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Center for Applied Geoscience (ZAG)

MASTER THESIS

Master of Science in Applied
Environmental Geoscience

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Method Development and Application of Compound-Specific Isotope Analysis for Source Allocation of Polycyclic Aromatic Hydrocarbons in Contaminated Soil.

Tuebingen 2004

Acknowledgments

Abstract

Refined petroleum products, mineral-oils and coal tars are common examples of contamination sources at former oil-manufacturing sites. As a result of processes like pyrolysis, rectification, cracking and burning, polycyclic aromatic hydrocarbons are formed and subsequently released to the environment.

PAHs are classified as highly toxic and carcinogenic compounds and are often the cause of costly site investigations and remediations. Also, significant political and economical interests are reasons why natural attenuation has been growing in importance as a remediation approach at PAH contaminated sites. Identifying the sources of PAH contamination at these sites is a crucial step towards elucidating the fate of the organic pollutants. This is usually supported through analytical tools; such as fingerprinting analyses with gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography (HPLC), to mention some classical examples.

This investigation focuses on the application of compound-specific isotope analysis (CSIA) of carbon, a highly powerful and less known analytical technology, in order to assess the PAHs source allocation at a site with a massive and highly diverse contamination. With this method, the assessment of the carbon isotope ratios of PAHs can be used as source indicator at highly contaminated sites.

The objective of this master thesis was to develop an extraction and purification method to isolate the PAHs from a complex contaminated soil matrix. Of special interest was to determine if the extraction and purification procedures affect the isotopic composition of the target analytes. This was of big importance since the main aim was the evaluation of the real isotopic composition of PAHs at the contaminated site. In order to achieve this objective, two enrichment and purification methods for the PAHs in the soil matrix were developed. The first method consisted of a liquid-solid extraction combined with a liquid-liquid extraction and a flash chromatography (LSE/LLE/FC), and the second method consisted of an accelerated solvent extraction combined with a liquid-liquid extraction and a final flash chromatography (ASE/LLE/FC). The comparison of the separated PAHs through these methods with respect to PAH standards showed that no detectable change in the isotopic composition of the target compounds occurred and therefore these purification methods can effectively be applied in highly PAH contaminated soil samples at the surveyed site through CSIA if soil samples contain a minimum concentration of PAHs of 11.1 mg/kg.

Abstract

Raffinierte Erdölprodukte, Mineralöle und Kohlenteere sind typische Beispiele für Ursachen einer Kontamination an ehemaligen Ölverarbeitungsstätten. Als Produkte von Prozessen wie Pyrolyse, Rektifikation, Cracking, und Verbrennung werden polyzyklische aromatische Kohlenwasserstoffe (PAKs) gebildet, die in die Umwelt gelangen können. PAKs werden als stark toxische und kanzerogene Verbindungen eingestuft und sind oft der Anlass für kostspielige Bodenuntersuchungen und –Sanierungen. Außerdem sind starke politische und volkswirtschaftliche Interessen die Gründe dafür, dass dem natürlichen Schadstoffabbau eine wachsende Bedeutung als Sanierungsansatz in mit PAKs verunreinigten Arealen zukommt. Die Quellenidentifikation der PAKs in diesen Arealen ist ein entscheidender Schritt, um das Schicksal dieser Kontaminationen aufzuklären. Dies wird üblicherweise mit analytischen Methoden durchgeführt wie z.B. einer Fingerprinting- Analyse.

Diese Master Arbeit konzentrierte sich auf die Anwendung der Komponentenspezifischen Isotopen analyse (CSIA) von Kohlenstoff, um die Schadstoffquelle der PAKs in einem stark und heterogen verschmutzten Gebiet zu charakterisieren. Mit dieser Methode kann durch die Kohlenstoffisotopenverhältnisse der PAKs der Ursprung der Verschmutzung in stark heterogen verunreinigten Arealen bestimmt werden.

Das Ziel dieser Master-Abschlussarbeit war es, eine Extraktions- und Aufreinigungsmethode zu entwickeln, um die PAK aus verunreinigten Bodenproben zu isolieren. Von speziellem Interesse war es herauszufinden, ob dabei eine Veränderung der isotopischen Zusammensetzung stattfindet. In diesem Forschungsprojekt wurden zwei Anreicherungs- und Aufreinigungsmethoden für PAKs aus Bodenproben entwickelt. Die eine Aufreinigungsmethode besteht aus einer Flüssig/Fest Extraktion kombiniert mit einer Flüssig/Flüssig Extraktion und einer Flash-Chromatographie (ASE/LLE/FC). Die zweite Metode besteht aus einer beschleunigten Flüssigextraktion kombiniert mit einer Flüssig/Flüssig Extraktion und einer abschließenden Flash-Chromatographie (ASE/LLE/FC). Der Vergleich dieser Methoden mit PAKs Standards zeigte keine Veränderung in der isotopischen Zusammensetzung der untersuchten Verbindungen. Beide Aufreinigungsmethoden für PAKs können verunreinigte Bodenproben verwendet werden, die anschließend mittels CSIA untersucht werden sollen.

Abstract

Productos refinados de petróleo, aceites minerales y alquitrán de hulla son ejemplos comunes de fuentes de contaminación en sitios donde anteriormente se ubicaban manufacturadoras de aceites. Como resultado de diversos procesos como pirólisis, rectificación, cracking y combustión, los hidrocarburos policíclicos aromáticos son formados y liberados en el medio ambiente. Estos compuestos son clasificados como altamente tóxicos y cancerígenos, los cuales son frecuentemente la causa de investigaciones y remediaciones costosas en los sitios contaminados. También, grandes intereses políticos y económicos son razones por las cuales la atenuación natural ha ido creciendo en importancia como alternativa de remediación en los sitios contaminados por estos compuestos. La identificación de las fuentes de contaminación en estos sitios es crucial para determinar el destino de estos contaminantes orgánicos. Esto es apoyado por muestreo analítico como el análisis por medio de cromatografía de gases-espectroscopía de masas (GC-MS) y cromatografía de líquidos de alta resolución (HPLC), por mencionar algunos ejemplos.

Esta investigación está enfocada en la aplicación de la técnica de análisis de compuestos específicos de isótopos (CSIA) de carbono, una instrumentación analítica altamente poderosa y poco conocida para determinar la fuentes de contaminación por hidrocarburos policíclicos aromáticos en un sitio donde la contaminación es masiva y altamente diversa. Por medio de esta técnica, la determinación de la proporción de isótopos de carbon de los hidrocarburos policíclicos aromáticos puede ser utilizado como indicador de fuentes de contaminación en sitios altamente contaminados. El objetivo de esta tesis de maestría es el de desarrollar un método de extracción y de purificación para aislar estos compuestos del suelo, el cual está caracterizado por tener una contaminación compleja. De gran interés es el determinar si un cambio en la composición isotópica se está llevando a cabo. Durante esta investigación, dos métodos de enriquecimiento y de purificación para estos compuestos en suelos fue desarrollado. Un método de purificación que consiste en una extracción líquido-sólido combinado con una extracción líquido-líquido seguido por una cromatografía flash (LSE/LLE/FC) y un segundo método que consiste en una extracción acelerada de solvente combinado con una extracción líquido-líquido y seguido por una cromatografía flash (ASE/LLE/FC).

Al comparar los compuestos aislados por estos dos métodos con respecto a una solución estándar de hidrocarburos policíclicos aromáticos fué posible determinar que no ocurre un cambio significativo en la composición isotópica de los compuestos de interés y que estos dos métodos de purificación pueden ser efectivamente aplicados en suelos altamente contaminados con estos compuestos en este sitio investigado por medio de CSIA.

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Symbols

δ	Delta value of carbon isotope composition relative to a reference sample	[‰]
ρ	Density	[g cm^{-3}]
i.d.	Internal diameter	[mm]
IS_{D200}	Internal standard with deuterated PAH (IS concentration 200 ppm)	[μg ml^{-1}]
λ	Wave length	[nm]
-log C_{iw}^{sat}	Negative logarithm of the aqueous solubility of a compound	[mol L^{-1}]
log K_{ow}	Logarithm of the octanol/water partitioning coefficient	[-]
MW	Molecular weight	[g mol^{-1}]
m/z	Mass to charge ratio	[-]
N	Number of neutrons	[-]
R_f	Retention factor	[-]
T_b	Boiling point	[°C]
T_m	Melting point	[°C]
v/v	Volume ratio	[-]
Z	Number of protons	[-]

Abbreviations

ASE	Accelerated solvent extraction
B11	Soil sample from borehole 11
B16	Soil sample from borehole 16
B17	Soil sample from borehole 17
B29	Soil sample from borehole 29
CH	Cyclohexane
CSIA	Compound-specific isotope analysis
DCM	Dichloromethane
DL	Detection Limit
FC	Flash chromatography
GC	Gas chromatography
GC-IRMS	Gas chromatography-isotope ratio mass spectrometry
GC-MS	Gas chromatography-mass spectrometry
L.A.	Low amplitude
HPLC	High performance liquid chromatography
LC	Liquid chromatography
LLE	Liquid-liquid extraction
LSE	Liquid-solid extraction
LVI	Large volume injection
MeOH	Methanol
mbgs	Meter below ground surface
PTFE	Polytetrafluorethylene
PTV	Programmable temperature vaporizer
SIM	Selected ion mode
TCM	Trichloromethane
TLC	Thin layer chromatography
UCM	Unresolved complex mixture
UV	Ultra violet

Abbreviation of Contaminants

Ace	Acenaphtene
Ace-d$_{10}$	Acenaphtene deuterated
AHC	Aliphatic hydrocarbons
Ant	Anthracene
Any	Acenaphthylene
BaA	Benzo(a)anthracene
BaP	Benzo(a)pyrene
Bb/Kf	Benzo(b)fluoranthene, benzo(k)fluoranthene
BghiP	Benzo(g,h,i)perylene
BTEX	Generic for benzene, toluene, ethylbenzene and xylene
CHC	Chlorinated hydrocarbons
Chr	Chrysene
Chr-d$_{12}$	Chrysene deuterated
DahA	Dibenzo(a,h)anthracene
Fln	Fluorene
Fth	Fluoranthene
Indeno	Indeno(1,2,3-cd)pyrene
MHC	Mineral-oil hydrocarbons
Nap	Naphthalene
Nap-d$_8$	Naphthalene deuterated
PAHs	Polycyclic aromatic hydrocarbons
PAH STD	Standard of polycyclic aromatic hydrocarbons
Perylen-d$_{12}$	Perylene deuterated
Phen	Phenanthrene
Phen-d$_{10}$	Phenanthrene deuterated
Py	Pyrene
VC	Vinyl chloride

Other Abbreviations

BBodSchV	Bundes-Bodenschutz und Altlastenverordnung
US EPA	United States Environmental Protection Agency

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1
Introduction

1.1 Historical Background and Description of the Contaminated Site

Since the end of the 19th century, an intensive production and consumption of mineral-oils and their derivatives took place at the former mineral-oil treatment facility in Stuttgart. Extensive groundwater pollution was detected at the end of 1960 and in 1984 the first site investigations were done by Landeshauptstadt Stuttgart, Amt für Umweltschuzt. In 1988 all production activities were stopped and in 2001 remediation including dismantling of the site was decided [1].

The former mineral-oil treatment facility is located over extensive contamination of soil and groundwater with multiple pollutants, such as mineral-oil hydrocarbons (MHC), polycyclic aromatic hydrocarbons (PAH), chlorinated hydrocarbons (CHC), aliphatic hydrocarbons (AHC) as well as monocyclic aromatic compounds (BTEX).

1.2 Geology and Hydrogeology of the Site

The geological profile characterizing the site is the following:
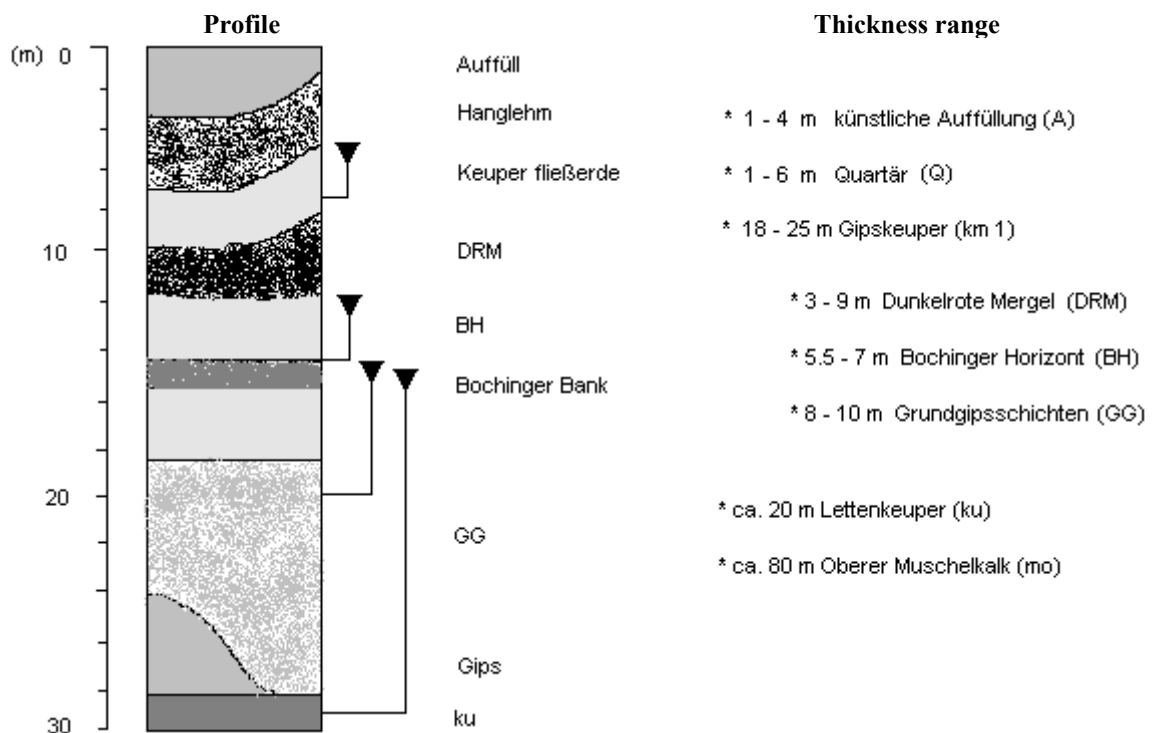
Figure 1. Geological profile of the contaminated site [1.]

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[1] Künstliche Auffüllung-anthropogenic filling, Quartär-Quaternary, Gypskeuper-clay dominated strata with relicts of leached anhydrite and gypsum, Dunkelrote Mergel-dark-red marls, Bochinger Horizont-Bochinger Horizon, Grundgipsschichten-clay dominated strata with gypsum, Lettenkeuper-Letten clay, Oberer Muschelkalk-calcite with fossils [2].

The groundwater flows basically through three main hydraulic storage zones:

1. Quartär / Dunkelrote Mergel (Q/DRM)
2. Bochinger Horizont (BH)
3. Oberer Lettenkeuper (ku)

The groundwater direction in these three hydraulic zones is from the Northwest to the Southeast (117°-130°). Important hydrogeology information is given in the following Table 1:

Table 1. Hydrogeology of the contaminated site.

Groundwater Storage	Groundwater Level [meters over sea level]	Unsaturated Zone Depth [m]	Transmissivity [m^2 / s]	Flow [L/s]
Q / DRM	234	6	3.4 E -5	0.1
BH	233.5	6.5	1.7 E -5	0.08
ku	228	12	1.3 E -4	0.9

After a groundwater analysis, the first Q/DRM and second BH aquifers were found to contain a "pollutant cocktail" or extensive mixture of several contaminants (PAH, MHC, CHC and BTEX) which were identified as the primary pollutant source. In the third hydraulic storage zone (ku) only CHC and PAH were found as contaminants. In this aquifer a vertical transport downwards happens. Due to this, the pollution has already affected the water quality in many wells and shafts.

1.3 Contaminant Description

The main focus of this thesis are the PAH contaminants at the surveyed site, on which analyses through CSIA are done in order to assess the contaminant source allocation.

PAHs are organic compounds containing more than one fused aromatic rings, which have drawn considerable interest of study in the environment because of their toxic, mutagenic and carcinogenic characteristics [3] .Several types of cancer are directly related with exposure to PAHs. Some examples are lung, bladder, skin and internal organs cancer. Also, anaemia can result from a long exposure to naphthalene. An effect resulting from PAH physical contact is dermatitis, meanwhile the effects due to inhalation or ingestion are headaches, dazzlement and vomit. In the worst cases, death resulting after kidney failure can occur [4], [5].

The released PAHs in the subsurface are adsorbed on fine particles and accumulated in sediments. The adsorption of the PAHs in different soil matrices results from their low vapour pressure [6], their hydrophobicity reflected by their low water solubility [4] and the matrix nature. Also, their hydrophobic and lipophilic properties result in a high tendency for bio-accumulation and persistance in the environment, a reason for high eco-toxicological concern [7].

Today, PAHs enter into the environment mainly from anthropogenic sources. Some examples are the discharges from petroleum spills, oil seepages and industrial production activities. The main direct inputs derive from tar-oil treatment facilities, disposal areas and polluted sites. They can also enter the

environment as a result of natural events, for example from natural thermical processes like volcanism, burning of forests and biosynthesis [8].

According to Hirner [4], PAH soil concentrations (considering the 16 PAHs used as a reference after US EPA) in Germany at gardens and playgrounds should be <0.1 μg/kg and in sport areas <1 μg/kg. Meanwhile PAH concentrations due to natural processes are in the range of 1 to 10 μg/kg. According to BBodSchV [9], the precautionary limit of PAH concentration in soil is 3 mg/kg for soils with less than 8 % of organic matter and 10 mg/kg for soils with organic matter above 8%. An example of a highly contaminated site with PAHs is 650 mg/kg [3].

1.4 Previous Investigations and Remediations at the Surveyed Site

Previous qualitative and quantitative chemical analyses at the polluted site started in 1998 where it was determined that the main contamination is focused in the first 2 m from the surface. Distribution of the contamination reaches up to 8 meters below ground surface (mbgs), reaching into the saturated zone. The main concentration profile of the contaminants is shown in the Table 2.

Table 2. Contaminant chemical analyses in soil at the polluted site on 1998 and 2002.

Contaminant	Main Contamination Depth (mbgs)	Maximum Concentration on 1998 [mg/kg] [11]	Maximum Concentration on 2002 [mg/kg] [12]	Contaminant Precautionary Limit in Soil [mg/kg] [9], [10]
PAH	2 - 3	970	3600	3 to 10
MHC	1 - 2	40400	890	
CHC	0 - 1	43	48	<1
Benzene	7.7 – 8	7.95	29	<1
Toluene	0 - 1	14.4	48	<1

The contamination of the leachate was determined to be above the accepted legal limits. Through investigations of the air content in the soil in 1998, concentrations of AHC (22 mg/m^3), benzene (4.4 mg/m^3), CHC (53 mg/m^3) and VC (15 mg/m^3) were found. The average contamination found in the leachate was mainly from mineral-oils (21936 μg/L), PAHs (6243 μg/L), naphthalene (647 μg/L), AHC (5883 μg/L), benzene (1425 μg/L), CHC (1685 μg/L) and VC (35 μg/L) [11].

This extensive contamination led to remediation at the site. In 1998, the first hydraulic zone Q/DRM (see Figure 1) was treated with Pump and Treat with the aim of removing the source of contamination. This solution was effective at that time because the pollution diminished extensively. In the year 2001, digging-out of the source from the unsaturated zone took place. Later in the same year, the isolation of the source and plume in the first hydraulic zone was attempted. Initially, it was planned that the remaining contamination at the first saturated zone would be treated again with Pump and Treat, but several aspects make remediation of the site very difficult, such as a complex fractured aquifer below the mineral-oil treatment facility, the long-term and the expensive remediation projects. Due to all the

disadvantages of the ordinary remediation approaches at the surveyed site, natural attenuation seems to be the most cost-effective alternative [1].

2

Aims

The assessment of the PAH source allocation represented the overall goal to achieve in this thesis work, which is of great importance for the monitoring and remediation of the contamination at the site. In this research, it was expected to identify the source allocation of PAH contaminants through the evaluation of the isotopic fractionation at the former oil-manufacturing site using the CSIA technique. Also the distinction between a source and a degradation process through the evaluation of the isotopic composition was expected to be clarified. In order to achieve this purpose, several other objectives concerning the development of a strategy for assessing the isotopic composition of PAHs at soil samples at the contaminated site were determined. The method consisted on the sample preparation prior to CSIA and the method development for analytical measurements.

The strategy involved the extraction of contaminants from the soil using either liquid-solid extraction or accelerated solvent extraction. This was followed by a liquid-liquid extraction and then by a selective separation of the PAHs from the other compounds through clean-up methods like flash chromatography.

Of special interest was to determine a possible influence on the isotopic fractionation that the extraction of PAHs from soil experiences after the clean-up methods, the liquid-solid extraction and the accelerated solvent extraction, the last one being performed under elevated temperature and pressure conditions. No literature references describing the influence that the accelerated solvent extraction, the liquid–solid extraction and clean-up with flash chromatography have on the isotopic fractionation were found, and thus probably no investigations referring to this effect in the mentioned extraction techniques have been reported. Therefore it was crucial to know the effect of the mentioned purification methods in the $\delta^{13}C$ value of the target PAH compounds in order to assess the real isotopic composition at the contaminated site. The importance of the developed procedures of purification and analysis provided a validated method that can be trustfully applied for further source allocation at highly contaminated sites.

Another objective of this study was to determine the PAH concentrations in soil samples at defined locations of the surveyed field site, evaluate and compare the extraction recovery between the LSE and ASE extraction methods. Gas chromatography-mass spectrometry (GC-MS) was the analytical instrumentation required to achieve these analyses.

3
Concepts of Isotopic Fractionation

3.1 Definitions

Since the discovery of the isotopes of hydrogen by Urey in 1932, an extensive investigation of stable isotopes and their application in many areas have been performed.

An *isotope* is a nuclide that presents mass differences due to a variation in the existing number of neutrons, but with the same number of protons, usually expressed with notations such as carbon-13 or ^{13}C, if we exemplify with carbon in this particular case. In Environmental Sciences, nowadays isotopes routinely contribute to investigations in Geochemistry, Hydrogeology, Environmental Forensics and Environmental Chemistry to mention some examples.

Naturally occurring nuclides can be classified as stable or unstable. Isotopes of a chemical element, which are not radioactive, are known as *stable isotopes*. In these nuclides, the greatest stability is achieved when the ratio between neutrons (N) and protons (Z) is around one. In contrast to the stable isotopes, unstable isotopes decay spontaneously to stable isotopes by emission of radiation [13], [14]. In the case of CSIA, only ratios of stable isotopes are measured.

3.2 The δ - notation

Stable isotope ratios of elements such as oxygen, hydrogen, carbon, nitrogen and sulphur are reported as δ values expressed in units of parts per mil (‰) relative to an international standard of known composition (e.g., Vienna Peedee Belemnite, VPDB for $^{13}C/^{12}C$). Mathematically, the δ value is defined by the equation [15]:

$$\delta_x = \left(\frac{R_x - R_{reference}}{R_{reference}} \right) \times 1000 \ [\text{‰}]$$

[Equation 1]

Where   R = ratio of heavy to light isotope ($^{13}C/^{12}C$),

       R_x= ratio of the heavy isotope to the light isotope ($^{13}C/^{12}C$) in a certain compound x,

       $R_{reference}$=ratio of the heavy isotope to the light isotope ($^{13}C/^{12}C$) in the international reference standard.

Indeed, the δ-value is reported as the difference of relative ratios in order to correct the mass-discriminating effects in instrumentation and to facilitate the comparison of published gas chromatography-isotope ratio mass spectrometry (GC-IRMS) data [16].

3.3 Isotopic Fractionation

Isotope effects result from the differences in physical and chemical properties when variations in the atomic mass of an element occur. These variations in the atomic mass can be explained through the potential energy that is a function of the distance between two atoms. The hydrogen isotopes and deuterium molecules are considered for the following example. Chemical bonds of a molecule containing heavier isotopes like H-D are more stable than molecules with lighter isotopes (H-H) due to the higher dissociation energy required for bond cleavage. Therefore, the H-D bond is more stable than the H-H bond because it requires a higher energy to dissociate their chemical bond [14]. In a potential energy curve vs. interatomic distance, the lowest possible energy in a molecule is known as the Zero Point Energies (ZPE) [15], which is different for the same molecule with isotopic differences. The isotopic fractionation between two molecules can be explained by differences in ZPE.
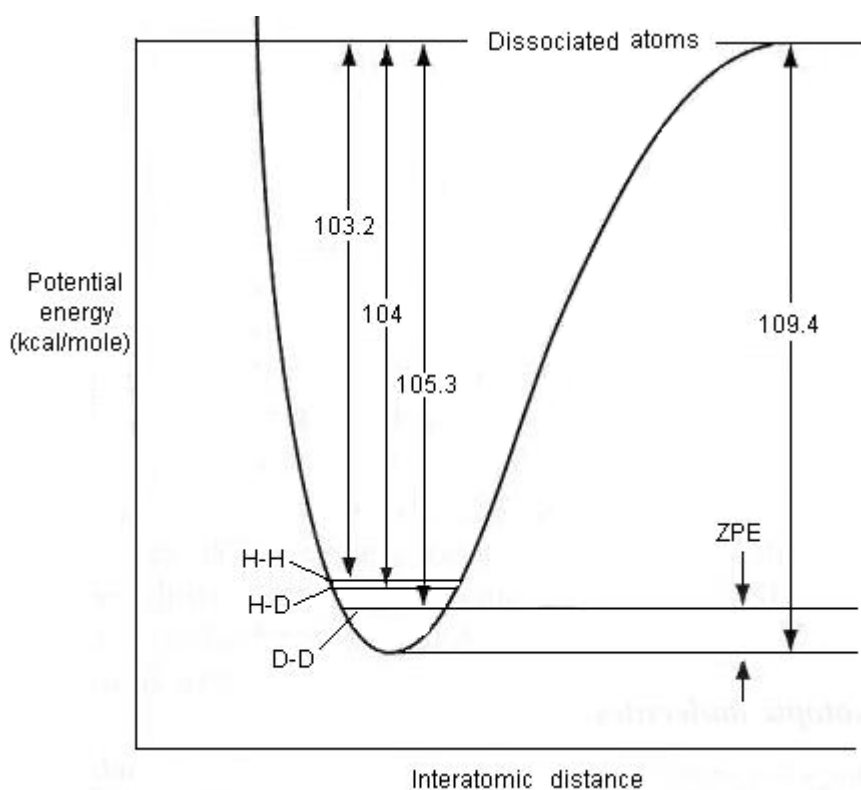
Figure 2. **Potential energy curve for hydrogen isotopes and deuterium molecules** [15.
Zero point energies (ZPE) are higher in molecules that are less stable.

An *isotopic fractionation* is a phenomenon that causes changes in the relative abundance of isotopes due to their difference in mass. The change in isotopic proportions can result in a mass difference originated by physical, chemical and biological processes [15]. Two different categories of isotopic fractionation effects are known: Kinetic processes and Thermodynamic processes.

Kinetic fractionations are irreversible processes depending mainly on isotope specific differences in the binding energies of the compound resulting in differences in the reaction rates of the molecules. During physical processes, such as evaporation and diffusion for example, isotopically lighter molecules have higher velocities and lower cohesion energies. In chemical processes including

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biological mediated reactions, light bonds involving isotopes (e.g. ^{12}C-H) can react faster than those formed between heavier isotopes (e.g. ^{13}C-H). This is known as a normal kinetic isotopic fractionation usually reflected in an increase on the isotopic composition of the remaining molecules of interest in the remaining phase (becoming more positive δ^{13}C values if we refer to carbon). If an opposite effect occurs, this is known as an inverse kinetic isotopic effect, where the δ^{13}C will be then decreased, showing more negative values [14].

Thermodynamic or *equilibrium fractionations* happen in case no net reaction occurs, but a change in the distribution between different chemical compounds, different phases or even between individual molecules occurs [16], [17].

4
Use of Compound-Specific Stable Isotope Analysis (CSIA) for Source Allocation

4.1 CSIA Concepts and Applications

CSIA is a powerful analytical tool that has been used in many fields to infer the origin and fate of organic contamination in diverse systems through the quantification of the isotopic composition. Several applications have recently been reported in Environmental Sciences. Examples to these are: [16], [17].

- Assessment of contaminated environments,
- Allocation of contaminant sources on a local, regional and global scale,
- Distinction between contaminant sources,
- Characterization and quantification of transformation reactions,
- Proof of *in-situ* degradation of organic pollutants.

Contaminants in the subsurface may present an isotopic composition that can behave either in a conservative (no contaminant reaction) or in a non-conservative (contaminant reaction) manner. On this thesis work, CSIA was focused on two possible applications:

- To apportion sources of conservative contamination by PAHs.
- The characterization of transformation processes or degradation of non-conservative contamination by PAHs.

4.2 Natural Attenuation Assessment

Several *in-situ* processes decrease the concentration of a contaminant in the liquid phase in the subsurface: dispersion/diffusion, dilution, sorption to soil [18], volatilization and degradation [19], [20] (see Figure 3).

However, in order to identify the contaminant attenuation at a polluted site, the intrinsic degradation needs to be assessed [21]. Usually the assessment of the contaminant mass depletion is very difficult due to the complexity of the subsurface and natural processes occurring herein. CSIA offers the great advantage of characterizing and sometimes even quantifying the degradation of the organic contaminants [22], [20] without the need of a mass balance. The low natural abundance of the heavier isotopes requires a very high precision that cannot be achieved with conventional analytical technologies, e.g. GC-MS.
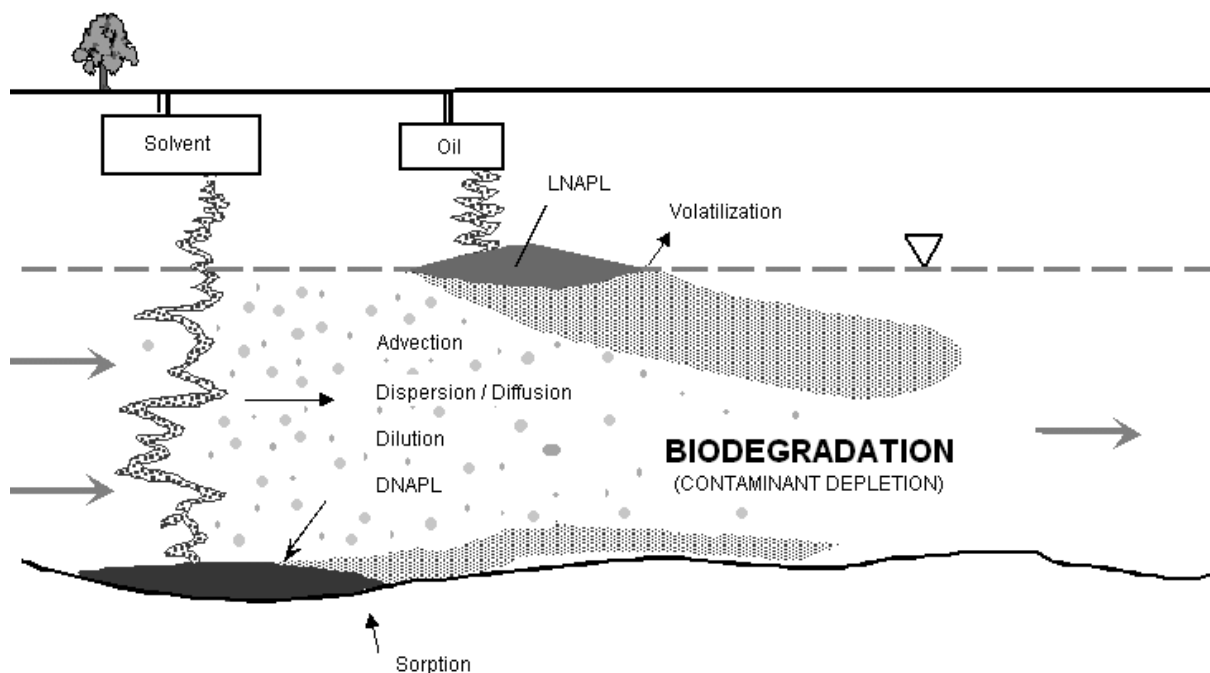
Figure 3. Processes occurring in the subsurface at a contaminated site.

4.3 CSIA Analytical Technology

4.3.1 CSIA Principle

CSIA uses an analytical method that measures the isotopic ratios of organic compounds with a very high precision. This technique is termed gas chromatography-isotope ratio mass spectrometry (GC-IRMS). It works under continuous flow and employs a separation method like a gas chromatography GC and very recently also liquid chromatography LC [23], [24], [25] via an on-line combustion/pyrolysis oven with a multicollector mass spectrometer [16], [26]. Until now, solely isotopic ratios of four elements can be measured with GC-IRMS: D/H, $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{18}O/^{16}O$.

4.3.2 GC-IRMS Operation

In this thesis, measurements of the isotopic composition of carbon in GC-IRMS were evaluated and thus here the instrumental operation is only described for carbon. The injection of the sample into the GC is the initial step in the GC-IRMS operation. The GC column separates the injected organic analytes. The carrier gas helium transports the effluent with the separated analytes into the combustion furnace loaded with CuO/Pt and held at a temperature of 940°C, where the organic compounds are quantitatively combusted to CO_2, N_2 and H_2O. A water trap removes the water from the effluent that otherwise might interfere with the measurement of CO_2 [27] because this will be later used for the $^{13}C/^{12}C$ measurements. This gas is then transferred to the IRMS where it is ionized. Afterwards the resulting ions are separated by the masses and simultaneously measured in fixed collectors in the

IRMS. In the case of carbon, the isotope ratios are determined by measurement of the ion current at masses 44 ($^{12}C^{16}O_2$), 45 ($^{13}C^{16}O_2$) and [26] and 46($^{12}C^{18}O^{16}O$) (See Figure 4).
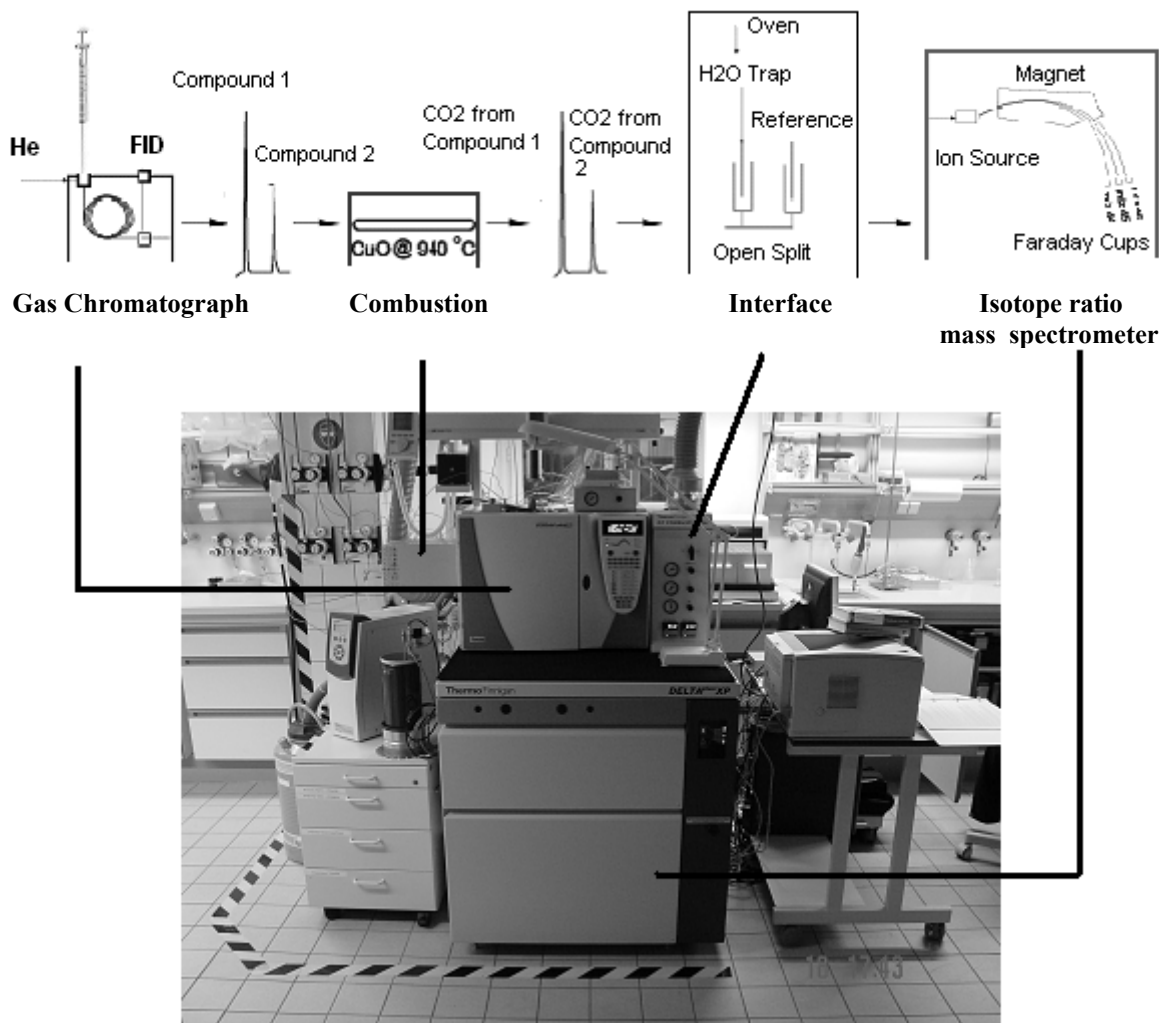
Figure 4. Set-up of a GC-IRMS. The upper picture shows the schematical steps for a carbon isotope measurement.

4.3.3 Application of CSIA at the Surveyed Site

In order to investigate and treat the PAH contamination at the former oil-manufacturing site, it is necessary to monitor the locations of the pollution sources. The application of CSIA for carbon promises to be a suitable technique for the assessment of PAH source allocation at a site [28], even if the evaluation of the isotopic ratio of $^{13}C/^{12}C$ is hampered by several factors [16]:

- The complex and extensive contaminant mixture.
- The highly heterogeneous contamination pattern.
- Existing limitations regarding the instrumental sensitivity.
- The lack of an appropriate extraction/clean-up method for the isolation of PAH from soil samples without altering the carbon isotopic composition of the compounds of interest. Also the development of a validated method in CSIA is crucial for reliable measurements.

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4.4 Existing Purification Methods for PAHs and Current Limitations

The precision and accuracy of the $\delta^{13}C$ determinations of target PAHs in CSIA is limited by the nature and extent of unresolved complex mixtures (UCM) [28]. Interfering compounds present in the extracts can show the same retention time as the target compounds or could form a background signal in the chromatograms that hampers the evaluation through CSIA.

Purification of the target analytes in the samples prior to GC-IRMS analyses can eliminate a significant portion of interfering compounds, and thus providing reliable isotopic composition values of the analytes of interest. Extraction and clean-up methods intend to isolate the target compounds from the soil samples at high concentrations in order to obtain peaks with high resolution and signal heights of the amplitude of mass 44 in CSIA.

Several extraction and clean-up methods have been applied for the recovery and isolation of the PAHs from soil samples. Some traditional extraction methods are the ultrasonic technique, the Soxhlet method [28], [6], [29], the liquid-solid extraction and the accelerated solvent extraction [30], being the last one of increasing use due to the high PAH extraction recovery reported from contaminated soils [31].

Other methods with a more specific application are the fixed bed pyrolysis under hydrogen pressure and the fluidized bed pyrolysis for PAH extraction applied to coal samples [32], [33], as well as the microwave assisted extractions for purification of methylphenanthrenes at marine sediments [34].

Clean-up methods reported for selective isolation of PAHs from the contaminated soils are treatments with alumina chromatography in order to separate aliphatic compounds (with n-hexane) from aromatics (with toluene) [32], [33], florisil columns to eliminate polar compounds from PAH extracts from marine sediments [34],purification of PAHs from soil samples with Sephadex and Silica Gel chromatography [28], [35], alumina and silica columns to separate saturated and unsaturated aliphatics from PAHs in lake sediments [29] and solid-phase extractions with silica gel-aluminium oxide columns for the separation of naphthalene with hexane and PAHs with toluene from tropical and temperate soils [29], [30].

Do the purification procedures of the PAH target compounds from soil samples alter the carbon isotopic composition? - a literature review-

Relevant investigations regarding the effect of the extraction and clean-up procedures on the isotopic composition of PAHs were done by O`Malley and Abrajano [28] in order to apportion PAH sources in environmental samples. This investigation employed a Sohxlet extraction with dichloromethane and clean-up with Cu-Sephadex and copper topped silica eluted with hexane (for aliphatics) and DCM:hexane (for aromatics). Results of the isotopic composition of the PAHs showed no significant

change in the δ^{13}C value due to extraction and clean-up. As well, isotopic alterations associated with evaporation, photolytic decomposition and microbial degradation were not apparent.

Investigations from Wilke [30] regarding clean-up of PAHs with solid-phase extraction with Silica Gel-aluminium oxide columns followed by a second purification with solid-phase extraction with 1 g of HR-P resin were studied. Advantages observed in this strategy was the selective separation of naphthalene and different types of perylenes from soil samples by allowing the separation of interfering compounds in hexane and hexane/dichloromethane from PAHs eluted in toluene. Results showed a shift of the δ^{13}C of the PAHs into more negative values (-0.3‰ for naphthalene and -0.5 ‰ for perylene), being naphthalene δ^{13}C values close to the method precision and therefore as insignificant considered. Only the isotopic composition of perylenes allowed distinguishing source apportionment.

Hydropyrolysis purification at 650°C of PAHs from coal by Mc.Rae [33] resulted in isotopically heavier values (less negative) than the extractions with fluidized bed pyrolisis (by 2 to 3 ‰) at 900°C. Advantages observed with the hydropyrolysis extraction were the high oil yields obtained from coals and petroleum source rocks, and a δ^{13}C shift similar to the initial coal isotopic composition. As well, relatively mild conditions were used for this extraction. In the case of the fluidized bed pyrolysis, the advantage observed was that the resulting δ^{13}C values resemble coal carbonization processes, even though the disadvantage was the high temperature required.

The investigations from Mazeas [34] regarding the evaluation of the isotopic composition of PAHs at marine sediments through a microwave extraction followed by a clean-up with a fluorisil column, showed the advantage of separating methylphenanthrenes from methyldibenzothiophenes. In this method the limitation was that it was not possible to measure the individual isotopic composition of the several methylphenanthrenes present, it only allowed to quantify the δ^{13}C of PAHs for source apportionment at a same location at different times.

Research in PAH source apportionment in sediments from Lake Erie done by Smirnov [29] did not refer to the effect that the Sohxlet extraction and clean-up with silica and alumina columns have on the isotopic composition of PAHs. It showed the advantage of this method on the separation of saturated and unsaturated aliphatics from PAHs and polar compounds. As well, investigations done by Stark [35] in the source apportionment of fire place soots and road sweeps using Sohxlet extraction and Sephadex-silica gel chromatography as clean-up method did not refer to the effect on the isotopic composition of PAHs that these purification methods have.

In the reviewed literature concerning purification methods for this thesis work (1994-2004) was not found the effect that the LSE and ASE extractions and flash chromatography with Silica Gel clean-up has on the isotopic composition of the isolated PAHs from soil samples.

Therefore, a beneficial contribution regarding the assessment of the influence of these extraction and purification methods in the $\delta^{13}C$, results very helpful in order to afterwards know the real isotopic composition of the PAHs at the surveyed site. Also, the chosen extraction techniques mentioned were decided due to the fact that ASE results in high extraction yields and LSE was required to compare the ASE extraction method.

5
Experimental Section

This section refers to the description of the chemicals employed, instrumentation and laboratory devices, the methodology and the soil description.

5.1 Reagents and Sorbents

Following chemicals were used: Methanol ($\geq$99.9%, Merck, Darmstadt), Cyclohexane ($\geq$99.9, Merck, Darmstadt), and Trichloromethane ($\geq$99.8%, Merck, Darmstadt), Acetone ($\geq$99.99%, Fisher Scientific), Millipore Water (ultra pure water, Milli-Q), Dichloromethane (99.8%, Acros Organics), Naphthalene (99%, Acros Organics), Acenaphtene (99%, Aldrich Chemicals Co.), Fluorene (98%, Aldrich Chemicals Co.), Phenanthrene (98-99%, Aldrich Chemicals Co.), Fluoranthene (98-99%, Aldrich Chemicals Co.) and Pyrene (99%, Aldrich Chemicals Co.).
Silica Gel 60 (0.063-0.2 mm ,Carl Roth) was used as sorbent for the flash chromatography.

5.2 Instrumentation and Laboratory Devices

GC-IRMS: The compound specific isotope ratios were determined using a Trace GC Ultra (Thermo Finnigan, Milan, Italy) coupled to an isotope ratio mass spectrometer (DELTA ^{PLUS} XP, Thermo Finnigan, Bremen, Germany) via a combustion interface (GC Combustion III, Thermo Finnigan, Bremen, Germany). The GC was equipped with a programmable temperature vaporizer PTV (Optic 3, ATAS GL International, Eindhoven, The Netherlands). Splitless and large volume injection (LVI) methods were used. The packed liner used for LVI was the liner (ATAS GL International, Eindhoven, The Netherlands) for the US EPA method 8270.
The GC was equipped with a CombiPAL autosampler (CTC Analytics, Switzerland). For the combustion of the compounds, a temperature of 940°C was used. For the settings used, see the Appendix in section 9 from A1 to A4.

GC-MS: Extracts in cyclohexane containing the PAH analytes were separated in a GC HP5890 Series II and detected in a HP5972 quadrupole mass spectrometer. Splitless injections of 1 μl from a 1 ml sample were done with an HP6890 autosampler and the chromatograms were analysed with the software on line data acquisition software packet G1034 Ver.C02.05 (Hewlett Packard).
An external calibration curve was done using different concentrations of the 16 EPA PAH analytes used for quantification (see Section 5.5.7 Table 8). An internal standard IS_{D200} containing 5 deuterated PAHs at a concentration of 200 μg/ml (see Section 5.5.7 Table 8) was used to correct the variations of the injection volume and fluctuations in the ionization efficiency of the mass spectrometer.

ASE: Extractions of the contaminated soil samples were carried out using 34 ml stainless steel extraction cells in an Accelerated Solvent Extractor 3000 (ASE DIONEX, Idstein, Germany) with a solvent controller. The software AutoASE^{TM}2.2 was used. Extraction parameters were the following: An extraction time of 30 min with a temperature of 100°C and pressure of 100 bar, using acetone as solvent, a vessel flushing of 60% and a purging with nitrogen during 100 s. Extracts were collected in 250 ml collection bottles with a teflon septum in the cap [36].

Clean-up System: The flash chromatography (FC) separations were performed with a system consisting of an adsorption chromatography column, a dropping funnel, and a 90° bend adapter linked to a rubber ball.

The adsorption chromatography column of 23 ml (DURAN glass, length: 300 mm, i.d.: 10 mm, joint NS 14/23 with a PTFE stopcock (Carl Roth) was connected in its upper joint to the dropping funnel of 100 ml consisting of 2 joints NS 14/23, glass stopcock and a venting system (Carl Roth), which as well was connected in its upper part to the 90° bend adapter with PTFE stopcock and joint NS 14/23 (Carl Roth) linked to the rubber ball. All joints were fastened with sealing rings 14/23(Carl Roth).
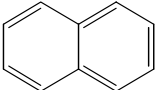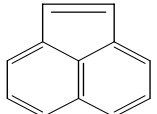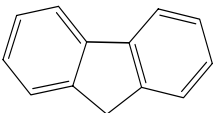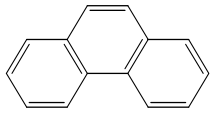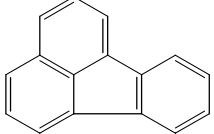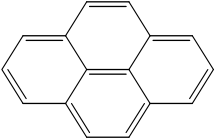
UV-Lamp: A UV-lamp (Konrad Benda) was used to visualize the PAH spots on the TLC plates, using a wavelength of 254 nm.

TLC: Thin Layer Chromatography precoated plates (ALUGRAM [R], Macherey-Nagel MN) were used. These are coated with Silica Gel 60 and have a size of 5 x 10 cm and a layer of 0.2 mm per sheet. TLC sheets were eluted in an elution chamber (DURAN, Carl Roth) with the size 60 x 50 x 100 mm. In each elution, a rounded paper filter with a diameter of 125 mm (Schleicher & Schuell) was required to saturate the chamber with the solvent vapor.

5.3 Standard Solutions

PAHs: The PAHs used were the following: Naphthalene, Acenaphtene, Fluorene, Phenanthrene, Fluoranthene and Pyrene. The properties of the 6 PAHs used as standards are shown in the following Table 3 [7].

Table 3. Properties of the 6 PAHs used as standards.

Compound Name	Structure	Molecular Formula	MW (g/mol)	ρ (g/cm^3)	T_m (°C)	T_b (°C)	-logC_{iw}^{sat} (s)	logK_{ow}
Naphthalene		$C_{10}H_8$	128.2	1.16	80.2	218	3.60	3.33
Acenaphthene		$C_{12}H_{10}$	154.2	1.05	96.2	278.0	4.61	4.20
Fluorene		$C_{13}H_{10}$	166.2	1.20	116.0	295.0	4.94	4.32
Phenanthrene		$C_{14}H_{10}$	178.2	0.98	101.0	339.0	5.2	4.57
Fluoranthene		$C_{16}H_{10}$	202.3	1.25	110.5	384.0	5.96	5.23
Pyrene		$C_{16}H_{10}$	202.3	1.27	156.0	403.0	6.16	5.13

Preparation: Individual PAH stock solutions of 3 mg/ml in cyclohexane were prepared. A nominal mass of 75 mg of the PAH was diluted to the mark into a 25 ml volumetric flask. 5 ml of each PAH stock solution was pipetted into a 100 ml volumetric flask and diluted to a nominal concentration of 150 ppm$_v$. This mixed PAH standard solution was stored at 4°C and was prepared monthly. Then, working solutions were filled in 1.5 ml vials before use.

A second standard solution in cyclohexane was subsequently prepared in order to have a nominal concentration of 1.5 ppm$_v$. One milliliter of the initial stock solution (150 ppm$_v$) was taken with a 1 ml syringe (Hamilton) and diluted with cyclohexane in a 100 ml volumetric flask. Storage of this PAHs mixed solution (1.5 ppm$_v$) was also at 4°C and was prepared monthly. A PAH standard solution in methanol with a nominal concentration of 150 ppm$_v$ was prepared for the spiking of soil samples, following the same procedure as mentioned before.

5.4 Soil Information and Treatment

5.4.1 Soil Description

Four collected soil samples from the former oil-manufacturing site located in the City of Stuttgart were used for this study. From fingerprinting analyses done in 1998, it was determined that from the four soil samples mentioned, the chosen samples were the 3 most polluted at the site: B11 followed by

B16 and then B29. Also B17 seemed to be the least contaminated soil sample of the polluted site [11] and therefore it was selected as a blank matrix. The sampled boreholes at the former oil-manufacturing site are shown in Figure 5.

Figure 5. The former oil-manufacturing site with the sampled boreholes. [1]

The samples analyzed in this research were the ones belonging to the boreholes B11, B16, B29 and B17. The soils were sampled at the field on the 2nd of February, 2002. The soils were stored in aluminium buckets. In Table 4, a description of the 4 sampled soils is given.

Table 4. Description of the 4 soil samples used for the extraction and clean-up method treatment.

Soil Sample	Description
B11	Light brown, mainly composed of clay with few silt. Some gravel is present. Strong odour.
B16	Dark brown, composed of clay with some silt. No silt and gravels present. Light odour. Homogeneous soil.
B29	Dark brown, mainly clay with some gravels and coal, few sand and silt. Light odour.
B17	Brown, mainly clay containing gravel, silt and sand. No odour.

5.4.2 Soil Pre-treatment to Extraction

Each soil sample had to be pre-treated before the extraction with the goal of having a representative sampling. The soil kept in its correspondent aluminium container was mixed in order to have a homogeneous sample. According to Fisher, a PAH contaminated soil with PAHs that was grinded into small particles resulted in better extraction recoveries [31]. Several portions of the homogenised soil were taken from different locations within the container in order to have a representative sample for the analysis.

5.4.3 Soil Spiking

After consulting a previous fingerprinting analysis done in 1998, it was found out from the bibliography [11], that B17 was the least contaminated soil. Subsequent determination of the PAH concentration in the 4 soils confirmed this previous investigation. The least contaminated soil sample B17 was used as matrix blank.

Spiking refers to the addition of an analyte of known concentration into a matrix blank for the estimation of the recovery [37]. In this thesis, the spiking was done in order to determine the PAH recovery after extraction from the soil. First, the sample was pre-treated as previously mentioned (see Section 5.4.2).The PAHs standard solution in methanol 150 ppm$_v$ was used for spiking.

5.4.4 Soil Spiking Previous to LSE

Soil spiking before the liquid-solid extraction was done using a nominal soil mass of 10 g and contained in a 15 ml glass vial with PTFE sealed screw top cap. 3.5 ml of the PAHs standard solution in methanol (150 ppm$_v$) were homogeneously spiked throughout the soil sample with a volumetric pipette. The pippetting was done quickly in order to minimize loss of volatiles. Additional 0.5 ml of pure methanol was added for a better stirring.

The soil was agitated for 24 hours in a shaker (IKA^R KS260basic) at a speed of 300 min^{-1}. When the mixing time concluded, the solvent was evaporated for 24 hours [38]. This step enhances the sorption of the PAHs to the soil matrix, with exception of naphthalene [7]. A significant loss of naphthalene was expected to occur, due to the high volatility that characterizes this compound. The vial with the spiked sample was closed with the cap and was ready to proceed with the ordinary liquid-solid extraction described in Section 5.5.1.

5.4.5 Non-spiked Soil Previous to LSE

The non-spiked soil was treated in the same way as mentioned before, except for the addition of the PAH standard solution. Instead of the addition of 3.5 ml of the PAH standard solution with 0.5 ml of pure MeOH, 4 ml of pure methanol were added. This step was included and applied in all the soils

samples with the aim of making a consistent and comparable method with the spiked one. Therefore, the same loss of volatiles in the extracts of each sample was expected. Samples receiving this treatment were B11, B16, B29 and B17.

5.4.6 Soil Spiking Previous to ASE

55 g of the soil B17 were accurately weighed in a 125 ml flask with a PTFE sealed screw top cap. Following, 19.25 ml of the PAHs standard solution were homogeneously spiked and 2.75 ml of pure methanol were added for a better stirring. The soil was stirred for 24 h at a speed of 300 min^{-1}, followed by 24 h evaporation time. At the end of the evaporation time, the flask was closed and ready to proceed with the packing of the stainless steel extraction cell for ASE [36].

5.5 Methods

5.5.1 Liquid-Solid Extraction

The purpose of the LSE was to extract the PAHs from the soil. Due to the good solubility of PAHs in methanol, this was used as solvent for the extraction. After the treatment previous to LSE (see Section 5.4.2), the vials containing the samples B11, B29, B16, B17 and the spiked B17 were treated with the same procedure:

8 ml of methanol were added in each vial containing a nominal weight of 10 g of soil and the samples were homogenised by mechanical stirring at room temperature for 24 h. Settlement by gravity of the soil was allowed for another 24 h. Afterwards, the extracts containing the PAHs were transferred with a 1 ml glass syringe (Hamilton) to a clean PTFE sealed vial. Vials containing the extracts were closed and cooled for storage at 4°C. This treatment was done for each sample. The following Table 5 shows the total volume of extract obtained from each soil sample. For a further LLE extraction, only 0.8 ml from each of these extracts was used.

Table 5. Weight of the samples and volume of the extracts in methanol recovered from the LSE method.

Soil Sample	Weight (g)	Volume of the Methanol Extracts (ml)
B11	10.01	4.95
B16	10.02	3.9
B29	9.98	6.17
B17	10.01	4.6
spiked B17	10.0	4

5.5.2 Accelerated Solvent Extraction

ASE is a technique that is based on the use of solvent or solvent mixtures to extract organic compounds at elevated temperature and pressure from a solid or semisolid matrix. This technique

offers the advantage of considerably shorter extraction times, increased extraction efficiency and lower solvent consumption than other extraction techniques by using high temperatures (from 25°C up to 200°C) and high pressures (up to 150 bar). The elevated pressures maintain the solvent, like acetone or toluene, below its boiling point [36], [39]. The Figure 6 shows an ASE (DIONEX300) device on the left side.
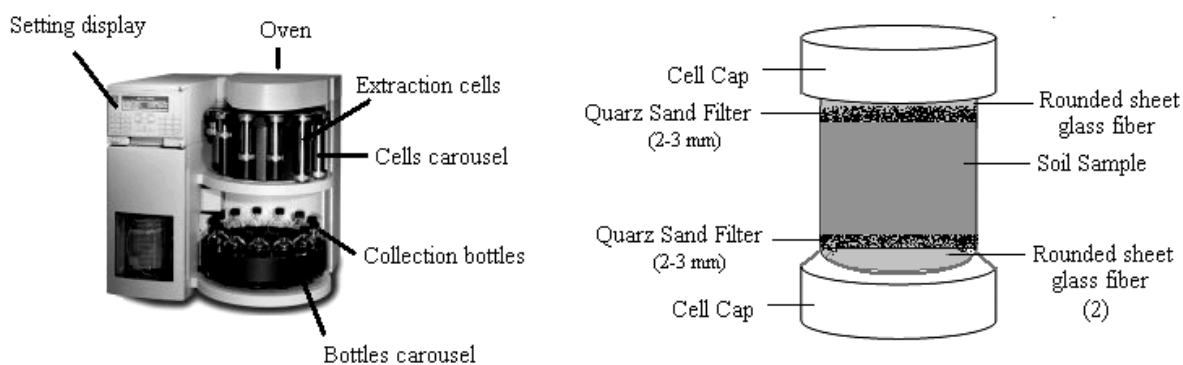
Figure 6. ASE300 DIONEX (left) and the packed extraction cell (right).

According to Hubert [40] ASE extraction efficiency of PAHs greatly depends on the composition of the matrix, the solvent used and the temperature selected. This technique has shown to be an effective extraction method for PAH contaminated sample material, in which similar or higher recoveries as in sonic bath and shaking methods are obtained from clay samples [31]. As we can see from a qualitative analysis of the soils (see Table 4), the samples from the former oil-manufacturing site have a high clay content. Therefore it was interesting to see if the extraction recoveries obtained agreed with previously reported results for these samples.

The contaminated soil sample already pre-treated to extraction (see Section 5.4.2) was contained in a 34 ml stainless steel extraction cell. Slight pressure from a rod helped to pack the soil in the extraction cell. In the case of the spiked sample, a homogeneous mixture of 91% spiked soil and 9% diatomaceous earth was used to fill in the extraction vessel. When PAHs have to be extracted from less solid soil samples (as in the case on spiked B17), it is recommended to use a mixture of diatomaceous earth with the soil matrix (3:97) in order to give a more solid consistency. Otherwise, solids could be contained in the extracts [36].

The ASE extraction cell consists of a column with two cell caps. Each cap has a frit in the base. A sealing band under high pressure holds the cap. In order to contain the soil in the column, one cell cap and the column are closed. Then, two rounded sheets glass fibre (diameter = 10 μm, Schechter & Shuell) are positioned at the base of the extraction cell. The coating retains particles that could infiltrate into the collection bottles.

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A quarzsand (grain size 1 to 2.5 mm) layer of 2 to 3 mm is used as filter. This layer contains the soil sample, which is filled up to 3 mm below the top of the column. 2 mm of the sand filter overlays the soil sample and one glass fibre sheet is positioned above the sand. Finally, the upper cell cap closes the extraction cell to finger tightness and is placed into the cell carrousel of the ASE 300 system. For a picture of the ASE instrumentation and the packed extraction cell, see Figure 6. Each soil was extracted with acetone twice. Therefore two extraction flasks were needed per sample. The glass collection bottles have a cap with an internal teflon septum.

The flasks were then placed in the bottle carrousel. After setting the extraction general conditions (see Section 5.2), a rinsing of the system was done automatically, flushing the solvent into a rinse bottle. The oven was preheated to 100°C. When this temperature was reached, the extraction cell in the carousel was brought into the oven. The pump filled the cell totally with acetone until a pressure of 100 bar was reached. Followed by a heating during 5 min, a static extraction cycle (where the pressure and temperature remain constant) during 10 min took place. Afterwards, a flushing phase continued, where 60% of the solvent with the extracted analytes contained in the cell were flushed into one collecting bottle. Then, a second static phase contained the remaining solvents in the cell. Finally, purging of the remaining solvent from the cell into the collection bottle and cleaning of the system with pressurized nitrogen for 60 s was done. After this step, the first extraction cycle ended and a second cycle identical as the one before described was ready to begin [36]. The extracts from each cycle were separately collected, corresponding each cycle to one flask with extract. In Table 6 the soil weight and total extract volumes obtained for each sample in ASE are presented. From these total acetone extracts, only 0.8 ml of each sample were used for the LLE extraction which is following described.

Table 6. Soil weight and total extract volumes for each sample with ASE.

Soil Sample	Soil Weight (g)	Volume of the combined acetone extracts from the 2 cycles (ml)
B11	56.20	59
B16	55.66	43.6
B29	47.98	60.2
B17	50.06	66.9
Spiked B17	32.3 (29.3 g of soil and 3 g of diatomaceous earth)	88.5

5.5.3. Liquid-Liquid Extraction

This extraction method was applied in order to separate the non-polar compounds from the polar compounds contained in the soil extracts. The PAHs partition in an organic phase composed of cyclohexane (upper phase) and the other polar compounds partition in the aqueous phase (lower phase) consisting of acetone or methanol and water. LLE was the second extraction method applied

after the LSE and ASE extractions. The LLE method consisted initially of a solution containing 0.8 ml of the aqueous phase from the LSE or ASE extracts containing the analytes diluted in 50 ml millipore water. The solution was contained in a 125 ml Müller-Krempel-bottle with a metallic cap with teflon lid.

Determination of the PAH soil concentration with GC-MS required the addition of 50 µl of deuterated internal standard 200 (IS_{D200}). In order to obtain high accuracy of the isotopic composition analyses for GC-IRMS, it was necessary to correct the measurements by external standards that contained the target analytes with known isotopic signatures because the fractionation effect is specific for each analyte [17]. The solution was stirred for 5 minutes with a speed of 300 min^{-1} and afterwards 15 ml of cyclohexane were added, followed by 1 h agitation with the same speed. Then, the flask was positioned under the fume chamber for 1 h to allow a separation of the two phases. In the aqueous phase (lower phase) no PAHs were expected to be present because the organic phase (upper phase) contained these target compounds. The cyclohexane extracts were collected separately in a 15 ml teflon sealed vial. Quantification of the PAH concentration in the soil through GC-MS was done using 1 ml of a solution containing 25% of the extract in cyclohexane with IS_{D200} and 75% of pure cyclohexane. For the GC-IRMS measurements, 1.5 ml of the extract in cyclohexane was used.

The following Figure 7. shows the general steps followed for the extraction and treatment of the PAHs from the sampled soils.
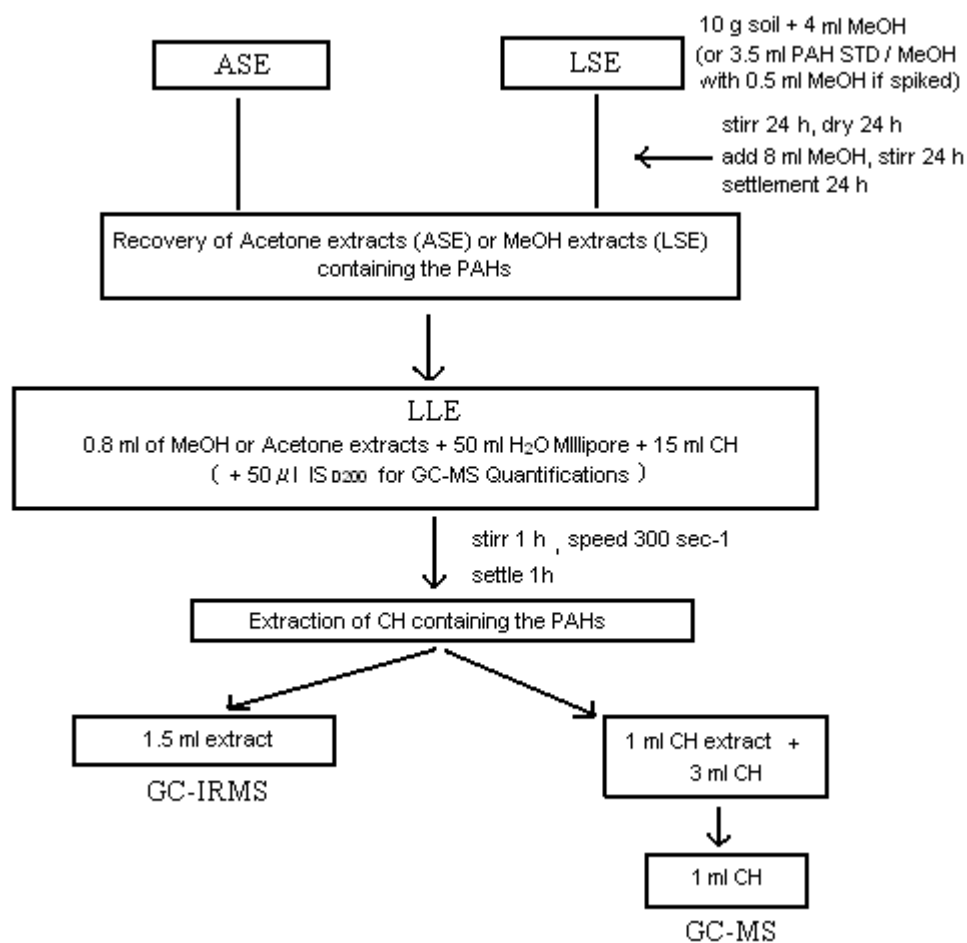
Figure 7. General Scheme: Extraction steps for the PAHs from the soil samples.

5.5.4. Thin Layer Chromatography

TLC is a tool that helped to isolate the six target PAHs from other compounds present in the LLE extracts. The difference in molecular structure that the organic compounds have result in a different interaction of them with a stationary and a mobile phase [41]. After the determination of the elution ratio that best isolated the target compounds from other contaminants in TLC, the LLE extracts were eluted in FC.

In this study, two elution methods were determined through TLC. One method was developed for the LSE/LLE extracts using cyclohexane (CH) and dichloromethane (DCM) as mobile phase. As well, an additional second method was developed for the ASE/LLE extracts using cyclohexane (CH) and trichloromethane (TCM) as mobile phase. This second mobile phase resulted from testing more polar mobile phases composed of different solvents.

The TLC plates are coated with Silica Gel 60, which is the same stationary phase used as in flash chromatography. Individual TLC plates used are 10 cm long and 1.5 cm wide. Two straight lines parallel to the plate width were drawn about 1.5 cm from the ends of the plate. One line corresponds to the application line and the other line to the solvent front. For each method, the spotting of the extracts and the reference PAH standard solution was done in each plate. After elution and drying of the plate, visualization of the spots on the TLC plate was done with UV light with a wavelength of 254 nm. Outlining of the revealed spots was done with a pencil.

Determination of the best separating elution ratio used as mobile phase was done in order to achieve the isolation of PAHs from other unknown extracted compounds for the LSE/LLE extracts [42]. Several preliminary elution ratios were applied. Extracts from B11 were used for this example (see Figure 7). Elution tests were performed using 10 ml of mobile phase consisting in each test on different solvent proportions: a first test, using CH:DCM 70:30 (v/v) with a R_f of the PAHs of 0.52, a second test using CH:DCM 80:20 (v/v) with a R_f of the PAHs of 0.45, a third test using CH:DCM 90:10 (v/v) with a R_f of the PAHs of 0.35 and a fourth test using CH:DCM 100:0 (v/v) with a R_f of the PAH of 0.17.
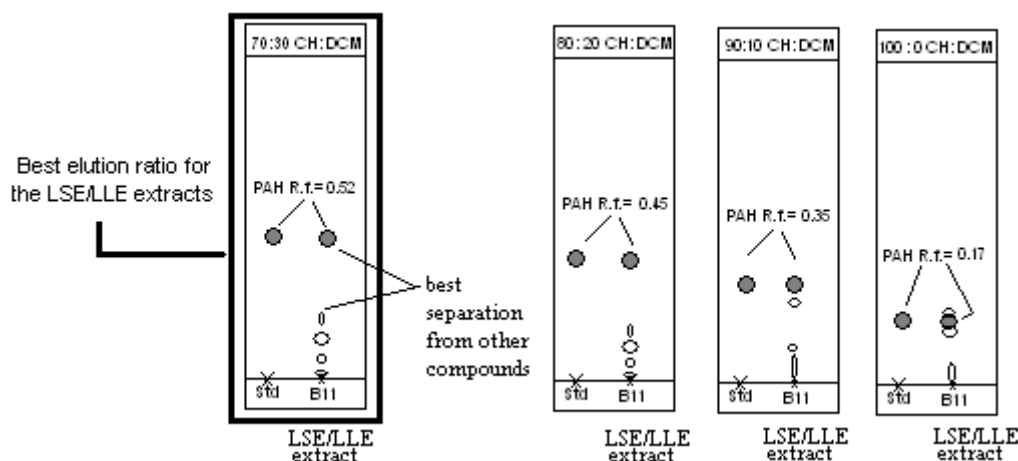
Figure 8. Elution tests for the LSE/LLE extracts from B11.

An elution ratio CH:DCM 70:30 (v/v) showed the best isolation of the target compounds, because the separation of other unknown contaminants (with an R_f smaller than 0.52) from the PAHs was very well achieved. Another aim of TLC was to separate selectively the polar contaminants in order to characterize these compounds in a full scan in GC-MS if possible. The PAH spots in the reference and in the LSE/LLE had a retention factor R_f of 0.52.

The second proposed purification method was applied for the ASE/LLE extracts. Several elution tests were done with 10 ml of a mobile phase with different elution ratios. The mobile phases used were CH:TCM 80:20 (v/v) with a R_f of the PAHs of 0.41, CH:TCM 90:10 (v/v) with a R_f of the PAHs of 0.38 and CH:TCM 95:5 (v/v) with a R_f of the PAHs of 0.33. The compounds separation for each case is here presented (see Figure 9.)
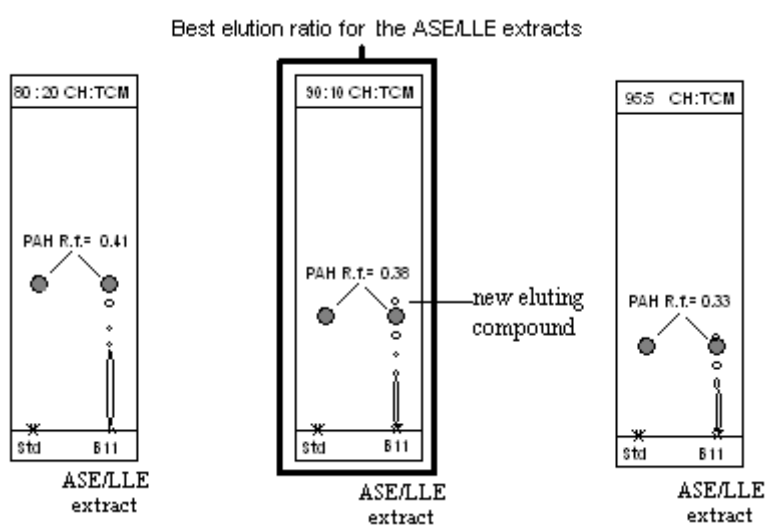
Figure 9. Elution tests for the ASE/LLE extracts from B11 .

The elution ratio CH:TCM 90:10 (v/v) was the mobile phase that best isolated the target compounds, because the separation of the PAHs from other two unknown compounds above and below the PAH spot (indicated in Figure 10. as a and c) was achieved (which had the same R_f as the PAH spot in the elution with CH:DCM 70:30 v/v). The R_f of the PAHs was 0.38. Even though these two unknown compounds are slightly separated from the PAH spot, it was intended to separate only the PAHs through the application of an elution sequence explained in section 5.5.5.in Table 7.
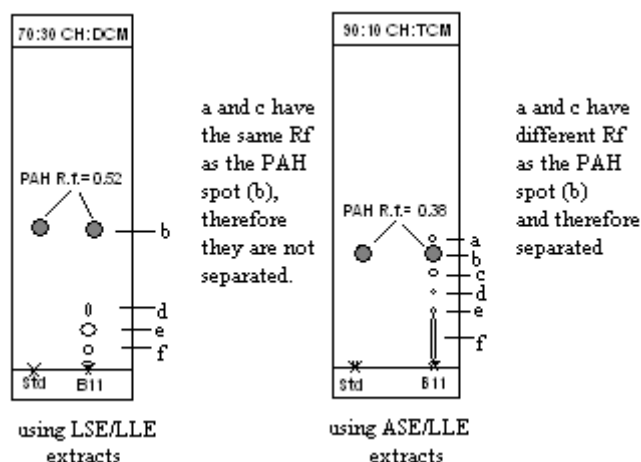
Figure 10. Comparison of the compounds separation for the LSE/LLE and ASE/LLE extracts
after the applied elutions.
The mobile phase applied to elute the ASE/LLE extracts (CH:TCM 90:10 v/v) showed the best separation of compounds, because the unknown compounds a and c are isolated from the PAH spot, meanwhile for the LSE/LLE extracts (CH:DCM 70:30 v/v) the unknown compounds a and c have the same R_f as the PAH spot.

Several oxidating agents were used in order to reveal the compounds present in the extracts. These were sprayed to the eluted and dried TLC plates. Examples of the employed reagents were:

- A solution of 0.5% $KMnO_4$ in NaOH 1N (used to reveal compounds containing unsaturated bonds).
- A solution containing 30% H_2SO_4 followed by 15 min heating at 150°C (special for revealing alkanes, alkenes and hydrocarbons).
- A solution containing 60% H_2SO_4 followed by 15 min heating at 150°C.
- Iodine sublimation in an elution chamber during 3 hours (universal reagent) [41].

None of these oxidating agents revealed the separated compounds in the TLC plates. A possible reason could be the low concentration of the compounds at the eluted plates, which was not sufficient for giving a color after the oxidation.

5.5.5. Clean-up with Flash Chromatography

The selective separation of the six PAH target compounds from a complex mixture was done by a clean-up method with flash chromatography using non-polar solvents. Basically, this separation consisted of the pre-packing of the column, the elution of the PAHs and the collection of fractions followed by the evaporation under N_2 at a temperature of 40°C [43]. The presence of the PAHs in different fractions was tested by TLC. A wet packing method was applied for filling the column placed vertically. The column was filled with a 2 cm sand layer followed by the loading of an 18 cm layer of Silica Gel 60 in cyclohexane. Settlement and packing of the Silica Gel solid support in the column was supported by pressure from a rubber ball. Special care avoiding the dryness of the

stationary phase, the appearance of bubbles and heterogeneousness in the solid support was always procured.

After packing homogeneously the stationary phase, a 2 cm sand layer was placed on top. 3 ml of the sample to be separated was directly given on the upper sand layer. Afterwards, the dropping funnel connected to the rubber ball was quickly put to the column. The extract was incorporated into the solid support by gravity. Then the rest of the column was filled with the mobile phase that with air pressure enables the sample to run through the column and become separated. The lower stopcock of the column controlled the flow and volume of the fractions.

Different elution sequences were applied to the LSE/LLE and ASE/LLE extracts, in order to find the elution serie that separated the PAHs more selectively. For all elution sequences, eluted fractions of 13 ml +/- 0.5 ml were sequentially collected in 23 ml glass test tubes. In total, 24 test tubes were required. The *labelling of tubes* was, for example, 1, 1b, 2, 2b and so on until 12b. The separation took less than 30 minutes. The following table shows the elution sequences applied to the extracts and the combined fractions where the PAHs were mainly contained. As well, it is indicated which ones were the best elution sequences in LSE/LLE/FC and ASE/LLE/FC in order to optimize the clean-up method.

Table 7. Flash chromatography preliminary elution sequences for LSE/LLE and ASE/LLE extracts.

Soil extracts LSE/LLE	Combined fractions with PAHs	Elution Sequence in LSE/LLE	Soil extracts ASE/LLE	Combined fractions with PAHs	Elution Sequence in ASE/LLE
B11 B17	2b to 4 or 2b,3,3b,4.	CH 100:0 50 ml CH:DCM 90:10 50 ml CH:DCM 80:20 50 ml CH:DCM 70:30 150 ml **Best elution sequence**	B29 B17 spiked	2b and 3-4 or 2b and 3,3b,4. 3-4 or 3,3b,4.	CH 100:0 20 ml CH:TCM 95:5 50 ml CH:TCM 90:10 200 ml DCM 100: 0 20ml **Best elution sequence**
B16 B17 spiked	1b-3 or 1b,2,2b,3.	CH 100:0 20 ml CH:DCM 90:10 30 ml CH:DCM 80:20 30 ml CH:DCM 70:30 150 ml	B11	3b, 4.	CH 100:0 50 ml CH:TCM 90:10 50 ml CH:TCM 80:20 50 ml CH:TCM 70:30 150ml
B29	2-4 or 2,2b,3,3b,4.	CH 100:0 30 ml CH:DCM 90:10 30 ml CH:DCM 80:20 50 ml CH:DCM 70:30 150 ml	B17 B16	3-4 or 3,3b,4. 2b-6 or 2b,3,3b,4,4b, 5,5b,6.	CH 100:0 20 ml CH:TCM 90:10 200 ml DCM 100: 0 50ml

Subsequently, the individual eluted fractions were transferred to 15 ml vials where the solvent of the eluent was evaporated under N_2 on a sand bed at 40°C up to a volume of 1 ml for the most contaminated samples (B11, B16, B29 and spiked B17) and up to 0.5 ml or less of the solvent for B17.

Composition analyses of the fractions after the FC clean-up were done by TLC. The extract volume employed for the spotting in TLC was not the same for all fractions. Application of the extracts to the TLC plates was done with a piece of a capillary column. The application was done several times until a spot was observed with UV light at 254 nm. Fractions derived from the LSE/LLE extracts were eluted with 10 ml of a mobile phase CH:DCM 70:30 (v/v), while the fractions derived from the ASE/LSE extracts were eluted with 10 ml of a mobile phase CH:TCM 90:10 (v/v). Afterwards, the PAHs visualization was only done with UV λ=254nm, and then the individual fractions were combined. This selective mixing was based on collecting the fractions with the separated components with the same R_f. Then, 5 ml of the selectively combined fractions were prepared on 15 ml glass vials. In the case that less than 5 ml resulted after the combination of fractions, additional cyclohexane was added until having 5 ml of extract in order to measure the extracts from a same volume in GC-IRMS.

Some of the reasons to decide the best elution sequence for LSE/LLE extracts (indicated in Table 7/ left elution sequence) were:

- An initial elution with 50 ml of pure CH allowed to first separate non polar compounds, like alkanes and alkenes from the PAHs. Due to the reason that no oxidating agents revealed the compounds separated in the TLC plates and also that these more non polar compounds were not observed in UV light, the 50 ml of CH were applied in order to make sure their initial elution. In this way it was pretended to eliminate them as interfering compounds in the CSIA chromatograms.
- The PAHs were mainly eluted in the 50 ml of CH:DCM 90:10 (v/v).
- The following elution with 50 ml of CH:DCM 80:20 (v/v) allowed a sufficient separation of the PAHs from other more polar compounds.
- The elution with 150 ml CH:DCM 70:30 (v/v) was used in order to selectively separate the unknown polar compounds in case their analyses were necessary (see Figure 10 / left TLC plate)

Some of the reasons to decide the best elution sequence for ASE/LLE extracts (indicated in Table 7/ right elution sequence) were:

- An initial elution with 20 ml of pure CH intended to separate alkanes and alkenes from the PAHs not possible to observe with oxidating agents in TLC.
- The 50 ml of CH:TCM 95:5 (v/v) allowed to elute remaining alkanes, alkenes and the compound a (see Figure 10 /right TLC plate) in fractions 2 and 2b after a FC. A very small amount of PAHs were contained in fraction 2b. The last 18 ml of this mobile phase started to elute PAHs.
- The first 21 ml from the CH:TCM 90:10 (v/v) mobile phase eluted mainly the PAHs and the remaining volume (182 ml) separates compounds c to f (see Figure 10 / right TLC plate).

- 20 ml of pure DCM was used for eluting all remaining compounds still adsorbed in the stationary phase.

The following Figure 11 shows a diagram of the steps followed in the clean-up with flash chromatography until the measurement of the isotopic composition in CSIA.
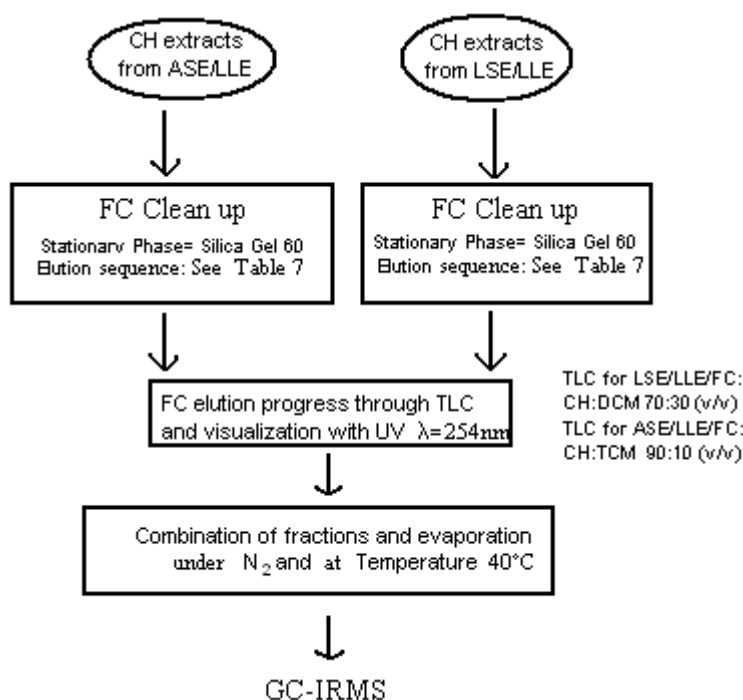
Figure 11. General Scheme: Clean-up procedures applied to the contaminated soil aliquots.

5.5.6 Determination of the Pure Analytes

An aliquot of the pure liquid and solid standards was introduced into the combustion chamber of an elemental analyzer (EA) (NC2500, Thermoquest, San Jose, CA) coupled to an IRMS (Delta XL, Thermo, Bremen). The isotopic signatures of the analytes were corrected in order to obtain $\delta^{13}C$ values relative to Vienna PeeDee Belemnite(VPDB). This correction was obtained using a linear regression derived from the $\delta^{13}C$ determination of three different solid reference materials (Acetanilid, Laaser Marmor, CaCO3 2059 and USGS 24) measured with the same instrumental setting and the same internal reference CO_2.

5.5.7 GC-MS

The extracts were analysed using a GC HP5890 Series II combined with a HP5972 mass spectrometer and a HP6890 auto sampler. A 30 m x 0.25 mm i.d. ZB-5 capillary column (Phenomenex) with a film thickness of 0.25 mm was used. The capillary column consisted of 5% phenyl and 95% dimethylpolysiloxane. The GC temperature program initiated with 65°C for 4 minutes and was ramped

with 10°C/min to 270°C. This temperature was then held for 10 minutes. Afterwards, the temperature was again ramped at 10°C/min to 310°C and held for 6.5 min. Vials containing 1 ml extracts in cyclohexane were analysed. The samples were injected in splitless mode at 250°C with an injection volume of 1 µl. The carrier gas was helium (purity 5.0) and was set at a constant flow of 0.7 ml/min. Following, the GC-MS transfer line was held at 315°C and the ion source at 175°C. For the measurements, SIM mode was used. In the following table the target compounds are shown, in addition to the standards and deuterated internal standards used:

Table 8. Target and qualifier ions, external standards and deuterated internal standards used
for the quantification with GC-MS

Target and Qualifier Ions (m/z)	**Nap-d8** (136), **Nap** (128, 64), **Any** (152, 76), **Ace-d10** (164), **Ace** (154, 76), **Fln** (166, 139), **Phen-d10** (188), **Phen** (178, 89), **Ant** (178, 89), **Fth** (202, 101), **Pyr** (202, 101), **BaA** (228, 114), **Chr-d12** (240), **Chr** (228, 114), **Bb/kF** (252, 126), **BaP** (252, 126), **Perylen-d12** (264), **Indeno** (276, 138), **DahA** (278, 139), **BghiP** (276, 138) .
External Standard	Calibrations for GC-MS were done using an external standard consisting of a solution of *16 EPA-PAH* compounds in cyclohexane with a concentration of 10 µg/ml (Dr. Ehrensdorfer): **Nap, Any, Ace, Fln, Phe, Ant, Fth, Pyr, Chr, BbF, BkF, BaA, DahA, Indeno, BghiP and Perylen.** The ratio external standard: deuterated standard was 10:4.
Deuterated Internal Standard IS$_{D200}$	This internal deuterated standard solution contains *5 deuterated PAHs*: **Nap-d8**, **Ace-d10**, **Phe-d10**, **Chr-d12**, **BghiP-d12** from a stock solution in dichloromethane with 4 mg/ml (Restek GmbH). A dilution in cyclohexane to 200 mg/ml is used for the PAH quantification in µg. The IS$_{D200}$ allows accurate quantifications because it corrects the loss of volatile analytes through considering the ratio PAH IS$_{D200}$ / PAH sample.

PAH Extraction Recovery Calculations

The determination of the percentage of PAHs recovered from the sampled soil B17 after the application of the two extraction methods involved three main calculations:

1. Calculation of the theoretical 100% PAH recovery contained in 0.8 ml of the extract after the extraction of the spiked blank soil (a).

For this first step, a certain volume of the PAH standard solution of nominal concentration of 150 ppm$_v$ (see Section 5.4.4 for ml used in LSE and 5.4.6 for ml used in ASE) was employed for spiking the blank soil B17. After the evaporation of the solvent for adsorption of the PAHs in the matrices and a subsequent addition of solvent for stirring and extraction (see Section 5.5.1 for the added ml in LSE),

a volume of solvent containing the PAHs was extracted (see Table 5 for LSE extracted ml and Table 6 for ASE extracted ml). From each extracted volume, 0.8 ml was used for the calculations.

2. Calculation of the real μg of PAH contained in 0.8 ml of the extracts from the spiked field sample B17. This value was directly evaluated in GC-MS (b).
3. Calculation of the real μg of PAH in 0.8 ml of the extracts from the non-spiked field sample B17. This value was directly evaluated in GC-MS (c).

The following expression was used to determine the recovery [37]:

$$\frac{\text{Real PAHs in μg in spiked B17 (b) - Real PAHs in μg in non-spiked B17 (c)}}{\text{Theoretical 100\% PAHs recovered in μg from spiked B17 (a)}} \times 100 = \text{PAH extraction recovery}$$

[Equation 2]

Results of the extraction recovery are presented in Figure 12.

PAH Clean-up Recovery Calculations

In order to evaluate the clean-up recovery of PAHs, three determinations were required:

1. The determination of the PAH concentration (μg) in 3 ml of the B11 ASE/LLE extract before clean-up. This was evaluated through GC-MS (a).

2. The determination of the PAH concentration (μg) in the 24 eluted fractions after the flash chromatography of the B11 ASE/LLE extracts (b).

3. The determination of the clean-up recovery for B11, which was calculated with the following expression:

$$\frac{\text{Total PAH concentration (μg) in the 24 eluted fractions after the FC (b)}}{\text{Total PAH concentration (μg) in the 3 ml of ASE/LLE extract (a)}} \times 100 = \text{PAH clean-up recovery}$$

[Equation 3]

Results for the recovery of the individual PAHs after clean-up are shown in Table 11.

5.5.8 GC-IRMS Program

The GC was equipped with a 30 m x 0.25 mm i.d. DB-5 capillary column (J&W Scientific, Agilent Technologies) with a film thickness of 0.25 μm. The capillary column consisted of 5% diphenyl 95% dimethylpolysiloxane. As carrier gas helium (purity 5.0) was used with a constant flow. Two different injection methods were used for the analyses: splitless injection and large volume injection (LVI). The injection volume for the splitless injection was 1 μl and the following oven temperature program was

used: 65°C held for 4 min, followed by a ramp to 300°C with 10°C / min with a hold time of 5.5 min. The injection volume for LVI was 50 µl and the following oven temperature program was used: 45°C held for 4 min, followed by a ramp to 300°C with 10°C / min that was held for 5.5 min.

Splitless injections did not result in signals of amplitude of mass 44 above the detection limits (200 to 700 mV). Therefore these results were not used.

The LVI main operation consists of introducing a higher amount of the extract by injecting 50 µl that is injected in a cold packed liner, which undergoes prior solvent evaporation resulting in more concentrated samples. The analytes remain in the packed liner that afterwards are thermodesorbed followed by a subsequent transfer of the sample to the GC column [44]. Description of the parameters employed in LVI can be found in the Appendix / Section 9 / A1 and A3. At this point it must be mentioned, that all measurements were done with this LVI method.

The parameters used in the GCII-III Interface conditions used for both injection methods are described in the Appendix / Section 9 /A1 and A2. The OPTIC 3 injector for splitless injection and LVI are described in the Appendix / Section 9 / A3. As well, the parameters used in the IRMS can be found in the Appendix / Section 9 / A4.

The standards used were 1.5 ml from the PAH standard solution in cyclohexane (nominal concentration of 1.5 ppm_v). For every eight measured real samples from the contaminated site, an external standard had to be evaluated. The requirement for the reproducibility conditions were based on triplicate measurements with <0.5‰ standard deviation. Measurements resulting above the minimum detection limit (200 mV) and exceeding 0.5 ‰ standard deviation were as well included in the isotopic composition graphs of each soil sampled. Differences between samples (error bars) were assigned to all the values obtained. These differences could be due to variations in the reproducibility, but also due to changes in the instrumental operation.

6
Results and Discussion

6.1 Determination of the PAH Concentration

After a LSE/LLE treatment, the concentration of the PAHs in the soil samples was determined. The determination was done for the 6 target PAHs and for the sixteen EPA PAHs [31] contained in the soil samples. These results which show that the most contaminated soil was B11, followed by B29 and B16 are indicated in Table 9. The sample with the lowest contamination was B17. For the methods used for the quantification, see Section 5.5.1 and 5.5.3.

Table 9. Concentration of the six target PAHs and of the sixteen EPA PAHs (may 2004).

Soil Sample (µg/kg)	Nap	Ace	Fln	Phen	Fth	Pyr	$\sum$6 target PAHs	$\sum$16 EPA PAHs
B17	16	14	0	15	25	54	124	219
spiked B17	19483	26691	28720	36236	34905	30367	176402	176508
B16	99	255	726	3607	12769	6254	23710	27622
B29	840	309	0	1818	6078	2006	11051	16647
B11	80569	168960	163664	498276	198700	87470	1197639	1333207

After comparison of the 6 target PAH concentration in soil provided on 2002 by the Amt für Umweltschutz, Landeshauptstadt Stuttgart [12] with respect to the ones determined for this thesis on may 2004, it was found that the concentration in each soil decreased after 2 years. Soil B17 showed a decrease in PAH concentration of 6 times, B16 showed a decreased concentration of 43 times, B29 decreased 67 times and B11 of 2.8 times less. The following Table 10. shows the comparison between the values obtained on 2002 with the ones obtained on 2004.

Table 10. Concentration of PAHs in soils sampled on 2002 and 2004.

Soil Sample (mg/kg)	$\sum$6 target PAHs On 2002 [12]	$\sum$6 target PAHs On 2004	Ratio $\dfrac{\text{PAH conc 2002}}{\text{PAH conc 2004}}$
B17	0.752	0.124	6
B16	1029	23.710	43.4
B29	746	11.051	67.5
B11	3350	1197.639	2.8

Possible explanations to this decrease in PAH concentration in 2004 for the sampled soils could be the different extraction method applied in each case and also possible processes (like microbiological or chemical effects) taking place in the soils in 2 years, even though they were kept under refrigeration.

6.2 Extraction Recovery in LSE/LLE and ASE/LLE Methods

Soil aliquots of sample B17 were used as a blank for the estimation of the extraction recovery by spiking the six PAH analytes applying the LSE/LLE as well as the ASE/LLE treatment. The obtained results showed that the recovery determined with ASE/LLE in all PAHs except naphthalene was higher than with LSE/LLE. The sample preparation for ASE and the filling of the extraction cell needed more time and this was probably the reason for the naphthalene losses. Figure 12. shows the recovery of the 6 target PAHs after the applied extraction methods. These results do not involve any flash chromatography treatment.

PAH	LSE/LLE %	ASE/LLE %
Nap	36.735	32.177
Ace	50.675	62.572
Fln	55.477	68.672
Phen	68.138	88.792
Fth	65.821	77.745
Pyr	58.32	72.002
TOTAL	55.861	66.993
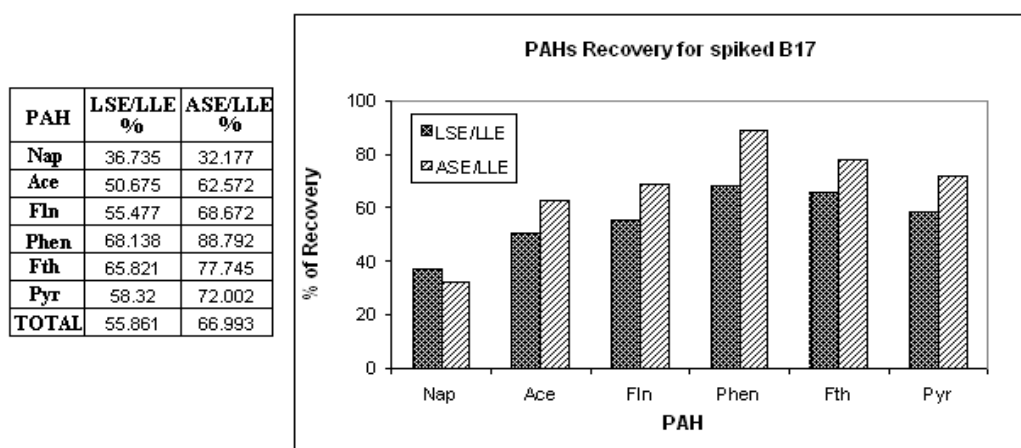
Figure 12. **PAH extraction recovery after a LSE/LLE and ASE/LLE in spiked B17.**
(These results show the PAH recovery after the extraction. No flash chromatography treatment was applied)

After comparing Fisher´s investigation [31] regarding PAH recoveries with ASE from contaminated soil samples with a high clay content to our results indicated in Figure 12, it was possible to conclude that our data agreed with the ones obtained by the mentioned author. Fisher´s PAH recovery were the same or higher than shaking extraction methods (comparable with LSE) for soil samples containing clays. Our results showed in all cases, with exception of naphthalene, that a higher recovery with ASE rather than with LSE can be obtained from this kind of soil samples.

6.3 Recovery of the PAHs after the Clean-up Step

PAH losses can easily occur before, during and after the flash chromatography purification. Processes that could decrease the concentration after the clean-up are: The volatilisation of the PAHs during their incorporation to the packed column before elution, non-eluted analytes that were still retained in the stationary phase, transfer of the extracts, solvent evaporation under N_2 at a temperature of 40°C and human errors. The individual recovery of the six analytes after the clean-up of the ASE/LLE extracts from B11 was determined including the 24 eluted fractions after the flash chromatography. Table 11 shows the obtained results and section 5.5.7 refers to the calculations [37] for these

determinations. These results refer to the recovery of PAHs after the clean-up with flash chromatography and not to the total method recovery (extraction/clean-up).

Table 11. Clean-up recovery of the individual PAHs.

PAH	Purification Recovery (%)
Nap	-
Ace	-
Fln	41.13
Phen	38.5
Fth	44.42
Pyr	43.46

6.4 TLC Results

The clean-up of the LSE/LLE extracts based on the elution sequence with CH:DCM (see Section 5.5.5 and Table 7 for a description of the elution sequence) showed that the PAHs were basically found in the fractions 2b-4 or 2b-3-3b-4. As shown in Figure 13a., the target analytes could be found in the fractions 2 to 4. The PAHs were present in very small amount in fraction 2 and mainly the target compounds were found in fractions 2b-4. This was concluded after qualitative observations in UV λ=254 nm. Unfortunately the comparison between fractions 2 and 2b to 4 through CSIA chromatograms was not possible, because fraction 2 was not measured in CSIA.The following Figure 13a. shows the purification for B11 with the elution sequence used for LSE/LLE. A clean-up for PAH separation that closely resembles the here applied mobile phase can be as well found in O´Malley investigations [28].
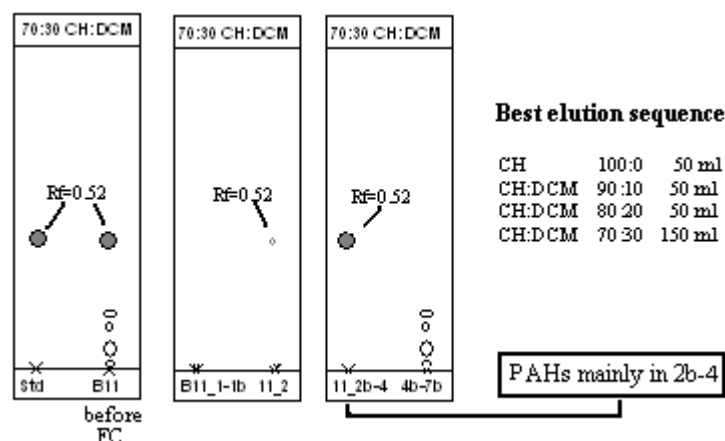
Figure13a. Determination of the PAHs in the fractions by TLC for B11 using LSE/LLE extracts.

After analyzing in CSIA the B11 fraction 2b-4 resulting from the clean-up of the LSE/LLE extract, it was observed in the chromatogram that this combined fraction containing the target PAHs was not selectively separated from several interfering compounds (41). Some reasons could be that the elution sequence is not the optimal. Unknown compounds have the same R_f as the PAHs and therefore they

elute in the same fraction. These provide a background signal and overlapping peaks on the target PAHs signals in the chromatogram. We have to remember that in general, the clean-up resulting from the LSE/LLE extracts present a higher concentration of PAHs than the ones from ASE/LLE extracts. This is because the LLE was done with 0.8 ml of approximately 50 ml of the acetone ASE extracts which are more diluted in comparison with the LSE/LLE treatment, where 0.8 ml of approximately 5 ml of methanol LSE extracts were used. This higher concentration of purified fractions from the LSE/LLE/FC can be as well an explanation to the additional interfering compounds with higher amplitude of mass 44. The CSIA chromatogram for this fraction is provided in the Appendix 9 / Section 5.1 and the data in Table 12.

Table 12. CSIA chromatograms information of B11 after clean-up of LSE/LLE extracts.

B11 combined fractions after clean-up (diluted 14 times)	Number of peaks in the CSIA chromatogram	Interfering compounds present in the chromatogram	Amplitude of mass 44 for the PAH target compounds [mV]
2b-4	46	41	Nap= 0 , Ace=1652 , Fln= 1569 Phen=4152 , Fth=1948 , Pyr= 942

Consecutively, a second optimized elution sequence was used for the ASE/LLE extracts (see Table 7), where the PAH target compounds were basically found in the fraction 3-4. In small amounts the PAHs were found in fraction 2b and in fractions 4b-5b. This was concluded after relating the observations in UV λ=254 nm and the comparison of CSIA chromatograms. The following Figure 13b.shows the purified fractions for spiked B17 ASE/LLE extract eluted with the optimized sequence in ASE/LLE.
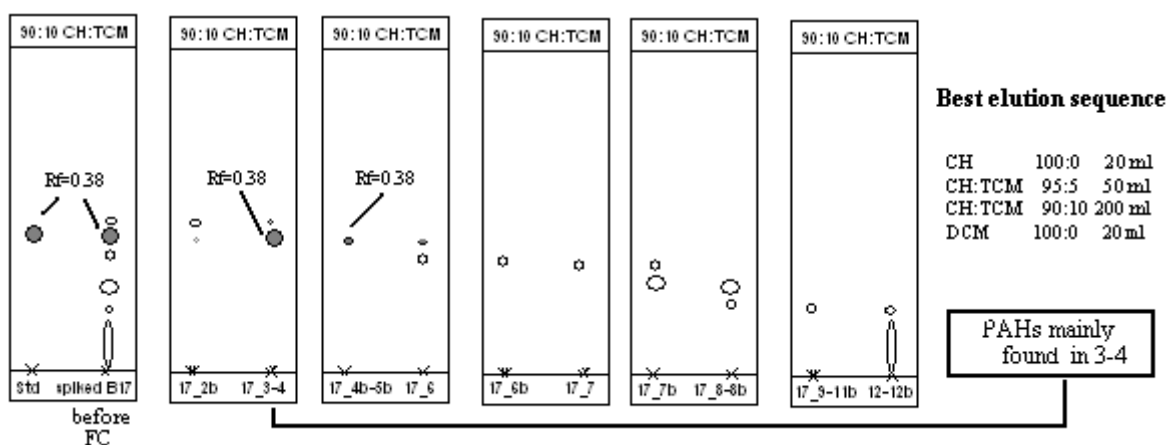

Figure 13b. Determination of the PAHs in the fractions by TLC for spiked B17 using ASE/LLE extracts.

The following Table 13 provides information of the spiked B17 ASE/LLE extract after the clean-up. A comparison in number of peaks and their amplitude of mass 44 between the fractions 2b, 3-4 and 4b-5b is provided and afterwards analyzed.

Table13. CSIA chromatograms information of the spiked B17 eluted fractions after clean-up of the ASE/LLE extracts.

Spiked B17 fractions after clean-up	Number of peaks in the CSIA chromatogram	Interfering compounds present in the chromatograms	Amplitude of mass 44 for the PAH target compounds [mV]
2b	25	19	Nap= **54** , Ace=**1372** , Fln= 140 Phen=507 , Fth=158 , Pyr= 398
3-4	20	15	Nap= 0 , Ace=774 , Fln= **1022** Phen=**1498** , Fth=**1304** , Pyr= **1327**
4b-5b	15	11	Nap= 0 , Ace=0 , Fln= 387 Phen=193 , Fth=709 , Pyr= 177

After comparing the amplitudes of mass 44 for each CSIA chromatogram, we observed that fraction 3-4 presented the highest PAH amplitudes with respect to the PAH amplitudes in the other analyzed fractions indicating the highest concentration of PAHs within these fractions. These amplitudes in fraction 3-4 are above the low amplitude range of 200 to 700 mV. The only exception was observed for acenaphthene and naphthalene, which were found in higher amplitude in fraction 2b. Also, the reduced number of peaks from other unknown compounds in fraction 3-4 and the qualitative observations in UV λ=254 nm allowed to conclude that the PAHs are mainly eluted in this fraction with presence of few interfering compounds (15). Thus, this elution sequence presented the best separation of the target PAHs.

The CSIA chromatograms for the eluted fractions of spiked B17 after the ASE/LLE/FC treatment are shown in the Appendix 9 / Section A5.2 corresponds to the CSIA chromatogram of fraction 2b, A5.3 corresponds to the fraction 3-4 and A5.4 to the CSIA chromatogram of 4b-5b.

6.5 Carbon Isotopic Composition Results

6.5.1 CSIA Measurements

The isotopic composition of the six target PAHs in the soil extracts were initially measured with GC-IRMS by splitless injections following a preliminary non-optimized extraction method using an LSE/LLE extraction. The isotopic compositions were not reported because of the erroneous values that could be determined for the individual analytes due to the fact that the presence of interfering compounds from the soil cause large peak uncertainties, as shown in the Figure 14.
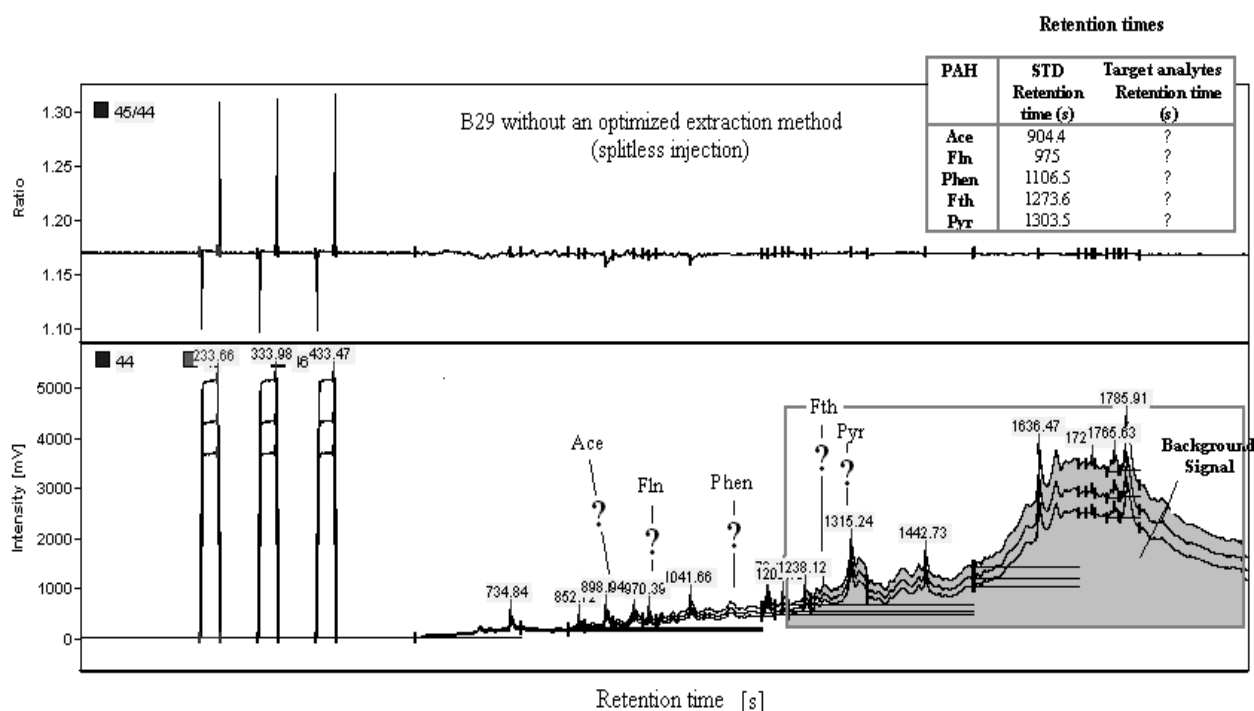
Retention times

PAH	STD Retention time (s)	Target analytes Retention time (s)
Ace	904.4	?
Fln	975	?
Phen	1106.5	?
Fth	1273.6	?
Pyr	1303.5	?

Figure 14. Chromatogram showing the separation of the analytes in soil B29 in a splitless injection following a non-optimized LSE/LLE method of extraction. None of the 5 target PAHs were clearly detected.

Further measurements were done to determine the carbon isotope composition of extracts from the optimized LSE/LLE method described in Section 5.5.1 and 5.5.3. A splitless injection was applied. Unfortunately, it was not possible to consider these results because all the resulting measurements were below the detection limit of 10 nmol/L for a GC-IRMS detection with splitless injection [16]. Therefore it was necessary to apply an alternative injection method, in which the $\delta^{13}C$ of the PAH analytes could be measured by GC-IRMS, being a possibility the application of a large volume injection [44].

6.5.2 Assessment of the Detection Limits for Large Volume Injections (LVI)

One of the main requirements to obtain reliable measurements of the PAH isotopic composition in the soil extracts was to obtain high quality results in the CSIA chromatograms at high and low detection limits of the analytes. These can be achieved through the elimination of the background signal, definition of a baseline after an underground separation as well as good peak resolution. Injections with LVI were done in order to achieve these characteristics.

For an estimation of the detection limit DL, the extract ASE/LLE of spiked B17 was used. The spiked nominal concentration of 150 ppm$_v$ corresponds to an average PAH amplitude of mass 44 of 4449.93 +/- 287.58 mV (without considering naphthalene due to its less amount in the packed liner during the solvent evaporation), which corresponds to a PAH concentration in soil of 247.23 mg/kg.

A plot of signal heights in LVI is given in the Figure 15. This graph was determined in order to know the relation between the amplitude of mass 44 of the analytes (proportional to the concentration of PAH) and their isotopic composition, as suggested by Schmitt [45]. The *detection limit* and a *low amplitude range* were determined for the target PAHs.
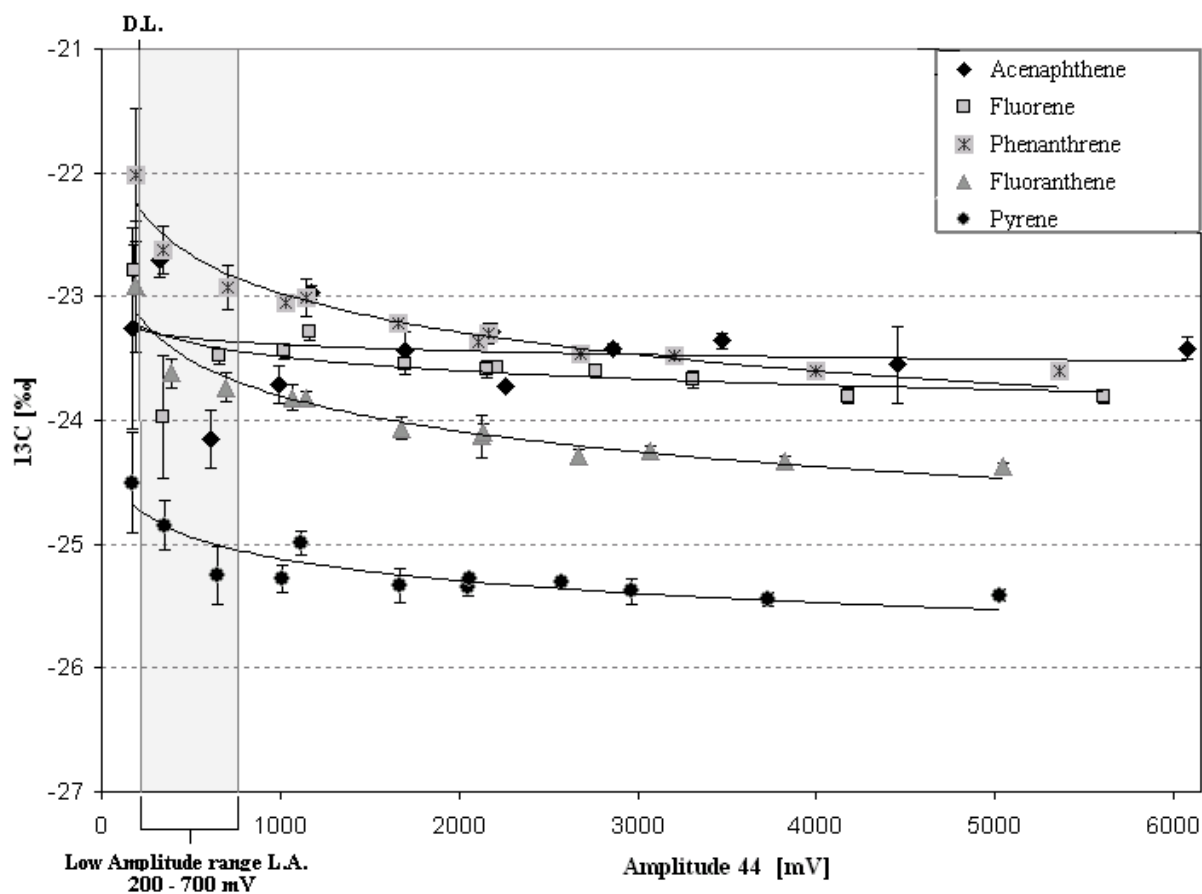
Figure 15. Signal height in LVI (50 μl injection)

Plot of the $\delta^{13}C$ vs. Amplitude of mass 44 for 50 μl injection of the PAH standard solution containing the 6 target PAHs.
Naphthalene measurements were not included because of the bad results obtained below the L.A. range.

The detection limit and the low amplitude range were assigned to the zone in the plot where the slope changes the most. This means that within and below the low amplitude range, high variations on the $\delta^{13}C$ of the PAHs are occurring, being the main reasons the small PAH concentrations sampled showing a higher standard deviation. Small variations on the isotopic composition of the PAHs and small standard deviations are expected in the region of the plot where the slope becomes constant (above the low amplitude range). Therefore, extracts with high PAH concentration will result in measurements that can be as reliable considered for the analyses.

The *DL* was assigned to an amplitude of mass 44 of 200 mV, which corresponds to a PAH concentration of the standard solution in soil of 11.1 mg/kg. All measurements below this amplitude were not considered, due to the high variation in the $\delta^{13}C$ values. A *low amplitude* range was assigned to the values fitting between 200 and 700 mV, corresponding to a concentration of 11.1 and 38.9

mg/kg respectively. Considered data fitting in this range are indicated in each isotopic composition graph as L.A.[2]

The overall trend observed in the plot for signal heights in LVI was that at if the amplitude of mass 44 increases (or analytes concentration increases), the $\delta^{13}C$ decreases until a constant isotopic composition is reached.

In order to show the importance of applying purification methods in the soil extracts, an example using Method 2 (ASE/LLE/FC) was selected. As previously shown, ASE is a highly effective extraction method with a disadvantage associated with it [31], [39]: the presence of additional impurities in usually high concentration that could even present a similar retention time to the target analytes, resulting in a highly disturbing background. The overlapping of peaks and a bad resolution in the following chromatogram corresponded to an ASE/LLE extract as shown in Figure 16. The peak resolution was only clearly achieved for fluorene and phenanthrene.
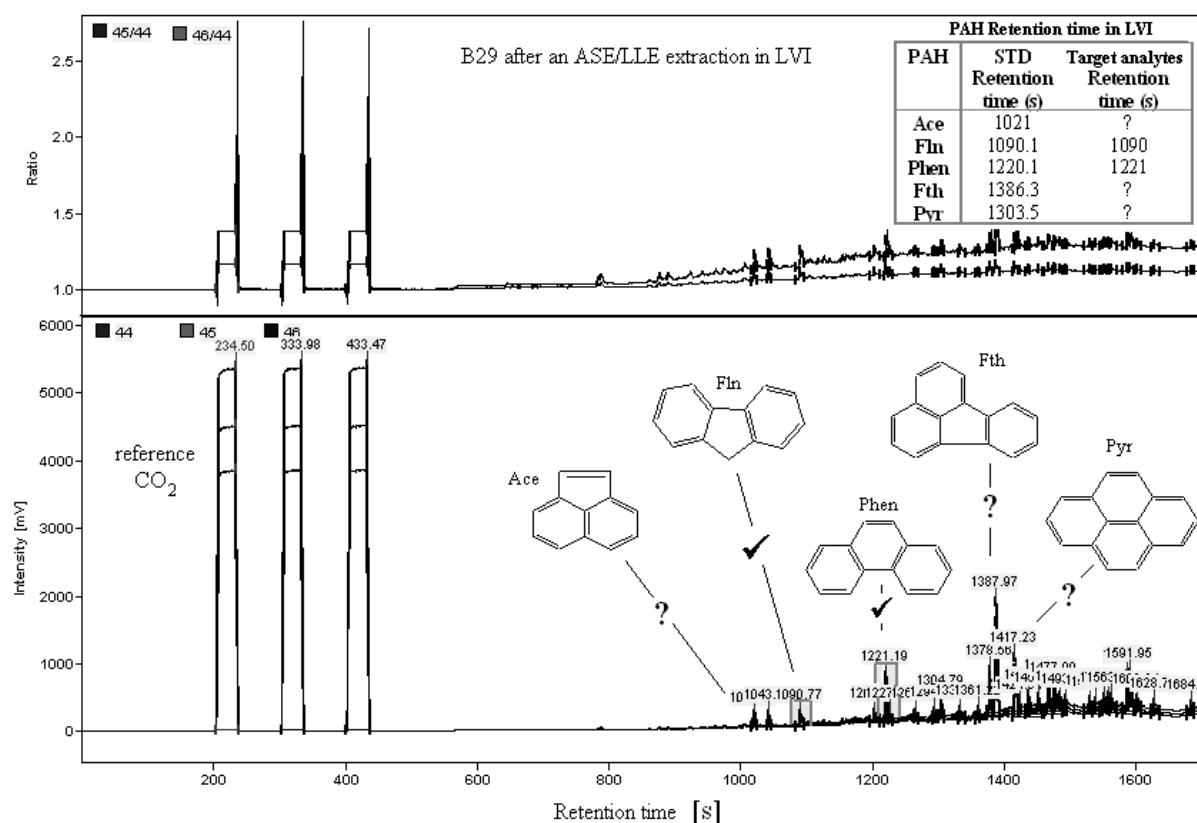

Figure 16. **The chromatogram shows a large volume injection of soil sample B29 after the ASE/LLE extraction.** Fluorene and Phenanthrene were the only compounds clearly identified in the CSIA chromatogram

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[2] L.A. is a range with a higher uncertainty for the δ-value.

After an ASE/LLE/FC treatment, it was possible to determine the peaks for 5 PAH analytes. The fraction shown is the fraction 3-4 after the clean-up, where mostly the target compounds are contained (see Figure 17).
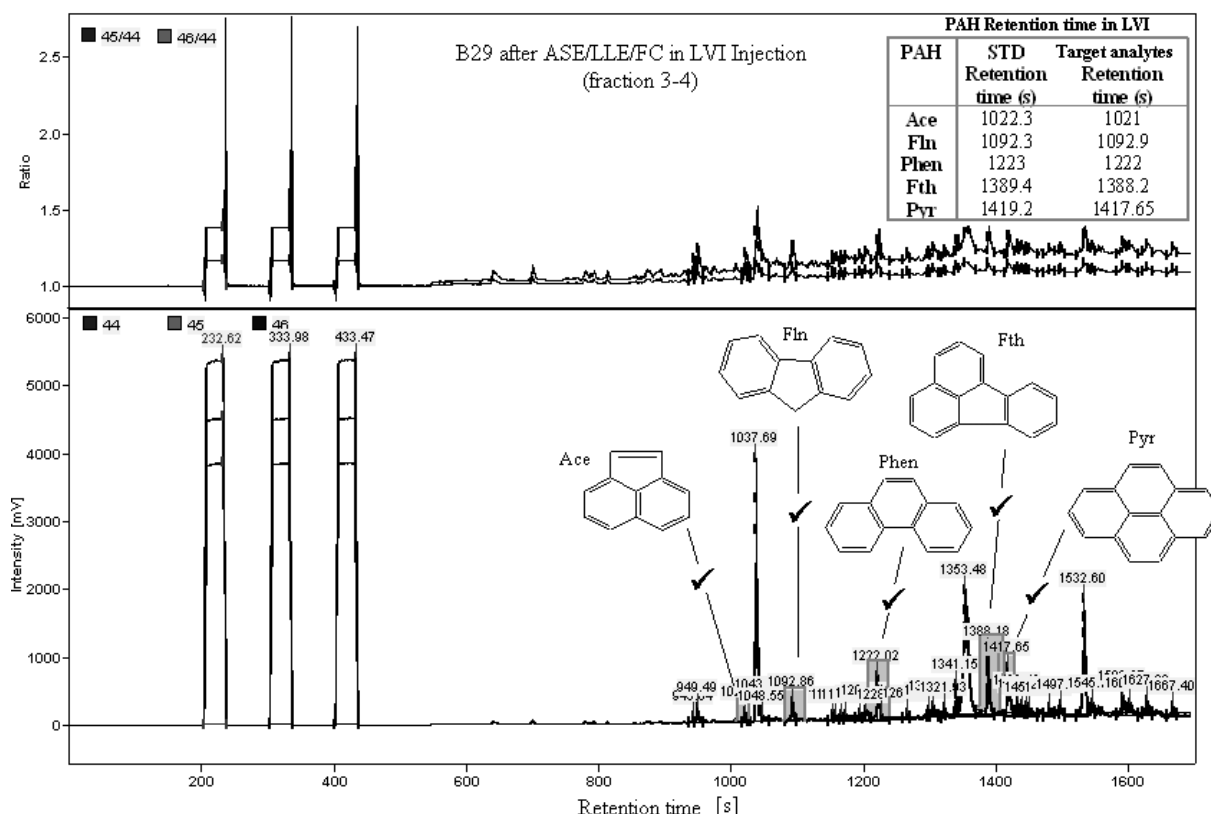
Figure 17. The chromatogram shows a large volume injection of soil sample B29 after ASE/LLE and a FC clean-up. The 5 target PAHs are clearly detected after the applied purification method.

6.5.3 Extraction and Clean-up Isotopic Analysis

The results of the isotopic fractionation are presented in the following graphs:

- Two-dimensional graphs, in which the $\delta^{13}C$ values are on the y-scale and the purification steps on the x-axis.
- Each graph shows two columns and for spiked B17 only two additional columns: In the 1st column (on the left), the $\delta^{13}C$ value of the pure liquids and solids from the elemental analysis of the PAH are shown, followed by a 2nd column corresponding to the value of the analysed standard PAH mix.
- The first column in the graphs of the soils B11, B16 and B29 shows the purification steps of method 1 (LSE/LLE/FC). The right column shows the purification steps of method 2 (ASE/LLE/FC). For the spiked B17, these two methods are represented as the 3rd and 4th column.
- Results of the purification treatment in method 1(left column) refer to two representative values: A 1st value (LSE/LLE) shows the $\delta^{13}C$ of the analysed PAH after a Liquid-Solid Extraction and a Liquid-Liquid Extraction. The 2nd value shows the $\delta^{13}C$ value of the

combined fractions containing the target PAHs after a clean-up with flash chromatography (FC) followed by evaporation under N_2 at 40°C. FC was done for the LSE/LLE extracts using an elution sequence (see Table 7) based on CH:DCM.

- Results of the purification progress for method 2 (right column) indicates two representative values: The first one (ASE/LLE) shows the $\delta^{13}C$ value of each analysed PAH in each sample after the accelerated solvent extraction and the liquid-liquid extraction. The second value shows the $\delta^{13}C$ value of the combined fractions containing the target PAHs after the clean-up with FC followed by the evaporation under N_2 at 40°C. The FC in method 2 was done with the ASE/LLE extracts, using an elution sequence with CH:TCM (see Table 7). An additional 3rd value is given for some PAHs, showing combined fractions which were at the end eluted in very low amounts.

The standard deviation of triplicate measurements of the elemental analysis for the respective PAHs is indicated as a bar in each graph of spiked B17. This value is the $\delta^{13}C$ of the pure liquids and solids and should indicate variations caused by the chromatography.

When the triplicates of a sample showed differences between them, error bars are assigned to each value obtained. Additionally, measurements with amplitude of less than 200 mV (D.L.) are not considered. The $\delta^{13}C$ data with amplitude within a range between 200 mV and 700 mV are considered and indicated in each graph as L.A. (low amplitude). As previously discussed, uncertainty in the $\delta^{13}C$ values on this range are due to the low signal intensities reflected in variations in the obtained precision (standard deviation). The main criterion to be fulfilled in order to consider a measurement for determining a general trend was that the standard deviations from triplicates result in variations lower than +/- 0.5‰. Measurements with standard deviations higher than +/- 0.5‰ were also plotted and indicated as L.A.. Results for B11, B16, B29 and spiked B17 are presented in the following figures.

Samples of a volume of one millilitre were measured in CSIA. For the measurements of B11 after a LSE/LLE/FC and ASE/LLE/FC treatments, volumes of 1 ml samples were also measured, which were previously diluted 14 times. This was done after observing the very high amplitudes of mass 44 in the CSIA chromatograms obtained with the non-diluted samples.

Spiked B17. From the results obtained, no PAHs were detected by GC-IRMS and GC-MS in soil B17. Therefore, this soil was used as a real matrix blank for the spiking procedure. Figure 18 shows the 6 PAHs evaluated with CSIA with an LVI injection for spiked B17. The results are the following:

(a)

(b)

(c)

(d)

(e)

(f)
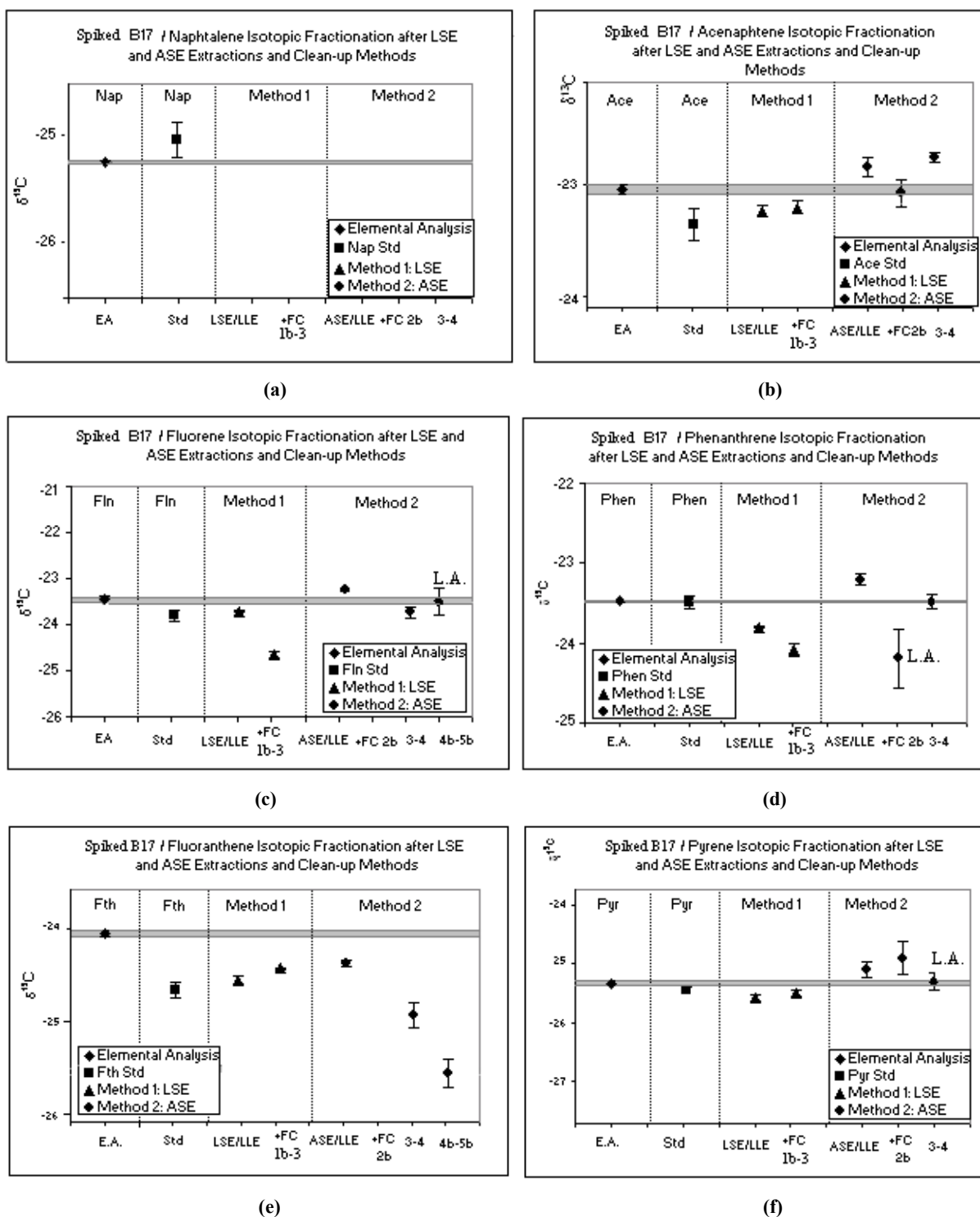
Figure 18. Individual PAH isotopic composition after an elemental analysis (E.A.), an evaluation of the PAH STD, the extraction and clean-up steps in method 1 (LSE/LLE/FC) and method 2 (ASE/LLE/FC) for spiked B17.

The graphs show for each PAH the $\delta^{13}C$ of the elemental analysis (1st column from left to right), the value for the PAH standard (2nd column), the purification steps in method 1 (3rd column) consisting of a 1st value that corresponds to the $\delta^{13}C$ after a LSE/LLE and a 2nd value that shows the isotopic fractionation of the combined fractions 1b-3 after the FC. (elution sequence with CH:DCM in Table 7). The 4th column shows the $\delta^{13}C$ after each purification step in method 2 consisting of a 1st value that shows the $\delta^{13}C$ after the ASE/LSE followed by a 2nd value (fraction 2b), a 3rd one (combined fractions 3-4) and in some PAHs a 4th one (combined fractions 4b-5b) after a clean-up with FC using CH:TCM (elution sequence with CH:TCM in Table 7).

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The overall observed trend for the 5 PAHs is that the extraction and clean-up procedures showed no significant change (<+/- 0.5‰) in the isotopic composition. Several specific analyses support these results:

- A comparison between the LSE/LLE vs. PAH STD showed that the change in the isotopic composition is slightly above the instrumental specification (< +/- 0.23‰) [46] meanwhile the comparison ASE vs. LSE showed a slight increase in $\delta^{13}C$ on ASE/LLE for the 5 PAHs of less than 0.4‰, which for both cases could be considered insignificant.

- The value for the combined fraction 1b-3 after the LSE/LLE/FC shows no significant decrease in $\delta^{13}C$ except for fluorene with respect to the LSE/LLE value. Experiments evaluating the effect that the evaporation by rotary distillation and evaporation under N_2 at 40°C have on the isotopic composition of PAHs showed no change in the $\delta^{13}C$ values of the analytes. Further details regarding these experiments can be provided by Michaela Blessing from the Environmental Mineralogy Research Group Therefore it is unlikely that the decrease in $\delta^{13}C$ for fluorene can be explained by inverse isotopic effect occurring due to the evaporation under N_2 promoted by a temperature of 40°C. No explanation was found to explain the result for fluorene.

- Measurement of the isotopic composition of the PAHs after a FC using ASE/LLE extracts over the low amplitude range show an isotopic fractionation less than 0.5‰, that could be also neglected. All values after the FC that show a change in the isotopic composition of more than 0.5‰ are values in the low amplitude range with a high standard deviation. Therefore we can conclude that the FC and evaporation with N_2 at 40°C do not affect the $\delta^{13}C$ value in the 5 PAHs detected and that the variations observed could be the result of the low signal intensity (description of the signal height in LVI in Figure 15).

Soil sample B11. Five PAHs from the soil sample B11 were measured with GC-IRMS in LVI injection. The following Figure 19 shows the results of the $\delta^{13}C$ measurements obtained for each compound:

(a)

(b)

(c)

(d)

(e)

Figure 19. Individual PAH isotopic fractionation in B11 after extractions and clean-ups.

The left column shows the $\delta^{13}C$ values after the purification steps in method 1 (LSE/LLE/FC). The 1st value corresponds to the LSE/LLE, the 2nd value to the PAHs combined fractions 2b-4 after the FC (elution sequence for B11 with CH:DCM in Table 7). The right column shows the $\delta^{13}C$ values after the purification steps in method 2 (ASE/LLE/FC). The 1st data (ASE/LLE) refers to the ASE/LLE value, the 2nd one to the $\delta^{13}C$ of PAHs on the fraction 4 after a FC (elution sequence for B11 with CH:TCM in Table 7), the 3rd value (4conc) to the same fraction but concentrated 14 times and the 4th value (4b-5b) to these combined fractions after the FC. All the B11 data are determined in a 14 times dilution with pure cyclohexane from the original extract.

The results obtained for B11 showed in general no significant isotopic fractionation for the methods 1 and 2 after the extraction and clean-up procedures. No difference in the δ values could be found between the LSE/LLE vs. ASE/LLE extracts.

Evaluation of the combined fractions 2b-4 after the LSE/LLE/FC followed by the evaporation under N_2 at a temperature of 40°C showed a slight decrease around 0.5‰ in the $\delta^{13}C$ value for the lighter PAHs (acenaphtene and fluorene). No explanation to this trend can be provided, because as mentioned before, studies of evaporation on the target PAHs showed to have no effect on the isotopic composition. Compounds with a heavier structure (phenanthrene, fluoranthene and pyrene) showed no variation with respect to the LSE/LLE extracts.

In general, the FC purification treatment for method 2 (ASE/LLE/FC) showed no change in the $\delta^{13}C$ for all PAHs except for fluoranthene. The measurement for fluoranthene in the fraction 4 fitted the low amplitude range and thus the reliability of this data was uncertain and not considered for the determination of an overall trend, meanwhile the decrease in $\delta^{13}C$ for the fraction 4b-5b was not completely understood.

Also, the concentration effect on the $\delta^{13}C$ was tested on this field sample B11 (from the treatment ASE/LLE/FC). A comparison between B11 samples and B11 samples fourteen times concentrated were done. The general trend observed showed that if the amplitude of mass 44 (proportional to the PAH analyte concentration) increases, the $\delta^{13}C$ decreases. Interestingly, this trend observed from the measurements of the field sample B11 mostly agreed with the trend observed in the plot of the signal heights for LVI (see Figure15.).

An exception to this trend was only observed in fluoranthene, where with the increase in amplitude of mass 44, an increase in the $\delta^{13}C$ occurred. Possible reasons to this result remain uncertain.

After no observed change in the isotopic composition occurring either in the reference spiked B17 and B11, we can assess that B11 is a contamination source and that the two developed purification methods in general, are effective to evaluate the real isotopic fractionation at heterogeneous and highly contaminated field samples. These results allowed us to distinguish between two possible processes undergoing at this sampled borehole: the source allocation of the contamination by PAHs and the biodegradation of this analytes.

Soil Sample B16. Five PAHs were detected by CSIA in LVI, as indicated in the Figure 20.
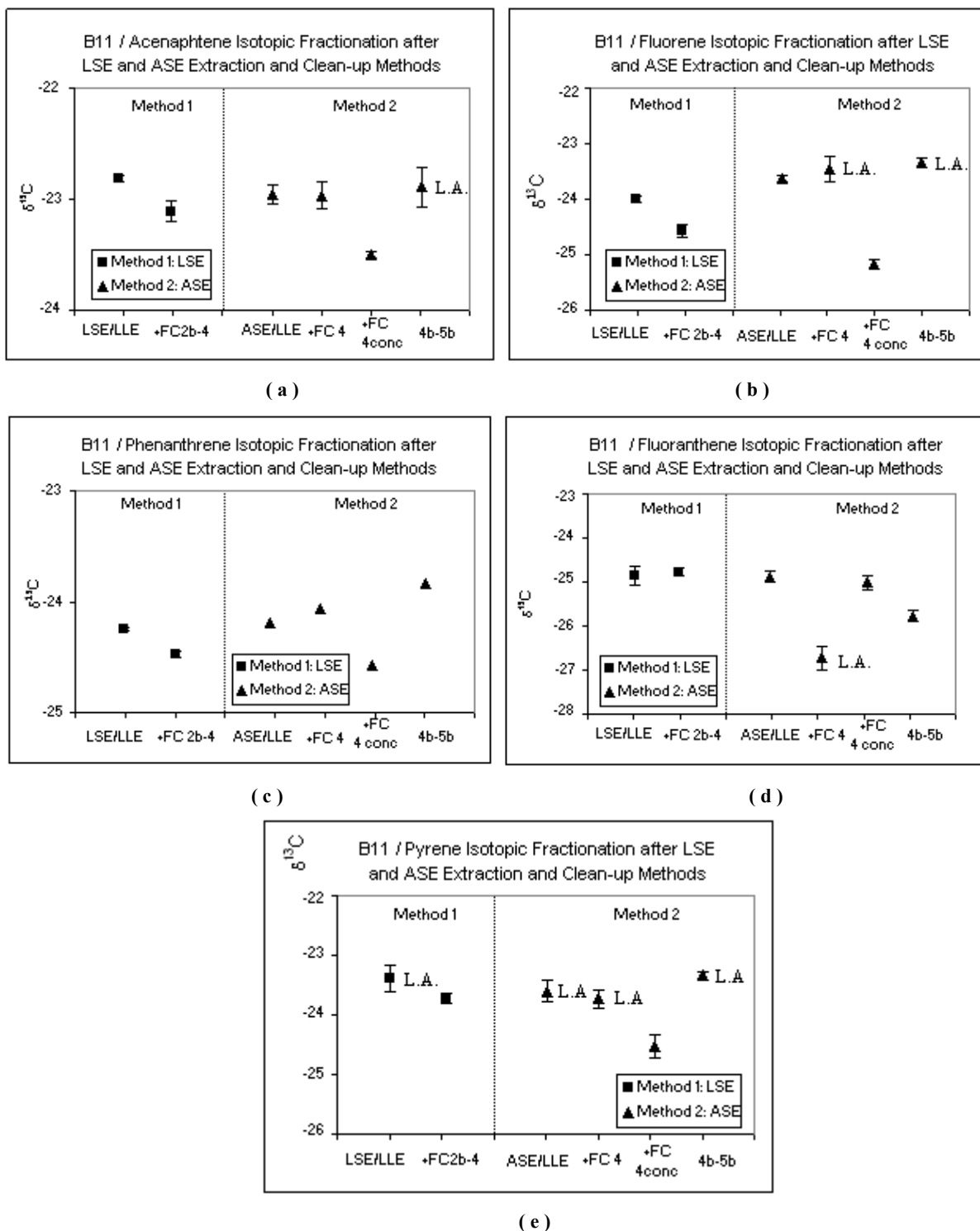

(a)

(b)

(c)

(d)

(e)

Figure 20. Individual PAH isotopic fractionation in B16 after extractions and clean-ups.
The left column shows the $\delta^{13}C$ values after each purification step in method 1 (LSE/LLE/FC). The 1st value corresponds to the LSE/LLE, the 2nd value to the PAHs combined fractions 1b-3 after the FC (elution sequence for B16 with CH:DCM on Table 7). The right column shows the $\delta^{13}C$ values after each purification step in method 2 (ASE/LLE/FC). The 1st one corresponds to the ASE/LLE value, and the 2nd one to the $\delta^{13}C$ of PAHs on the fractions 2b-6 after a FC (elution sequence for B16 with CH:TCM in Table 7).

Negligible changes in the isotopic composition (< 0.3‰) were observed for fluoranthene and pyrene after extraction and clean-up treatments in the methods 1 (LSE/LLE/FC) and method 2 (ASE/LLE/FC). Phenanthrene showed no isotopic fractionation after the extractions of both methods (LSE/LLE and ASE/LLE). The δ^{13}C values of the FC in methods 1 and 2 showed a significant decrease (>0.5‰) for phenanthrene and fluorene. Possible explanations to these variations could be that these values fitted into the range of low amplitude, making these data not completely reliable. It was also not possible to identify if the isotopic fractionation for fluorene after the LSE/LLE treatment was the real δ^{13}C value. A big standard deviation was observed on this data. In the case of acenaphthene, it is not possible to assess a trend due to the lack of information. The effect on the δ^{13}C due to the FC purification treatment in method 2 was not elucidated, because all the fractions containing the target compounds were combined in fractions 2b to 6 (one has to keep in mind that different elution sequences were tested in the extracts, therefore sometimes it is not possible to exactly compare the same combined fractions. To see the elution sequence here employed, see Table 7.

Soil Sample B29. Four PAHs were measured from B29 samples in CSIA in LVI injection.

The results in Figure 21. showed that no significant change in the isotopic composition (< 0.5‰) was observed between LSE/LLE and ASE/LLE for fluoranthene and pyrene. For all PAHs, the FC fractions from the 2 methods showed a significant isotopic fractionation (>0.5‰), except for pyrene in ASE/LLE/FC. All measurements of the detected PAHs after the FC of both methods fitted the low amplitude range.

Inexplicable trends were observed for the FC after the LSE/LLE and ASE/LLE treatments for pyrene and fluoranthene, in which enrichment was observed for both compounds after the FC in LSE/LLE, meanwhile a decrease in the δ^{13}C occurred in the FC after ASE/LLE. Phenanthrene showed also a decrease in the δ^{13}C after the FC. It was not possible to elucidate a trend of the effect that the clean-up itself has on the isotopic composition of the PAHs in this sample B29, because only one additional combined fraction was observed for one PAH (fluoranthene), which presents a decreased δ^{13}C value in the low amplitude range with a high standard deviation. In general, the methods 1 and 2 applied for B29 were effective to determine the isotopic fractionation in the LSE/LLE and ASE/LLE extracts, but not in the FC fractions from the 2 methods, being the main reason the low signal intensity associated with higher a standard deviation, as observed in the LVI signal height plot in Figure 15.
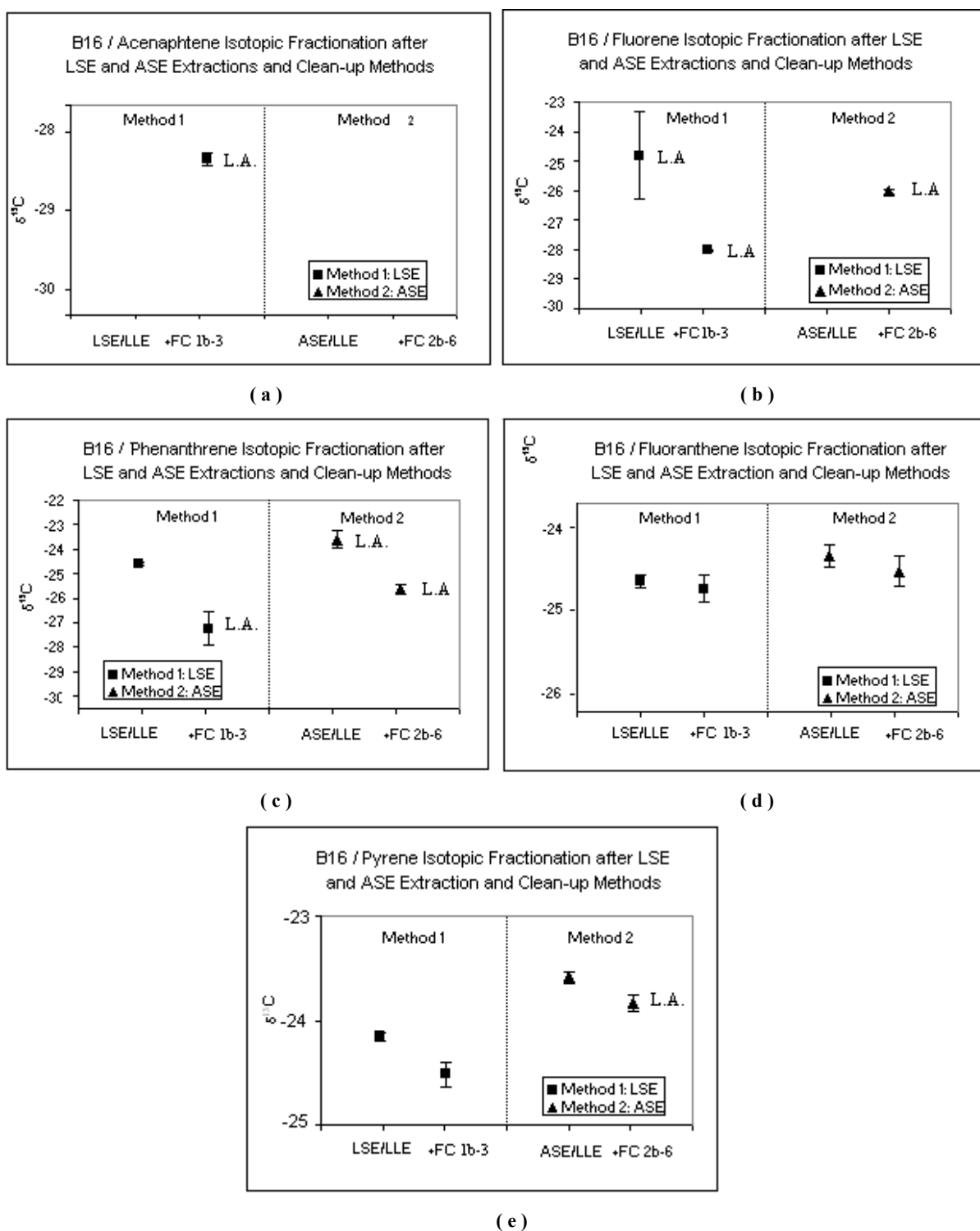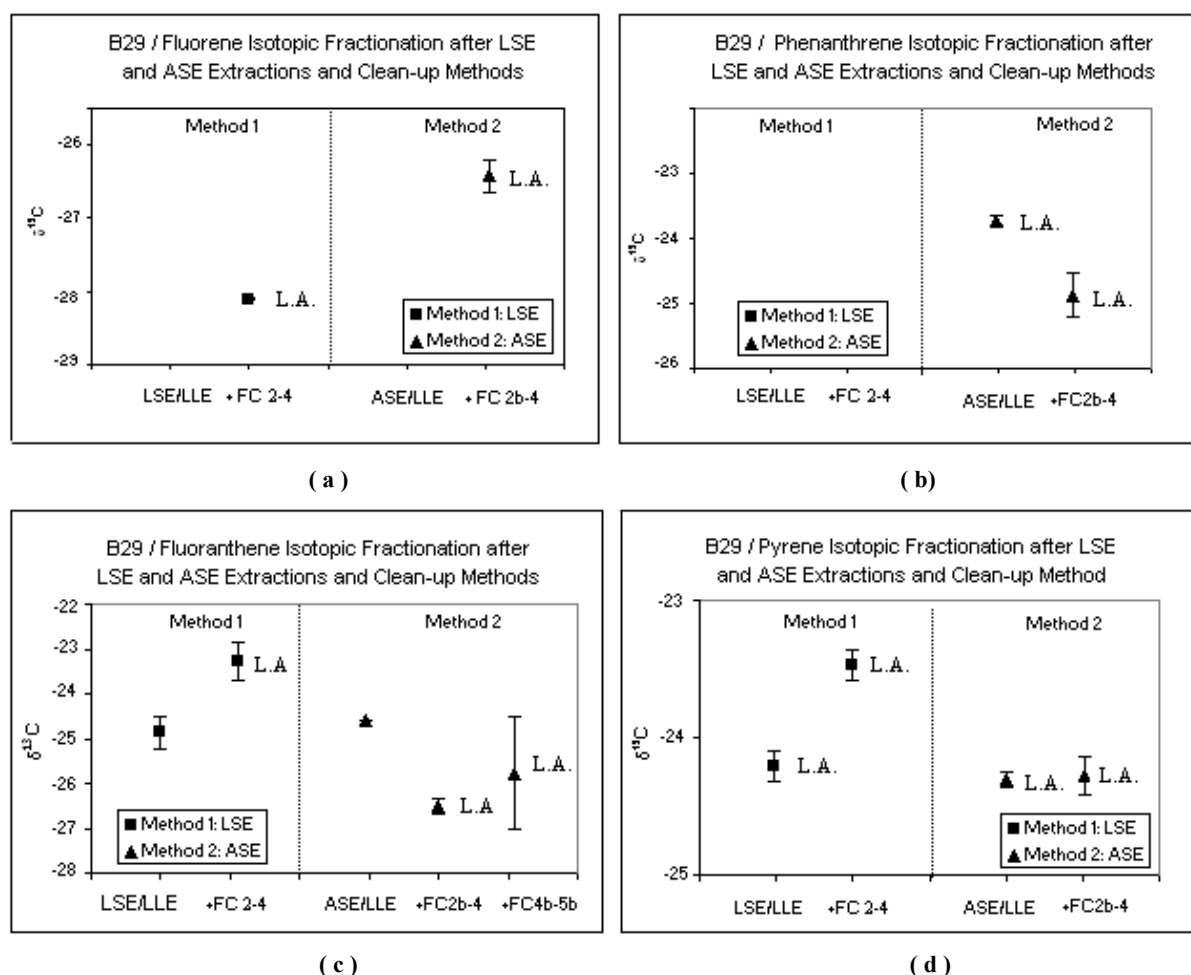
Figure 21. Individual PAH isotopic fractionation in B29 after extractions and clean-ups.

The left column shows the $\delta^{13}C$ values after each purification step in method 1 (LSE/LLE/FC). The 1st value corresponds to the LSE/LLE, the 2nd value to the PAHs combined fractions 2-4 after the FC (elution sequence with CH:DCM in Table 7) . The right column shows the $\delta^{13}C$ values after each purification step in method 2 (ASE/LLE/FC). The 1st one corresponds to the ASE/LLE value, and the 2nd one to the $\delta^{13}C$ of PAHs on the fractions 2b-4 after a FC (elution sequence with CH:TCM in Table 7). A 3rd value shows the combined fraction 4b-5b.

6.6 Source Allocation at the Surveyed Site

The measurements of the isotopic composition obtained after the ASE/LLE/FC method were used in order to assign values of the $\delta^{13}C$ of PAHs for source allocation at the surveyed site. One has to remember that this method resulted in the best elimination of interfering compounds but as well most of the $\delta^{13}C$ measurements after the FC clean-up fitted into the low amplitude range. Recommendations to obtain measurements above the L.A. range are described in Section 8.

Simultaneously, a comparison with the $\delta^{13}C$ values obtained from the LSE/LLE extracts was analyzed for a better evaluation because these results were mostly above the low amplitude range and also no significant difference in the isotopic composition with respect to the ASE/LLE extracts was observed. The map site showing the $\delta^{13}C$ values from both treatments is in the following Figure 22. presented.

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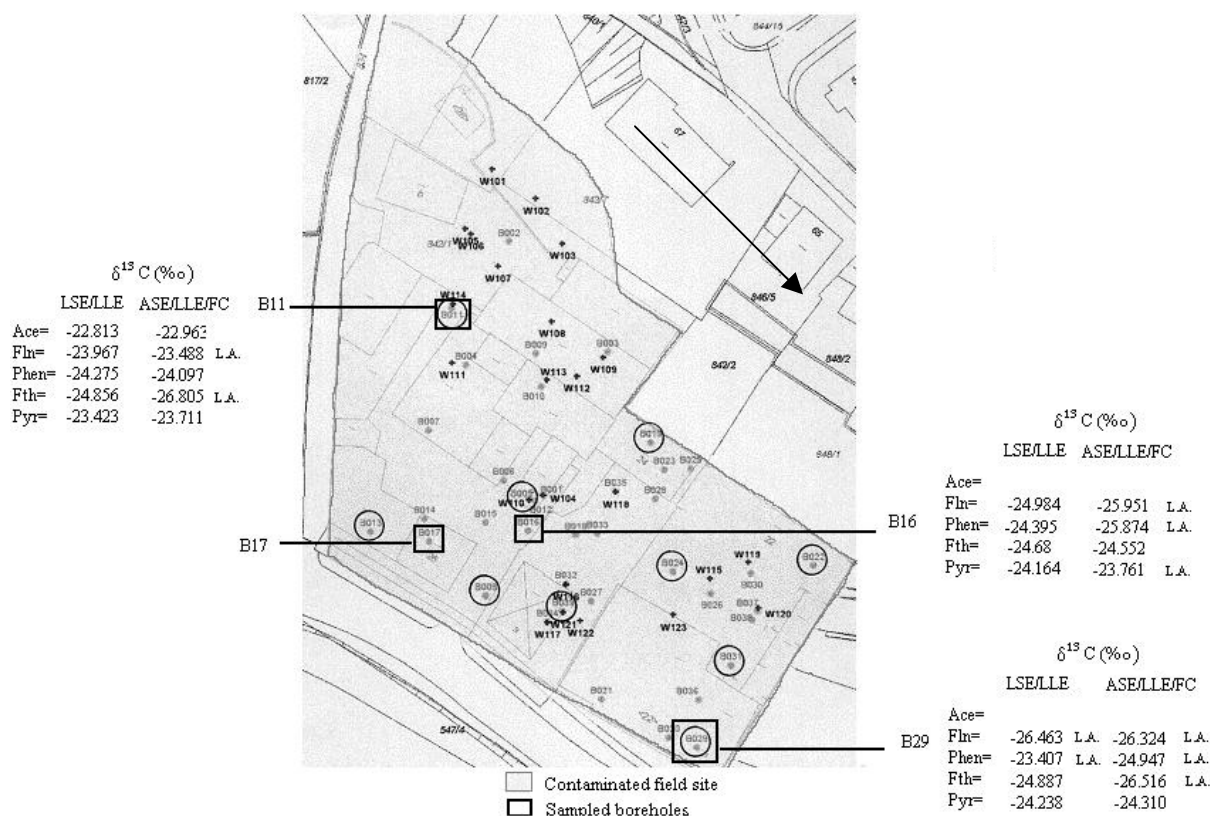
Figure 22. Isotopic composition of the PAHs at the investigated boreholes.

The results from the LSE/LLE extracts presented isotopic fractionations of <1‰ and amplitudes of mass 44 above the low amplitude range. A possible interpretation to this obtained trend could be that the contamination could come from the same source.

After analyzing the results from the ASE/LLE/FC treatment, variations >1‰ were observed in the PAHs. It is believed that these results were mainly due to the low signal intensity resulting in uncertain information. This low signal intensity resulted after the poor recovery from FC clean-up. In order to conclude that such $\delta^{13}C$ significant variations result from different sources at the site, it is necessary to repeat the extraction and clean-up method making sure that higher PAH concentrations (above the L.A. range) are sampled (see Section 8 for application of this recommendation).

After relating the information obtained from LSE/LLE and ASE/LLE/FC measurements, it was possible to neglect the possibility of a biodegradation as possible interpretation of the change in more than 1 ‰ in the isotopic composition of these soils at these specific borehole locations.

7
Conclusions

The objective of this investigation was to develop a purification method that isolates the PAHs from the complex contaminated soil samples without altering the carbon isotopic composition of the analytes and also to evaluate if the $\delta^{13}C$ stable isotope measurements of the PAH contaminated soil could be used as a monitoring technique for their source allocation in CSIA.

The ^{13}C isotopic composition results presented here support the application of the two developed purification methods; LSE/LLE/FC and ASE/LLE/FC at extraction recoveries of 56% and 67% respectively, and as well the use of CSIA with LVI injection method because the extraction and clean-up treatments did not introduce a significant isotopic fractionation on the measurements at the contaminated field sources.

A second important point to note is that the determination of the detection limits for PAHs in CSIA using a LVI injection method is defined as well. The detection limit for isotopic composition measurements corresponds to an amplitude of mass 44 of 200 mV equivalent to a PAH soil concentration of 157.9 mg/kg when extracting 29.3 g of the soil sample with 88.5 ml of solvent with the ASE/LLE treatment. As well, a low amplitude range is defined, corresponding to 200 to 700 mV of amplitude of mass 44 at a PAH soil concentration of 11.1 to 38.5 mg/kg, where the measurements are uncertain but considered. Some variations in the isotopic composition of the extracts from soils not highly contaminated, like B29 and B16, are mainly encountered after the FC clean-up on each extraction method. The probable interpretation to this behaviour is the poor signal intensity of the measurements and thus fitting in the low amplitude range.

In regard to the extraction methods, ASE proves to be an effective, promising and fast extraction technique that does not affect the isotopic composition of the PAH target analytes which as well yield better extraction recoveries for all the target compounds with the exception of naphthalene, the recovery of which in LSE/LLE is slightly higher.

After relating two important pieces of information; GC-MS and GC-IRMS results for the 4-monitored soil samples, it is determined that B11 is a source and B17 can be used as a soil blank, meanwhile it is still not clear if B29 and B16 belong to the same source of contamination or if they correspond to different ones. It was possible to determine that no biodegradation is taking place at the sampled soils. In this way the purification and CSIA developed methods may be used in future endeavors to determine the source of PAH contamination at this specific site.

8
Recommendations

Suggestion for the extractions in ASE would be the concentration of the acetone extracts with a rotary distillation, followed by an acetone/water-cyclohexane extraction. This is in order to obtain higher analyte concentrations, resulting in measurements in LVI above the low amplitude range. The acetone extracts would be evaporated to 5 ml and then dissolved in 50 ml H_2O Millipore water. The amount of cyclohexane used for the liquid-liquid extraction would be 15 ml. This cyclohexane extracts could as well follow the FC clean-up proposed in this thesis.It is also important to consider, that the concentration of the acetonic extracts could as well present another additional organic cosolvents that may change the solvation properties of the aqueous phase [7].

Another approach to obtain high signal intensities would be as well the concentration of the obtained cyclohexane extracts (after the liquid-liquid extraction) through N_2 evaporation at 40°C. This step would increase the analyte concentration before the proposed clean-up method, which after the purification would still be sufficient for resulting in signals with high amplitudes of mass 44 in CSIA.

A third alternative for increasing the PAH analyte concentration before the CSIA measurements after the flash chromatography clean-up would be to evaporate under N_2 the combined fractions to 50 μl instead of 1 ml as done for this research.

An alternative extraction method that could result in an efficient isolation with high extraction recoveries (84 to 94%) of the PAHs separating the aliphatic compounds from complex contaminated soil samples can be achieved using a DMF-pentane extraction, as described by Mandalakis [47]. The application of this method would allow measurements in CSIA in LVI over the low amplitude range, and thus making possible the monitoring of sources and less contaminated soil samples.

The proposed method would initiate with an ASE extraction with toluene, the extract of which would be concentrated in a rotary distillation (that does not affect the isotopic composition) and thereafter treated twice consecutively with a dimethylformamide (5%H_2O)-n-pentane binary system, that would concentrate the PAHs in the DMF phase and the aliphatic compounds in the pentane phase. The DMF phase could then be extracted with cyclohexane, which afterwards could follow the proposed FC purification progress done in this thesis.

9
Appendix

A1 ISODAT METHOD FOR LVI MEASUREMENTS

A1.1 Instrument information LVI.

Method	Gas CO_2		
Experiment	Continuous		
Configuration	GCC Interface		
Gas Configuration	CO_2		
Pre Script			
Main Script	Acquisition is1		
Post Script			
Isotope MS			
Integration time[s]	0.200	Peak center predelay [s]	5.0
Peak Center Cup	3	Peak center postdelay [s]	5.0
Reference Port			
GCII-III Interface	Reference		

A1.2 Time event List LVI.

Time Events

Time [s]	Reference	Oxidize	Backflush	Split
0.0	Off	Off	On	Out
200.0	On			
230.0	Off			
300.0	On			
330.0	Off			
400.0	On			
430.0	Off			
500.0			Off	In
1700.0			On	Out
Acquisition Time [s]	1700.0			

A1.3 Evaluation information LVI.

Ion Correction Type				CICA_CO$_2$_SSH		
Ref. Nr.:	**Ref.Time:**	**Ref. Name:**	**d13C/d12C**	**vs.**	**d18O/16O**	**vs.**
1	330.00	CO_2 Lab. Tank	-25.960	VPDB	0.00	VSMOW

A1.4 Peak detection LVI.

Peak find methods

Start Slope	0.20
End Slope	0.40
Peak Min. Height	50
Peak Resolution	50.00
Max. Peak Width	180.00
Detection on Mass	44
BGD Type	Individual BGD
Peak Detection	1
BGD Detection	1
BGB History	5.000000
Time shift	1
Square Peak Detection	0
Square Peak Det. Fac.	0.550000
Smoothing	Standard Smoothing 5 Points

A2 ISODAT METHOD FOR SPLITLESS INJECTIONS

A2.1 Instrument information for splitless injection.

Method	Gas CO_2		
Experiment	Continuous		
Configuration	GCC Interface		
Gas Configuration	CO_2		
Pre Script			
Main Script	Acquisition is1		
Post Script			
Isotope MS			
Integration time[s]	0.200	Peak center predelay [s]	5.0
Peak Center Cup	3	Peak center postdelay [s]	5.0
Reference Port			
GCII-III Interface	Reference		

A2.2 Time event list for splitless injection.

Time Events Time [s]	Reference	Oxidize	Backflush	Split
0.0	Off	On	Out	Off
200.0				On
230.0				Off
300.0				On
330.0				Off
400.0				On
430.0				Off
500.0		Off	In	
1400.0		On	Out	
Acquisition Time [s]	1400.0			

A2.3 Evaluation information for splitless injection.

Ion Correction Type					CICA_CO$_2$_SSH		
Ref. Nr.:	Ref.Time:	Ref. Name:	d13C/d12C	vs.	d18O/16O	vs.	
1	330.00	CO$_2$ Lab. Tank	-25.960	VPDB	0.00	VSMOW	

A2.4 Peak detection data for splitless injection.

Peak find methods	
Start Slope	0.20
End Slope	0.40
Peak Min. Height	50
Peak Resolution	50.00
Max. Peak Width	180.00
Detection on Mass	44
BGD Type	Individual BGD
Peak Detection	1
BGD Detection	1
BGB History	5.000000
Time shift	1
Square Peak Detection	0
Square Peak Det. Fac.	0.550000
Smoothing	Standard Smoothing 5 Points

A3 OPTIC 3 INJECTOR SETTINGS

A3.1 OPTIC 3/ Splitless injection (1 µl).

Method Name	EppleFlu2
Equilibration time	00:30 mm:ss
End time	1500 s
Initial temperature	300°C
Final temperature	300°C
Temperature Control	Keep current temperature
Solvent Cooling Effect	No
Cooling Valve Mode	No
Transfer Column Flow	3.0 ml/min
Transfer Time	01:00 mm:ss
Initial Column Flow	2.0 ml/min
Split Flow	40 ml/min

A3.2 OPTIC 3 / Large volume injection (50 µl).

Method Name	TESTLVI
Equilibration time	00:05 mm:ss
End time	26:40 min
Initial temperature	60°C
Ramp Rate	15.0°C/s
Final temperature	300°C
Temperature Control	Keep current temperature
Solvent Cooling Effect	Yes
Cooling Valve Mode	No
Sample Sweep Column Flow	1.0 ml/min
Transfer Column Flow	3.0 ml/min
Transfer Time	01:00 mm:ss
Initial Column Flow	2.0 ml/min
Final Column Flow	2.0 ml/min
Vent Mode	Solvent level
Solvent Monitor Level	10
Vent Flow	100 ml/min
Split Flow	25 ml/min

A4 GENERAL IRMS SETTINGS

Table A4.1 Mass spectrometer information.

High Voltage [KV] 2.998	Magnet [Steps] 10770
HV 3.08 KV	Box 0.8 mA
Vac 1.2E-006 mBar	Trap 0.72 mA

Table A4.2 Focus delta information.

|	
Emission	1.50 mA
Trap	40.00 V
Electron Energy	150.000 V
Extraction 1	64.98 %
Extraction 2	77.12 %
X-Focus 1	15.21 %
X-Focus 2	13.11 %
X-Deflection	46.74 %
Y-Deflection 1	58.44 %
Y-Deflection 2	52.67 %
SE-Supressing	87.35 %

A5. CSIA Chromatograms

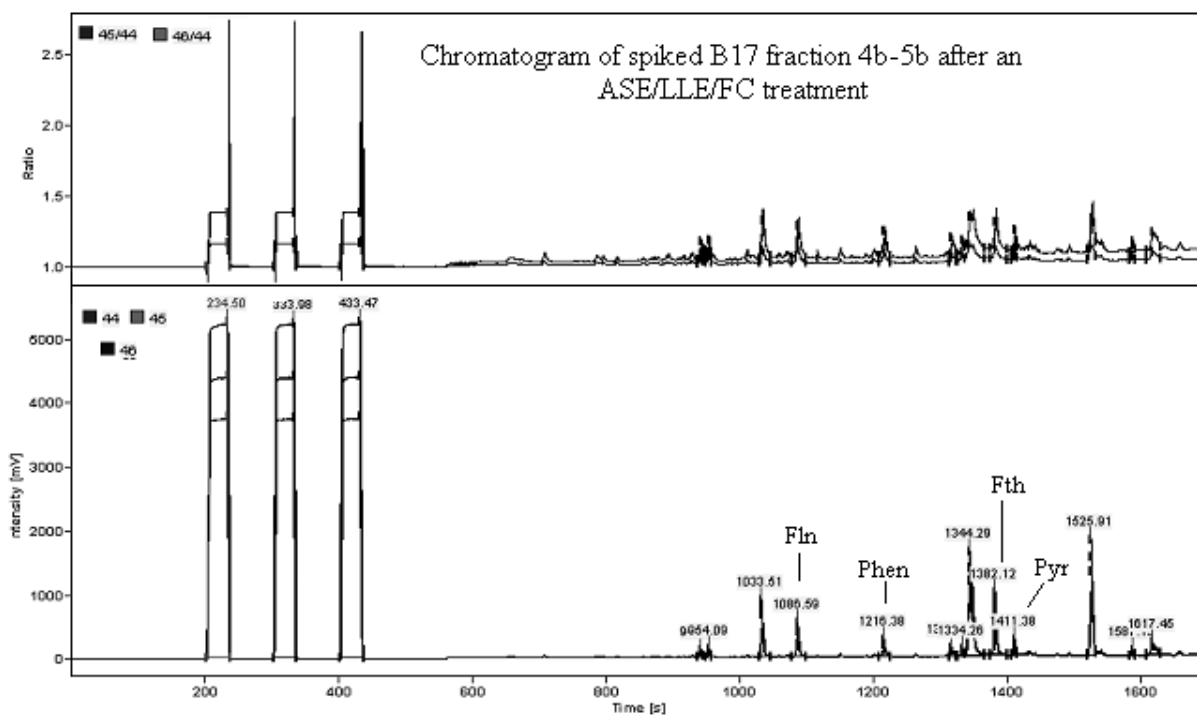A5.1 Chromatogram of B11 fraction 2b-4 after an LSE/LLE/FC treatment.
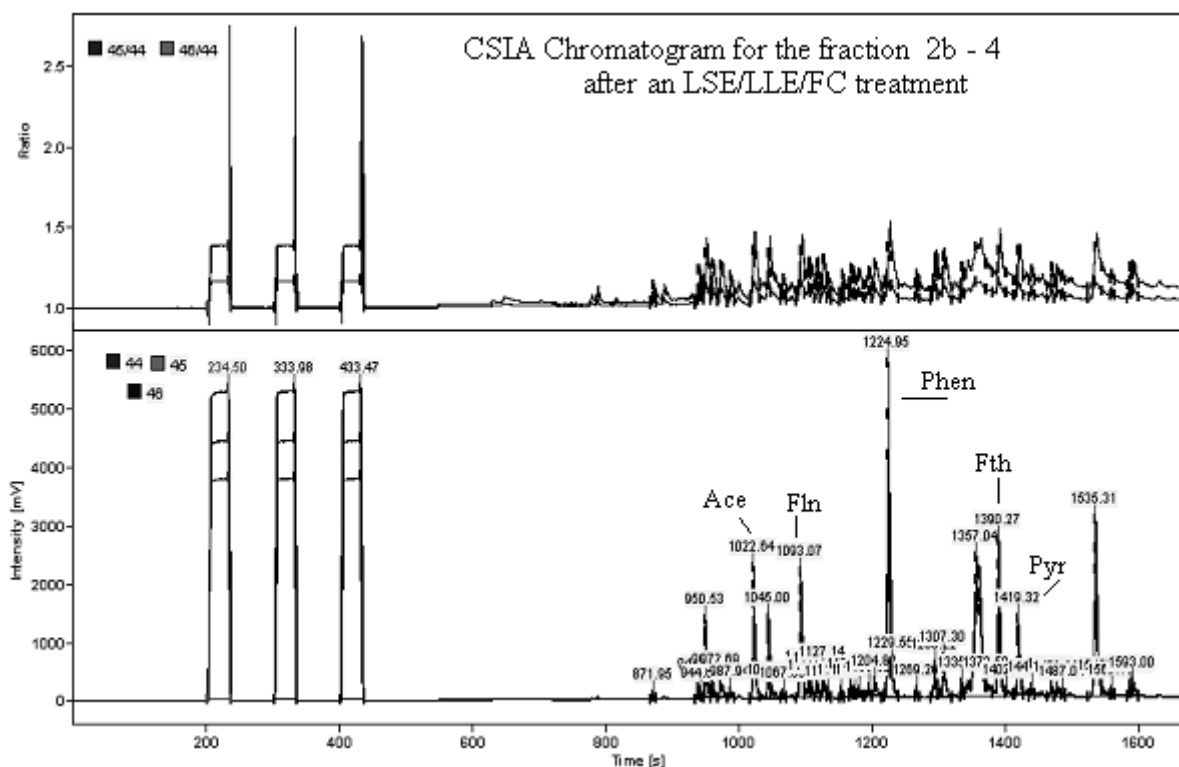
A5.2 Chromatogram of spiked B17 fraction 2b after an ASE/LLE/FC treatment.
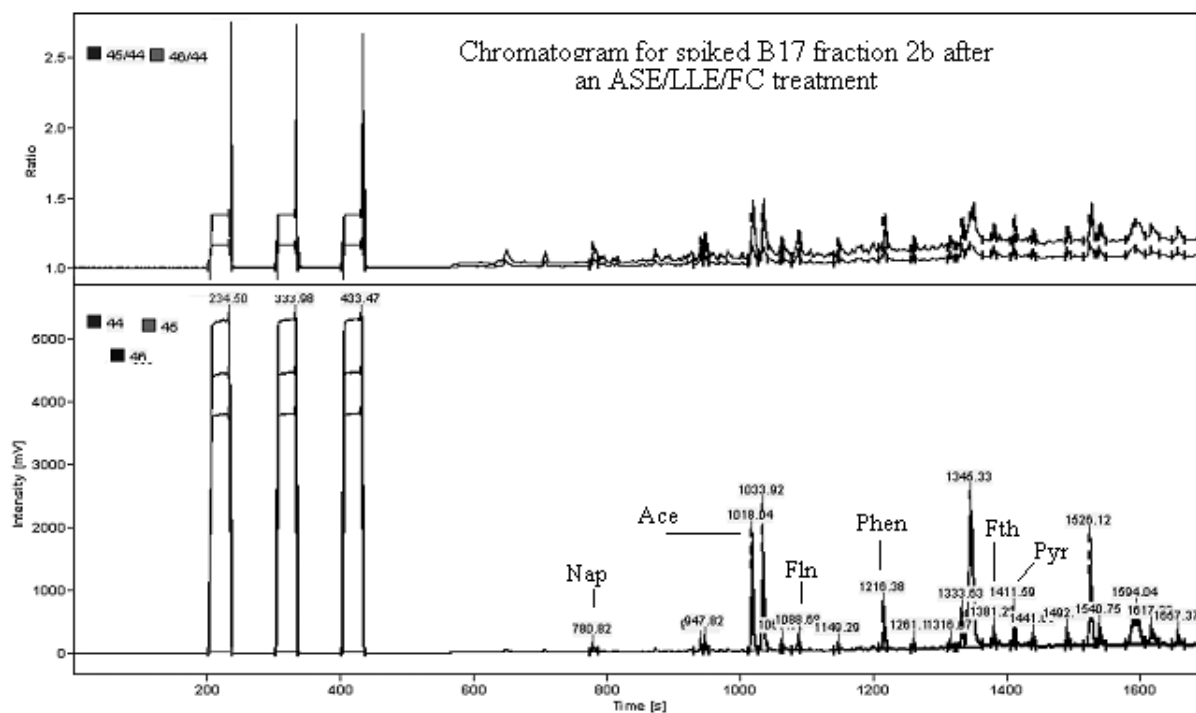
A5.3 Chromatogram of spiked B17 fraction 3-4 after an ASE/LLE/FC treatment.
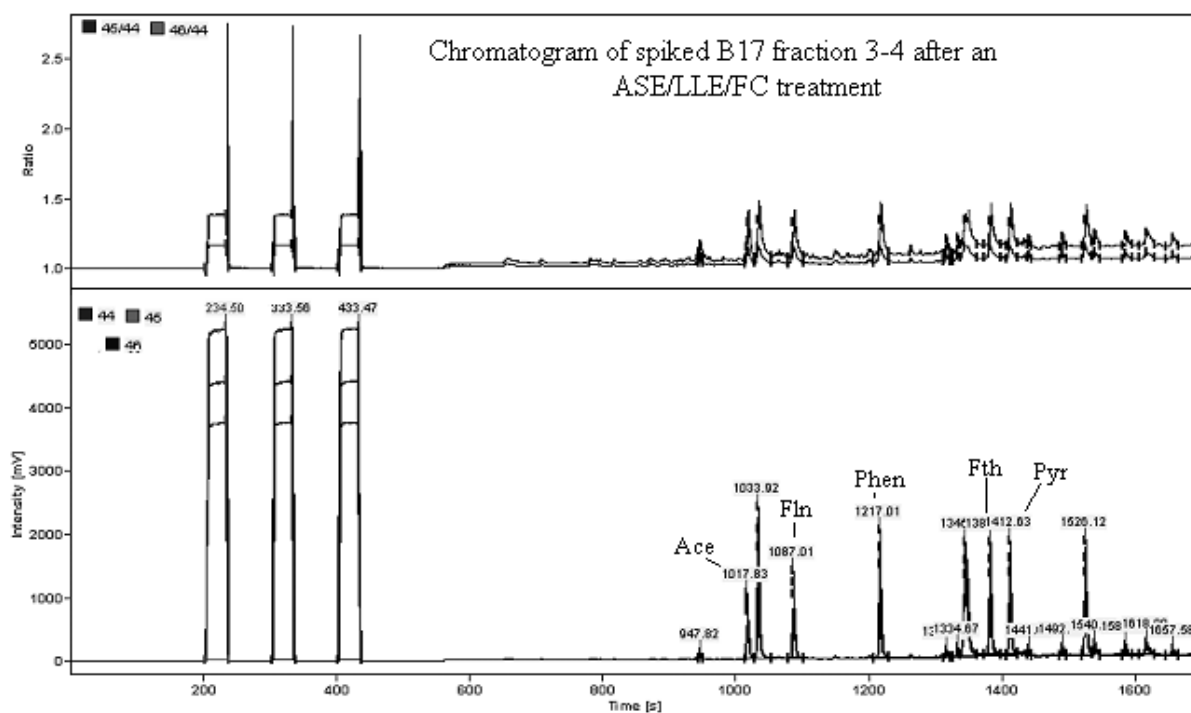
A5.4 Chromatogram of spiked B17 fraction 4b-5b after an ASE/LLE/FC treatment.

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