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Review

Stable carbon isotopic ratio measurement of polycyclic aromatic hydrocarbons as a tool for source identification and apportionment—A review of analytical methodologies

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ABSTRACT

The measurement of the ratio of stable isotopes of carbon ($^{13}C/^{12}C$ expressed as a $\delta^{13}C$) in the individual components of a sample may be used as a means to identify the origin of these components. This article reviews the approaches and reports on the successes and failures of source identification and apportionment of Polycyclic Aromatic Hydrocarbons (PAHs) with the use of compound-specific isotope analysis (CSIA). One of the conditions for a precise and accurate analysis of isotope ratios with the use of GC–C–IRMS is the need for well separated peaks, with no co-elutions, and reduced unresolved complex mixture (UCM). Additionally, special care needs to be taken for an investigation of possible isotope fractionation effects introduced during the analytical treatment of samples. With the above-mentioned problems in mind, this review discusses in detail and compares current laboratory methodologies, mainly in the extraction and subsequent clean-up techniques used for environmental samples (air particulate matter, soil and sediments). Sampling strategies, the use of isotopic internal standards and the ranges for precision and accuracy are also reported and discussed.

Contents

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1. Introduction

Analysing the ratio of stable isotopes of carbon ($^{13}C/^{12}C$ expressed as a $\delta^{13}C$) in the individual components isolated from various environmental samples is one of the means to identify the origin of these components [1]. In contrast, the bulk $\delta^{13}C$ value of a given sample represents an average of all carbon compounds present in the sample, and thus averaging of all their sources. The allocation of the contaminants to a specific source allows an appropriate method of risk reduction to be taken, or can be used to identify the parties responsible for the contamination [2].

Some of the first studies on source identification applying gas chromatography coupled to isotopic ratio mass spectrometry (GC–IRMS), were published by Matthews and Hayes [3], Freeman et al. [4] and Riely et al. [5]. In this technique the components of the sample are converted to a desired gas (e.g. CO_2, H_2, N_2) as they elute from a chromatographic column whilst the effluent is continuously analysed by IRMS for isotopic composition. For this reason, it is often referred to as Gas Chromatography–Combustion–Isotopic Ratio Mass Spectrometry (GC–C–IRMS). Later, following technical and software improvements, the technique became widely applied. GC–C–IRMS is described in detail elsewhere, e.g. by Ricci et al. [6] or a review by Meier-Augenstein [7].

The variations in isotopic abundances are normally very small, thus the carbon isotopic data are given as per mil (‰) difference from the reference [8]. The reference for carbon is Vienna Peedee Belemnite (VPDB) which $\delta^{13}C$ defines 0‰ on the VPDB scale. The conventional δ-notation is expressed as

$$\delta^{13}C = \frac{^{13}C/^{12}C_{sample}}{^{13}C/^{12}C_{VPDB}} - 1$$

VPDB is a hypothetical standard substitute for PBD (Peedee Belemnite), a cretaceous belemnite, *Belemnitella Americana*, from the Peedee formation of South Carolina [9] which was originally used as an international standard. The equation above originally contained a term ∗1000 which denoted per mil. The convention, however, requires the user to additionally place the symbol ‰ after the numerical value [10] and thus, according to the latest IUPAC guidelines [10], to minimise the confusion the term ∗ 1000 should be deleted from the equation.

Polycyclic Aromatic Hydrocarbons (PAHs) are a class of compounds that contain two or more fused benzene rings. They are widely spread in the environment (e.g. atmosphere, sediments, soil) and can be classified into three general categories: petrogenic-derived from slow maturation of organic matter under geothermal gradient conditions; pyrogenic-derived from incomplete combustion of organic matter; and short-term diagenetic products derived from biogenic precursors [11]. Major sources for PAHs are: combustion of fossil fuels (coal, oil, natural gas and wood), arising from vehicle exhausts, power and heat generation plants, residential heating, incinerators, several industrial processes (e.g. coke production, aluminium smelting); fuel oil or gasoline spills, natural seeps and creosote releases. The anthropogenic emissions contribute the greater proportion of PAHs [12]. They are suspected carcinogens and the US EPA (US Environmental Protection Agency) has identified 16 parental PAHs as priority pollutants. Because of the hydrophobic and lipophilic properties of PAHs they are adsorbed on particulate matter and eventually accumulate in sediments or soils [13].

The investigation of molecular ratios is the most common approach for source identification of PAHs [14–17]. Such an approach suffers, however, from many complications. In addition to the non-uniqueness of some source signatures [13,18,19] and the fact that molecular compositions of PAHs depend on various factors such as source material, combustion temperature, air/fuel ratio [1,20], various processes, including physical, chemical and microbial transformations may alter these molecular compositions, so that they may no longer resemble the original fingerprint of the source [1,12,13,21–24]. Thus, source investigations using molecular considerations, especially for heavily weathered samples, can often be difficult. In such cases, the use of additional information, e.g. carbon isotopic ratios, are needed to improve the studies of the origin or fate of PAHs [25]. The stable carbon isotope composition of individual PAHs is expected to reflect those of the organic matter after processes such as combustion [1] and is relatively resistant to environmental degradation. It can complement the molecular methods, providing an effective additional tracer [26]. As an example, the use of compound-specific isotope analysis (CSIA) (PAHs extracted from the sediments of Elisabeth River, VA, USA) made it possible to separate the two signatures derived from similar sources—coal and coal gasification [11]. The sole use of isomer ratios (fluoranthene/pyrene and benzo(a)anthracene/chrysene) did not allow the source contributions to be differentiated.

Compound-specific isotope analysis of carbon isotopes has been successfully applied as an alternative [12] or complementary method to molecular fingerprint investigations as a means of source identification and apportionment of PAHs pollutants [11,13,19,27–36]. One of the conditions for a reliable analysis of isotope ratios when applying GC–C–IRMS is to obtain well separated peaks, free of co-elutions and unresolved complex mixture (UCM) [26,37]. During the chromatographic separation, an isotope effect associated with the partitioning of the analyte between the mobile and stationary phase occurs. The isotopically heavier molecules elute slightly earlier, thus, the isotope ratios vary significantly across the widths of peaks. The beginning of the peak is strongly enriched in ^{13}C whilst the end of the peak is depleted [6]. In the case that compounds of interest are not well resolved from each other or from the impurities present in the sample, an artificial enrichment of the preceding peak in ^{13}C would occur and the depletion of the following compound during the separate integration of peak areas. If the carbon isotope ratio of a co-eluting compound, even of a minor one, is significantly different from the target component, it can have a significant effect on its isotope ratio [26]. Samples, particularly these contaminated by oil, contain UCM, which usually appears as a broad hump in the gas chromatogram and can degrade the precision and accuracy of the isotope measurement of PAHs [38].

The aim of this paper is to review the existing publications on carbon stable isotope measurements in PAHs isolated from environmental samples such as soils, sediments and air particulate matter in order to facilitate the source identification and apportionment of these toxic organic pollutants. We focus mainly on the methodologies used for extraction of PAHs from these samples and further steps performed such as sample clean-up and class separation in order to accurately measure the isotopic ratios

Table 1
The details of procedures used for extraction of PAHs from the environmental/sources samples.

Sample type and size (if specified)	Extraction method	Use of drying agent	Solvent used	Additional remarks	Reference
Dried and crushed sediment (15 g [12,29], 15–40 g [13]), 35 g dry of soil [28], fireplace soot (at least 5 g [12,29], 2–4 g [13], 20 g [28]), road sweep (2–4 g [13] from 1 m^2 asphalt), 1 g ground tyre [28]	Soxhlet (24 h)	2 g Na_2SO_4 [12,13,29]; 10 g aliquot of Na_2SO_4 [28]	140 ml dichloromethane (DCM)		[12,13,28,29]
Soil samples, dried, sieved to 2 mm and ground	Ultrasonic extraction (3 × 30 min, 30 °C)	ns[a]	Chloroform/methanol (MeOH) (3:1; v-v)		[30]
2 cm slices (10 cm diameter) of sediment, freeze-dried and sieved at 2 mm [31,47], 10 g of sediments [25]	Microwave assisted extraction (10 min, 30 W)	ns	DCM	Sample filtered after extraction	[25,31,47]
Sediment	Soxhlet (24 h)	2 g Na_2SO_4	DCM + MeOH (10%)		[48]
Sediment	ns	ns	DCM		[49]
10 g of wet sediment	Soxhlet (16 h)	10 g Na_2SO_4	DCM	Extract added dropwise to *n*-hexane under stirring, the precipitated vinyl polymers were centrifuged off	[50]
Sediment homogenised to a fine powder	Soxhlet (overnight)	ns	Acetone/*n*-hexane (60:40; v-v)		[27]
Dried and sieved (< 2 mm) soil; 25–500 g	Accelerated solvent extraction (ASE)	ns	*n*-Hexane/acetone (2:1)		[58]
Diesel particulates collected on a filter paper, soil (ground to 75–212 μm [19]), fly-ash, soot samples; coal tars [19,71], jet fuel and gasoline particulates [71]	Reflux (10 h or 16 h [19])	ns	DCM		[19,43,45,53,71]
Coal	Reflux (10 h)	ns	Chloroform		[44]
Ashes from laboratory burns	Soxhlet (24 h)	Na_2SO_4	150 ml DCM		[18]
Aerosols collected on filters	Soxhlet (16 h)	ns	150 ml DCM		[46]
Sediment	ASE	ns	Acetone/DCM		[11]
10 mg of soot, 20 mg of sediment, aerosol filter and PUF cartridges	Sonication (sediment [38] or aerosol filters [33])	ns	Benzene/MeOH (3:1); reduced in volume and liquid–liquid extracted with *n*-hexene/ether (9:1, v-v)	0.2 g of grained copper was activated by 1 M HCl and added to the extraction solvent of soot and sediment	[1,20,33,38,62,63]
	Soxhlet (20 h) (aerosol filter [33,38,63] + PUF cartridge [38] or sediment [62]) Soot (method ns) [38] Soxhlet (20 h) or sonication in acetone (gasoline exhaust particles and diesel exhaust particles [20])		Acetone; reduced in volume and liquid–liquid extracted with *n*-hexene/ether (9:1, v-v) [31], or solvent exchanged to *n*-hexane by adding 3 ml and washing by 3 ml of water [1,20,63]		
Aerosol filters and PUF cartridges	Soxhlet (24 h)	Na_2SO_4	DCM or acetone		[65]
Aerosol filters	Ultrasonic extraction (5 × 15 min)	Na_2SO_4	5 × 30 ml DCM		[70]
15 g of partially dried sediment (or 50–100 g when low PAHs content sediments were sampled)	ASE	Na_2SO_4	DCM		[26,34,35]
Certified reference material (LGC 6140) and a contaminated soil (BG CLR 17)	Soxhlet (8 h)	ns	150 ml *n*-hexane/acetone (2:1 v-v)	US EPA 3540C	[37]
	Soxtherm	Na_2SO_4	160 ml *n*-hexane/acetone (2:1, v-v)	US EPA 3541	
	Sonication	Na_2SO_4	40 ml DCM/MeOH (1:1, v-v) + 20 ml DCM, 18 ml elga water added and shaken	UA EPA 3550C	
	ASE	ns	30 ml DCM	US EPA 3545	
Ambient aerosol filters and soot collected from vehicle exhaust and coal combustion	Soxhlet (20 h)	ns	DCM		[66]
30–100 g of sediment	ASE	ns	DCM/ MeOH (9:1, v-v)		[51]

Table 1 (*continued*)

Sample type and size (if specified)	Extraction method	Use of drying agent	Solvent used	Additional remarks	Reference
100 g of dry soil	ASE	Na_2SO_4	*n*-Hexane/acetone (2:1)		[60]
Aerosol filters	Ultrasonic	ns	DCM 15 ml		[36]
100 or 300 g of soil (depending on sample depth)	ASE	ns	Cyclohexane (2 cycles at 100 °C)		[57]
20 g of freeze-dried sediment	Soxhlet (48 h)		Acetone/DCM (v:v, 1:1)	Activated copper was added prior to extraction	[52]
0.38–0.42 g of dust sample	Ultrasonic	ns	*n*-Hexane/acetone (1:1)		[67]
20–25 g of sediment sample	Surface-extraction followed by Soxhlet (72 h) or ASE	ns	DCM/MeOH (9:1, v-v)		[55]
Sediment	ASE	Na_2SO_4	Toluene (150 °C, 15 MPa, 10 min, two cycles)		[56]
PM2.5 filters	ASE		DCM/MeOH (9:1, v-v) (100 °C,1000 psi)		[68]

[a] Not specified.

in the specific compounds. We have chosen to focus on these kind of samples because of the comparable nature of the sample matrix which implies similar methodologies of sample treatment: extraction, clean-up and class separation procedures. However, the variation within the "similar sample treatment" is still fairly large. And whilst some authors use just one clean-up step, others present more complicated procedures incorporating up to three different techniques to separate the compounds of interest from the sample matrix. The need for a clean-up procedure also depends on the components of the sample matrix, as the variation of the matrix and the concentration of compounds which disturb the analysis of isotope ratios of PAHs may be large even within the same sample type. The composition of an environmental sample is determined to a large extent by its geographic origin and by the proximity of pollution sources e.g. harbour sediments vs. open water sediments [39,40] urban vs. rural aerosols [23,41,42].

Some of the publications which are reviewed here, describe in detail every single step of the analytical procedures that were applied. In many, however, the purification steps are only vaguely mentioned and in some not mentioned at all. In this review we will indicate these different steps, procedures or amounts of chemicals (Tables 1 and 2), which were not stated in the specific literature as "ns" (non-specified) rather than leaving a blank field in order not to imply to the reader that this particular step was omitted.

2. Potential of CSIA for source identification and apportionment

Most of the studies cited here aimed at verifying the proposal that PAHs generated from various processes have isotopic values and patterns which are source specific, and investigated the potential of compound-specific carbon isotope analysis (CSIA) as a tool for PAH source identification.

2.1. Combustion and/or pyrolysis of materials—determination of the isotopic signature of sources

McRae et al. [43] have reported stable carbon isotopic compositions for individual PAH compounds extracted from diesel particulates and products of coal and biomass pyrolysis and compared them with the bulk carbon isotope values of the primary source materials. Bulk values were determined by conventional sealed-tube combustion-IRMS. They found that the $^{13}C/^{12}C$ isotopic ratios of PAHs derived from coal and wood pyrolysis and diesel particulates vary over the range of 8‰. Further experiments conducted by the same group [44] were intended to investigate the isotopic variations in both PAHs and *n*-alkanes as a function of coal rank (six types of coal) and process conditions (fixed-bed hydropyrolysis at 650 °C and fluidised-bed hydropyrolysis at 900 °C). Whilst no significant variation in stable isotope signature was seen for *n*-alkanes, neither as a function of rank nor conversion method (hydropyrolysis vs. fluidized-bed pyrolysis), for PAHs large variations were observed as a function of process conditions. Those from hydropyrolysis were close to bulk values and were isotopically heavier than their counterparts from high temperature fluidized bed pyrolysis [44] and were also heavier than PAHs extracted from diesel particulates. Additionally McRae et al. [45] analysed coal-derived PAHs from a number of processes, including low and high temperature carbonisation, gasification and combustion and assessed the applicability of compound-specific isotope analysis for PAHs source apportionment in soil and vegetation samples from the vicinity of a low temperature carbonization plant. O'Malley et al. [18] have

Table 2
The details of procedures used for clean-up of PAHs extracts.

Clean-up method step 1	Elution	Clean-up method step 2	Elution	Clean-up method step 3	Elution	Additional remarks	Reference
5 g Sephadex (gel permeation)-+0.5 g acid-washed sand	3 × 2 ml MeOH/DCM (1:1, v-v)	**10 g Silica gel (in 50 ml DCM) displaced by hexane**	30 ml of *n*-hexane (aliphatics)+60 ml of DCM/*n*-hexane (1:1, v-v) (aromatics)			1.5 g activated copper added on top of the column in each step	[12,13,29]
Fine grain activated neutral alumina (grade I according to Brockmann)	*n*-Hexane (aliphatics) +toluene (aromatics)						[19,43,44,53]
2.5 g silica (40–140 mesh)+1.6 g alumina (80–200 mesh)	18 ml *n*-hexane (aliphatics)+45 ml *n*-hexane/toluene (2:1, v-v) (aromatics)					Adsorbents were activated prior to use at 200 °C for 4 h	[46]
Silica gel impregnated with KOH followed by HCO_2H acidification (in order to obtain neutral and acid fractions)	ns	**TLC using dibenzo(a,c)anthracene as standard** **TLC on hydrocarbon fraction with 2-methyphenanthrene and dibenzo(a,c)anthracene as standards**	**DCM as developer** ***n*-hexane as developer**				[30]
Florisil	ns	**HPLC (aminosilane phase) to separate methylphenanthrenes from interfering methyldibenzotiophens**	*n*-pentane				[31,47]
Silica+alumina columns	40 ml of *n*-hexane (saturated aliphatics)+30 ml DCM/*n*-hexane (1:9, v-v) (unsaturated aliphatics)+30 ml DCM/ *n*-hexane (2:8, v-v) (PAHs)+30 ml DCM (remaining polar compounds)						[48]
Silica column	PAHs eluted with DCM/ *n*-hexane (40:60; v-v) [49], ns [71]						[49,71]
10 g silica gel (60 mesh)	25 ml *n*-hexane (aliphatics) +35 ml DCM+25 ml DCM (PAHs were present in the 2^{nd} – 25 ml DCM fraction)					Silica gel was activated for 24 h at 135 °C; 1 g copper powder (3 μm particle size) was added to the top of the column together with the Na_2SO_4	[50]
1.4 g alumina micro-column (150 basic, type *T*, 0.063–0.2 mm particle size); conditioned by 10 ml DCM prior to use	10 ml DCM	**0.8 g silica gel (silica gel 60, 0.063–0.2 mm particle size); conditioned by 10 ml of pentane prior to use**	6 ml *n*-pentane (aliphatics) + 10 ml *n*-pentane/DCM (65:35; v-v)	**HPLC on aminosilane phase; fractionation based on ring number, into: monoaromatics, diaromatics (naphthalenes and biphenyls), dibenzotiophenes+fluoranes, phenanthrenes, fluoranthenes+pyrenes,**	*n*-pentane as mobile phase	Alumina and silica were washed with DCM, activated and maintained at 150 °C; copper (40 mesh) added on top of alumina column for marine sediments extracts; activated previously with 7 M HCl; washed	[21,25,32]

Table 2 (*continued*)

Clean-up method step 1	Elution	Clean-up method step 2	Elution	Clean-up method step 3	Elution	Additional remarks	Reference
				chrysenes+benzo(a)anthracene and penta-+hexaaromatics [25,32]; separation of phenanthrenic fraction from dibenzotiophenes [21]		subsequently with water, acetone and DCM and stored in DCM prior to use	
Alumina column	ns	**Silica column**	*n*-Hexane (saturated hydrocarbons and PCBs) + DCM (PAHs and pesticides)			Activated copper added after alumina cleanup	[27]
Silica column	ns	**HPLC**	*n*-Hexane elution at a rate of 0.3 ml min^{-1} for 18 min and 0.7 ml min^{-1} for 24 min			Aim was to separate the fraction 2 containing PAHs from fraction 1 which contained UCM and naphthalene	[11]
3 g aluminium oxide (5% deactivated)+3 g silica (5% deactivated) SPE column	40 ml of *n*-hexane (fraction 1 containing naphthalene); + 30 ml hexane (fraction 2 containing other PAHs)	**SPE with 1 g of HR-P resin (polystyrene divinylbenzene copolymer)**	8 ml *n*-hexane (interfering compounds in fraction1) + 20 ml toluene (naphthalene); 15 ml *n*-hexane/DCM (4:1, v:v) (interfering compounds in fraction 2) + 20 ml of toluene (PAHs)				[58]
2 g aluminium oxide (5% deactivated)+2 g silica (5% deactivated)	15 ml *n*-hexane, 5 ml *n*-hexane/DCM (9:1, v-v), 20 ml *n*-hexane/DCM (4:1, v-v)	**3 g HR-P resin**	20 ml toluene				[28]
Silica gel deactivated with 5% (w/w) distilled water	2 ml *n*-hexane, + 2 ml *n*-hexane/benzene (3:1, v-v) [38], or 2 ml hexane/DCM (3:1 v-v) [1,20,33,62,63]	**Automatic SPE system using 1 g aminopropylsilane (NH2) as stationary phase**	*n*-hexane/benzene (97:3, v-v) at a flow-rate of 0.03 ml min^{-1} (PAHs eluted in 3 fractions: 0–2,5 ml Fr1, 2.5–4.5 ml for Fr 2 and 4.5 ml–15 ml Fr 3)				[1,20,33,38,62,63]
Silica gel (10% deactivated; 63–200 μm particle size)+Na_2SO_4; or Florisil	60 ml of *n*-hexane	**Dimethylformamide (DMF)-pentane cleanup done on pooled aerosol extracts**	Solvent exchanged to pentane and partitioned between DMF-5% H_2O, subsequently mixed with water and partitioned with *n*-hexane and eluted through silica column with 8 ml *n*-hexane	**Preparative capillary gas chromatography (PCGC)**		Most abundant PAHs pooled together using PCGC; 10% of resulting CO2 was reserved for $\delta^{13}C$ determination, rest reduced to graphite for radiocarbon analysis	[65]
Silica gel (12 cm in 1 cm ID x 25 cm column; 3% deactivated) + aluminium oxide (6 cm; 3% deactivated) + Na_2SO_4 (1-2 cm)	40 ml *n*-hexane (aliphatics)+50 ml *n*-hexane/DCM (1:1, v-v) (aromatics)	**TLC (20 cm × 20 cm) plates coated with 0.25 mm layer of silica gel or 0.25 mm of aluminium oxide**	*n*-hexane/chloroform (45:5, v-v)				[70]
Alumina (1% deactivated), silica (5% deactivated)	100 ml *n*-pentane (aliphatics)+200 ml pentane/DCM (1:1, v-v)	**Gel permeation chromatography (GPC) using HPLC equipped with two size exclusion**	DCM used as mobile phase at a flow rate of 7 ml min^{-1}	**TLC silica gel plates (60 Å, 500-μm thickness)**	Prewashed by DCM/MeOH(1:1, v-v), activated at 120 °C, developed with about 50 ml of	Alumina (150 mesh) was activated at 400 °C for 4 h, and silica gel (100–2000 mesh)	[26,34,35]

		columns (22,5 × 250 mm, Phenomenex Phenogel 100 Å)			cyclohexane/toluene (3:2, v-v), PAHs were scrapped off the plate and extracted with DCM by sonication	was activated by heating at 170 °C for 12 h	
Silica gel column	40 ml hexane (saturated aliphatics)+30 ml DCM/ hexane (1:9, v-v) (unsaturated aliphatics)+30 ml DCM/ hexane (2:8, v-v) (PAHs)+30 ml DCM (remaining polar components)					activated at 240 °C for 2 h	[66]
Silica gel	*n*-hexane (saturated aliphatics)+20% DCM in *n*-hexane (aromatic hydrocarbon fraction)					Silica was activated at 150 °C overnight	[51]
SPE with 2 g alumina and 2 g silica gel (both 5% deactivated with deionized H_2O)	15 ml *n*-hexane+5 ml hexane/DCM (9:1, v-v)+20 ml *n*-hexane/ DCM (4:1, v-v)	**SPE with 1 g HR-P resin**	ns				[60]
Silca gel	40 ml hexane (aliphatics)+120 ml DCM/hexane (1:1, v-v) (PAHs)+50 ml DCM (polar compounds)					Silca gel activated at 450 °C for 4 h	[36]
Alumina/silica (both - 5% deactivated)	ns	**100% activated silica; Humic layer soil: 17 cm silica (1 cm diameter column), sub-soil layer: 4 cm silica (230 mm Pasteur pipette)**	Humic layer soil: 40 ml *n*-hexane (aliphatics), 100 ml *n*-hexane/DCM (aromatics) (9:1, v-v). Sub-soil: 4 ml *n*-hexane (aliphatics), 10 ml *n*-hexane/DCM (9:1, v-v) (aromatics)			Silica was heated at 250 °C overnight	[57]
Silica/alumina (2:1, v-v) + anhydrous Na_2SO_4	15 ml n-hexane (aliphatics)+70 ml DCM/ *n*-hexane (3:7, v-v) (PAHs)	**Gel permeation chromatography (GPC)**		**TLC**			[52]
Silica gel SPE column	10 ml hexane+10 ml hexane/DCM (1:1, v-v) Both fractions were combined	**Silica gel SPE column**	3 ml n-pentane (aliphatics), 2 ml pentane/ DCM (9:1) (aromatics)+4 ml pentane/DCM (4:6, v-v) (aromatics); fraction 2 and 3 were combined				[67,69]
Silica gel; smaller columns (5 × 0.5 cm), bigger (15 × 1.5 cm)	2 or 30 ml n-hexane (aliphatics), 2 or 30 ml DCM in hexane (20%) (aromatics), 30 ml DCM (aromatic/polar fraction- on longer column) and 2 ml DCM:methanol mixture (polar fraction)					Silica was activated for 8 h at 150 °C	[55]
Silica gel cartridge	3 ml n-hexane (perylene fraction), 9 ml DCM/ hexane (5:95, v:v) (alcohol fraction), 6 ml *n*-hexane:acetone (friedelin fraction)						[56]

Table 2 (*continued*)

Clean-up method step 1	Elution	Clean-up method step 2	Elution	Clean-up method step 3	Elution	Additional remarks	Reference
Silica gel (5% deactivated)	*n*- hexane (aliphatics), *n*-hexane/DCM (1:1, v-v) (aromatics), DCM/MeOH (9:1, v-v)(polar fraction)	**Dimethylformamide (DMF)-pentane cleanup done on pooled aerosol extracts, drying with Na_2SO_4**		**Preparative capillary gas chromatography (PCGC); PAHs grouped together based on ring number**		10% of resulting CO2 was analysed for δ13C, rest reduced to graphite for radiocarbon analysis	[68]

determined the carbon isotopic ratio of PAHs derived from the combustion of biomass material (such as C3 and C4 plants) and evaluated the influence of altering combustion conditions and biomass fuel material. No notable difference in alkene/alkane isotopic ratio of gaseous and condensate compounds was found and although PAHs were not detected in the gaseous fraction, the same result was assumed. The alkanes originating from C3 plants were significantly more ^{13}C depleted than those originating from C4 plants. It further supports the observation that the isotopic composition of the original material is preserved in the combustion products, and the temperatures used in the study did not significantly affect the isotopic composition of the individual combustion products. Large variations were found in PAHs; those formed in C3 plant combustion exhibited delta values in the range of −28.8 to −28.0‰ whilst those originating from C4 plant combustion had delta values of −15.9 to −17.1‰. It is expected that the observed C3 and C4 plant isotopic signatures will be preserved in natural tropical and boreal biomass fires and serve as indicators of fuel source or origin and frequency of past fire activity [18]. Because of the high chemical stability of aromatic compounds, these molecules, present in modern environments e.g. sediments, can have ancient sources such as ancient vegetation fires [30]. Ballentine et al. [46] have characterised the isotopic composition of fatty acids and PAHs extracted from air particulate matter collected during laboratory and field burns of sugar cane (unfortunately PAHs were not detected in laboratory burns) and assessed their utility as suitable tracers compounds for large-scale biomass burning. The results of isotopic determinations of PAHs during the field burns of sugar cane (C4) were not consistent with values published previously [18] (−15.9 to −17.1‰), i.e. they exhibited $\delta^{13}C$ values between −22.9 and −25.4‰. The possible explanation for this difference was given as higher fractionation resulting from the higher intensity of the field burn relative to the controlled burns. High amounts of substituted PAHs were present in experiments done by O'Malley et al. [18] thus temperature might have been not conducive enough for PAHs formation [46]. This explanation was also supported by the fact that the ^{13}C isotopic depletion of fatty acids relative to those from unburned vegetation was more pronounced for species collected from the field burns than for the laboratory burns [46].

2.2. Application to environmental samples

2.2.1. Sediments

O'Malley et al. [12] have investigated the potential of CSIA for PAH source apportionment in sediments from St John's harbour and Conception Bay, Newfoundland, Canada. The authors found that PAHs arising from wood burning and vehicle emissions exhibited significantly different isotopic signatures, with 4- and 5-ring PAHs originating from vehicle emissions (gasoline engines) being significantly enriched in ^{13}C, particularly pyrene. Variations in the isotopic signature did not seem to be related to the fuel type (gas vs. diesel). The PAHs isolated from crankcase oils were significantly depleted in ^{13}C [12,29]. Somewhat different results were obtained in 1996 by the same group [29], where the values for car soot and wood burning soot were comparable, only with the exception of pyrene in car soot which was consistently characterised by a high ^{13}C δ value. In this article, a more quantitative approach (two component Langmuir mixing equation) was successfully performed to assess the contribution of these three sources: fireplaces soot, car soot and crankcase oils on the PAH content of harbour sediments. The delta values of 4- and 5-ring PAHs were used and their molecular abundances. Mazeas and Budzinski [31,47] have evaluated the potential of isotopic PAHs source identification strategy using phenanthrene and

methylphenanthrenes in an in-situ oil simulation experiment. In this study, a known quantity of petroleum was introduced at the top of the sedimentary column, thus in the samples both natural and petrogenic PAHs were present. The results showed that it was possible to quantitatively source apportion the petrogenic methylphenanthrenes in the contaminated sediment [47] and thus study their behaviour [31]. A combined molecular and isotopic approach was used by Smirnov et al. [48] for PAHs extracted from the sediments in Lake Erie. The authors performed principal component analysis on this data set. Three distinctive zones were statistically identified within the lake, characterised by both different concentrations and isotopic signatures. McRae et al. [49] and Fabbri et al. [50] reported extremely low delta values for PAHs (varying between −31 and −62‰) in lagoon sediments that are characteristic of a natural gas source. The biogenic methane in the reservoirs in the neighbourhood area has been utilised by the local chemical industry since the late 1950s; the $\delta^{13}C$ of this methane ranged between −69 and −73‰ [49,50]. It was concluded that the exceedingly low values found for PAHs could only be explained by the PAHs having their source in the natural gas of a biogenic rather than petrogenic origin, which is isotopically heavier. Mazeas et al. [25] determined the stable carbon isotope signature of parental PAHs and methylphenanthrenes of a sediment suspected to have been contaminated by petroleum products spilled by the Erica tanker. The tanker sank in 1999, releasing thousands of tons of heavy fuel oil into the sea around the Bay of Biscay [32]. The isotopic ratios were measured in the contaminated sediment, in a control sediment and in the oil transported by the Erica tanker. Except for the fluoranthene, the isotopic ratios of all other PAHs measured in the contaminated sediment were similar to ones exhibited by PAHs in oil, whilst PAHs in the control sediment were significantly ^{13}C-enriched compared to both polluted sediment and Erica oil. Fluoranthene was much less abundant in the oil than other PAHs and this was given as a cause for fluoranthene having the same isotopic composition as the control sediment. Thus, the isotopic results represented a proof of contamination of sediments by the oil spilled by Erica tanker. Unfortunately, after the Erica spill, other tankers took advantage of the event and cleaned their tanks along the Atlantic coast of France. In 2002, the same group [32] investigated both the molecular and stable carbon isotopic composition of alkanes, parental and alkylated PAHs (phenanthrenes) of oil residues (tar balls) found in different places along the Atlantic shoreline of France and samples of oils collected from the feathers of birds, in order to identify their sources. Similar conclusions were obtained from the molecular results and carbon stable isotopic ratios of alkanes and PAHs, and also when bulk carbon isotopic ratios of oils were considered, thus proving that the molecular carbon isotopic ratio can be used as a source identification tool. Within the samples collected on-shore, those located at the north Atlantic coast were of Erica oil spill, whilst others showed distinct molecular and isotopic compositions and hence were of other origins, confirming the infamous practice of other oil tankers taking advantage of the catastrophe. The oils collected from the birds' feathers clearly came from the Erica spill.

Stark et al. [13] combined the information from molecular and isotopic compositions in clarifying the distribution and sources of PAHs in the St. Lawrence River from the outflow of Lake Ontario to the Massena/Cornwall area. The isotopic composition of wood-burning sources (fireplace soots) in the St. Lawrence basin was determined and additionally the PAH carbon isotopic ratio of road sweep particles to obtain the molecular and isotopic signature of urban surface runoff in the region. The results were compared to the previous results for similar samples [12]. The values for fireplace soot generally overlapped with the previously published results, with a few exceptions. Actually some differences were to be expected, since the PAHs $\delta^{13}C$ composition of wood-burning sources will reflect changes in the type of wood used in different regions. The isotopic results from road sweeps were distinctly narrow (<2‰ total range per compound) despite the fact that the samples came from widely differing geographic locations and environments (urban through industrial and fairly rural). With the exception of benzo(a)pyrene and benzo(e)pyrene, the results correlated well with the $\delta^{13}C$ values reported by O'Malley for vehicular exhausts, being dominated by combustion PAHs. In sediment samples the molecular ratios approach (including alkylated PAHs) agreed well with the expected isotopic signatures. For the industrial area, comparison of compound $\delta^{13}C$ values of PAHs in upstream, effluent and downstream sediments showed distinct variations which were attributed to the impact of an aluminium smelter contribution to the river sediments. Yan et al. [27] have used the $\delta^{13}C$ value of pyrene (similar stable isotopic patterns of other major PAHs were observed among samples) to quantify the contributions of petroleum-related PAHs and combustion-related PAHs in sediments from the New York/New Jersey harbour complex using a mass balance model and investigated the temporal trends of PAHs within the cores. The authors correlated the pyrene $\delta^{13}C$ value with other molecular source indicators (e.g. fluoranthene/(fluoranthene+pyrene)) and found moderate to strong positive correlations. It was thus concluded that pyrene $\delta^{13}C$ value and three other well correlated molecular indicators can be used for successful source elucidation of sediment PAHs. Walker et al. [11] applied CSIA (PAHs extracted from the river sediments), successfully to separate the signatures derived from two similar sources—coal and coal gasification. The use of isomer ratios (fluoranthene/pyrene and benzo(a)anthracene/chrysene) alone did not allow the source contributions to be distinguished. This was possible, however, when additional $\delta^{13}C$ analysis was conducted. The authors reported values obtained for sediments contaminated with coal gasification products as significantly different (depleted in ^{13}C in the range of −25.8 to −27.0‰) from other contamination sources such as coal and creosote and similar to values from gasification of coal published earlier [45]. PAHs (chrysene/triphenylene, benzo(e)pyrene and 9-methylphenanthrene) delta values from sediments spanning the Permian-Triassic boundary were analysed by Grice et al. [51]. The depleted delta values (−30 to −33‰) of PAHs from basal Triassic levels were comparable to the values obtained in an earlier study for phytane (phytoplankton origin) from the same samples. They were also more depleted than the PAHs measured for samples of the Permian age. In Triassic samples a strong correlation was also evident for $\delta^{13}C$ values of PAHs, $\delta^{13}C$ of kerogen and hydrogen index confirming that $\delta^{13}C$ are mainly governed by the organic matter type rather than the thermal maturity and that PAHs are of the algal origin. The authors postulated that PAHs derived from Permian age were on the contrary most likely to arise from the combustion of terrigenous material. Ou et al. [52]determined the carbon isotope ratios of PAHs extracted from the Yangtze estuarine sediments in order to help identify their sources in this complicated environment. Kim et al. [26] collected the samples of sediments from different environments, e.g. urban lake, a shipping waterway, harbour and a relatively remote lake. Also, some terrestrial samples and marine sediments from Antarctica were analysed. Some differences but also similarities were found between the samples both for low and high molecular weight PAHs. Similar values were found for high molecular weight PAHs in urban lake, shipping water ways and harbour sediments suggesting common source vs. depleted PAHs in the remote lake. For low molecular weight PAHs in the urban lake, ^{13}C enriched stable carbon ratios were visible, distinct from all other sites. The delta values of naphthalene and methylnaphthalene in terrestrial samples from locations near fuelling stations, old oil tanks and the

helipad were similar whilst ones located close to the machine shop exhibited clearly lower $\delta^{13}C$ values. The carbon isotopic composition of individual sources was unknown, thus making it difficult to explain in detail the variations between the samples. The detailed investigations of above mentioned samples from Antarctica may be found in [34]. Here, the authors complemented the carbon isotopic investigations with the PCA. Good agreement was found between the two methods with the exception for marine sediments, in which PAHs were more depleted in ^{13}C than the terrestrial samples despite the similar chemical composition suggesting different or additional sources of naphthalene and methylnaphthalenes. This most probably reflected the previous spills or contamination from ship operations over many years, in addition to the contribution from the seasonal run off from the adjacent land areas [34]. Comparable work was done on lake sediments [35]. Also here, similar conclusions could be drawn from various source apportion tools, such as PAHs molecular ratios, principal component analysis, and stable carbon isotopic ratios.

2.2.2. Soils

Lichtfouse [30] have investigated the origin of PAHs by analysing $^{13}C/^{12}C$ of the total aromatic fraction extracted from soils after they have been cultivated with C4 plants (Zea mays). The results clearly showed that PAHs present in the soil were not biological products from plant, soil biomass and their humification products, despite the fact that the fraction of maize-derived C of the bulk soil organic carbon reached 43% after 23 years of cultivation (the delta value of the bulk organic carbon increased by +6‰), whilst PAHs value remained unchanged; instead, two other main sources have been suggested: the pyrolytic products from the combustion of fossil fuels and minor contribution of uncombusted fossil fuels. In 2000, McRae et al. [53] have reported on the PAHs formation in domestic combustion and the resulting isotopic composition, in order to trace PAHs from such a combustion source. Soil samples were collected from the area of Lochwinnoch, a village in the neighbourhood of Glasgow, UK, where large portion of households still used coal as a main source of a heating. It was shown that the PAHs were isotopically heavy (in the range of −25‰) and these values were consistent with the results for PAHs originating from coal burning. A sample which came from outside the village showed a different distribution of PAHs and also the $\delta^{13}C$ of the heavier PAHs was close to −30‰; input from biomass combustion for this sample was suggested. In order to investigate the fate of PAHs (including the isotopic composition) in the environment after emission, the authors have also conducted two weathering (leaching) experiments using low temperature coal carbonisation tar from the Coalite process [53]. In these experiments rainwater was allowed to pass through a bed of soil impregnated with tar under as natural conditions as possible. After as little as 80 days, parent PAHs, particularly, fluoranthene and pyrene, became more prominent, suggesting that these compounds may survive the oxidation/weathering to a greater extent than their alkylated counterparts. Glaser et al. [28] used carbon compound-specific ratio analysis for the apportionment of the PAHs extracted from soil, located at different distances from the highway, in order to estimate the contribution of traffic-derived PAHs. In this case the investigators reported that the carbon CSIA of PAHs failed for source apportionment, most probably, because of a high number of possible contamination sources and the low difference between their stable carbon isotope signatures. The black carbon analysis and concentration analysis of PAHs, on the other hand, confirmed the significant contribution of tyre abrasion and tailpipe exhaust to the soils adjacent to the motorway. Sun et al. [19] used the isotopic signatures in conjunction with molecular fingerprints of PAHs derived from contaminated soil samples. Such an approach resolved coal-derived PAHs from those emanating from petroleum sources, which was a major input source of two soils investigated. Evidence also was provided that tar contaminating the Glasgow Green (UK) soil sample was a high temperature carbonisation tar as opposed to one originating from gas works, despite the similar molecular distributions.

2.2.3. Origin of perylene

Perylene origin has been discussed in recent years, as its abundance and occurrence differ to those of other, combustion derived, PAHs [54–56]. Grice et al. [55] have measured carbon and hydrogen isotopic ratios of perylene and performed a detailed analyses of sediments (originating from the Holocene era), i.e., investigation of perylene and other PAHs profiles, palynological analyses, analyses of lignin. They hypothesised that perylene originated from the activity of wood-degrading fungi. A similar conclusion was made by Itoh and Hanari [56], who isolated perylene from the Biwa lake sediment in Japan. Based on the $\delta^{13}C$ values of perylene (−27.8‰) and $\delta^{13}C$ ranges of Japanese plants (−26.0 to −28.3‰), they concluded that possible precursors of perylene originated from C3 gymnosperms. The $\delta^{13}C$ analysis of soil perylene was also done by Gocht et al. [57]. The samples originated from the Northern Black Forest in Germany. Soils that showed an increasing concentration of perylene with depth, in comparison to other PAHs, were analysed for carbon isotopic ratio. Values found for perylene were 5.7 per mil more depleted than other PAHs in the top layer of the soil. Atmospheric deposition was ruled out by calculations of necessary leaching time from top-soil. It was thus concluded that in-situ generation of perylene was the most likely scenario for high perylene concentrations. Wilcke et al. [58] demonstrated that the determination of $\delta^{13}C$ value of perylene may allow the PAHs sources such as combustion-derived (in temperate soils) and biologically produced (in tropical soils) [58] to be distinguished. Perylene in tropical soil (−29.7 to −35.1‰) and termite nests (−30.3 to −32‰) originating from biological processes was significantly depleted compared to perylene isolated from the soils from temperate environments (e.g. house garden, gas work or roadside soils) (−26.7 to −27.5‰). This interpretation was however criticised in a review by Glaser [59], who hypothesised that pyrolized soil organic matter, from forest fires, would produce similar values. Aichner et al. [60] measured the concentrations and delta values of PAHs derived from soils from various locations in Kathmandu, Nepal. The high concentration of perylene relative to other PAHs, in some of the samples, was remarkable. The authors postulated a biological origin (crop rotation practice) and the result was confirmed by the significantly enriched in ^{13}C perylene in those samples.

2.2.4. Air particulate matter

Preliminary results of the investigation of the potential of carbon stable isotope source apportionment of PAHs in atmospheric aerosols have been reported by Norman et al. [61]. The samples were obtained from both urban and rural locations in Canada. The comparison of values for individual PAHs between these two environments suggested that the isotopic difference may be as large as 6‰. Okuda et al. [33] reported the measurements of $\delta^{13}C$ value of PAHs extracted from Malaysian aerosol collected in the vicinity of the forest-fire sites in Indonesia. Some source samples were also collected for comparison. The sampled sources were gasoline and vehicle exhaust and muffler soot, smoke from artificial combustion of wood, and soot from domestic incinerators in which wood was burned. Both molecular

and isotopic ratios (wood-burning PAHs were significantly more depleted in ^{13}C than the air samples and no correlation of delta values versus the molecular weight of PAHs was visible, in contrast to the environmental samples) revealed that the PAHs found in Malaysian air are not significantly affected by the neighbouring forest-fires, even under heavy haze conditions; instead automotive exhaust was suggested as the main contribution source to aerosol PAHs. A quantitative estimation indicated a 25 to 35% contribution from wood burning, with a considerably higher, 65% to 75%, contribution from automotive exhaust. In 2002, the same group of researchers [62] examined whether the change in major combustion sources from large biomass and coal to petroleum, which occurred globally over the last 100 years would be recorded in the PAHs isotopic composition. The results showed however very small variation through the sediment core in weighted averages of the $\delta^{13}C$ values and little correlation between PAHs concentration and the $\delta^{13}C$ of PAHs. This unexpected homogeneity was attributed to complexity of the local combustion sources [62]. Okuda et al. [1] separated the three Chinese cities studied into two groups according to the carbon isotopic profile: one affected mainly by automotive exhausts (Beijing) and the other affected by coal combustion (Chongqing and Hangzhou). A clear trend was not found using molecular profiles; these were similar for all three cities (with an exception of one of the diagnostic ratios). In 2004, Okuda et al. [63] estimated the contributions of automotive sources to aerosol PAHs at 1 m, 10 m and 200 m away from the road using compound-specific carbon isotope ratio analysis and molecular fingerprints. The authors estimated that the contributions ranged from 33–80% at the site closest to the road, 11–74% at a distance of 10 m and 0–52% at 200 m. The last position was located in a residential area and considered as suburban background. The authors compared the results with the findings of Nielsen [64] and obtained an agreement between the results of these two studies.

Mandalakis et al. [65] measured the $\delta^{13}C$ of PAHs compounds, harvested together, that were extracted from air particulate matter samples and polyurethane foam cartridges from three European background locations. Up to 50 24 h-samples were pooled together to obtain one sample for radiocarbon analysis. 10% of the resulting CO_2 from each sample was reserved for $^{13}C/^{12}C$ determination. The $\delta^{13}C$ values of isolated PAHs (combined PAH compounds combusted offline to CO_2) exhibited low variability between sites, thus making the source apportionment using stable carbon isotopes difficult. Peng et al. [66] determined the carbon isotope values of airborne PAHs in two Chinese cities, and found some differences especially for high MW PAHs. Some samples of sources, e.g. soot from coal combustion or gasoline or diesel vehicle exhaust were also analysed. The differences in delta values between the two cities were explained by differences in contribution to PAHs emissions from cars and coal combustion. Zhang et al. [36] investigated the delta values of particulate matter PAHs emitted by two indoor sources: environmental tobacco smoke (ETS) and cooking oil fumes. The authors found notable differences between the delta values of phenanthrene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene and indeno(1,2,3-cd)pyrene which could be used for source identification of these pollutants. Mikolajczuk et al. [67] investigated the differences in $\delta^{13}C$ of PAHs extracted from two rural dust samples (dust collected from the filters of a building ventilation system in Belgium) and urban dust (collected from road tunnel walls in Poland). Significant differences between the rural and urban isotopic ratios could be seen in acenaphthene and anthracene, with urban values being enriched in the heavier isotope. This was not evident for other PAHs investigated however. Xu et al. [68] used stable carbon isotope ratio analyses together with the radiocarbon data analyses on PAHs groups extracted from PM2.5 filters collected in North Birmingham, Alabama (USA) for differentiating between two important sources of PAHs: fossil fuel and biomass burning. The authors found out that samples collected in the vicinity of coke plant (source samples) had characteristic and consistent values ($-24‰$) through all PAHs groups whilst ambient samples were more depleted, especially those collected in winter. The data from radiocarbon analyses showed that the samples from vicinity of the coke plant were most depleted in ^{14}C carbon, thus originating from fossil fuel combustion whilst those collected in urban locations in winter contained more modern carbon and thus a higher percentage of biomass burning PAHs. Quantitative interpretation of the results was done using radiocarbon data.

3. Fractionation issues

The common goal of all sample treatment procedures is to isolate PAHs from the samples without altering the isotopic composition of the compounds of interest. Therefore, when elaborating a new method or modifying an existing one, tests are required which provide the proof of absence of significant isotopic fractionation during any of the extraction and sample cleanup steps. Most of the time this is performed by comparing the delta values of a solution of standards, which has been subjected to the whole procedure (extraction and cleanup) and delta values of standards dissolved in appropriate solvent and analysed directly without any treatment.

3.1. Investigation of the possibility of isotopic fractionation during the laboratory procedures

O'Malley et al. [12] dissolved 4 mg of each PAHs standard in distilled hexane. 10 μl of the solution were added to 15 g portions of sterile sediments that had been previously extracted. The spiked sediments were subsequently extracted and the extracts were purified using the same procedure as for real samples. The analytical procedure did not significantly affect the isotopic composition of the standard compounds, even when the recovery was below 50%.

Mazeas and Budzinski [47] have measured the composition of commercial 2-methylphenanthrene before and after application of the analytical procedure. The results were $-25.04 \pm 0.12‰$ before the extraction and compound class separation procedure and $-25.15 \pm 0.15‰$ afterwards. Mazeas and Budzinski [25] checked the absence of fractionation on a mixture of standards containing methylphenanthrenes. The isotopic compositions of the standards were measured before and after the application of the analytical procedure (extraction, clean-up, class separation on silica gel and applying HPLC). The uncertainty was below 0.5‰. No significant difference in isotopic ratios was observed before and after the application of the whole protocol. SRM petroleum material was subjected to clean-up and GC–IRMS analysis in three independent assays [25]. Also here, no significant differences in isotopic compositions were observed between the three assays showing that the analytical procedure allows reproducible measurements. The effect of HPLC was further investigated on PAHs isotopic composition extracted from marine sediment (SRM 1944) which showed a dominant pyrolytic contamination. The extracted PAHs were measured without any HPLC fractionation, after a simple fractionation (collection of all the aromatics after naphthalene fraction) and after complete fractionation into monoaromatics, diaromatics (naphthalene and biphenyls), dibenzothiophenes and fluorenes, phenanthrenes, fluoranthene and pyrenes, chrysenes and benzo(a)anthracene and penta- plus

hexa-aromatics. It should be noticed that when the additional step of HPLC was not carried out on the aromatic extracts, an important background signal was observed due to the unresolved complex matrix (UCM). The UCM was partly removed by the simple fractionation and totally removed when the complete fractionation was performed. Here also no important isotopic differences were observed, except for fluoranthene and therefore it was concluded that for this type of sample (marine sediment) the application of HPLC fractionation is not so determinant. However, after the full HPLC fractionation, the uncertainties were lower than when compared to two other conditions (no fractionation or simple HPLC fractionation). They were lower than 0.3‰, whilst in case of none or simple HPLC, for some compounds the uncertainties were higher than 0.5‰ (most probably due to the presence of high background signal). In the case of fluoranthene, significant ^{13}C-enrichment was measured after the whole HPLC procedure. This was explained by the possibility of co-elution of fluoranthene with some alkylated phenanthrenes or dibenzotiophenes, which elute in the same range as fluoranthene and which are removed during the complete HPLC fractionation procedure. Yan et al. [27] have also investigated the effect of HPLC to improve the measurement precision but this effect appeared to be insignificant (<0.3‰). A negative effect however, was the incomplete recovery of some low molecular weight PAHs which led to dramatic discrepancies in GC–IRMS measured δ^{13}C values and the actual value (measured by conventional IRMS). Cleanup tests using size-exclusion (Phenomenex Phenogel, 100 Å) and TLC (silica gel, 60 Å, 500 μm thick) were performed on a standard harbour sediment extract (NRC HS3B, National Research Council, Canada). The authors report that the repeat analyses of pyrene showed improvement in multiple injection precision ($1\sigma < 0.2$‰); the actual measured values (conventional IRMS) are identical to the δ^{13}C pyrene measured without this added cleanup to within 0.2‰. Consequently, the GC–C–IRMS analyses were performed without the additional HPLC cleanup. On the contrary, Walker et al. [11] report obtaining simpler chromatograms and increased signal for PAHs relative to the baseline when the additional HPLC clean-up step was used after the silica solid–liquid extraction precleanup of sample extracts. Wilcke et al. [58] found out that the purification step resulted in a mean shift to more negative values of naphthalene by 0.3‰ and perylene by 0.5‰ compared to an unprocessed standard. This bias was close to the precision of the method, therefore the results were not corrected for it. Okuda et al. [38] found differences of ± 0.5‰ before and after the application of their cleanup procedure for all PAHs, except for phenanthrene for which a depletion of -0.8‰ was observed. All differences were within the standard deviation of GC–C–IRMS measurement for the corresponding PAH and it was concluded that there was no significant isotopic difference before and after the separation procedure. Gocht et al. [57] also found differences of less than 0.5‰ for perylene standard solutions before and after the sample purification. Kim et al. [26] found ^{13}C enrichments in some of the analytes after the sample purification. The authors speculated that the concentration step could be responsible for preferential loss of isotopically lighter PAH compounds; alternatively, other sources and processes could be responsible for this enrichment since it was demonstrated by O'Malley et al. [12], that evaporation had no effect on the measured stable isotope ratios of PAHs. The differences were however minimal and fell in the range of 2σ of the mean isotope ratios of unprocessed standards. Four different extraction procedures (US EPA procedures for Soxhlet, Soxhterm, Sonication and ASE extractions) and two clean-up protocols of PAHs from a certified reference material and from a contaminated soil were compared by Graham et al. [37]. The authors examined the stable carbon isotope signature of PAHs extracted with different methods prior to clean-up, and subsequently, investigated the effect of clean-up procedures on the stable carbon isotope signature. For the concentration determination of PAHs from the reference material, the results were comparable and no method was better than any other. Similar results were found for a contaminated soil sample; discrepancies in higher molecular weight PAHs occurred, and sonication gave values which were the closest to the traditional soxhlet protocol. Two of the extraction methods (Soxhlet and Accelerated solvent extraction (ASE)), for which there was less co-extracted material, were chosen for further experiments. Alumina/hexane/toluene and silica/DCM clean-up methods were subsequently compared. The former gave higher recoveries of PAHs than the latter one. The stable carbon analysis of recovered PAHs showed clear differences between the values obtained before and after the clean-up. In general the post-clean-up values were more negative. The effect of clean-up was much smaller for ASE, compared with soxhlet, and the standard deviations associated with replicate measurements of delta values were generally lower for ASE extracted PAHs. Mikolajczuk et al. [69] investigated isotopic fractionation during cleanup using a solution of six PAH compounds (measured previously with Elemental Analyzer–Isotope Ratio Mass Spectrometry (EA–IRMS)) and solution of 16 EPA mixture PAHs. The authors optimised an LVI (Large Volume Injection) technique and investigated the effects of isotopic fractionation by comparing the delta values of 100 and 50 ml injections with those of 1 μl splitless injection and compared them to previously determined EA–IRMS values. No significant differences were found in their experiments (before and after the silica cleanup procedure or when comparing LVI to splitless injection and EA–IRMS measurements), except for benzo(a)pyrene and benzo(b)fluorene. For benzo(b)fluorene incomplete chromatographic resolution from benzo(k)fluorene was given as possible explanation, whilst for benzo(a)pyrene the fractionation remained unexplained.

3.2. Investigation of the possibility of isotopic fractionation of PAHs in the environment

O'Malley et al. [18] have investigated the consequences of evaporation, photolytic decomposition and microbial degradation of PAHs. Two separate experiments were carried out. In the first one, several mg of 5 standard solid PAHs were exposed separately to sunlight and air for 30 h at 27 °C. Afterwards, the PAHs were mixed, taken up in DCM and analysed. In the second experiment PAHs were dissolved in cyclohexane (3 mg of each in 5 ml solvent). This mixture was divided into 12 portions and exposed to sunlight for 0, 4, 8, 24, 36 and 48 h at 12–14 °C. Microbial degradation was assessed on pure naphthalene since low molecular PAHs are more susceptible to microbial degradation than higher molecular weight species. A culture of naphthalene degrading bacteria *Pseudomonas putida* was used for this experiment. After completion of the experiment, naphthalene was extracted and concentrated before applying GC–C–IRMS analysis. The various tests showed no significant alteration in the δ^{13}C of individual standard PAHs, even in the case of naphthalene where 95% reduction in concentration was observed. Anthracene showed shifts up to 2‰ after 8 h of exposure to direct sunlight. This was accompanied by significant photolytic decomposition. However, subsequent experiments failed to verify this shift at similar degrees of decomposition.

Mazeas et al. [21] investigated the possible isotopic fractionations of *n*-alkanes and polycyclic aromatic hydrocarbons (phenanthrene compounds) induced by bacterial degradation. The authors tested the isotopic effect of aerobic biodegradation on crude oil (portion of 30 mg) by a bacterial community isolated previously from marine sediment and a standard 2-methylphenanthrene (around 3 mg) biodegradation by a pure bacterial strain (*Sphingomonas sp.*) isolated as well from marine sediment. In both cases the isotopic composition remained constant during the 16-days experiment although around 90% of the methylphenanthrenes were degraded.

4. Environmental sample type

PAHs have been extracted from various types of environmental samples such as sediments [11–13,25–27,29,34,35,37,38,47,48,50–52,55,56,62], soil [19,28,30,34,45,53,57,58,60], aerosols [1,33,36,38,46,61,63,65–68,70] or vegetation [45,46].

5. Sample size

O'Malley et al. [12] extracted portions of sediments up to 15 g and at least 5 g of soot. Stark et al. [13] used 15–40 g of sediments, 2–4 g of fireplace soot and road sweeps samples. Fabbri et al. [50] used portions of approximately 10 g of wet sediment. Mazeas and Budzinski [25] also used 10 g amounts of sediments and 20 mg of petroleum for extractions. Wilcke et al. [58] subjected between 25 and 500 g of soils to ASE extraction. Glaser et al. [28] extracted 35 g of dried soil and 20 g of domestic soot or 1 g of ground tyre. Kim et al. [26,34,35] used approximately 15 g of sediments for extraction of PAHs; sample weights of 50–100 g were used for sediments with low PAHs contents. Grice et al. [51] ground between 30 and 100 g of each sediment sample to a fine powder. Aichner et al. [60] extracted 100 g of dry soil sample whilst Gocht et al. [57] used 100 g or 300 g of soil, depending on the sampling depth, and thus the concentration of compounds. Grice et al. [55] used 20–25 g of sediments and Ou et al. [52] 20 g of freeze-dried sediments. Okuda et al. [38] used approximately 10 mg of soot, about 20 mg of freeze-dried sediment and aerosol samples collected with the use of a high-volume sampler over a 24 h period with a flow rate of 22.6 $m^3\,h^{-1}$. Okuda et al. [33] sampled the air particulate matter in the vicinity of forest fires sites in Indonesia with the use of a high volume sampler having a flow rate of 0.5 $m^3\,min^{-1}$, thus $\approx$700 m^3 were sampled over a 24 h sampling period. Samples of aerosol collected in the vicinity of a road (1 m and 10 m) during 8 h of high-value sampling (0.5 $m^3\,min^{-1}$) in a Japanese suburban area and 24 h in a calm forest, 200 m distant from the road, not affected directly by the vehicle emissions from the roads were collected by Okuda et al. [63]. Liu et al. [70] collected the aerosol samples over a 72 h sampling period (the sampling flow was 1.05 $m^3\,h^{-1}$). Peng et al. [66] reports 24 h sampling period with a flow rate of 100 L min^{-1} for total suspended particulate matter (TSP) and PM10. The individual TSP and PM10 samples for every sampling location were combined as one sample for analysis. Mikolajczuk et al. [67] took between 0.38 and 0.42 g of dust samples.

6. Sampling of sources

Samples of various sources are often taken from locations close to where environmental samples have been taken. These include soot material from fireplaces or chimneys [12,13,28], car mufflers [12,13,28,29], motor bike mufflers [38], domestic furnaces [12,29], domestic incinerator [33], a power generating plant [12], road-sweep samples obtained by sweeping 1 m^2 of asphalt and cemented surfaces (stones and twigs were removed) [12,13,52] and samples of car tyres [28]. O'Malley et al. [29] have additionally sampled crankcase oil directly from car engines, waste oil sumps of garages in the area and other petroleum related sources such as crude oil, fuel oil, gasoline, outboard motor effluents and virgin crankcase oils. McRae et al. [43,44] have subjected samples of coal, biomass (wood) to high temperature pyrolysis, and collected the liquid products and tar. Additionally diesel particulates [43] collected on a filter were analysed for carbon isotopic ratio. O'Malley et al. [18] have collected the ash material from controlled laboratory burns of C3 and C4 plants. Ballentine et al. [46] used low volume and high volume air samplers to collect aerosol samples on filters from controlled laboratory burns of sugar cane leaves and field burns, respectively. The field burns were performed on a farm in a rural area, so that airborne pollutants originating from vehicular and industrial emissions were not significant. Xu et al. [68] collected fence line samples of PM2.5 in the vicinity of a coke plant. Samples of other possible sources of PAHs such as coal [44], the condensates and ashes of controlled laboratory burns of coal [43], wood [18,33,43], crops [30], tars from various coal processing [19,45,71], carbon black [43,50], samples of vehicle soot [12,13,33,53], gasoline vehicle exhaust [33], diesel vehicle exhaust [33], particulate matter from the combustion of jet fuel [71], crude oil from the Erica tanker oil spill [25,32] have been used in order to identify and/or apportion the sources by measuring stable isotopic ratios. Walker et al. [11] took sediment samples which were located close to suspected or potential source areas, such as wood treatment facilities (contamination with creosote), a coal-fired power plant, petroleum storage/transport areas, a former coal gasification site and a coal terminal in order to compare their signatures to the sediments collected in the main stem and the southern branch of the Elisabeth River, VA, USA. Peng et al. [66] collected the exhaust of different vehicles (diesel and gasoline) with the use of "Vehicles Carrying Samplers with Impactors". Particulate matter generated under different working conditions was collected. The soot of coal combustion from industrial and domestic uses was sampled with a flue-gas diluting sampler. Zhang et al. [36] sampled directly the aerosol of Environmental Tobacco Smoke (ETS) from the mainstream and sidestreams of a smoking machine in an environmental microchamber. In every sampling procedure, 3–4 cigarettes were inhaled. The authors also sampled aerosols originated from cooking oil. 100 ml of oil was heated to 275 °C and particulate matter arising from the pot was collected for 60 min.

7. Sample treatment prior to extraction

Most of the authors store all of the samples prior to extractions at −20 °C; [12,13,20,29,33–35,38,52] or −30 °C [62]. Before the extraction sediments may be air-dried in ambient temperatures (in a darkened fume-hood) [12,13,48,56–58], in aluminium boxes at 35 °C [28], at 35 °C under a flow of air filtered through Florisil [27] or freeze-dried [38,52]. McRae et al. [45,53] and Sun et al. [19] dried soil and soot samples in a vacuum oven overnight at 40 °C and ground them to 75–212 μm particle size. O'Malley et al. [18] crushed the ash material in a mortar and pestle to a fine powder and so did Yan et al. [27] and Grice et al. [55] with their sediments. Lichtfouse [30] and Gocht et al. [57] dried and sieved the soil samples to 2 mm prior to the extraction. Mazeas and Budzinski [47] freeze-dried 2 cm slices of sediments and sieved at 2 mm. Wilcke et al. [58] sieved the soil sample to <2 mm. Fabbri et al. [50] mixed 10 g of wet sediment with 10 g of anhydrous sodium sulphate. Aichner et al. [60] air-dried soil samples for 4 days and subsequently sieved them (<2 mm). Itoh and Hanari [56] air-dried the sediments and then pulverised them, sieved to remove particles larger than 106 μm, sterilised by γ-irradiation with ^{60}Co and stored them at 4 °C.

8. Extraction

The Soxhlet extraction of PAHs from various samples is commonly applied [1,12,13,19,20,27–29,33,38,46,48,50,52,55,62,63,65,66]. Another way of extraction is by reflux [19,43–45,53,71] and ultrasonic extraction [20,30,33,36,38,67,70]. Also, microwave assisted extraction [25,47] has been used. Several researchers used ASE [11,26,34,35,51,55–58,60,68]. The extracts are usually

concentrated to a small volume or evaporated to dryness (e.g. when solvent exchange is necessary) and subjected to the clean-up procedure.

9. Sample clean-up and class separation

Column chromatography (e.g. using glass chromatographic columns or SPE microcolumns) is often the preferred type of sample clean-up. Gel permeation using conditioned Sephadex prior to silica gel adsorption columns was used for the elimination of a significant portion of interfering materials [12,13,18,29]; silica-gel columns impregnated with KOH followed by HCO_2H acidification [30]; silica-gel columns [11,36,49–51,55,56,66–69,71]; neutral alumina [19,43–45,53,65]; a combination of silica plus alumina, either in one column or two separate columns e.g. SPE cartridges [21,25,27,28,31,32,46,48,52,57,58,60,70]; Florisil [26,34,35,47,65]; a combination of silica gel clean-up and chemically bonded aminopropylsilane phase [1,20,33,38,62,63].

Aliphatic hydrocarbons are usually found in high concentrations in environmental samples. Since they co-elute with PAHs during the GC analysis, they need to be separated from the PAHs fraction before the IRMS analysis [26]. The aliphatic fraction of the sample is eluted with solvents such as *n*-hexane [1,12,13,18–20,27–29,33,36,38,43–46,48,51–53,55,57,60,62,63,66,68,70] or *n*-pentane [21,25,26,31,32,34,35,67,69] and the PAHs fraction with solvents of higher polarity such as toluene [19,28,43–45,53,58], DCM [27] or a mixture of solvents, e.g. DCM/*n*-hexane [1,12,13,18,20,28,29,36,48,51,52,55,57,60,63,66–69], *n*-hexane/toluene [19,46,70], *n*-pentane/DCM [21,25,26,31,32,34,35], *n*-hexane/benzene [1,20,33,38,62,63]. Copper is often added on top of the clean-up columns [12,13,25,27,29] or added together with the extraction solvent [33,38]. This is done especially for sediments, in order to remove elemental sulphur.

In addition to the use of column chromatography (or alternatively SPE), other methods, such as automated gel permeation chromatography (GPC) [26,34,35,52] or thin layer chromatography (TLC) [26,34,35,52,70] are often used for further fractionations or purifications. Another technique used for specific class separation is HPLC [11,21,25,47].

Fabbri et al. [50] needed to purify the extracts from vinyl polymers present in the sample, mostly poly-vinylchloride (PVC), which could interfere with the clean-up procedure. In the case of crude oil, Mazeas and Budzinski [25] have separated the maltene and asphaltene fractions by dissolving 20 mg petroleum in 10 ml pentane and recovering the supernatant (maltene fractions, thus a mixture of hydrocarbons and resins) with a Pasteur pipette. The separated fraction was reduced in volume and subjected to clean-up. Glaser et al. [28] and Wilcke et al. [58] have used a column filled with HR-P adsorption resin as an additional cleanup after the alumina/silica purification. Mandalakis et al. [65] after the first step of silica-gel or Florisil cleanup further treated the sample with a dimethylformamide (DMF)-pentane cleanup followed by a preparative capillary gas chromatography programmed to trap selected PAHs. The isolated PAHs (the most abundant PAHs harvested together) were further processed in order to perform radiocarbon and stable carbon ratio analysis. A similar procedure was followed by Xu et al. [68].

10. Clean-up experiments

Okuda et al. [38] have examined the use of various solvents, stationary phases for automatic solid phase extraction (chemically bonded aminopropylsilane and cyanopropylsilane phases) and elution rates for the best method of separation of PAHs from UCM in various environmental samples (sediments, aerosol and a sample of motor-bike soot). Chemically bonded aminosilane phase appeared superior to cyanopropylsilane, since better separation of 2, 3, 4 and 5-ring PAHs was obtained, and was adopted in further experiments. Three different setups of solvents were tested *n*-hexane, *n*-hexane/DCM (97:3, v/v) and *n*-hexane/benzene (97:3, v/v). The last mixture provided a much better separation than *n*-hexane/DCM but similar to that of *n*-hexane and lower solvent amounts were needed to separate PAHs. When the separation of PAHs from UCM was considered, the mixture of *n*-hexane/benzene gave better results than pure *n*-hexane. Also flow rates of 0.006–0.6 ml min^{-1} were tested with 0.03 ml min^{-1} chosen as the optimal flow rate. Liu et al. [70] tested the influence of an additional clean-up: thin layer chromatography (TLC) (after the initial cleanup by alumna/silica gel column chromatography). The authors used several different solvent mixtures in order to optimise the separation of PAHs from impurities. In addition a clean-up using 3-aminopropyl bonded silica gel column was compared. The most efficient solvent mixtures for TLC was *n*-hexane/chloroform (45:5, v-v) as the UCM, that was still visible after the alumina/silica gel cleanup, was almost completely removed, and no co-elutions with PAH peaks were observed in the chromatogram. This clean-up procedure also proved to be superior to a 3-aminopropyl bonded silica gel cleanup.

11. Internal standard

O'Malley et al. [12] prior to GC–IRMS and GC–MS injection added appropriate volumes of phenyl hexane to the sample extract. Ballentine et al. [46] used naphthalene-d8 as an internal standard of known composition. Fabbri et al. [50] added *n*-dodecane (C12) to each sample. If the isotopic value of this standard was within 0.3‰ relative to its established offline value, the isotopic data was then considered being accurate within the error range. *n*-Dodecane was also used by Ou et al. [52]. Smirnov et al. [48] have used acenaphthylene, pentacosane and triacontane as isotopic internal standards. Stark et al. [13] have used acenaphthene, pentacosane (C25) and triacontane (C30). Walker et al. [11] co-injected Eicosane (*n*-C20) with every sample. Over the course of these analyses, the $\delta^{13}C$ values for *n*-C20 were $\pm$0.2‰ (standard error). Samples exceeding 0.6‰ (standard deviation) were not used in the data analysis.

Glaser et al. [28]used 11:0 fatty acid methyl ester (FAME) as an internal standard prior to the GC–C–IRMS. Okuda et al. [20,33] used alkanes and perdeuterated alkanes as internal isotopic standards. The same group also used acenaphthene-d_{10}, *p*-terphenyl-d_{14} and *n*-$C_{34}H_{70}$ (tetratriacontane) [1,20,63].

The details of all procedures reviewed in this paper are placed in Tables 1 and 2.

12. Analytical column

McRae et al. [43–45,53] used the WCOT 25 m, CP-Sil 5CB column. Injection was performed in split mode 30:1. Sun et al. [19] used 25 m fused silica capilary column, SP-Sil 5CB, 0.22 mm ID and 0.25 μm film thickness.

Liu et al. [70] and Itoh et al. [56] used HP-5 capillary column of 60 m × 0.25 mm ID and 0.25 μm film thickness. Kim et al. [26,34] used DB-5 MS column (30 m × 0.32 mm ID, 0.5-μm film thickness). Peng et al. [66] used the HP-1 MS silica capilary column (60 m, 0.32 mm ID and 0.25 μm film thickness). Grice et al. [51] used 60 m × 0.25 mm ID × 0.25 μm film thickness, 5% phenyl–methyl silicone phase. Aichner et al. [60] used 5-MS fused silica column (60 m × 0.25 mm ID × 0.25 μm film thickness). Zhang et al. [36] and Ou et al. [52]used HP-5 MS 60 m × 0.32 mm ID × 0.25 μm film thickness. Gocht et al. [57]used DB-5 MS column, 30 m × 0.25 mm ID × 25 μm film thickness. Mikolajczuk

et al. [67,69], DB-5 MS column (60 m × 0.25 mm ID, 0.25-μm film thickness), whilst Grice et al. [55] used DB-1 (60 m × 0.25 mm ID × 0.25-μm film thickness).

13. Precision and accuracy

O'Malley et al. [12,29] have used one aromatic (acenaphthalene) and two aliphatic standards (C21 and C25 *n*-alkanes) of known isotopic values in order to assess the instrument performance and reliability of background subtraction in complex sample matrices. Precision measurements (repeat injections) on standards (max deviation from the mean) ranged from 0.17‰ for fluoranthene to 0.31‰ for perylene [12]. O'Malley et al. [29] gives a precision (2σ) for a standard mix of PAHs ranging from 0.25‰ to 0.39‰. The accuracy (maximum deviation from the true value) measured by conventional IRMS was between 0.09–0.59‰ and 0.01‰–0.57‰. For samples, the precision of some of the co-eluting species, ranged 0.17–1.16‰ for benzo(a)anthracene/chrysene and below 0.30‰ for well separated components, and 0.85‰ for some co-eluting isomers. Stark et al. [13] report a precision of 0.2‰ and accuracy standards (absolute deviations from conventional ^{13}C delta values of the isotopic standards) better than 0.3‰. Walker et al. [11] report obtaining a difference of 0.48‰ ± 0.2‰ between GC–C–IRMS analyses and an offline established value of internal standard (eicosane) which was co-injected with every sample ($n=42$). In some cases, when compounds of interest were co-eluting (e.g. methylphenanthrenes) [25,47], it was possible to measure the isotopic composition of co-eluting doublets or even the sum of four compounds with a good reproducibility [47]. Okuda et al. [38] report a precision of ±0.5 to 1.2‰ for PAH standards, except for coronene which was ±1.8‰. For aerosol samples, the SD of triplicate measurements were from 0.2‰ to 1.2‰ for most of the PAHs and up to 1.7–2.0‰ when a target peak was interfered by another peak. A precision (SD of replicate measurements) of ±1.1‰ for benzo(g,h,i)perylene, ±1.6‰ for coronene and ±0.7‰ for other PAHs was observed by the same authors [33]. The same group [1,20,63] reported the range of SDs of individual PAHs standards from 0.4 to 0.7‰ and the accuracy which was determined by comparing the obtained data with the conventional closed-tube, off-line method ranged from 0.0 to 0.4‰. Sun et al. [19] reported a reproducibility of 0.2–0.4‰. Kim et al. [26] report that the precision ranged between 0.1 and 0.4‰. The SDs of isotope ratios for multiple injections of environmental samples after purification fall in the range of 0.1–0.7‰. The authors only used peaks which were greater than 10 V in area for the precise IRMS measurements whilst Liu et al. [70] used peak heights (amplitude above 0.5 V) to guarantee the accuracy of the result. Peng et al. [66] report standard deviations of less than 0.6‰ for the stable carbon ration measurements. The same SD was reported by Zhang et al. [36]. Gocht et al. [57] report precisions of triplicate measurements better than ±0.5‰. Mikolajczuk et al. [67] calculated an expanded uncertainty for PAHs extracted from three different dust samples. For tunnel dust the uncertainties were below 1‰, for winter rural dust higher than 1‰ in case of fluorene, anthracene and benzo($b+k$)fluoranthenes and for summer dust samples they ranged 0.35–1.3‰, with the exception of fluorene and benzo($b+k$)fluoranthenes for which uncertainties reached even 3.8‰. Grice et al. [51] only took the results with standard deviations lower than 0.4‰ into consideration. Ou et al. [52] rejected the samples if precision (SD) exceeded 0.6‰.

14. Summary and conclusions

This paper presented the approaches of the CSIA of carbon in PAHs in order to allocate the sources of these pollutants. Special attention was paid to methodological procedures because obtaining high purity extracts is an important concern for isotopic ratio determinations. In case of the GC–MS analysis alone it is less crucial because of a capability of single ion monitoring mode. This is not possible when IRMS is applied and therefore the chromatographic peaks must be well resolved and the UCM substantially reduced before reliable results of isotopic analyses are obtained. Promising results were achieved when using the isotopic data in order to find out the origin of PAHs but also difficulties in the interpretation of results could be seen. This was the case especially when a mixture of many sources was contributing to the total PAH content of the sample or when the isotopic signatures of the sources were not known. Combining the diagnostic ratios of PAHs together with the isotopic data seemed to be one of the ways helpful in overcoming some of these problems. Another solution, not yet well examined for PAHs, might be the combination of the isotopic data of hydrogen with the data for carbon. Future experiments to investigate the usefulness of this approach are needed.

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