and BghiPer. Moreover, HPLC with fluorescence detection is more selective and sensitive than HPLC with UV detection for the determination of PAHs and, therefore, is more suitable for determining them in sewage sludge-amended soils.

XAD-2 clean-up split the PAHs into two fractions: the low-fused PAHs were eluted with pentane and the high-fused PAHs with toluene.

The silica gel column provided all PAHs in the second fraction together with alkylbenzenes, but these monoaromatic hydrocarbons did not interfere with HPLC analysis. Further, the concentration of low-fused aromatic hydrocarbons, namely Na and Fl/Ace, isolated with XAD-2 clean-up was lower than that obtained with silica gel clean-up. Losses were probably due to irreversible interactions of PAHs with the XAD-2 polymer and volatilization during toluene evaporation. Moreover, chromatograms of XAD-2 extracts presented more interferences, probably owing to the elution of monomers and oligomers of the polymer together with PAHs, although XAD-2 had been carefully cleaned. Hence the use of silica gel to clean up extracts appeared more suitable for PAH determinations.

Saponification improved the fluorescence profiles and enhanced the determination of PAHs. Associations between minor PAHs and lipid sludge fraction are reduced when the raw extract is submitted to a basic treatment, and liquid–liquid partitioning allows fatty acid removal and, therefore, extract clean-up is made easier.

The effectiveness of the methods was assessed with the analysis of a sludged soil spiked with PAHs. The recoveries were better for Soxhlet plus saponification extraction with silica gel clean-up (Table 1). Again, fluorescence detection was shown to perform better than UV detection in HPLC analyses. When the recoveries were calculated with UV chromatograms, Fa, Pyr and BkFa presented anomalous values, probably owing to difficulties in the quantification. In conclusion, Soxhlet plus saponification, silica gel clean-up and HPLC with fluorescence detection are suitable for PAH identification and quantification in sewage sludge-amended soil.

The precision of the selected procedure was studied with the analysis of a sludged soil sample in quadruplicate. The repeatability of analyses within one day was 17–31% with the exception of BghiPer (56%). The reproducibility of analysis from day-to-day was 25–37% with the exception of Na (81%). The accuracy of the method was tested by a standard addition analysis. Three different levels of addition were made in order to
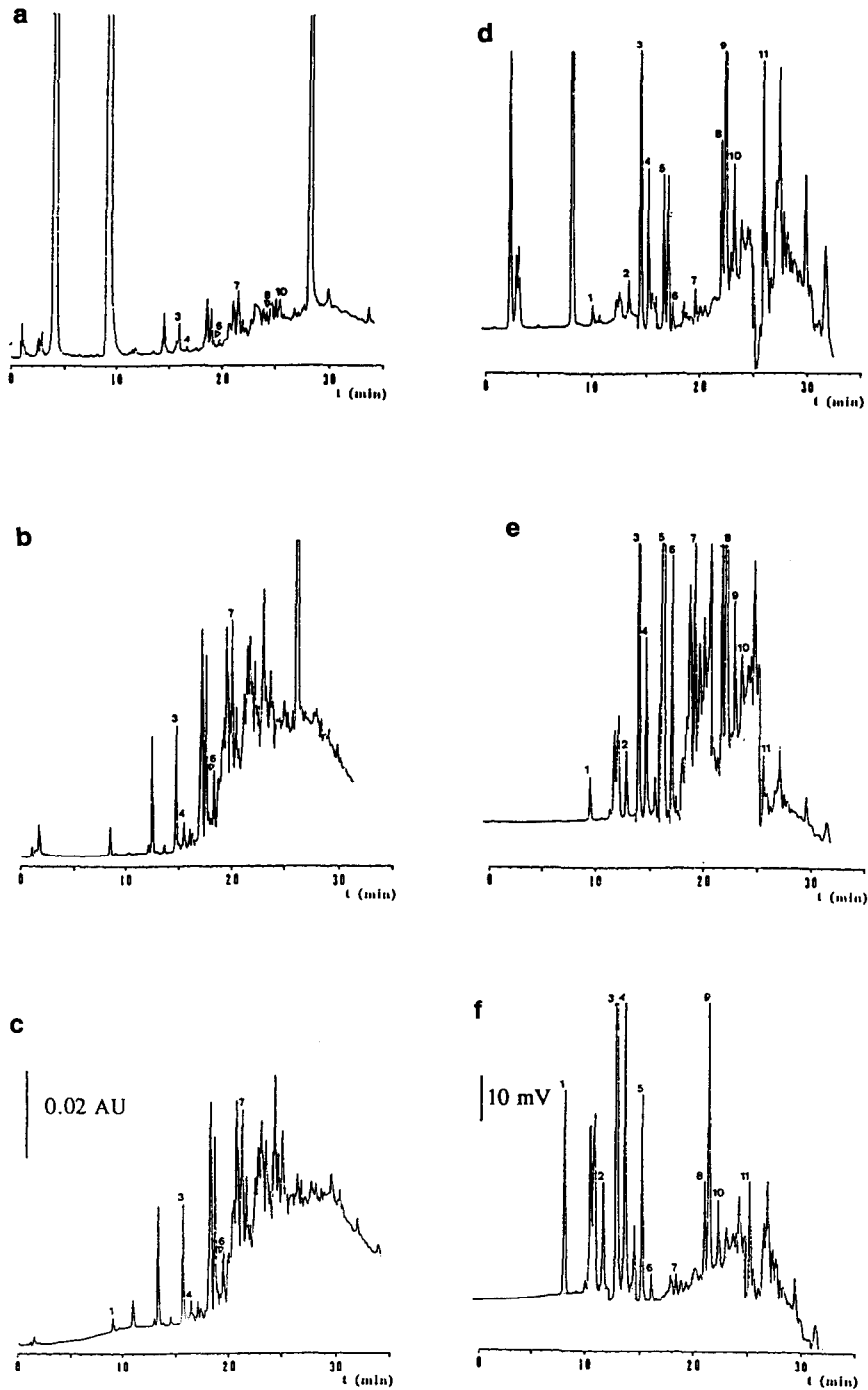
Fig. 1. (a–c) HPLC–UV and (d–f) HPLC–fluorescence detection of PAHs isolated from sewage sludge-amended soil by both extraction and clean-up methods: (a and d) Soxhlet with XAD-2 clean-up; (b and e) Soxhlet with silica gel clean-up; (c and f) Soxhlet plus saponification with silica gel clean-up. Identified PAHs: 1 = Na; 2 = Fl/Ace; 3 = Pa; 4 = An; 5 = Fa; 6 = Pyr; 7 = Chry/BaAn; 8 = BbFa; 9 = BkFa; 10 = BaPyr; 11 = BghiPer.

Table 1
Recoveries (%) of PAHs added to sewage sludge-amended soil obtained with different analytical methods

PAH	Soxhlet				Soxhlet + saponification			
	XAD-2		Silica gel		XAD-2		Silica gel	
	UV	Fluorescence	UV	Fluorescence	UV	Fluorescence	UV	Fluorescence
Na	0	0	46	41	0	2	74	80
Aci	7	–[a]	48	–[a]	21	–[a]	106	–[a]
Fl/Ace	63	8	64	53	42	21	128	119
Pa	70	70	40	46	117	115	108	102
An	41	41	50	50	96	95	106	106
Fa	159	65	240	61	162	124	256	108
Pyr	7	62	–9	212	79	107	–19	89
Chry/BaAn	76	61	43	52	146	132	95	96
BbFa	63	71	59	63	140	148	94	105
BkFa	111	65	174	59	185	142	233	106
BaPyr	59	50	80	41	107	120	121	82
diBahAn	44	62	0	56	120	148	108	101
I-P	0	–[a]	0	–[a]	172	–[a]	119	–[a]
BghiPer	0	54	46	54	145	114	103	93

[a] Do not have fluorescence at the programmed wavelengths.

increase the PAH concentration in the sewage sludged soil by two, five and ten times. The mean recoveries obtained were over 85%, except for Na (60%), Fl/Ace (44%), BaPyr (21%) and BghiPer (70%).

3.3. PAH losses from sewage sludge-amended soil

Significant losses of PAHs from sewage sludge-amended soil were achieved over the whole experimental period. Fig. 2 shows the results for HPLC with fluorescence detection at days 0, 8 and 141 for sample extracts taken from treatment CON B.

The losses of Na, Fl, Pa, An, Fa and Pyr were statistically different from zero, according to an analysis of variance at the $\alpha = 0.1$ level of confidence. Moreover, differences between loss percentages of these PAHs were very important. The low-molecular-mass PAHs appeared to be eliminated faster than the high-molecular-mass PAHs. The compound structure seems to in-

fluence PAH losses strongly. Losses of PAHs with the highest molecular mass, *i.e.*, Chry, BbFa, BkFa, BaPyr and BghiPer, were not statistically different from zero ($\alpha = 0.1$). These observations were consisted with other reported findings [12–14].

Fig. 3 shows the evolution of the total PAH concentration during the experimental period. This concentration also decreased statistically ($\alpha = 0.1$ level of confidence).

Half-lives were calculated for individual and total PAHs, as shown in Table 2. The expected increase in half-life with increasing molecular mass was observed in all the experiments. Further, half-life derived for experiments with the highest initial concentration of PAHs (PAH 10) are lower than those for the experiments with lower initial concentrations (CON and PAH 2). Losses of PAHs were statistically more rapid when the initial concentration was higher. Overall, PAHs added to the sludged soil appeared to be more susceptible to loss mechanisms and did not mimic the behaviour of sludge-applied PAHs.

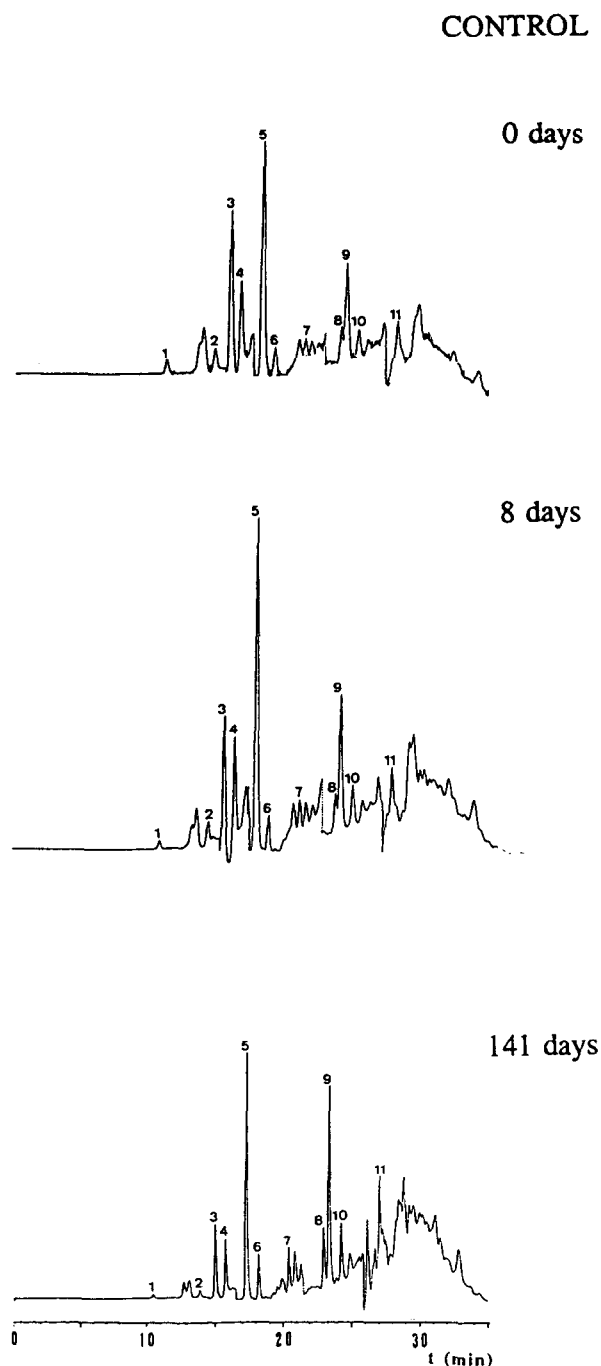CONTROL

0 days

8 days

141 days

Fig. 2. HPLC–fluorescence detection of PAHs from CON experiment (sewage sludge-amended soil without addition) after 0, 8 and 141 days of evolution. Identified PAHs: 1 = Na; 2 = Fl/Ace; 3 = Pa; 4 = An; 5 = Fa; 6 = Pyr; 7 = Chry/BaAn; 8 = BbFa; 9 = BkFa; 10 = BaPyr; 11 = BghiPer.

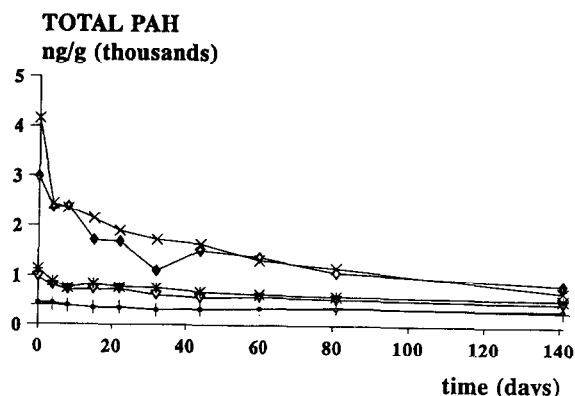**TOTAL PAH**
ng/g (thousands)

time (days)

Fig. 3. Variation of total PAH concentration in sewage sludge-amended soil for each experiment *versus* time elapsed from the start of the experiment. □ = CON A; + = CON B; ∗ = PAH 2A; ∇ = PAH 2B; × = PAH 10A; ◇ = PAH 10B.

3.4. Biotic and abiotic losses

Losses of PAHs from the sludged soils biologically inactived with $HgCl_2$ (2%) were due to abiotic mechanisms (mainly volatilization), whereas losses from the sludge-amended soil were due to the combination of both abiotic and biotic mechanisms. Biotic losses were then calculated by subtracting the abiotic losses from inactive soils from the total losses from biologically active soils. Fig. 4 shows the estimated abiotic and biotic loss percentages of PAHs in the experiment with double addition (PAH 2 and PAH 2Hg).

Biological loss processes were significant for some of the low-molecular-mass PAHs (Na, Fl, Pa and An). Losses for all PAHs occurred partially due to non-biological processes, and these were the main mechanisms of losses for Fa and Pyr.

As expected, the rate of biodegradation decreased with increasing number of benzene rings in the PAH molecules, probably owing to changes in the aqueous solubility, bioavailability and structural stability with increasing molecular mass. In literature it is reported that low-molecular-mass PAHs, such as naphthalene and phenanthrene, can act as the sole carbon/energy sources for microbes, while the multi-ringed

Table 2
PAH half-life derived for sewage sludge-amended soils

PAH	$t_{1/2}$ (days)					
	CON A	CON B	PAH 2A	PAH 2B	PAH 10A	PAH 10B
Na	3	3	3	3	2	2
Fl	16	13	7	11	3	7
Pa	14	18	6	7	4	7
An	24	29	20	2	4	14
Fa	260	175	100	102	46	69
Pyr	n.d.[a]	n.d.[a]	189	383	68	78
PAH	623	648	107	131	17	25

[a] Not determined.

species cannot act as sole sources, but may be degraded slowly in the environment by co-oxidation processes [7,14].

On the other hand, the percentage of abiotic losses decreased only slightly with increasing molecular mass. Other studies have shown greater differences between abiotic PAH losses, due to decreasing volatilization with increasing molecular mass [14].

Biodegradation seems to be an important process leading to the loss of low-molecular-mass PAHs in the soil system. This is feasible because the application of sludge to soil causes a period of enhanced microbial activity due to the addition of an available substrate and essential nutrients.
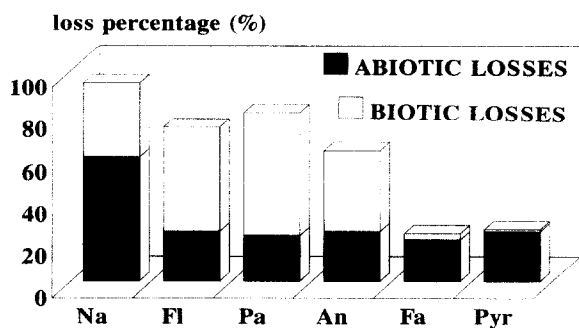
4. Conclusions

Soxhlet plus saponification and HPLC with fluorescence detection are suitable for assessing PAH weathering in sewage sludge-amended soil. Losses of low-molecular-mass PAHs were significant over the experimental period, but high-molecular-mass PAHs were persistent. Moreover, the loss rates were faster for PAHs added at high concentrations to the sludged soil. The results suggested that biological degradation and abiotic processes were important for some of the low-molecular-mass PAHs.

Acknowledgements

Fig. 4. Estimated abiotic and biotic loss percentages of some PAHs in 20 days from the experiment with double addition of PAHs (HID 2 and HID 2Hg).

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