Oxygen-containing polycyclic aromatic hydrocarbons (OPAHs) and their parent-PAHs in soil

- analysis, sources and fate -

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Benjamin Acham Musa Bandowe, M. Sc.,

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List of abbreviations

AAS	Atomic Absorption Spectroscopy
ACEN	Acenaphthene
1-ACENOL	1-Hydroxyacenaphthene
ACENY	Acenaphthylene
1,2-ACQ	1, 2-Acenaphthylenequinone
9,10-ANQ	9, 10-Anthraquinone
ANTH	Anthracene
ASE	Accelerated solvent extraction
B(A)A	Benz[a]anthracene
7,12-B(A)A	Benzo[a]anthracene-7,12-dione
B(A)P	Benzo[a]pyrene
B(BJK)	Benzo[b+j+k]fluoranthenes
B(E)P	Benzo[e]pyrene
B(GHI)	Benzo[ghi]perylene
BAM	Bundesanstalt für Materialforschung [German Federal Institute for Materials Research and Testing]
BC	Black Carbon
2-BPCD	2-Biphenylcarboxaldehyde
BP-D5	Benzophenone-2,3,4,5,6-D_5
BSTFA	N,O-bis-(trimethylsilyl)trifluoroacetamide
C/N	Carbon to nitrogen ratio
CHRY	Chrysene+triphenylene
COR	Coronene
C_{org}	Organic carbon
CR	Concentration ratio between topsoil and subsoil
DAD	Diode array detector
DIBE	Dibenz[a,h]anthracene
2,6-DIISOPNAPH	2,6-Diisopropylnaphthalene
1,3-DMNAPH	1,3-Dimethylnaphthalene
1,7-DMPHEN	1,7-Dimethylphenanthrene
2,6-DMPHEN	2,6-Dimethylphenanthrene
3,5-DMPHEN	3,5-Dimethylphenanthrene

3,6-DMPHEN	3,6-Dimethylphenanthrene
DOM	Dissolved organic matter
ECEC	Effective cation-exchange capacity
EI	Electron impact ionization
ERM	European reference material
EU	European Union
9-FLO	9-Fluorenone
FLUA	Fluoranthene
FLUO	Fluorene
9-FLUORENOL	9-Hydroxyfluorene
GC	Gas chromatography
GPC	Gel permeation chromatography
HPLC	High performance liquid chromatography
IND	Indeno [1,2,3-cd]pyrene
1-INDA	1-Indanone
IUSS	International Union of Soil Science
K_{OC}	Organic carbon-water partition coefficient
K_{OW}	Octanol-water partition coefficient
LC	Liquid chromatography-mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
LRAT	Long-range atmospheric transport
MAE	Microwave-assisted extraction
1-MNAPH	1-Methylnaphthalene
2-MNAPH	2-Methylnaphthalene
1-MPHEN	1-Methylphenanthrene
2-MPHEN	2-Methylphenanthrene
MS	Mass spectrometry
1,8-NAA	1,8-Naphthalic anhydride
NAPH	Naphthalene
2-NAPHTHOL	2-Hydroxynaphthalene
NCI	Negative chemical ionization
1-NLD	1-Naphthaldehyde

1,4-NQ	1, 4-Naphthoquinone
OPAH	Oxygen-containing PAH derivatives and metabolites
OPAK	Sauerstoffhaltiger polyzyklischer aromatischer Kohlenwasserstoff
PAH	Polycyclic aromatic hydrocarbon
PAK	Polyzylischer aromatischer Kohlenwasserstoff
PBDE	Polybrominated diphenyl ethers
PCB	Polychlorinated biphenyls
PERY	Perylene
PHEN	Phenanthrene
PLE	Pressurized liquid extraction
POP	Persistent organic pollutant
PP	2-Hydroxybiphenyl
PYR	Pyrene
RETENE	1-Methyl-7-isopropylphenanthrene
RSD	Relative standard deviation
SALYD	2-Hydroxybenzaldehyde
SCAN	Full scan mode
SIM	Single ion monitoring
SOM	Soil organic matter
SPE	Solid phase extraction
1,2,3,4-THNAPH	1,2,3,4-Tetrahydronaphthalene
TMCS	Trimethylchlorosilane
2,3,5TMNAPH	2,3,5-Trimethylnaphthalene
UK	United Kingdom
UNECE	United Nations Economic Commission for Europe
USEPA	United States Environmental Protection Agency
WRB	World Reference Base of Soil Resources

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Summary

In spite of the higher toxicity of oxygen-containing polycyclic aromatic hydrocarbons (OPAHs) than of their parent-PAHs, there are only a few studies of the concentrations, composition pattern, sources and fate of OPAHs in soil, the presumably major environmental sink of OPAHs. This is related to the fact that there are only few available methods to measure OPAHs together with PAHs in soil.

The objectives of my thesis were to (i) develop a GC/MS-based method to measure OPAHs and their parent-PAHs in soils of different properties and pollution levels, (ii) apply the method to soils from Uzbekistan and Slovakia and (iii) investigate into the fate of OPAHs, particularly their vertical transport in soil

I optimized and fully evaluated an analytical method based on pressurized liquid extraction, silica gel column chromatographic fractionation of extracted compounds into alkyl-/parent-PAH and OPAH fractions, silylation of hydroxyl-/carboxyl-OPAHs with N,O-bis(trimethylsilyl)trifluoracetamide and GC/MS quantification of the target compounds. The method was targeted at 34 alkyl-/parent-PAHs, 7 carbonyl-OPAHs and 19 hydroxyl-/carboxyl-OPAHs. I applied the method to 11 soils from each of the Angren industrial region (which hosts a coal mine, power plant, rubber factory and gold refinery) in Uzbekistan and in the city of Bratislava, the densely populated capital of Slovakia.

Recoveries of five carbonyl-OPAHs in spike experiments ranged between 78-97% (relative standard deviation, RSD, 5-12%), while 1,2-acenaphthenequinone and 1,4-naphthoquinone had recoveries between 34-44%% (RSD, 19-28%). Five spiked hydroxyl-/carboxyl-OPAHs showed recoveries between 36-70% (RSD, 13-46%), while others showed recoveries <10% or were completely lost. With the optimized method, I determined, on average, 103% of the alkyl-/parent-PAH concentrations in a certified reference material.

The ∑OPAHs concentrations in surface soil ranged 62-2692 ng g^{-1} and those of ∑alkyl-/parent-PAHs was 842-244870 ng g^{-1}. The carbonyl-OPAHs had higher concentrations than the hydroxyl-/carboxyl-OPAHs. The most abundant carbonyl-OPAHs were consistently 9-fluorenone (9-FLO), 9,10-anthraquinone (9,10-ANQ), 1-indanone (1-INDA) and benzo[a]anthracene-7,12-dione (7,12-B(A)A) and the most abundant hydroxyl-/carboxyl-OPAH was 2-hydroxybenzaldehyde. The concentrations of carbonyl-OPAHs were frequently higher than those of their parent-PAHs (e.g., 9-FLO/fluorene >100 near a rubber factory in Angren). The concentrations of OPAHs like those of their alkyl-/parent-PAHs were higher at locations closer to point sources and the OPAH and PAH concentrations were correlated suggesting that both compound classes originated from the same sources. Only for 1-INDA and 2-biphenylcarboxaldehyde sources other than combustion seemed to dominate. Like those of the alkyl-/parent-PAHs, OPAH concentrations were higher in topsoils than subsoils. Evidence of higher mobility of OPAHs than their parent-PAHs was provided by greater subsoil:topsoil concentration ratios of carbonyl-OPAHs (0.41-0.82) than their parent-PAHs (0.41-0.63) in Uzbekistan. This was further backed by the consistently higher contribution of more soluble 9-FLO and 1-INDA to the ∑carbonyl-OPAHs in subsoil than topsoil at the expense of 9,10-ANQ, 7,12-B(A)A and higher OPAH/parent-PAH concentration ratios in subsoil than topsoil in Bratislava.

With this thesis, I contribute a suitable method to determine a large number of OPAHs and PAHs in soil. My results demonstrate that carbonyl-OPAHs are more abundant than hydroxyl-/carboxyl-OPAHs and OPAH concentrations are frequently higher than parent-PAH concentrations. Furthermore, there are indications that OPAHs are more mobile in soil than PAHs. This calls for appropriate legal regulation of OPAH concentrations in soil.

Zusammenfassung

Bislang gibt es nur wenige Arbeiten zu Gehalten, Mustern, Quellen, Verbleib, Umsatz und Transport von sauerstoffhaltigen polyzyklischen aromatischen Kohlenwasserstoffen (OPAK) in Böden, ihrer wahrscheinlich größten Senke in der Umwelt, obwohl einzelne OPAK teilweise sogar toxischer sind als ihre Mutter-PAK. Dies hängt mit der Tatsache zusammen, dass es nur wenige verfügbare Methoden zur Gehaltsbestimmung von ausgewählten OPAK zusammen mit ihren Mutter-PAK gibt.

Die Ziele meiner Doktorarbeit waren (i) eine GC/MS-basierte Methode zur Bestimmung der OPAK- und PAK-Gehalte in Böden unterschiedlicher Eigenschaften und Schadstoffbelastung zu entwickeln, (ii) diese Methode auf Böden aus Usbekistan und der Slowakei anzuwenden und (iii) das Verhalten der OPAK in Böden, insbesondere den vertikalen Transport, zu untersuchen.

Ich optimierte und testete eine analytische Methode, die aus beschleunigter Lösemittelextraktion, Silika-Gel-Festphasenextration, Fraktionierung der extrahierten Verbindungen in Alkyl-/Mutter-PAK und OPAK, Silylierung von Hydroxyl-/Carboxyl-PAK mit N,O-bis(trimethylsilyl)trifluoracetamid und GC/MS-basierte Quantifizierung der Ziel-Substanzen. Die Methode umfasste 34 Alkyl-/Mutter-PAK, 7 Carbonyl-OPAK und 19 Hydroxyl-/Carboxyl-OPAK. Ich wendete die Methode auf jeweils 11 Böden aus dem Angren-Industriegebiet (mit Kohlemine, Kraftwerk, Gummifabrik und Goldhütte) in Usbekistan und aus der dicht bevölkerten Hauptstadt der Slowakei, Bratislava, an.

Die Wiederfindungen von fünf Carbonyl-OPAK in Aufstockungs-Experimenten lagen zwischen 78 und 97% (relative Standardabweichung, RSD: 5-12%), während von 1,2-Acenaphthenchinon und 1,4-Naphthochinon 34-44% (RSD: 19-28%) wiedergefunden wurden. Von fünf Hydroxyl-/Carboxyl-OPAK wurden 36-70% (RSD: 13-46%) wiedergefunden, während andere Hydroxyl-/Carboxyl-OPAK Wiederfindungen <10% aufwiesen oder ganz verloren gingen. Mit der optimierten Methode bestimmte ich im Mittel 103% der Alkyl-/Mutter-PAK-Gehalte in einem zertifizierten Referenzmaterial.

Die ∑OPAK-Gehalte in Oberböden variierten zwischen 62 und 2692 ng g^{-1} und diejenigen der ∑Alkyl-/Mutter-PAK zwischen 842 und 244870 ng g^{-1}. Die Carbonyl-OPAK wiesen höhere Gehalte auf als die Hydroxyl-/Carboxyl-OPAK. Die häufigsten Carbonyl-OPAK waren konsistent 9-Fluorenon (9-FLO), 9,10-Anthrachinon (9,10-ANQ), 1-Indanon (1-INDA) und Benzo[a]-anthracen-7,12-dion (7,12-B(A)A) und das häufigsten Hydroxyl-/Carboxyl-OPAK war 2-Hydroxybenzaldehyd. Die Gehalte der Carbonyl-OPAK lagen häufig höher als diejenigen ihrer Mutter-PAK (z.B. 9-FLO/Fluoren >100 nahe der Gummifabrik in Angren). Die OPAK- und die PAK-Gehalte waren in der Nähe von Schadstoff-Punktquellen erhöht, was auf gemeinsame Quellen von OPAK und PAK schließen lässt. Lediglich für 1-INDA und 2-Biphenylcarboxaldehyd schienen andere Quellen als Verbrennungsprozesse zu dominieren.

Die Gehalte von OPAK und PAK waren in Oberböden höher als in Unterböden. Die höheren Unter- zu Oberboden-Gehaltsverhältnisse von Carbonyl-OPAK (0.41-0.82) als von ihren Mutter-PAK (0.41-0.63) in Usbekistan wiesen auf eine höhere Mobilität von OPAK als von PAK in Böden hin. Diese Vermutung wurde durch die durchgängig höheren Anteile der besser wasserlöslichen 9-FLO und 1-INDA auf Kosten der Anteile von 9,10-ANQ und 7,12-B(A)A an der ∑Carbonyl-OPAK-Gehalte im Oberboden und durch höhere OPAK/PAK-Gehaltsverhältnisse im Unter- als im Oberboden in Bratislava gestützt.

Mit dieser Dissertation trage ich eine geeignete Methode zur Bestimmung einer großen Zahl an OPAK und PAK in Böden bei. Meine Ergebnisse belegen, dass Carbonyl-PAK häufiger als Hydroxyl-/Carboxyl-PAK sind und das OPAK-Gehalte höher liegen können als diejenigen ihrer Mutter-PAK. Außerdem gibt es Hinweise darauf, dass OPAK in Böden mobiler sind als PAK. Die OPAK sollten daher in gesetzliche Umweltschutzregelungen aufgenommen werden.

A Summarizing overview

1 Introduction

Persistent organic pollutants (POPs) in soil are a threat to soil quality, because POPs might impair the health of soil organisms and plants, enter the food chain, and contaminate air and groundwater with negative effects on wildlife and humans. To this end, several national laws and international conventions have been enacted to protect soils from the effects of pollutants including POPs (Keith and Telliard, 1979; BBodSchV, 1999; COM, 2006). In addition, understanding the behavior of POPs in soil is critical to our overall understanding of the fate and global recycling of POPs in the environment (Meijer et al., 2002; Ockenden et al., 2003). The oxygen-containing derivatives and metabolites of polycyclic aromatic hydrocarbons (OPAHs) are produced together with polycyclic aromatic hydrocarbons (PAHs) from combustion sources or by post-emission photooxidation, chemical oxidation or biological degradation of PAHs (Lundstedt et al., 2007). However, the sources, concentrations, composition patterns and fate of OPAHs in soil are not well studied. Therefore, my thesis focuses on OPAHs in soils.

1.1 Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are a group of semi-volatile POPs with at least two fused benzene rings (Blumer, 1976). These compounds are known to be ubiquitous, persistent, toxic, bioaccumulative, long-range transported and accumulated even in remote regions of the world such as the poles. Polycyclic aromatic hydrocarbons are therefore included amongst the compounds that are regulated by both the USEPA and the EU (Keith and Telliard, 1979; Menzie et al., 1992; Masclet et al., 2000; Wenzl et al., 2006; Berrojalbiz et al., 2009; Hellou et al., 2009). The sources of these compounds are mainly combustion of fossil fuels and other organic materials with minor and location-specific sources from volcanic exhalations, diagenesis, and petroleum spillage and forest and prarie fires (Nikolaou et al., 1984; Baek et al., 1991; Lima et al., 2005). In the tropics, there are contributions of biological sources of naphthalene, phenanthrene and perylene related to the activities of termites and associated microorganisms (Wilcke et al., 2000; Bandowe et al., 2009).

Polycyclic aromatic hydrocarbons released by combustion activities into the atmosphere are deposited in soil by means of gas-soil exchange, dry and wet deposition or in association with falling litter from vegetation (Cousins et al., 1999a; Wilcke, 2000). Therefore, soil is a major sink for PAHs and other POPs. It is estimated that in the UK 90% of emitted PAHs are finally deposited in soils (Wild and Jones, 1995). Particularly for western

Europe, North America, and Japan, PAH concentrations, patterns and fate in soil have been intensively studied (Edwards, 1983; Sims and Overcash., 1983; Wilcke, 2000; Wilcke, 2007). Polycyclic aromatic hydrocarbon concentrations are generally higher at locations closer to point sources (industries, power plants, roads, waste deposition sites), in forest than cropped/grassland soils, and in urban than rural areas (Jones et al., 1989; Stalikas et al., 1997; Horstmann and McLachlan, 1998; Howsam et al., 2000; Krauss and Wilcke, 2003; Chrysikou et al., 2008). In urban areas, PAHs may originate from point sources and a diffuse background contamination because of domestic heating and vehicular transport (Johnsen et al., 2007) while remote regions receive PAHs mainly from long-range transport after repeated air-soil exchanges ("grass hopping", Hoyau et al., 1996; Wania and Mackay, 1996; Cousins et al., 1999a; Masclet et al., 2000; Gouin et al., 2004). After deposition to soils, PAHs mainly partition to soil organic matter and soot-like "black carbon" (Sims and Overcash, 1983; Chiou et al., 1998; Cornelissen et al., 2005). The strength of the sorption defined by the organic carbon-water partition coefficient (K_{OC}) is proportional to the hydrophobicity of a compound defined e.g., by its octanol-water partition coefficient (K_{OW}, Means et al., 1980; Karickhoff, 1981). Polycyclic aromatic hydrocarbons undergo a combination of processes including volatilisation, degradation, and transport (leaching, bioturbation) that determine their vertical distribution (Fig. A-1, Guggenberger et al., 1996; Pichler et al., 1996; Cousins et al., 1999b; Krauss et al., 2000; Moeckel et al., 2008).

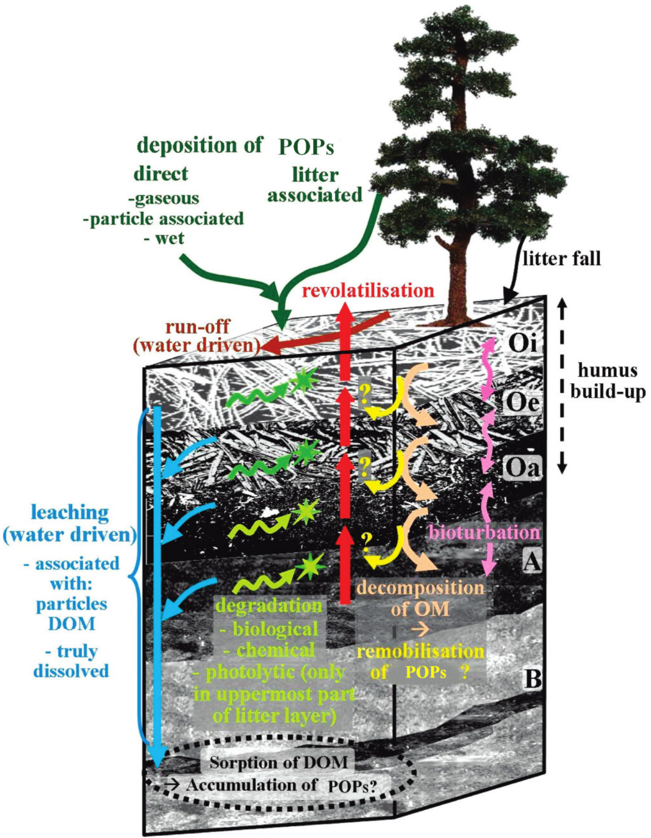

Figure A-1: Description of the main processes affecting the fate of POPs in soil (modified from Moeckel et al., 2008).

1.2 Oxygen-containing polycyclic aromatic hydrocarbons (OPAHs)

An emerging group of PAH-related pollutants which are little studied in soils are the oxygen-containing PAHs derivatives and metabolites (OPAHs). The OPAHs may contain functional groups like carbonyl, hydroxyl, carboxylic, coumarines, anhydrides and combinations of them (König et al., 1983; Lundstedt et al., 2007). These compounds have been identified as major constituents of atmospheric samples, coal, coal smoke, diesel emissions, wood smoke, ground water, sediments, and biological samples and as degradation products formed during bioremediation of PAH-contaminated sites and soil (Ramdahl, 1985; Oda et al., 1998; McKinney, 1999; Jakober et al., 2007; Lundstedt et al., 2007; Simoneit et al., 2007; Schlanges et al., 2008; Johnson-Restrepo et al., 2009).

Oxygen-containing PAHs are produced from combustions processes and released together with parent-PAHs (primary sources) or they are produced from post emission photooxidation, chemical oxidation, or microbial degradation of parent-PAHs (secondary sources, Nikolaou et al., 1984; Cerniglia, 1992; Kochany et al., 1994; Lundstedt et al., 2007).

Photooxidation of PAHs occurs in the environment as a result of strong absorption of UV radiation (300-420 nm) leading to rapid photolysis and formation of OPAHs (Fig. A-2, Nikolaou et al., 1984). Photooxidation reactions take place in the atmosphere, water bodies and in soils even though photodegradation in soils is slow and limited to the uppermost soil layer of a few mm (Nikolaou et al., 1984; Matsuzawa et al., 2001; Krauss and Wilcke, 2002).

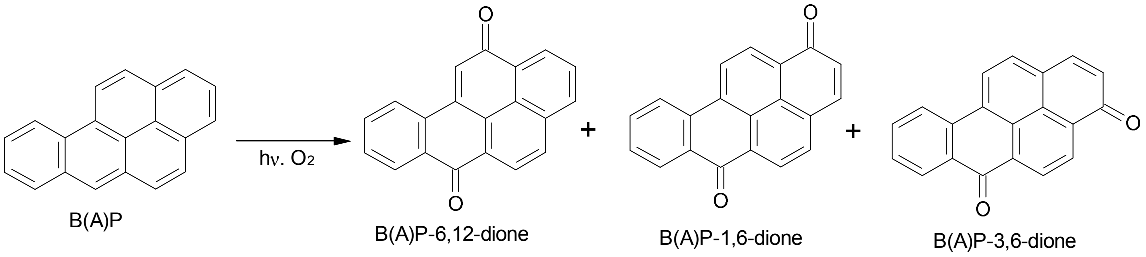

Figure A-2: Photoxidation of benzo[a]pyrene leading to formation of OPAHs (Nikolaou et al., 1984).

Chemical oxidation of alkyl/parent-PAHs in the environment with oxidants such as O_3, $^{\cdot}OH$, and NO_x can also lead to the formation of OPAHs (Atkinson and Arey, 1994; Perraudin et al., 2007; Lee and Lane, 2009). The concentrations of the oxidants depend on complex photochemical processes in the atmosphere. Hydroxyl radicals in soils may further be generated by the reactions of naturally present Fe in a Fenton-like mechanism as shown in Fig. A-3 to form $^{\cdot}OH$ (Jonsson et al., 2007).

Fenton reaction: $H_2O_2 + Fe^{2+} \rightarrow {}^{\cdot}OH + {}^{-}OH + Fe^{3+}$

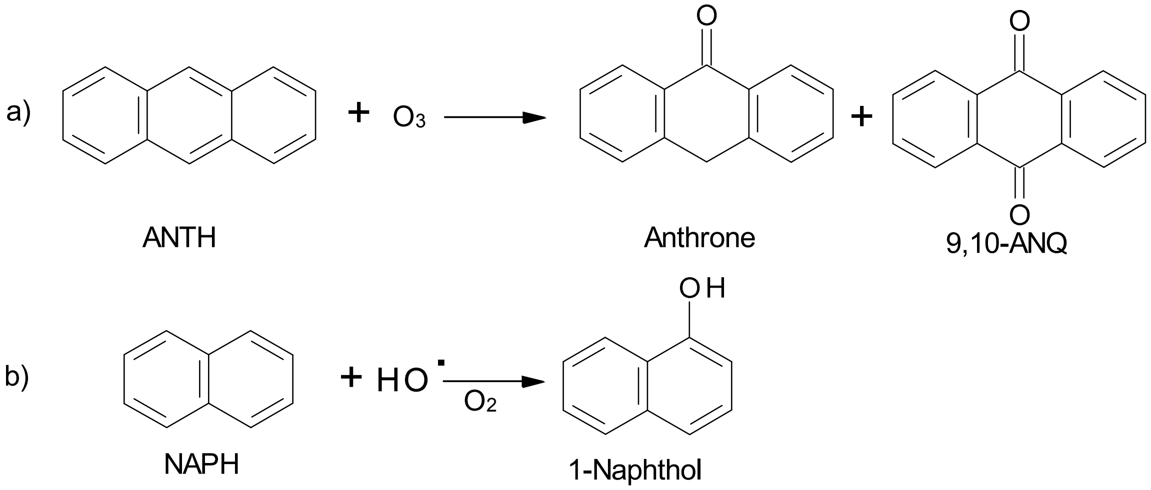

Figure A-3: Reaction of anthracene (ANTH) with O_3 (a) and naphthalene (NAPH) with ${}^{\cdot}OH$ (b) to form OPAHs (anthrone, 9,10-anthraquinone (9,10-ANQ), and 1-naphthol, Perraudin et al., 2007; Lee and Lane, 2009).

Microbial degradation of PAHs by bacteria and fungi both under aerobic and anaerobic conditions is another pathway of OPAH production in the environment (Fig. A-4, Cerniglia, 1992; Meckenstock et al., 2004).

Unlike the PAHs, national and international regulatory agencies do not yet include OPAHs in the list of compound groups that need to be monitored in soils. However, several properties and the reactivity of these compounds render them particularly relevant as emerging contaminants which warrant serious attention. Oxygen-containing PAHs can be more easily spread in the environment than parent-PAHs because of their higher water solubility. Batch experiments have shown that these compounds migrate vertically more easily than parent-PAHs (Weigand et al., 2002; Lundstedt et al., 2007). Weigand et al. (2002) postulated that the mobility of OPAHs compared to their parent-PAHs in soils increased in the order anthracene < anthraquinone < <2, 3-dihydroxynaphthalene < 3-hydroxy-2-naphthoic acid < 4-(3-hydroxy-(2)naphthyl)butyric acid. Weigand et al. (2002) and Novoszad et al. (2005) indicated, however, that the mobility of OPAHs in soils are also controlled by factors including anion sorption, ligand exchange, complexation with Al and Fe oxides, electrostatic potential and not only hydrophobic interactions as in the case of alkyl/parent-PAHs.

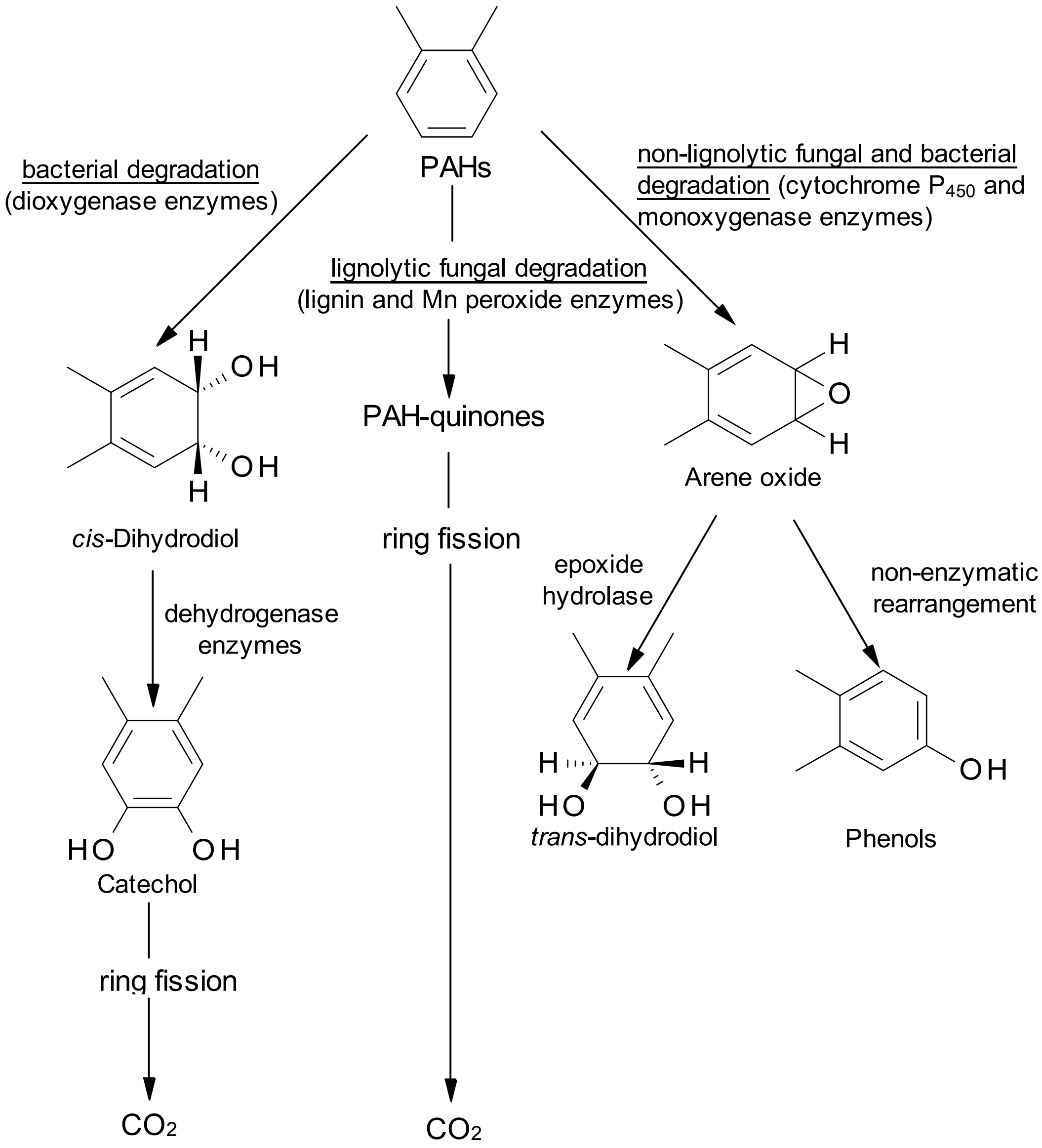

Figure A-4: Microbial degradation pathways of PAHs under aerobic conditions (Cerniglia, 1992).

The *toxicity* of OPAHs is another relevant reason to study them. The OPAHs are direct-acting mutagens compared to PAHs which require enzymatic activation to exhibit their toxicity; OPAHs also produce reactive oxygen species responsible for oxidative stress (Bolton et al., 2000; Xue and Warshawsky, 2005; Lemieux et al., 2008). The processes by which parent-PAHs get activated to exert their mutagenic and carcinogenic properties include the formation of electrophic epoxide using cytochrome P450 and epoxide hydrolase enzyme. The resulting electrophilic epoxide can form adducts with DNA. The other pathways involve dehydrogenation of the initially formed dihydrodiol to catechol using dihydrodiol dehydrogenase and the oxidation of catechols to form PAH-o-quinones. These o-quinones can form stable and damaging adducts with DNA. The processes of redox conversion between catechol and PAH-o-quinones generates reactive oxygen species (hydrogen peroxide, and superoxide ions that can can be responsible for DNA damage and cell toxicity. As the intermediates of PAH activation are OPAHs, OPAHs in environmental samples are expected to exibit higher and direct toxicity. This was already confirmed by studies showing that some OPAHs and OPAH-containing fractions of atmospheric samples exhibited higher toxicity to both microorganisms and human cells than PAHs (Alsberg et al., 1985; Wilson et al., 1996; Xia et al., 2004).

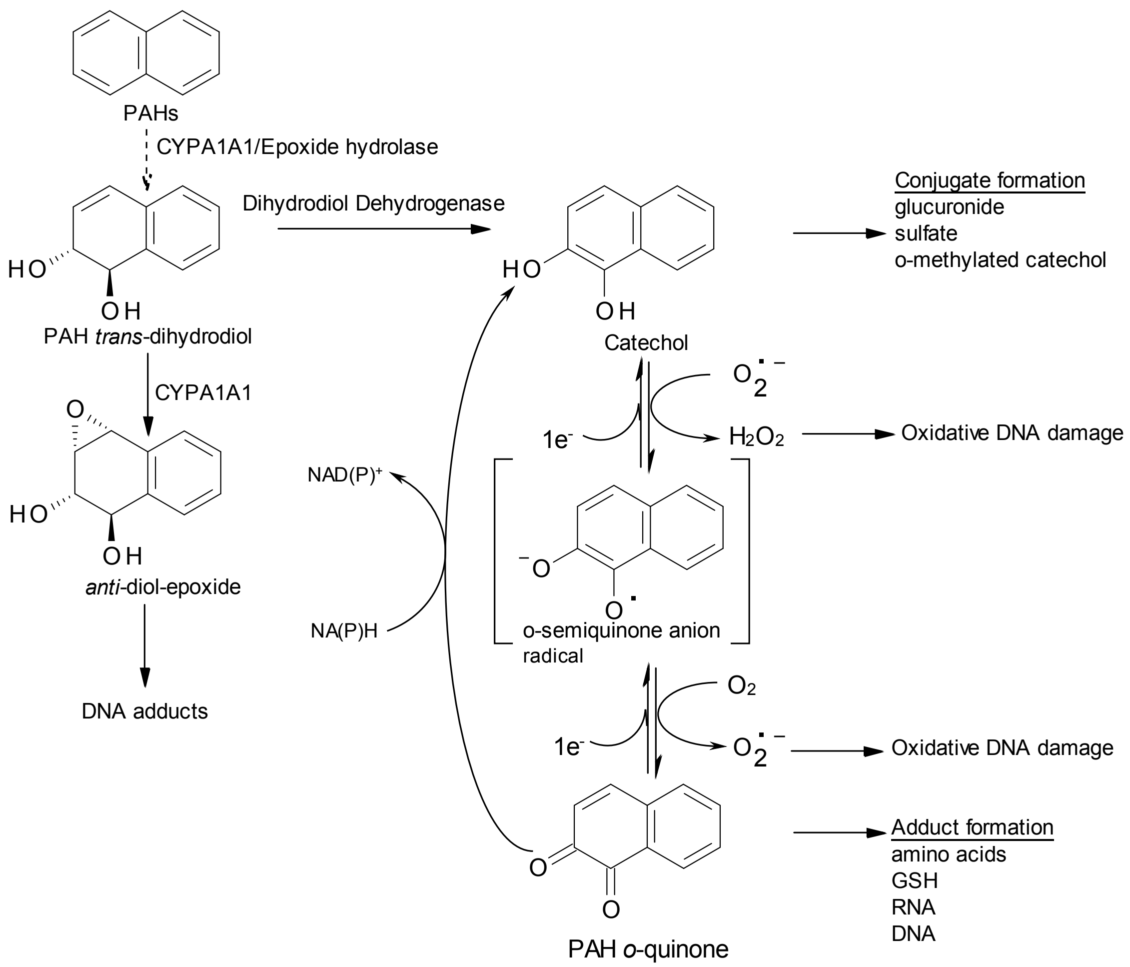

Figure A-5: Schematic representation of the mechanism of toxicity of OPAHs (Bolton et al., 2000).

Beyond risk assessment, the determination of OPAHs together with parent-PAHs in soils might be useful for source apportionment as well as for the study of natural attenuation of PAHs in soils (McKinney et al., 1999; Reineke et al., 2007).

The concentrations, composition of OPAH mixtures, sources (primary/secondary, biotic/abiotic), and vertical transport of OPAHs in soil remains up to now little known. However, it was shown that OPAH fractions significantly contribute to the toxicity of PAH-contaminated soils (Lemieux et al., 2007). Limited measurements in highly polluted soils have shown that soils polluted with PAHs also contain significant quantities of OPAHs and OPAH concentrations were sometimes higher than those of parent-PAHs (Lundstedt et al., 2007). There is a lack of standardized and validated procedures for the joint analysis of a number of OPAHs and PAHs. The available analytical methods to determine OPAH and PAH concentrations in environmental samples are based on extraction with soxhlet, by ultrasonication or with pressurized liquid extraction (PLE) followed by clean-up/fractionation using column chromatograpy/solid phase extraction (SPE) and separation/quantification with gas chromatography/mass spectrometry (GC/MS) or high pressure liquid chromatography-diode array detector (HPLC/DAD, Wischmann et al., 1996; Meyer et al., 1999; Lundstedt et al., 2006). The analyses were in most cases limited to PAHs and carbonyl-OPAHs. In the few studies in which hydroxyl/carboxyl-OPAHs were analyzed, the target compounds were quantified using HPLC/DAD (which has a high limit of detection, Meyer et al., 1999; Lundstedt et al., 2006). The analysis of OPAHs is more complicated and challenging than that of parent-PAHs. This is because OPAHs are more reactive, labile, polar, and unstable and subject to many more interferences originating in the soil matrix compared to the relatively less reactive parent-PAHs (Cho et al., 2004; Lintelmann et al., 2005). Frequently, several additional steps need to be included to analyze OPAHs (e.g, derivatization because of their low volatility and stability problems) if the established and widely available GC/MS is to be used for detection (Zink et al., 1995). Furthermore, there is a lack of reference materials, standards and labelled OPAHs to be used as internal standards in contrast to PAHs for which all is easily available (Lundstedt et al., 2006; Lundstedt et al., 2007).

From the state of knowledge, the following research questions arise:

1. Is it possible to develop a multi-compound method to determine a large group of PAHs, alkyl-PAHs and OPAHs of all functional groups (carbonyls, anhydrides, coumarines, hydroxyls and carboxylics) in one analytical run with sufficient accuracy, precision and limits of determination to be used for soils with different properties and pollution levels (background and unpolluted)?
2. How are the concentrations of OPAHs in soils relative to alkyl/parent-PAHs? Which OPAHs are most abundant? Can the composition of the OPAH mixtures be used for source apportionment?
3. What is the relative importance of primary (combustion) and secondary (photooxidation, chemical oxidation and microbial transformation) sources of OPAHs in soil?
4. Is the vertical transport of OPAHs in soils related to hydrophobicity? Can the octanol-water partition coefficient (K_{OW}) correctly predict OPAH vertical migration?

1.3 Objectives

Based on these research questions, the main objectives of my thesis included to:

1. modify and evaluate a multi-compound analytical protocol based on PLE, clean-up/column chromatographic separation and GC/MS for the determination a wide range of alkyl/parent-PAHs (34) and OPAHs of 2- and 3-ring PAHs covering a wide range of functional groups (carbonyl, hydroxyl/carboxyl and combinations) known for OPAHs **(Section B,** Bandowe and Wilcke., 2010**).**
2. apply the method developed in Section B to determine the spatial distribution of alkyl/parent-PAHs and OPAHs in soils along a linear 20 km-long transect in the Angren industrial region (Uzbekistan). In this study, I sought to explore the effect of the various industries (coal production, power plant, rubber production, gold refinery) on the spatial distribution of alkyl/parent-PAHs and OPAHs. Furthermore, I explored the correlations between alkyl/parent-PAHs, OPAHs and anthropogenically emitted heavy metals as indication of their possibly common emission sources. I tested the hypotheses that (i) at locations closer to the emission sources, OPAH concentrations are strongly correlated with those of alkyl/parent-

PAHs while at locations far from emission sources correlations are weaker and (ii) OPAHs will increasingly dominate in deeper layers because of their higher solubility in water compared to parent-PAHs **(Section C, Bandowe et al., in press)**.

3. further apply the method to soils from the densely populated city of Bratislava, the capital of Slovakia with 450,000 inhabitants at the border with Austria and Hungary. This was to represent soils in a typical urban environment. The specific aims were to (i) assess the state of contamination of soils from this geographically important city with OPAHs and alkyl/parent-PAHs, (ii) identify the sources of OPAHs (iii) assess the importance of OPAHs in soil relative to their corresponding parent-PAHs and (iv) to investigate into vertical transport of OPAHs in soils with the help of subsoil/topsoil concentration ratios and change in the composition of OPAH mixtures with increasing soil depth **(Section D)**.

4a. The fourth part of the thesis is placed in the appendix because it had a slightly different objective. It was known that naphthalene, phenanthrene and perylene can be produced biologically in the tropics by the activity of termites. However, there was not yet direct evidence of this. A microcosm experiment was therefore set-up in Brazil in which we held termites under controlled conditions and monitored all fluxes of PAHs and alkyl-PAHs **(Appendix E-1)**. The experimental set-up as well as the major findings of this study are in the attached published paper (**Appendix E-I, Bandowe et al., 2009**) and will not be further discussed in this thesis. A next step that could not yet be realized in my thesis is the search for precursors of the biological synthesis of naphthalene, phenanthrene and perylene in soils with the help of the new method developed in Section B.

4b. To broaden the number of POPs that can be determined by limited modifications of the analytical method described in Section B, a method including PLE, gel permeation chromatography (GPC), column chromatographic clean-up and detection with GC/NCI-MS and GC/EI-MS was optimized to measure polybrominated diphenyl ethers (PBDEs) – added as flame retardants to many products until recently. The optimized PLE-GPC-SPE-GC/NCI-MS method was then applied to the same soils as used in Section D **(Appendix E-2, Thorenz et al., 2010)**. The result of this work will not be further discussed in my thesis.

2 Materials and methods

2.1 Standards and chemicals

The target compounds included the OPAHs and PAHs shown in Tables B-2 and B-3 (Supplemental Information to Section B, p. 65) which were obtained from Dr. Ehrenstorfer (Augsburg, Germany), Ultra Scientific (North Kingston, RI, USA), Chiron (Trondheim, Norway), Acros Organics (New Jersey, USA), Sigma-Aldrich group (Steinheim, Germany), Cambridge Isotope Laboratories (CIL, Andover, MA, USA), and C/D/N Isotopes Inc. (Pointe-Claire, Quebec, Canada). All solvents were picograde from Promochem (Wesel, Germany). Diatomaceous earth was from Dionex (Sunnyvale, CA, USA). The derivatization reagent N,O-bis-(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane [BSTFA+TMCS (99:1)], silica gel 60 (70-230 mesh) and trifluoroacetic acid were from Sigma-Aldrich (Steinheim, Germany) and Na_2SO_4 (p.a, water-free) from Carl Roth (Karlsruhe, Germany). Silica-gel, Al oxide and Na_2SO_4 were heated at 300°C for 12 h. Deactivated silica gel (10%) was prepared by adding 0.3 g water to 2.7 g silica gel (cooled to room temperature) and mixed. Nitrogen (purity > 99.996%) used as purge gas in ASE-200 (Dionex, Sunnyvale, USA), He (purity > 99.9993) used as carrier gas in GC/MS were from Linde (Munich, Germany).

2.2 Study sites and sampling

For method development (**Section B**) soil samples (0-0.1 m) were collected from the Botanical garden of the University of Mainz, Germany and the Terrra firme region, 50 km from the Brazilian city of Manaus, Brazil. The third soil used for method development was a certified European Reference Material “ERM®-CC013a-Polycyclic Aromatic Hydrocarbons in Soil” (Federal Institute for Materials Research and Testing [BAM], Berlin, Germany). The latter soil was sampled from a former gasworks site in Berlin-Mariendorf, Germany. ERM®-CC013a is delivered with certified values for the 16 EPA-PAHs and was used to validate the PAH measurements. Certified OPAH concentrations were, however, not provided and there was no other reference material available for which certified OPAH concentrations were given.

In **Section C** of my thesis, the method developed in Section B was applied to soils from the Angren industrial region (Uzbekistan). The study area was located 114 km south of Tashkent, the capital of the Republic of Uzbekistan, central Asia. This region is a semi-arid mountain valley located along the Akhangaran River between 41°01’ and 40°58’ N and

70^o10’ and 69^o57’ E, at 900-950 m a.s.l. Soil samples were collected at two depths (0-0.1 m and 0.1-0.2 m) from 11 locations including sites near a coal mine, a power plant, a rubber factory and a gold mine along a 20-km transect downwind of the coal mine and adjacent power plant.

In **Section D**, nine soils with up to five mineral horizons and two additional surface horizons were sampled from different locations in Bratislava (Slovakia). All soil samples were air-dried and sieved to <2 mm, placed in aluminum containers and stored in a fridge at 4°C until analysis.

2.3 Physico-chemical characterization of soils

Soil type was determined according to IUSS Working Group WRB (2006). Soil texture was analyzed with the pipet method after organic matter, lime and pedogenic Fe oxides were destroyed and sand fractions removed by sieving. Soil pH was determined in 1 M KCl solution for the Manaus soil, in 0.01 M $CaCl_2$ (soil:solution ratio 1:2.5) for the Mainz and Bratislava soils and in water (soil:solution ratio 1:2) for the Angren soils. Effective cation-exchange capacity (ECEC) in Manaus, Mainz, Angren and Bratislava soils was the sum of the charge equivalents of Al, Ca, K, Mg, Mn, and Na measured by flame AAS (AA240FS, Varian Inco., Palo Alto, CA) after extraction with 1 M NH_4NO_3 (soil:solution ratio 1:25). Total C and N were measured with an elemental analyzer (Elementar vario EL, Elementar Analysensysteme GmbH, Hanau, Germany). Total organic C (C_{org}) in the Mainz soil was determined as the difference between total C and carbonate-C (measured in soil after destruction of C_{org} at 550°C for 15 h in a furnace). The C_{org} in the Angren soil was determined by oxidation with K-dichromate in H_2SO_4 without additional heating (Rowell, 1994) or with the elemental analyzer.

2.4 Calculations, compound properties and statistical analysis

The sum of the concentrations of all parent- and alkyl-PAHs is ∑34PAHs, all parent-PAHs alone ∑21PAHs, the 16 EPA-PAHs ∑EPA-PAHs, the combustion-derived PAHs [FLUA, PYR, B(A)A, CHRY, B(BJK), B(E)P, B(A)P, IND and B(GHI)] ∑COMB-PAH (Hwang et al., 2003), 1- and 2-methylnaphthalenes ∑C1-NAPH, 1- and 2-methylphenanthrenes ∑C1-PHEN and 3,6-, 3,5-, 2,6- and 1,7-dimethylphenanthrenes ∑C2-PHEN. The octanol-water partition coefficients (K_{OW}) of PAHs and alkyl-PAHs were taken from Neff et al. (2005) and Mackay et al. (2006), and those of OPAHs from Hansch et al.

(1995) or estimated with KOWWIN v1.67 EPI Suite^{TM} version 4.0 (USEPA: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm, verified on 05/08/2009). Statistical analysis was performed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Normality of the data was checked with the Kolmogorov-Smirnov test. Data were log-transformed to improve normality before correlation analysis.

2.5 Analysis of polycyclic aromatic hydrocarbons and their oxygen-containing derivatives and metabolites in soil (Section B)

I optimized a method to determine 34 alkyl/parent-PAHs, 7 carbonyl-OPAHs and 19 hydroxyl/carboxyl-OPAHs in a single analytical run. These compounds are known metabolic products if naphthalene, 1-methylnaphthalene, acenaphthylene, acenaphthene, fluorene, biphenyl, phenanthrene and anthracene (Bandowe and Wilcke, in press). I further tested the suitability of several deuterated-OPAHs (1-hydroxynaphthalene-D_7, transcinnamic acid-D_6, 1,4-naphthoquinone-D_6, benzophenone-2,3,4,5,6-D_5) as processing internal standards. The optimized method was based on pressurized liquid extraction (PLE), column chromatographic clean-up/separation (based on Bodzek et al., 1993) of target compounds into the alkyl/parent-PAHs and OPAHs fractions. The alkyl/parent-PAH fraction was measured with GC/EI-MS using seven deuterated PAHs spiked to the soil before extraction as internal standards. The OPAH fraction was spiked with FLUA-D_{10} split into two fractions of which one was measured directly using GC/MS to quantify carbonyl-OPAHs and the other fraction was derivatized using BSTFA+TMCS and measured with GC/MS to quantify hydroxyl/carboxyl-OPAHs. To test the performance of the method, I conducted a spiking experiment in which 20 g of the Manaus soil was thoroughly mixed with 4-6 g of diatomaceous earth and placed in the 33 ml ASE extraction cell. Into the soil-diatomaceous earth mixture, 100 µl of 20 µg ml^{-1} of carbonyl-OPAHs and hydroxyl/carboxyl-OPAHs were spiked leading to a concentration of 150 ng g^{-1} of each target compound. In addition, 1-hydroxynaphthalene-D_7, transcinnamic acid-D_6, 1,4-naphthoquinone-D_6, benzophenone-2,3,4,5,6-D_5 (2 µg each) and seven deuterated PAHs (250 ng as internal standard) were spiked to the mixture and allowed to stay for 12 h to equilibrate. Recoveries of all spiked OPAHs, deuterated OPAHs and PAHs, precision, accuracy, limits of detection (LOD). The accuracy of the PAH measurement was checked by measuring ERM$^{®}$-CC013a. A schematic description of the optimized procedure for analysis of PAHs and OPAHs in soil is shown in Figure A-6.

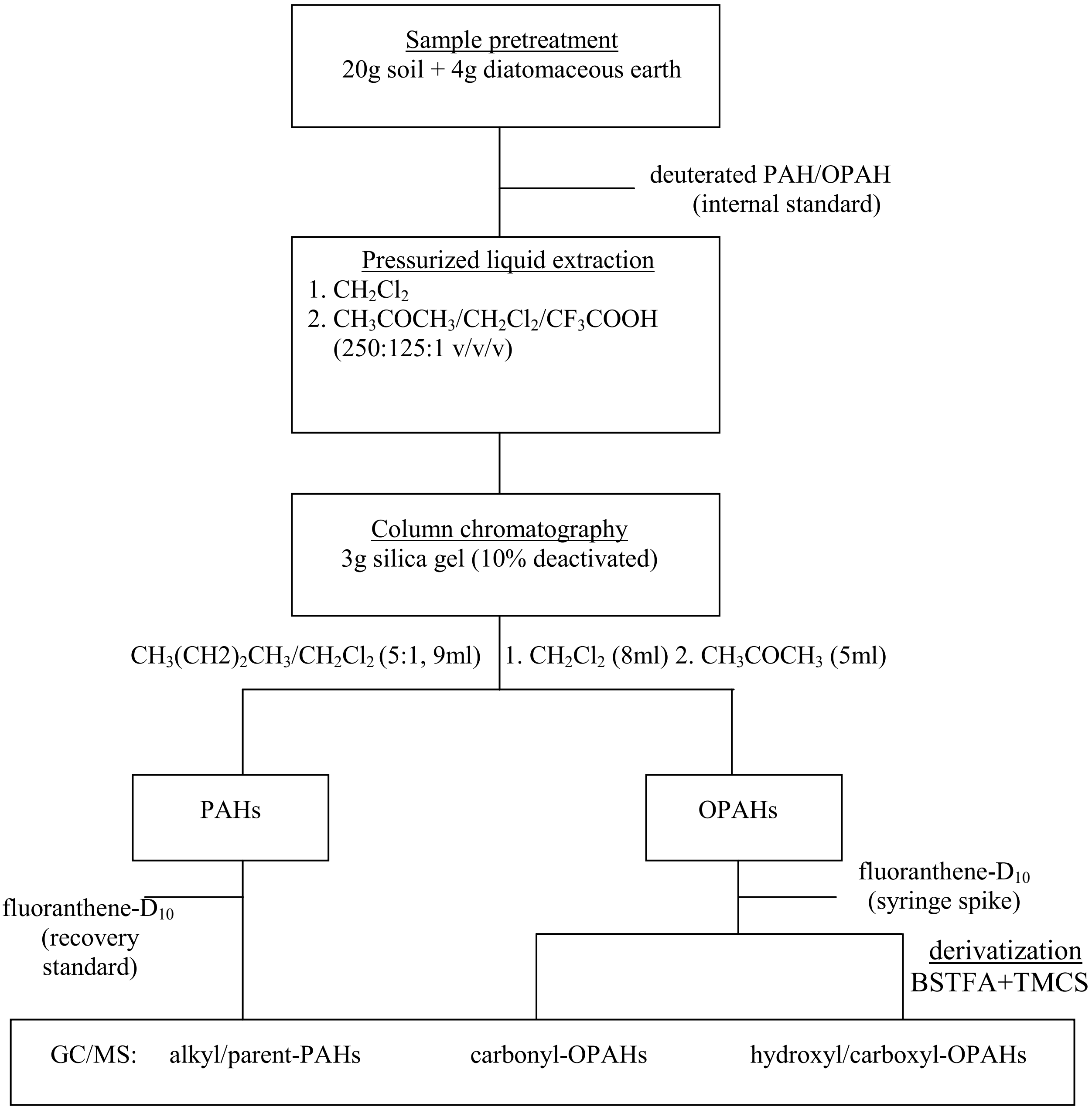

Figure A-6: Schematic description of the optimized analysis procedure for alkyl/parent-PAHs and OPAHs in soil.

2.6 Polycyclic aromatic hydrocarbons and their oxygen-containing derivatives and metabolites in soils from the Angren industrial Region, Uzbekistan (Section C)

The optimized method (Section B, Fig. A-6) was applied to soil samples from the Angren industrial region, to determine 31 alkyl/parent-PAHs, the seven carbonyl-OPAHs as in Section B and the five hydroxyl-OPAHs with recoveries from 36-70 (in spike experiment, Section, B). I calculated the percentage contribution of each individual carbonyl-OPAH and hydroxyl-OPAHs to their respective sums and individual alkyl/parent-PAH to their sum to investigate if their composition is related to different possible sources and if changes in the composition of the mixtures with depth can be used as indication of transport. To investigate into possible joint sources and air-soil organic matter partitioning I correlated the soil concentrations of individual and sums of selected compounds with each other and with those of C_{org} and heavy metals reported from this same site in an earlier study (Shukurov et al., 2009). I further calculated the concentration ratios of selected OPAHs to their parent-PAHs (1-INDA/FLUO, 1,4-NQ/NAPH, 1-NLD/1-MNAPH, 2-BPCD/PHEN, 9-FLO/FLUO, 1,2-ACQ/ACENY, 1,2-ACQ/ACENY and 9,10-ANQ/ANTH) in both topsoils and subsoils as indication of the importance of OPAHs relative to parent-PAHs, vertical transport of OPAHs and the effect of transformation processes. To test if the vertical distribution of OPAHs and PAHs was related to their transport by leaching, I calculated the concentration ratio [CR] defined as the concentration of a compound in the lower soil layer (0.1-0.2 m) to that in the upper soil layer (0-0.1 m). I then regressed the log CR on log K_{OW} (octanol-water partition coefficient) for all carbonyl-OPAHs and alkyl/parent-PAHs.

2.7 Oxygen-containing polycyclic aromatic hydrocarbons in urban soils of Bratislava, Slovakia: patterns, relation to PAHs and vertical distribution (Section D)

In **Section D,** the method developed in Section B was again applied to soil samples from Bratislava, Slovakia. Three new compounds not included in the method presented in Section B were added (BP, 1,8-NAA and 7,12-B(A)A). The addition of these compounds required a slight modification of the GC oven temperature program for carbonyl-OPAHs used in Section B. I correlated the log-transformed soil properties (texture, pH, C_{org} concentrations) and log transformed concentrations of individual alkyl/parent-PAHs, ∑34 alkyl/parent-PAHs, ∑COMB-PAHs, ∑carbonyl-OPAHs, ∑hydroxyl-OPAHs and individual OPAHs with each other in order to investigate into the influence of soil properties on concentrations of OPAHs and alkyl/parent-PAHs, air-organic matter partitioning, and possible common sources. I used the composition of the OPAH and alkyl/parent-PAH mixtures in both, topsoil and subsoil horizons as further indication of OPAH and PAH sources and vertical migration. OPAH/parent-PAH ratios were also calculated in topsoil and subsoil as indication of the importance of OPAHs relative to PAHs and subsoil:topsoil changes as indication of relative vertical migration. To test if the vertical distribution of OPAHs and alkyl/parent-PAHs was related to their transport by leaching, I again regressed the log CR of individual alkyl/parent-PAHs and OPAHs on their log K_{OW}.

3 Results and Discussion

3.1 Analysis of polycyclic aromatic hydrocarbons and their oxygen-containing derivatives and metabolites in soils (Section B)

There is a lack of validated methods for the joint analysis of alkyl/parent-PAHs and OPAHs in soil. In Section B, a method based on PLE, silica gel clean-up and fractionation, derivatization and GC/MS quantification of target compounds was optimized and validated to quantify 34 alkyl/parent-PAHs, nine carbonyl-OPAHs and 19 hydroxyl/carboxyl-OPAHs. Mean recoveries of seven of the nine target carbonyl-OPAHs ranged from 78 to 97% (RSDs, 5-12%) but two quinones, 1,2-acenaphthenequinone and 1,4-naphthoquinone, showed lower recoveries (34-44%, RSDs 19-28%). Five hydroxyl-OPAHs had recoveries between 36-70% (RSD, 13-46%), six others had between 2-7% (RSD, 8-25%) and eight were completely lost in sample preparation. The accuracy of my parent-PAH determination was proven by the measurement of the European Reference Material (ERM®-CC013a, for which certified PAH concentrations are provided). My analysis recovered 103% (n =3, RSD, 6%) of the certified sum of 16 PAH concentrations in the reference soil material. Limits of detection ranged between 0.1-1.6 ng g^{-1} for OPAHs and 0.01-0.56 ng g^{-1} for PAHs. The protocol was applied to soils from a former gasworks site in Berlin and an urban soil from Mainz, both in Germany, and a forest soil from near Manaus, Brazil. The sums of 34 alkyl/parent-PAH concentrations were 107000, 4490, and 38 ng g^{-1}, those of seven carbonyl-OPAHs were 15690, 170, and 7 ng g^{-1} and those of eleven hydroxyl/carboxyl-OPAHs 518, 36, and 16 ng/g for Berlin, Mainz, and Manaus soils, respectively. The concentration of ∑21 parent-PAHs was very similar to that measured in the same soil by a different method confirming the accuracy of our new method (16-38 ng g^{-1}, Krauss et al., 2005). The concentrations of OPAHs followed a similar trend like that of alkyl/parent-PAHs indicating that the same anthropogenic source responsible for alkyl/parent-PAH contamination is responsible for OPAHs. The most abundant carbonyl-OPAHs were 9,10-ANQ, 9-FLO and hydroxyl-OPAHs were quantitatively dominated by SALYD. Several OPAHs (frequently: 9,10-ANQ and 9-FLO) had concentrations higher than their parent-PAHs showing the importance of including OPAHs in monitoring programs.

3.2 Polycyclic aromatic hydrocarbons and their oxygen-containing derivatives and metabolites in soils from the Angren industrial area, Uzbekistan (Section C)

I applied the optimized and validated method (Section B) to measure the concentrations of 31 parent/alkyl-PAHs and 12 highly recovered OPAHs in soils sampled a 20-km transect in 2-km intervals at the depths of 0-0.1 and 0.1-0.2 m in the Angren industrial region near Tashkent, Uzbekistan in order to study the concentrations, spatial variation, sources, composition of the compound mixtures and vertical distribution of OPAHs and PAHs. The ∑31 alkyl/parent-PAHs concentrations in the 0-0.1 m depth ranged from 118 to 5913 ng g^{-1}. The ∑31 alkyl/parent-PAHs concentrations in the 0.1-0.2 m depth was lower than in the topsoil and ranged from 104 to 3852 ng g^{-1}. The composition of the alkyl/parent-PAH mixtures in topsoils was dominated by NAPH (9-35%, mean: 17%), PHEN (9-18%, 15%), and B(BJK) (5-15%, 10%). The ∑7 carbonyl–OPAHs concentrations ranged from 54 to 1848 ng g^{-1}. The ∑7 carbonyl-OPAHs concentrations were consistently higher in topsoil than in subsoil where they ranged from 29 to 595 ng g^{-1}. The carbonyl-OPAHs mixtures were dominated by 9-FLO (mean contribution to the ∑7 carbonyl-OPAHs concentrations in topsoil: 47%/subsoil: 41%) and 9,10-ANQ (38%/41%); 1-INDA contributed on average 10% to the ∑7 carbonyl-OPAHs concentrations in topsoil and subsoil. The ∑5 hydroxyl-OPAHs concentrations ranged from 8 to 63 ng g^{-1} in topsoil and from 7 to 23 ng g^{-1} in subsoil. The mixture of hydroxyl-OPAHs was dominated by SALYD with mean contributions to the ∑5 hydroxyl-OPAHs concentrations of 65% in topsoil and 64% in subsoil. The highest concentrations of alkyl/parent-PAHs and OPAHs occurred in areas near industrial emitters (coal mine, power plant, rubber factory). Thus, the alkyl/parent-PAHs and OPAHs originated from coal combustion (power plant), industrial emissions (rubber factory) and coal dust from coal mining activities. This is in line with other findings that coal dust contains high PAHs concentrations, and combustion is a known source of PAHs and OPAHs (Oda et al., 1998; Simoneit et al., 2007; Achten and Hofmann, 2009).

There was a strong positive correlation of the concentrations of ∑7 carbonyl-OPAHs with those of the ∑31 alkyl/parent-PAHs ($r = 0.98$, $p < 0.01$), the ∑5 hydroxyl-OPAHs ($r = 0.64$, $p < 0.05$). The ∑5 hydroxyl-OPAHs also significantly correlated with ∑31 alkyl/parent-PAHs ($r = 0.72$, $p < 0.05$). Both ∑7 carbonyl-OPAHs and ∑31 alkyl/parent-PAHs further significantly correlated with industrially emitted heavy metals (Ba, Pb, Th, U and Zn; Shukurov et al., 2009, $0.70 \leq r \leq 0.82$, $0.01 \leq p \leq 0.05$). Most individual alkyl/parent-PAHs and carbonyl-OPAHs correlated significantly with each other in the topsoil except PERY, RETENE, INDA and 2-BPCD, while only 1-INDA and SALYD correlated significantly with C_{org}. The above cited correlations all suggest that most of the OPAHs and parent/alkyl-PAHs originate from joint sources (combustion and coal dust). In contrast, PERY, RETENE, 1-INDA and 2-BPCD had other sources such as e.g., biological transformation, photoxidation or chemical oxidation (Sims and Overcash, 1983; Venkatesan, 1988; Wang et al., 1995; Casellas et al., 1997).

At several sampling sites, the ratios of OPAHs to their parent-PAHs (1-INDA/FLUO, 9-FLO/FLUO, 1,2-ACQ/ACENY, 1,2-ACQ/ACEN, 9,10-ANQ/ANTH) were > 1 in both topsoils and subsoils underlining the importance of OPAHs measurements. In topsoils, the ratio of 1-INDA/FLUO was 0.3-12 (mean: 4), that of 9-FLO/FLUO 13-126 (mean: 31) and of 9,10-ANQ/ANTH 13-44 (mean: 29). The ratios 1,4-NQ/NAPH, 1-NLD/1-MNAPH and 2-BPCD/PHEN were consistently < 1. The OPAH/parent-PAH ratios were generally higher in topsoils closer to an emission source, which is attributable to the higher contribution of industrial emissions containing more OPAHs relative to PAHs, and a higher deposition of the OPAHs closer to their emission source because of stronger association with atmospheric particles and thus, shorter travel distances while post-depositional microbial transformation was minimal.

Vertical migration was investigated by comparing concentration ratios (CR: concentration of a compound in the subsoil divided by the concentration of the same compound in topsoil). The mean CR (for all sites) was mostly < 1 ranging from 0.43-0.83 and 0.41-0.82 for individual PAHs and carbonyl-OPAHs, respectively, indicating that both groups of compounds were mostly accumulated in topsoil after deposition from the atmosphere because of strong sorption to organic matter. The OPAHs/parent-PAH ratios were not consistently higher in subsoils than topsoils. The CRs of carbonyl-OPAHs (log K_{OW}; 1.71-3.58) were only marginally higher (0.41-0.82) than those of the low molecular weight-PAHs NAPH, 1-MNAPH, ACENY, ACEN, FLUO, PHEN, ANTH (0.41-0.63) in spite of similarly log K_{OW} (3.3-4.5). This is attributable to a more complex interaction of carbonyl-OPAHs with soil constituents (hydrophobic interaction, anion sorption, ligand exchange and complexation with Al and Fe oxides (Weigand et al., 2002; Novoszad et al., 2005). The CRs of both, PAHs and carbonyl-OPAHs were negatively correlated with K_{OW} at the site closest to the power plant, r = -0.42 and -0.77, respectively, showing that leaching played only at this site a significant role in vertical transport (Guggenberger et al., 1996).

3.3 Oxygen-containing polycyclic aromatic hydrocarbons in urban soils of Bratislava, Slovakia: patterns, relation to PAHs and vertical distribution (Section D)

The concentrations of ∑14 OPAHs in the surface soil horizons of 10 of the 11 sampling sites ranged from 88 to 509 ng g^{-1} which is similar to those measured in other cities such as Mainz, Germany (mean of 206 ng g^{-1} of the ∑18 OPAHs) and Basel, Switzerland (29-440 ng g^{-1} of the Σ4 OPAHs) but higher than near Manaus, Brazil (23 ng g of the ∑18 OPAH) (Niederer, 1998; Bandowe and Wilcke, in press). The highest ∑18 OPAHs concentrations of 2692 ng g^{-1} for the Σ14 OPAHs) occurred near a chemical waste dump and was similar to 1938 ng g^{-1} measured near the Angren power plant (Fig. C-2) but lower than in soils from other industrial sites in Sweden and Germany (Meyer et al., 1999; Lundstedt et al., 2007). The ∑34 PAHs concentration ranged from 842 to 9842 ng g^{-1} except at the chemical waste dump which contained a much higher ∑34 alkyl/parent-PAH concentration of 244870 ng g^{-1}. Although ten of the study soils could be classified as contaminated or highly contaminated, the PAH concentrations in Bratislava soils were comparable to those in other urban areas of Europe and the USA (Tab. D-4). The site near the abandoned waste dump, areas near a former chemical factory which was destroyed during World War II, housing gardens and an anti-flooding embankment were the highest polluted areas while current or former agricultural soils even when close to roads showed a low level of PAH contamination. The higher polluted areas where dominated by combustion-derived PAHs (>50%) except the anti-flooding embankment which was dominated by NAPH and PHEN, likely attributable to pollution from petrogenic sources which reached the soil via inundations with oil-containing river water. The concentrations of ∑carbonyl-OPAHs ($r = 0.92$, $p < 0.001$) and ∑hydroxyl-OPAHs ($r = 0.73$, $p = 0.01$) correlated significantly with those of ∑34 alkyl/parent-PAHs showing that they originated from the same sources. The OPAH mixtures were dominated by 1-INDA, 9-FLO and 9,10-ANQ while a few highly polluted areas (e.g., near an abandoned waste dump) were dominated by 7,12-B(A)A. The coincidence between the occurrence of the COMB-PAHs (contributing 98% to the Σ31 alkyl/parent-PAHs) and 7,12-B(A)A indicate that soils polluted by combustion sources may contain high concentrations of high molecular weight OPAHs. Again, 1-INDA, 9-FLO and 9,10-ANQ were frequently much higher concentrated than their parent compounds FLUO and ANTH.

The OPAH and alkyl/parent-PAH concentrations were higher in topsoil than subsoil because of deposition from the atmosphere and strong sorption to soil organic matter. The concentration ratios of OPAHs to parent-PAHs increased with increasing depth at several sites,and the contribution of 1-INDA and 9,10-ANQ to ∑carbonyl-OPAHs increased with depth at the expense of the contributions of the more hydrophobic 7,12-B(A)A. This provided evidence of faster vertical mobility of OPAHs than parent-PAHs (because of higher water solubility of OPAHs) and also suggested that a dominant transport mode of OPAHs was by leaching as truly dissolved solute. This was further backed by the significant negative correlation of subsoil:surface soil concentration ratios with K_{OW} at several sites.

3.4 Concentrations, composition of the mixtures, sources and vertical transport of OPAHs in soils

Results obtained in sections 3.1-3.3 and taken from the literature (Tab. A-1) show that soils contaminated by alkyl/parent-PAHs also contain significant concentrations of OPAHs, especially carbonyl-OPAHs. The concentrations of OPAHs and alkyl/parent-PAHs in soils from industrial sites (gasworks sites, coal power plants, coal mines, waste dumps) and urban areas are higher than at remote sites. This indicates that OPAH concentrations in soil follow the same known land use-related spatial distribution pattern as those of alkyl/parent-PAHs. The most abundant OPAHs in soils were 1-INDA, 9-FLO, 9,10-ANQ, SALYD and 7,12-B(A)A. The two compounds, 9-FLO and 9,10-ANQ are frequently the most abundant OPAHs in atmospheric samples (Albinet et al., 2007; Tsapakis et al., 2007). The concentrations of 9-FLO and 9,10-ANQ in soils were frequently higher than the concentrations of their legislatively regulated and often measured parent-PAHs (e.g., 9-FLO/FLUO = 126 and 9,10-ANQ/ANTH = 44 at different sites in Angren, Tab. C-4). This illustrates the need to include OPAHs in monitoring programs because of their potentially higher toxicity and ability to spread and pollute groundwater (Schlanges et al., 2008)

Correlation analysis shows that OPAH concentrations in soils are strongly correlated with those of alkyl/parent-PAHs (Fig. A-7) suggesting that OPAHs in soils - like the alkyl/parent-PAHs - are mainly the result of primary sources including combustion and industrial processes. Other evidence supporting direct emission of OPAHs is the strong positive correlation of carbonyl-OPAH concentrations with those of trace metals (Zn, Ba, Pb, Th and U) emitted by industrial activities in the Angren industrial region.

Table A-1: Comparison of OPAH and alkyl/parent-PAH concentrations [ng g^{-1}] of OPAH and alkyl/parent-PAHs measured in my thesis with those from other sites taken from the literature.

Region	∑OPAHs	∑alkyl/parent-PAHs	Reference
Manaus, Brazil (rural area)	16±4	38±13	Bandowe and Wilcke, 2010 (this thesis, Section B).
Mainz, Germany (urban site)	196±42	4490±1159	
Berlin, Germany (gasworks site)	16064±1871	106454±6095	
Angren, Uzbekistan (agricultural to industrial sites)	62-1893	118-5913	This thesis, Section C
Bratislava, Slovakia (urban site)	88-2692	842-244870	This thesis, Section D
Basel, Switzerland (urban Site)	29-440	11100±11900	Niederer (1995, 1998)
North Germany (wood processing plant)	58000	142000	Meyer et al. (1999)
Husarviken, Sweden (gasworks site)	427000	2779000	Lundstedt et al. (2007))
Northeast France (coking plant sites)	113120-543360	1378320-1435520	Biache et al. (2008)

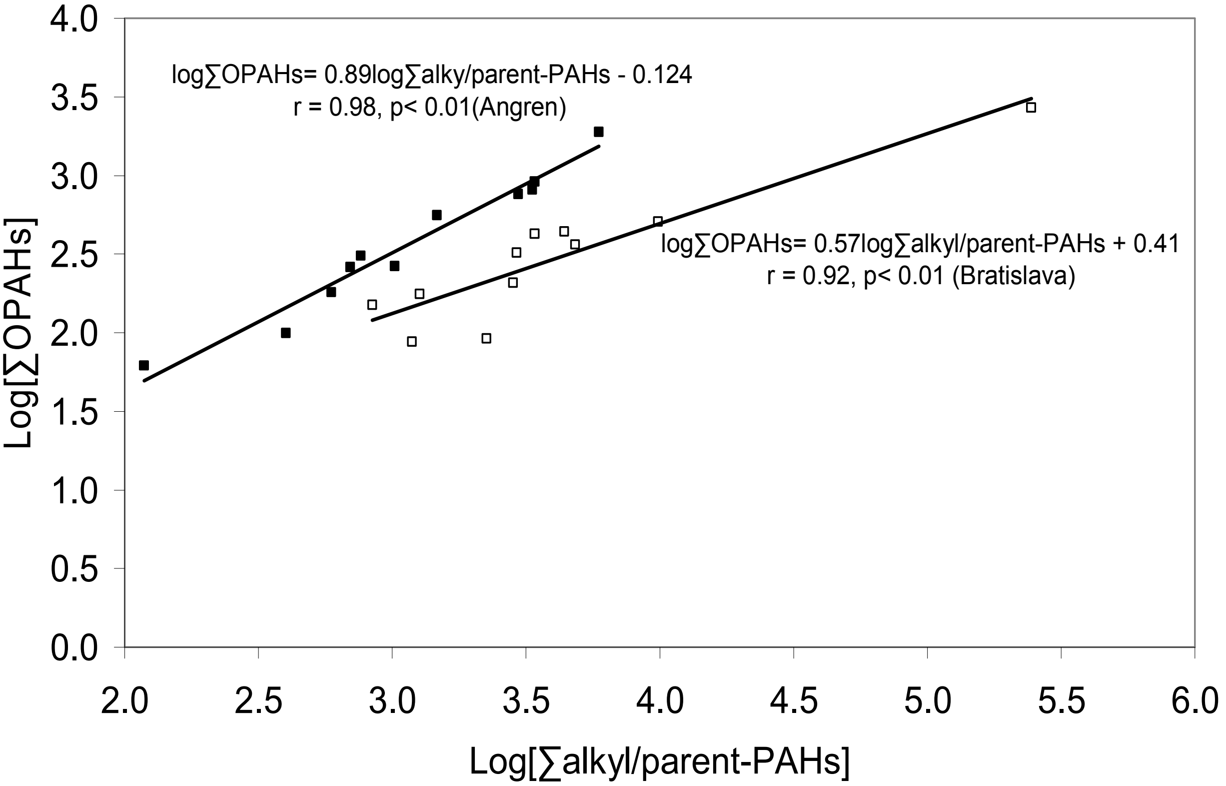

Figure A-7: Relation between ∑OPAHs and ∑alkyl/parent-PAHs concentrations in Angren surface soils (closed boxes) and Bratislava surface soils (open boxes).

Most individual carbonyl-OPAH concentrations were significantly correlated with those of individual alkyl/parent-PAHs because of their common origin except for 1-INDA and 2-BPCD. This I interprete as indication that 2-BPCD is of a secondary source while 1-INDA had a homogeneous atmospheric concentration produced by an unknown source and was partitioned from the atmosphere to soil organic matter as illustrated by the significant correlation between the 1-INDA and C_{org} concentrations.

Evidence of faster vertical transport of carbonyl-OPAHs by leaching could be deduced from the vertical distribution of OPAHs in both the Angren and Bratislava soils. Therefore, OPAHs might be a bigger threat of groundwater than the less mobile parent-PAHs. This again calls for including OPAHs in regular monitoren programs together with PAHs.

3.5 Quality control and error discussion

I am aware that the organic trace analysis in complicated environmental matrices like soil is potentially subject to contamination from several sources as well as errors during sample processing. Because of this, several quality control and assurance steps were taken from sampling until analysis to reduce the potential for contamination and analytical errors and to produce reliable results. All soil samples were stored in closed and cleaned aluminum containers. The samples were then air-dried in a separate storage room (at room temperature) away from all laboratory activity to avoid contamination. After drying and sieving to < 2mm), samples were returned into closed aluminum containers and stored in fridges at 4°C until analysis. All used glassware was rinsed with acetone, machine-washed and baked at 250°C overnight. Before use, all glassware was rinsed with picograde acetone or toluene. All solvents used for extractions, sample and standards preparations were of the picograde-for-residue-analyses quality (Promochem, Wesel, Germany).

I furthermore included several blanks to track and correct for contamination. The blanks were made of diatomaceous earth filled in the ASE extraction cell and passed through the entire analysis process from extraction until GC/MS measurement. The blanks were extracted and analyzed at the beginning, in the middle and at the end of each batch of 10 soil samples. The mean amount of target compounds measured in blanks was subtracted from each sample to correct for background contamination.

To avoid false identifications during GC/MS measurements, a minimum of two ions was monitored in my SIM measurements and a compound was only accepted as identified in the sample or blank if the ratio of the monitored ions was within ± 20% of that measured for the same compound in the calibration standards and also the difference in retention time was < 0.2 min for OPAHs and < 0.1 min for alkyl/parent-PAHs. I selected the molecular ion as one of the monitored ions for OPAHs and also avoided the selection of ions originating from the derivatization reagent (e.g., m/z = 73) during analysis of hydroxyl/carboxyl-OPAHs even if it was the base peak (most intense peak) to avoid false identifications.

To quantify alkyl/parent-PAHs, I spiked seven deuterated-PAHs (2-6 rings) to the samples prior to extraction, and for carbonyl-OPAHs, benzophenone-D_7 which were all added to the sample prior to extraction and used for internal quantification thereby automatically correcting for losses of target compound during sample processing leading to accurate quantification. However, for hydroxyl-OPAHs a suitable processing internal standard could not be found and therefore fluoranthene-D_{10} was used as syringe spike. The five hydroxyl-OPAHs with recoveries higher > 36% were subsequently analyzed in Section C and D.

In all batches of soil samples, I also included several replicates (n = 3) of the certified European Reference Material “ERM®-CC013a-Polycyclic Aromatic Hydrocarbons in Soil” (Federal Institute for Materials Research and Testing [BAM], Berlin, Germany) to check for the accuracy of PAH analysis. The recovery of the sum of PAHs for which certified and indicator values was provided ranged from 86-110%.

The precision of the determination of individual carbonyl-OPAH ranged from 5 to 28 % (n= 8) and of hydroxyl-OPAHs from 8 to 46% (n = 8) in spiked samples, and for individual parent/alkyl-PAHs from 0.2 to 13%, n = 3 in ERM®-CC013a. Limits of detection (Tab. B-1, B-2) of both, OPAHs (0.1-1.6 ng g^{-1}) and alkyl/parent-PAHs were low (0.01-0.56) allowing quantification in background soils.

4 General conclusions

A method based on pressurized liquid extraction, column chromatographic clean up with silica gel and fractionation during elution from the columns and derivatization with BSTFA+TMCS of part of the column eluate was optimized and fully evaluated for the measurement of 34 parent/alkyl-PAHs, nine carbonyl-OPAHs and five hydroxyl-OPAHs. The final method showed high recovery (78-97%) and precision (RSDs 5-12%) for all carbonyl-OPAHs except 1,4-napthoquinone and 1,2-acenaphthenequinone (34-44, RSD, 19-28%), five hydroxyl-OPAHs also showed lower recoveries 36-70% (RSD, 13-46) while others showed recoveries < 10%. The accuracy of alkyl/parent-PAH analysis was confirmed by the recovery of 103% of the certified concentration of the sum of PAHs from a certified reference material. Limits of detection of OPAHs (0.1-1.6 ng g^{-1}) and alkyl/parent-PAHs (0.01-0.56 ng g^{-1}) were low allowing quantification in background soils.

The concentrations of ∑carbonyl-OPAHs ($0.92 \leq r \leq 0.98$, $0.05 \geq p \geq 0.001$) and ∑hydroxyl-OPAHs ($0.72 \leq r \leq 0.81$ and $0.05 \geq p \leq 0.01$) correlated closely with those of ∑alkyl/parent-PAHs and with anthropogenically emitted trace metals at Angren indicating that OPAHs mainly originated from the same direct anthropogenic sources as their parent-PAHs in the studied samples (mainly from urban and industrialized areas). Individual parent/alkyl-PAHs and OPAHs positively correlated with each other and the sum of concentrations of selected compounds except PERY, RETENE, 1-INDA, 2-BPCD suggesting that only PERY, RETENE, 1-INDA and 2-BPCD originated from different sources such as biological or inorganic chemical transformations. The 1-INDA further correlated with C_{org} showing that it existed mainly in gaseous phase in equilibrium with soil organic matter and may have been long-range transported.

Carbonyl-OPAHs had generally higher concentrations in all soils than hydroxyl-OPAHs and were dominated by 9,10-ANQ, 9-FLO, 1-INDA, 7,12-B(A)A while the hydroxyl-OPAHs were dominated by SALYD, which however is a metabolite not only of PAH degradation but also of the degradation of other aromatic constituents of soil organic matter.

Concentrations of OPAHs (1-INDA, 9-FLO, 9,10-ANQ) were frequently higher than their parent-PAHs: FLUO and ANTH illustrating the need to include OPAHs in monitoring programs because of their toxicity and higher tendency to pollute groundwater.

Alkyl/parent-PAH and OPAH concentrations were generally much higher in topsoils than in subsoils because of the deposition of these compounds from the atmosphere and the scavenging effect of soil organic matter which is usually accumulated in the topsoil.

Evidence of faster vertical transport of OPAHs than their parent-PAHs in field soils was given by higher OPAH/parent-PAHs concentration ratios in subsoil than topsoil, increasing contributions of less hydrophobic compounds to the sum of OPAH concentrations in subsoil and a significant negative correlation of subsoil/topsoil concentration ratios with the respective octanol-water partition coefficients of the same compounds in some soils. This also indicates that the major transport mechanism of OPAHs is transport as truly dissolved solute.

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B Analysis of polycyclic aromatic hydrocarbons and their oxygen-containing derivatives and metabolites in soils[a]

Benjamin A. Musa Bandowe[1] and Wolfgang Wilcke[2]

[1] Earth System Science Research Center, Geographic Institute, Professorship of Soil Geography/Soil Science, Johannes Gutenberg University Mainz, Johann-Joachim-Becher-Weg 21, 55128 Mainz, Germany.

[2] Geographic Institute, University of Berne, Hallerstrasse 12, 3012 Berne, Switzerland.

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1 Abstract

Although polycyclic aromatic hydrocarbons (PAHs) have been extensively studied, the knowledge of their oxygen-containing derivatives and metabolites (OPAHs) in soils is limited. We modified and tested an existing analytical protocol involving pressurized liquid extraction of soil followed by fractionation of target compounds into PAHs and OPAHs on a silica gel column and GC/MS-based separation and quantification. Polycyclic aromatic hydrocarbons and carbonyl-OPAHs were quantified directly after separation on silica gel columns while hydroxyl/carboxyl-OPAHs were quantified after silylation with N,O-bis(trimethylsilyl)trifluoroacetamide. Recoveries between 78 and 97% (relative standard deviation [RSD], 5-12%) were obtained for six carbonyl-OPAHs, whereas 1,2-acenaphthenequinone and 1,4-naphthoquinone showed lower recoveries of 34 and 44% (RSD, of 19 and 28%, respectively). Five hydroxyl/carboxyl-OPAHs had recoveries between 36 and 70% (RSD, 13-46%), six others had between 2 and 7% (RSD, 8-25%), and nine were lost in sample preparation. Limits of detection ranged from 0.1 to 1.6 ng g^{-1} for OPAHs and from 0.01 to 0.56 ng g^{-1} for PAHs. The protocol was applied to soils from a former gasworks site, Berlin, an urban soil from Mainz, both in Germany, and a forest soil from near Manaus, Brazil. The sums of 34 PAH concentrations were 107000, 3505, and 21 ng g^{-1}; those of seven carbonyl-OPAHs were 15690, 170, and 7 ng g^{-1}, and those of eleven hydroxyl/carboxyl-OPAHs 518, 36 and 16 ng g^{-1} for Berlin, Mainz, and Manaus soils, respectively. Several OPAHs were present at concentrations higher than or equal to their parent-PAHs demonstrating the importance of OPAH measurement for the assessment of PAH-related environmental risks.

2 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of frequently monitored persistent organic pollutants included in the USEPA list of priority pollutants (Keith and Telliard, 1979). Combustion of fossil fuels is the main source of anthropogenic PAHs contamination in the industrialized temperate environment (Baek et al., 1991). Most of the PAHs released from combustion sources are transported in air or associated with particulate matter (e.g., aerosols) and finally deposited in soil. In the UK, 90% of the PAHs released to the environment is estimated to be stored in soils (Wild and Jones, 1995).

Oxygen-containing PAH derivatives and metabolites (OPAHs) are transformation products of PAHs containing carbonyl, hydroxyl, carboxyl, or combinations of these functional groups (Lundstedt et al., 2007). These OPAHs can be products of incomplete combustion and emitted together with PAHs from a point source or produced by post-emission photo- or chemical oxidation (with e.g., OH, O_3, NO_x) and microbial transformation (Nikolaou et al., 1984; Baek et al., 1991; Cerniglia, 1992). Oxygen-containing PAHs are ubiquitous, persistent and potentially more mobile in the environment compared with parent PAHs (Lundstedt et al., 2007). Oxygen-containing PAHs derivatives and metabolites are direct-acting mutagens and carcinogens, compared with parent PAHs, which require enzyme activation; OPAHs also produce reactive oxygen species responsible for oxidative stress which causes adverse health effect (Bolton et al., 2000; Lemieux et al., 2008). Oxygen-containing PAHs derivatives and metabolites or OPAH-containing fractions in environmental samples are more toxic to human and bacteria cells than their parent PAH or PAH-containing fractions (Alsberg et al., 1985; Wilson et al., 1996; Lemieux et al., 2008). It is therefore important to measure the OPAHs in addition to PAHs. The measurement of intermediates of PAH degradation under field conditions in soil will be useful for the study of natural attenuation of PAHs, monitoring of remediation processes and for source apportionment (McKinney et al., 1999; Lundstedt et al., 2007; Reineke et al., 2007). The available studies of OPAH concentrations in the environment focus on atmospheric samples: aerosols and gas phase (Allen et al., 1997; Albinet et al., 2006; Lintelmann et al., 2006; Delhomme et al., 2008), waste water (Pojana and Marcomini, 2007), sediments (McKinney et al., 1999), sewage sludge (Bodzek et al., 1997), human and animal fluids (Smith et al., 2002; Grova et al., 2005) and fish biles (Johnson-Restrepo et al., 2008). The few studies of soils are limited to highly contaminated soils (creosote/gaswork/wood impregnation/coke production sites) or spiked soils (at concentration levels of $\mu g\ g^{-1}$ to $mg\ g^{-1}$ ranges) used for recovery and degradation studies (Wischmann et al., 1996; Meyer et al., 1999; Lundstedt et al., 2006). In these soils, hydroxyl/carboxyl-OPAHs are completely left out or quantified with high performance liquid chromatography/diode array detection (HPLC/DAD). To the best of our knowledge only one study in Europe has reported the OPAH concentrations in background soils but these reports only included carbonyl-OPAHs (Niederer, 1998). Soil matrix can be more complicated because it contains a complex mixture of organic compounds compared with atmospheric samples. Background soils present further challenges because of the low concentrations of analytes. The classical procedures for the joint extraction of PAHs and OPAHs from soil involve solvent extraction with soxhlet, ultrasonication, or pressurized liquid extraction sometimes after pre-acidification. Thereafter, the raw analyte is usually cleaned-up and separated into different polarity groups with solid-phase extraction or column chromatography using, for example, silica gel and different solvent combinations. After extraction and clean-up/fractionation, the analytes are quantified by HPLC/DAD or GC with flame ionization detector or mass spectrometric detectors (Meyer et al., 1999; Lundstedt et al., 2006a), capillary electrophoresis (Copper, 2003) and liquid chromatography-mass spectrometry (Galceran and Moyano, 1996; Letzel et al., 2001; Lintelmann et al., 2006; Delhomme et al., 2008). Some problems that have been encountered in past analyses of OPAHs include low recoveries, matrix interferences, complicated and time-consuming sample preparation steps, high cost of analysis, shortage of authentic reference/labelled standards and lack of certified reference materials (Meyer et al., 1999; Albinet et al., 2006; Lundstedt et al., 2006; Lundstedt et al., 2007).

The objectives of our study were (1) to modify and evaluate a multi-compound analytical protocol based on pressurized liquid extraction with an accelerated solvent extractor, column chromatographic separation/clean-up and gas chromatography/mass spectrometry (GC/MS) to determine PAHs/alkyl-PAHs and OPAHs of 2- and 3-ring PAHs covering the entire range of functional groups (carbonyl, hydroxyl/carboxyl and combinations) known for OPAHs; (2) to test the suitability of several commercially available deuterated OPAHs for potential use as processing internal standards in an isotope-dilution quantification procedure; and (3) to apply the developed method to soils with different properties and PAH pollution levels. Even though such procedures have been previously used to analyze PAHs, and carbonyl-OPAHs in soil, we are not aware of any previous study that has evaluated the analytical suitability (e.g., recovery, limit of detection [LOD], and precision) of such procedures for the determination of a large number of PAHs and OPAHs including hydroxyl/carboxyl-OPAHs. It is predictable that many more measurements in the future will include PAHs and OPAHs. Therefore, it is of critical importance that analytical procedures are validated, reference materials are measured for comparison and many of the previous problems stated above solved

3 Materials and methods

3.1 Soil samples

The soil samples used for the method modification and evaluation were from the Terra Firme of the Amazon basin in Brazil, approximately 30 km north of the city of Manaus in the Brazilian State of Amazonas. This soil was chosen for the spike and recovery experiments of the OPAHs because tropical soils are in general known to be less polluted with PAHs (Krauss et al., 2005). A second soil was sampled from the Botanical Garden of the University of Mainz. Both soil samples were mineral topsoils (0-10 cm). The samples were air-dried and sieved (< 2 mm). The third soil was a certified European Reference Material (ERM) (ERM®-CC013a-Polycyclic Aromatic Hydrocarbons in Soil; Federal Institute for Materials Research and Testing [BAM], Berlin, Germany). This soil was sampled from a former gasworks site in Berlin-Mariendorf, Berlin, Germany. The ERM was air-dried and sieved (≤ 63 µm) prior to receipt. This reference material has certified values for the 16 EPA-PAHs and was used to validate the PAH measurements. Certified OPAH values were not provided.

Soil properties (Tab. BS-1, Supplemental Information) were determined for the Manaus and Mainz soil. pH was determined in 1 mol L^{-1} KCl solution for the Manaus soil and in 0.01 mol L^{-1} $CaCl_2$ solution for the Mainz soil (soil/solution ratio, 1:2.5). Effective cation-exchange capacity (ECEC) was the sum of the charge equivalents of Al, Ca, K, Mg, Mn and Na measured by flame atomic absorption spectrometry (AA240FS; Varian Inco., Palo Alto, CA) after extraction from soil with 1 mol L^{-1} NH_4NO_3 solution (soil/solution ratio: 1:25). Total organic C (C_{org}) and total N were measured with an elemental analyzer (Elementar Vario EL, Elementar Analysensysteme GmbH, Hanau, Germany). Total organic carbon (OC) in the Mainz soil was determined as the difference between total carbon and carbonate-C (measured in soil after destruction of organic carbon at 550 ^{0}C for 15 hours in a furnace). The properties of the Berlin soil (ERM®-CC013a) were provided by the BAM (Tab. B-3, Supplemental Information) except the soil type.

3.2 Chemicals and reagents

The target OPAHs and PAHs shown in Tables B-4 and B-5 were obtained from Dr. Ehrenstorfer (Augsburg, Germany), Ultra Scientific (North Kingston, RI, USA), Chiron (Trondheim, Norway), Acros Organics (New Jersey, USA), Sigma-Aldrich group (Steinheim, Germany), Cambridge Isotope Laboratories (CIL) (Andover, MA, USA) and C/D/N Isotopes Inc. (Pointe-Claire, Quebec, Canada). All target OPAHs have been identified/proposed as a biological/abiotic degradation products of biphenyl, naphthalene, 1-methylnaphthalene, 2-

methylnaphthalene, fluorene, acenaphthylene, acenaphthene, phenanthrene and anthracene. 4,5-Dihydroxyphenanthrene was analyzed because of its similarity to other identified dihydroxy-phenanthrene metabolites of phenanthrene and its detection in fuels (Tittmann and Lingens, 1980; Cerniglia, 1984; Cerniglia, 1992; Green et al., 1994; Mahajan et al., 1994; Wang et al., 1995; Casellas et al., 1997; Meckenstock et al., 2004; Bamforth and Singleton, 2005; Zeinali et al., 2008). All solvents were picograde from Promochem (Wesel, Germany), diatomaceous earth was from Dionex (Sunnyvale, CA). Derivatization reagents N,O-bis-(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane [BSTFA+TMCS (99:1)], silica gel 60 (70-230 mesh) and trifluoroacetic acid were from Sigma-Aldrich group (Steinheim, Germany) and Na_2SO_4 (p.a, water free) from Carl Roth (Karlsruhe, Germany). Silica-gel and Na_2SO_4 were heated at 300°C for 12 h. Deactivated silica gel (10%) was prepared by mixing 0.3 g water with 2.7 g silica gel (cooled to room temperature).

3.3 Pressurized liquid extraction

All samples were extracted using an accelerated solvent extractor (Dionex ASE 200, Sunnyvale, CA), equipped with 33 ml stainless steel extraction cells. A cellulose filter was placed at the bottom of the extraction cell followed by 20 g of soil thoroughly mixed with diatomaceous earth (4-6 g). Diatomaceous earth serves as a drying, bulk and dispersing material. A deuterated OPAH mixture of 1-hydroxynaphthalene-D_7, transcinnamic acid-D_6, 1,4-naphthoquinone-D_6 and benzophenone-2,3,4,5,6-D_5 (2 µg each, dissolved in acetone) as surrogates and seven deuterated PAHs as internal standards (250 ng in hexane) were added to the soil in the cell with any extra space filled by more diatomaceous earth followed by one more cellulose filter before the cell was closed. Samples were allowed to stand for 12 h for equilibration. All samples were first extracted with dichloromethane. The cells were filled with solvent, pressurized to 14 MPa, heated to 120°C within 6 min. Pressure and heat were held for 5 min (static extraction) followed by rinsing with more solvent (60% of cell volume) and purging with nitrogen for 90 s. The above conditions (except solvent) were previously used to extract PAHs and PCBs (Wilcke et al., 1999; Bandowe et al., 2009). To ensure complete extraction of more polar compounds, the extraction cycle was repeated a second time with $CH_3COCH_3/CH_2Cl_2/CF_3COOH$ solution (250:125:1 v/v/v). This second solvent system is one of the recommended solvent systems by the USEPA method 3545A-1 (US EPA, 2007) for extraction of chlorinated herbicides from soil by pressurized liquid extraction.

3.4 Clean-up/fractionation and derivatization

The two extracts from each sample were combined and dried by filtration through Na_2SO_4. Hexane (1 mL) was added and the mixture was rotary evaporated to about 1 mL at 35°C. Hexane (10 mL) was added, and the solution evaporated to a volume of about 1 mL to ensure complete exchange of solvent to hexane. An 8-mL borosilicate glass column (J.T. Baker, Deventer, The Netherlands) was self packed with 3 g silica gel (10% deactivated) in between two pieces of glass wool. The packed columns were conditioned with 10 mL hexane. Extracts were transferred onto the column and sequentially eluted with 9 ml hexane/CH_2Cl_2 (5:1, v/v) to elute the alkyl/parent-PAH fraction, and 8 ml CH_2Cl_2 followed by 5 ml acetone to elute the OPAHs. This fractionation/clean-up procedure was adapted and modified from previous qualitative separation studies on silica column (Bodzek et al., 1993). The PAH and OPAH fractions were collected into separate 50-mL pointed flasks. Two drops of toluene were added to the PAH fraction, and the solvent was evaporated until approximately 0.5 mL remained. Then 25 µl of 4 µg ml^{-1} fluoranthene-D_{10} (in toluene) were added as recovery standard before the solution was transferred into a 1.5-mL GC vial for GC/MS measurements. The OPAH fraction was evaporated to about 1 mL, and 10 ml acetone was added. The solution was evaporated to about 0.5 mL and transferred into a 1.5 mL GC vial. To this, 25 µl of 4 µg ml^{-1} fluoranthene-D_{10} dissolved in acetone was added as syringe spike. A portion of this mixture was transferred into a micro insert to measure the carbonyl-OPAHs with GC/MS. The remaining portion of the OPAH fraction was used for derivatization of the polar and therefore little volatile hydroxyl/carboxyl-OPAHs by adding 150 µL of BSTFA:TMCS (99:1). The mixture was then homogenized, heated at 60°C for 15 min, and left at room temperature for 60 min to derivatize the hydroxyl/carboxyl-OPAHs before GC/MS measurements. Derivatization with BSTFA:TMCS (99:1) converts hydroxyl/carboxyl-OPAHs to their more volatile $–Si(CH_3)_3$ derivatives, which improves their chromatographic performance, sensitivity and thermal stability (Li et al., 2001; Halket and Zaikin, 2003).

3.5 Gas Chromatography/Mass Spectrometry

The target compounds were analyzed using an Agilent 6890N gas chromatograph coupled to an Agilent 5975B inert mass selective detector equipped with electron impact ionization (EI) ion source, autosampler and a split/splitless injector. All reported data were acquired using the EI mode at 70 eV. The instrumental parameters for the analysis of all compounds were: injection port temperature at 280°C, transfer line at 310°C and the ion source at 230°C. Analytes (µL) were injected into the gas chromatograph in the splitless mode using the autosampler. Compounds were separated on a fused silica capillary column (30 m x

0.25 mm i.d. x 0.25 µm film thickness) (HP-5MS; J&W Scientific, Folsom). Helium was used as carrier gas with a constant flow rate of 1.2 mL min^{-1}.

For the OPAHs, the oven temperature was initially set at 80°C, held for 6 min, increased to 145°C at the rate of 5°C min^{-1}, held for 3 min and finally increased to 200°C at the rate of 5^{o}C min^{-1} and held for 7 min. The mass spectrometer was operated in full scan mode to obtain the mass spectra of single compounds from which ions were selected for quantification in the single ion monitoring (SIM) mode. For each compound, two ions were selected to be monitored, one for quantifying and the other as a qualifier. The monitored ions for the measured compounds are shown in Table B-4 (Supplemental Information). Chromatograms for the standard mixtures are shown in Figure B-2 (Supplemental Information).

The oven temperature for the measurement of PAHs/alkyl-PAHs was initially set at 80°C, held for 4 min, increased to 160°C at a rate of 14°C min^{-1}, held for 1.5 min, increased to 225°C at a rate of 5°C min^{-1}, held for 5 min, and then increased to 300°C at a rate of 5°C min^{-1} and held for 11 min (Bandowe et al., 2009). The monitored ions for the measured compounds are shown in Table B-5 (Supplemental Information). Chromatogram of the standard mixture is shown in Figure B-2. All GC/MS instrumental control and data recording and processing was done with Agilent MS ChemStation.

3.6 Quantification of target compounds

Ten calibration standards with concentration ranges of 5 to 15,000 ng mL^{-1}, 10 to 15,000 ng mL^{-1} and 5 to 5000 ng mL^{-1} were used for the quantification of hydroxyl/carboxyl-OPAHs, carbonyl-OPAHs, and PAHs respectively. Each OPAH calibration standard was spiked with 100 ng mL^{-1} of fluoranthene-D_{10}, and each PAH calibration standard was spiked with 250 ng mL^{-1} of seven deuterated PAHs as internal standards. The hydroxyl/carboxyl-OPAHs standards were derivatized as described previously before GC/MS measurement. The relative response (peak area ratios) of each OPAH to fluoranthene-D_{10} and each PAH to their respective deuterated PAH internal standard (Tab. B-4, Supplemental Information) measured in the standards was then plotted against their known concentration ratios to generate the calibration functions. The correlation coefficients established for a five-point linear calibration curve was > 0.98 for OPAHs and > 0.99 for PAHs. The relative response of target compounds to that of their corresponding deuterated internal standard measured in our final analyte (samples) were used to quantify target compounds using the calibration functions. We set up the post-measurement internal standard quantification procedure using the Agilent MS ChemStation software.

3.7 Recovery experiments and measurement of certified reference material

To assess the accuracy and precision of the method for the determination of OPAHs, the Manaus soil was used. After 20 g of soil mixed with diatomaceous earth had been placed in the ASE extraction cell, the sample was spiked with 100 µL of 20 µg mL^{-1} of carbonyl-OPAH and hydroxyl/carboxyl-OPAH target compounds and their deuterated surrogates, leading to a concentration of about 100 ng g^{-1}, which realistically mimics concentrations in background soils (Niederer, 1998). In addition, deuterated PAHs (internal standard) were added. Spiked samples were then left for 12 h to equilibrate and the whole procedure as described previously was applied. For each extraction procedure, in which target compounds were spiked, a set of the same unspiked soil samples was passed through the same procedure, and the their final concentration was subtracted from that of the spiked sample before recovery was calculated. The mean recovery of spiked soil samples is a measure of the accuracy of the entire analytical procedure to determine OPAHs, and the RSD of measurements of spiked samples after replicate extraction is a measure of the precision (repeatability) of the analytical method. A total of eight replicates of spiked samples and six replicates of unspiked samples were extracted during the spike and recovery experiment.

The analysis of ERM®-CC013a followed the procedure as described above except that only 1 g of soil sample was used during extraction of three replicates. The accuracy of PAH determination is reported as the closeness of the determined value to the certified value, and the precision is reported as the RSD of the three replicates. Because the whole procedure was applied, OPAHs were also determined in this reference material.

3.8 Method application

The method as described above was used to screen mineral surface soils (0-10 cm) for the entire range of OPAHs and PAHs sampled from a forest in the University of Mainz, Botanical Garden in addition to the forest soil from Manaus (initially used for spike experiments) and certified European reference material ERM®-CC013a (also used to validate PAH measurements) (Tab. B-3). This was to represent low, medium and highly polluted soils respectively and also soils of different characteristics. The measurement of OPAHs in commercially available ERM®-CC013a could provide useful data for comparison in other studies.

3.9 Quality assurance/control

Strict quality control measures were used during sample preparation and quantification. Glassware used in our sample preparations was rinsed with acetone and baked at 250°C overnight, rinsed again with "picograde solvent" before use. Solvents used were of "picograde" for residues analysis. Several blanks were run to check for background contamination during sample preparation as well as memory effects during the GC/MS measurements. Our blank included the cell filled with diatomaceous earth and prepared and measured at the beginning, middle, and end during sample preparation and GC/MS measurements. The mean of blank measurements were used to correct for contamination during sample preparation and measurements. During GC/MS measurement, a compound was only accepted as identified in the sample or blank if the ratio of the two monitored ions was within ±20% of that measured in the calibration standards and with a retention time difference of < 0.2 min for the OPAHs and < 0.1 min for the PAHs/alkyl-PAHs. As much as possible, we selected the molecular ion as one of the monitored ions for OPAHs and avoided selecting ions originating from the use of the derivatization agent (e.g., m/z = 73) for hydroxyl/carboxyl-OPAHs even if it was the base peak to avoid false identifications.

4 Results and discussion

4.1 Method performance

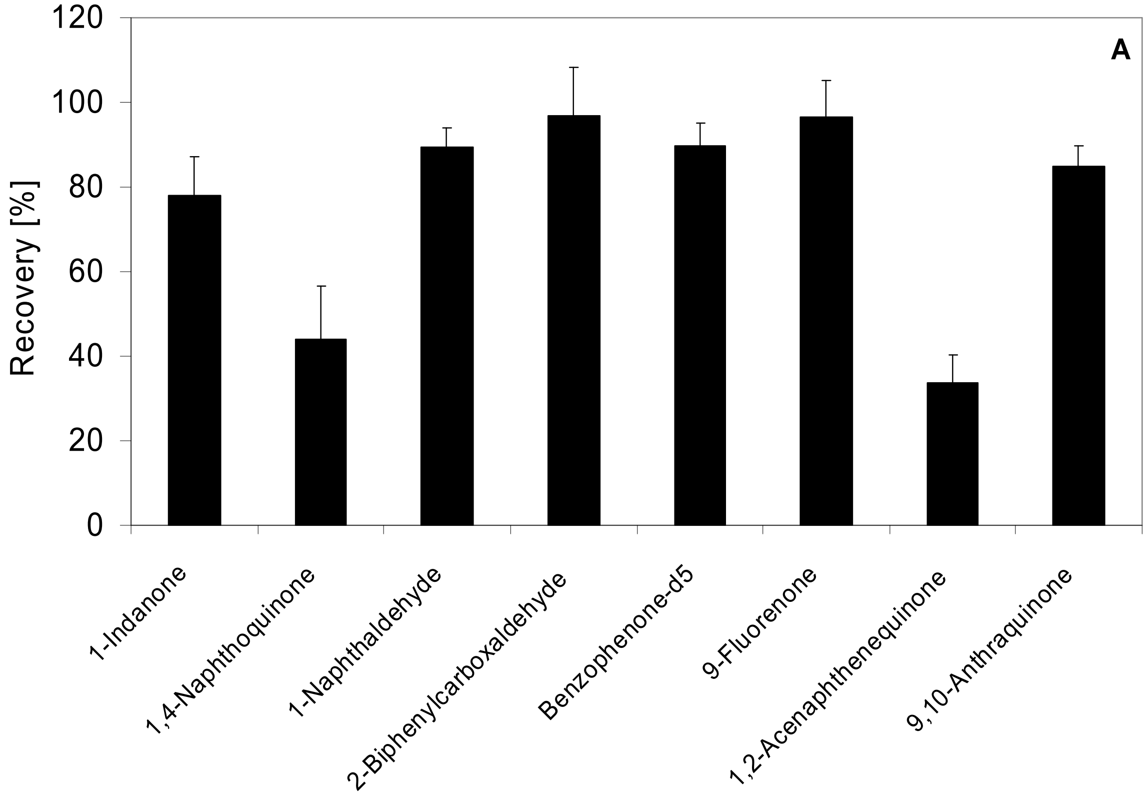
4.1.1 Accuracy and precision of the carbonyl-OPAHs analysis

The recoveries (mean ± standard deviation) of spiked carbonyl-OPAHs ranged between 78 ± 9% for 1-indanone and 97 ± 11 % for 2-biphenylcarboxyaldehyde while the quinones 1,2-acenaphthenequinone (34 ± 7%) and 1,4-naphthoquinone (44 ± 13%) had recoveries below 50% (Fig. B-1A). Our recoveries for the carbonyl-OPAHs are either in the range or better (for some compounds) than in other reported methods used for soil and where compounds were spiked at much higher concentrations (Wischmann et al., 1996; Meyer et al., 1999; Lundstedt et al., 2006a). Low recovery of 1,2-acenaphthenequinone (45%) similar to ours has been reported in a previous study (Meyer et al., 1999). Test measurements of carbonyl-OPAHs in the PAH fraction recovered only negligible quantities of these compounds, demonstrating that carbonyl-OPAHs are not eluted with the PAHs fraction. We propose that instability of 1,4-naphthoquinone and 1,2-acenaphthenequinone during GC/MS measurement is the reason for the lower mean recovery and higher standard deviation associated with their measurement. In our studies with standards of 1,4-napthoquinone and 1,2-acenaphthenequinone, we observed low and variable response factors, while 1,2-

naphthoquinone and 9,10-phenanthroquinone were lost. The analytical sensitivity for 1,2-naphthoquinone and 9,10-phenanthroquinone was therefore so low and their general behavior unpredictable that we excluded them from our targets. This is in line with the observations of other researchers (Cho et al., 2004; Liu et al., 2006). Liu et al. (2006) reported that 9,10-phenanthroquinone is converted to 9-fluorenone during GC/MS injection. To overcome low sensitivities and instability during GC/MS, Cho et al. (2004) propose derivatizing quinones with acetic anhydride. However, for these compounds, using liquid chromatography/mass spectrometry (LC/MS) might be the better alternative.

The precision of the analytical method for carbonyl-OPAHs was determined as the RSD of replicates of analyte recoveries in the spiked samples. It ranged between 5 and 28% (Tab. B-1) for the carbonyl-OPAHs, with the quinones 1,2-acenaphthenequinone and 1,4-naphthoquinone showing the highest RSD.

In our experiments, we tested two commercially available deuterated carbonyl-OPAHs for possible use as processing internal standard and found that benzophenone-2,3,4,5,6-D_5 showed good recovery (90 ± 6%) and stability when using our method. The recovery of benzophenone-2,3,4,5,6-D_5 is similar to that of most of the target carbonyl-OPAHs, except for 1,4-naphthoquinone and 1,2-acenaphthenequinone. Therefore, benzophenone-2,3,4,5,6-D_5 is recommended for use as processing internal standard for all the target carbonyl-OPAHs except 1,4-naphthoquinone and 1,2-acenaphthenequinone. Benzophenone-2,3,4,5,6-D_5, if added to the sample before extraction, allows for correction of incomplete extraction and losses during clean-up and fractionation leading to accurate quantification of all target carbonyl-OPAHs except 1,4-naphthoquinone and 1,2-acenaphthenequinone. The additionally tested 1,4-naphthoquinone-D_6 had sometimes unrealistic recoveries (159 ± 44%) and was sometimes completely lost, underlining our assumption that the low molecular weight PAH-quinones (2-ring) were not stable in our method. However, in principle 1,4-naphthoquinone-D_6 remains the best compound to be used as internal standard for 1,4-naphthoquinone.



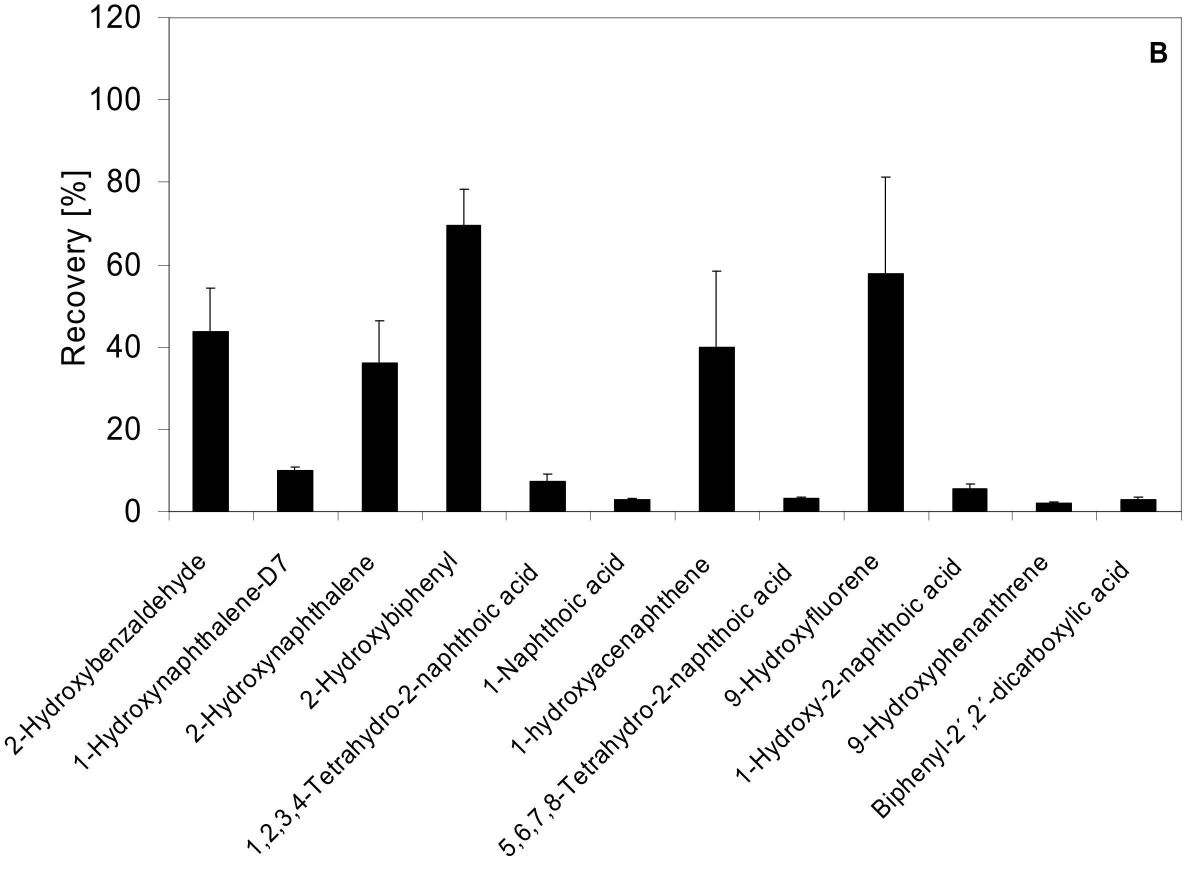


Figure B-1: Recoveries of carbonyl-OPAHs [A] and hydroxyl/carboxyl-OPAHs [B] from spiked soils. Error bars indicate the standard deviation of replicates (n=8).

Table B-1: Concentrations (mean±standard deviation) of OPAHs in Manaus, Mainz and Berlin (ERM®-CC013a) soils, precision (RSD, n = 8) and limits of detection (LODs) of the used method. Only compounds with accepted recoveries are reported (see text).

OPAH	Manaus	Mainz	ERM®-CC013a	RSD†	LOD
	-------------ng g^{-1}------------------------			%	ng g^{-1}
1-Indanone	1.7 ± 0.8	3.0 ± 0.4	1508 ± 135	11	0.18
1,4-Naphthoquinone	n.d‡	n.d	26 ± 4	28	1.00
1-Naphthaldehyde	1.1 ± 0.2	6.0 ± 1.8	103 ± 21	5	0.10
2-Biphenylcarboxaldehyde	n.d	29.1 ± 12.1	969 ± 188	12	0.10
9-Fluorenone	1.7 ± 1.4	47.2 ± 14.0	5089 ± 386	9	0.10
1,2-Acenaphthenequinone	n.d	4.9 ± 3.3	163 ± 4	19	0.75
9,10-Anthraquinone	2.1 ± 1.3	80 ± 15.2	7824 ± 608	6	0.10
∑carbonyl-OPAHs	6.6 ± 3.5	170.2 ± 57.0	15681 ± 1835		
2-Hydroxybenzaldehyde	6.2 ± 3.6	9.7 ± 1.8	41 ± 9	24	0.2
2-Hydroxynaphthalene	n.d	1.2 ± 0.2	24 ± 1	29	0.1
2-Hydroxybiphenyl	0.5 ± 0.2	4.6 ± 0.0	98 ± 1	13	0.1
1,2,3,4-Tetrahydro-2-naphthoic acid	4.5 ± 0.7	4.0 ± 0.0	86 ± 1	25	0.3
1-Naphthoic acid	n.d	n.d	n.d	8	0.2
1-Hydroxyacenenaphthene	n.d	6.1 ± 0.7	130 ± 24	46	0.3
5,6,7,8-Tetrahydro-2-naphthoic acid	2.0 ± 0.2	n.d	n.d	11	0.1
9-Hydroxyfluorene	2.6 ± 0.1	4.3 ± 0.5	90 ± 10	41	0.3
1-Hydroxy-2-naphthoic acid	n.d	6.4 ± 0.2	n.d	19	0.3
9-Hydroxyphenanthrene	n.d	n.d	n.d	9	0.3
Biphenyl-2’,2’-dicarboxylic acid	n.d	n.d	49 ± 3	14	1.6
∑hydroxyl/carboxyl-OPAHs	16 ± 4	36.3 ± 2.3	518 ± 47		

†RSD = relative standard deviation calculated from the spiked samples.

‡ n.d. = not detected

4.1.2 Accuracy and precision of the hydroxyl/carboxyl-OPAHs analysis

The recoveries of spiked hydroxyl/carboxyl-OPAHs were in general lower than those of the carbonyl-OPAHs. Five of our target hydroxyl/carboxyl OPAHs showed recoveries between 36 and 70% which is at the lower end of the range found for carbonyl-OPAHs, and six others showed low mean recoveries between 2 and 7% (Fig. B-1). Eight others (1,2-dihydroxybenzene, 2-hydroxybenzoic acid, 1-hydroxynaphthalene, 2,5-dihydroxybenzoic acid, 2-naphthoic acid, 2,3-dihydroxynaphthalene, 4,5-dihydroxyphenanthrene and, 2,3-naphthalenedicarboxylic acid) were lost. Nearly all lost compounds contained two hydroxyl/carboxyl functional groups making them particularly polar. Analytical protocols including solid-phase extraction, derivatization and GC/MS to determine hydroxyl/carboxyl-OPAHs (in aerosols, urine, and fish bile) have shown similar low recoveries (6-81 %) (Moyano et al. 1997; Smith et al., 2002; Johnson-Restrepo et al., 2008) while in soil others reported slightly higher recoveries (17-102%) using an HPLC/DAD method (Wischmann et al., 1996; Meyer et al., 1999). In our set-up, the possible reasons for the low recoveries were strong sorption to soil particles and therefore reduced extractability, or strong sorption to the silica column which prevented elution, degradation or transformation to other products and reduced efficiency of the silylation reaction (as a result of competing reactions from soil matrix compounds).

Initial ASE extractions were done with dichloromethane (2 cycles). In the current protocol, we used for the second cycle extraction an acidified acetone/dichloromethane mixture as recommended by the USEPA for extraction of chlorinated herbicides by pressurized liquid extraction (USEPA, 2007). This change improved the recoveries of the five most completely recovered hydroxyl/carboxyl-OPAHs by 2 to 3% but did not improve the others. We therefore included the acidified acetone/dichloromethane extraction cycle in our current protocol. However, these results demonstrate that polarity/acidity of the extraction solvent is not a major cause of the low recovery or loss of the most polar compounds.

To solve the problem of incomplete elution from the silica column we did several tests in which we eluted the column with methanol and acidified methanol. When eluted with this solvent, eluates showed strong color differentiation, turbidity, and heavy matrix. Attempts to completely dry methanol (by bubbling with N_2) and to exchange the solvent to a non-protic solvent (a requirement for successful derivatization with BSTFA) were slow and unworkable. Thus, the dichloromethane and acetone elution (as used in the current protocol) gave the best results. Also important is its easy adaptability to the derivatization procedure, which works best in acetone (Li et al., 2001). The use of acidified methanol might be required to improve the recovery of hydroxyl/carboxyl-OPAHs from silica columns during column chromatography (Wischmann et al., 1999). However, in the soils used for our spike experiment (4% organic C) the use of acidified methanol for column elution should be preceded by substantial post-ASE extraction matrix reduction using, for example, gel permeation chromatography.

Other researchers have reported that phenols are lost in the ASE tubing through which analytes are delivered to the vial during PLE (Berkner et al., 2004). Further tests are needed to establish stability of these compounds in during ASE extraction.

Several tests using standards dissolved in acetone showed that heating to 60°C for 15 mins and leaving the standard at room temperature for 1 h was the optimum condition for derivatization. There is, however, the possibility that compounds co-extracted from soil (e.g., humic and fulvic acids) might interfere with the derivatization of our target compounds. This was reported for the determination of acidic pharmaceuticals in aqueous samples (Togola and Budzinski, 2008). If interfering humic substances were the reason for the low recoveries of hydroxyl/carboxyl-OPAHs, matrix calibration curves might help to improve the measurement.

The precision of our analytical method for the measurement of hydroxyl/carboxyl-OPAHs was determined as the RSD of replicates of analyte recoveries in the spiked samples. It ranged between 8 and 46% (Tab. B-1).

The recovery of 1-hydroxynaphthalene-D_7 was low (10%), and transcinnamic acid-D_7 was completely lost. 1-Hydroxynaphthalene-D_7 is nevertheless the best internal standard to quantify 1-hydroxynaphthalene. These tests showed that there is strong variation in compound recoveries. Therefore, it is important that several labelled compounds are simultaneously used as internal standards.

4.1.3 Accuracy and precision of the parent-PAHs

We determined the absolute recovery of deuterated PAHs used as internal standards. The recovery ranges for the seven deuterated PAHs were 36 to 84%, 51 to 80%, 40 to 127%, 38 to 80%, 19 to 83%, 20 to 93% and 14 to 98% for naphthalene-D_8, acenaphthene-D_{10}, phenanthrene-D_{10}, pyrene-D_{10}, chrysene-D_{12}, perylene-D_{12} and benzo[ghi]perylene-D_{12}, respectively. The higher-molecular-weight deuterated internal standards had lower mean recoveries of 36, 38 and 31% for chrysene-D_{12}, perylene-D_{12} and benzo[ghi]perylene-D_{12} respectively, than the lower-molecular-weight compounds. There were a few extraction runs in which the recoveries of chrysene-D_{12}, perylene-D_{12} and, benzo[ghi]perylene-D_{12} were even <10%. Similar low recoveries of 32 to 92% and 21 ± 12 to 90 ± 18% of deuterated PAH internal standard were reported in other published methods for soils (Krauss et al., 2000; Bucheli et al., 2004). In these other studies, the lowest recoveries were always reported for naphthalene-D_8. The loss of naphthalene-D_8 during rotary evaporation because of its high volatility is often cited as reason for the low recovery, which was partly observed in our current study. In our method, naphthalene-D_8 had lower recovery compared with other low molecular weight deuterated-PAHs but heavier PAHs than pyrene-D_{10} show decreasing recovery with increasing molecular mass for all spiked soils and the diatomaceous earth used as blank. Low recovery of higher-molecular-weight deuterated PAHs compared with lower-molecular-weight ones observed in other studies were attributed to the fact that the ASE cell might serve as a chromatographic column that increasingly retains the higher molecular weight deuterated-PAHs (Lundstedt et al., 2000). We observed high recoveries (50-70%) for all deuterated PAHs when they were spiked onto diatomaceous earth, extracted and measured directly without column chromatographic fractionation. We therefore conclude that the low recoveries of the deuterated higher molecular weight PAHs are largely because of the fractionation procedure on the silica gel columns. Although the fractionation procedure needs further optimization, the use of the isotope dilution method in which the deuterated PAHs were added to the ASE extraction cell with the soil and passed through the entire extraction and clean-up greatly reduced errors, leading to accurate quantification of PAHs as confirmed by the analysis of the certified reference material. There was a close match between the certified values and our measured values (Tab. B-2). The mean recovery of the sum of PAHs (for which certified values are provided) compared with the provided certified concentration was 103% and very close to the 99% measured for the same reference material with our previous method (Bandowe et al., 2009).

Method precision for PAHs was determined from measurements of aliquots of certified European reference material with precision calculated as RSD of triplicate determinations. Precision was always better than 10% (Tab. B-2), except for three compounds that had RSDs between 10 and 13%. The precision of our method for determination of PAHs is similar or even better than results reported in other published work using a similar method where RSDs of 2 to 24% are reported (Maliszewska-Kordybach et al., 2008).

4.1.4 Limits of detection (LOD)

Limits of detection were determined as three times the standard deviation of the method blanks divided by 20 g to represent the standard mass of extracted soil in our measurements. For those compounds which were not detected in the blanks, the absolute value of the lowest calibration point divided by 20 was used as LOD.

The LODs for the carbonyl-OPAHs ranged from 0.1 to 1 ng g^{-1}. The LODs of the hydroxyl/carboxyl-OPAHs (0.1-1.6 ng g^{-1}) were usually lower than those of the carbonyl-OPAHs, showing that derivatized OPAHs are in general more sensitively measured than the carbonyl-OPAHs whose polar and yet underivatized nature restricts volatilization in the GC (Tab. B-1). We found only one report of LODs for OPAHs in which LODs ranged from 0.01 to 1.55 µg g^{-1} which is higher than in our method (Meyer et al., 1999). The values of the

LODs for PAHs ranged from 0.01 to 0.56 ng g^{-1} (Tab. B-2), which is similar to the LODs of 0.02 to 0.75 ng g^{-1} reported for soils in other studies (Sanchez-Brunete et al., 2006; Barco-Bonilla et al., 2009). The LODs of PAHs not detected in blanks was 0.25 ng g^{-1}.

Table B-2: Concentrations (mean±standard deviation) of PAHs in Manaus, Mainz and Berlin (ERM®-CC013a) soils with certified values, precision (RSD, n = 3) and limits of detection (LODs) of the used method. Reference values in parentheses are non-certified indicative values without uncertainty statement.

Name	Manaus	Mainz	ERM®-CC013a	Certified value†	RSD^{e}(%)	LOD
	-------------ng g^{-1}-----------------		--------------µg g^{-1}-----------------		%	ng g^{-1}
1,2,3,4-Tetrahydronaphthalene	n.d.	1.5± 0.6	n.d.‡			0.25
Naphthalene	16.2 ± 2.2	23.5±3.8	3.0± 0.1	2.4 ± 0.5	5	0.10
2-Methylnaphthalene	1.8 ± 0.1	17.8±4.7	1.1± 0.1		5	0.08
1-Methylnaphthalene	1.4 ± 0.4	18.2±5.6	1.2± 0.0		3	0.07
1,3-Dimethylnaphthalene	3.3 ± 1.1	48.0±13.6	1.1± 0.0		3	0.05
Acenaphthylene	n.d.	2.9±0.4	1.2± 0.1	(0.77)	6	0.25
Acenaphthene	0.4 ± 0.1	6.5±1.3	0.7± 0.0	(0.75)	1	0.25
2,3,5-Trimethylnaphthalene	1.0 ± 0.4	27.0± 6.6	0.4± 0.0		2	0.03
Fluorene	0.7 ± 0.3	9.5±2.4	1.0± 0.1	1.14 ± 0.11	5	0.02
2,6-Diisopropylnaphthalene	4.1 ± 4.2	12.8±2.7	nd		6	0.56
Phenanthrene	2.3 ± 0.9	213.4±36.1	13.7±1.4	12.0 ± 0.6	10	0.25
Anthracene	n.d.	12.7±2.8	0.7± 0.0	1.41 ± 0.22	0.1	0.25
2-Methylphenanthrene	0.4 ± 0.1	129.1±27.3	3.5± 0.1		3	0.13
1-Methylphenanthrene	0.2 ± 0.1	26.1± 3.0	0.6± 0.0		0.2	0.03
3,6-Dimethylphenanthrene	n.d.	10.7±2.0	0.2± 0.0		2	0.25
3,5-Dimethylphenanthrene	n.d.	22.1±5.2	0.5± 0.0		8	0.25
2,6-Dimethylphenanthrene	n.d.	15.0±2.3	0.3± 0.0		0.3	0.25
1,7-Dimethylphenanthrene	1.1 ± 0.0	26.0±3.5	0.4± 0.0		5	0.25
Fluoranthene	0.3 ± 0.1	415.7±111.7	8.8± 0.1	12.9 ± 0.7	1	0.01
Pyrene	n.d.	444.4±110.3	10.1± 0.1	9.6 ± 0.3	1	0.25
Retene	0.9 ± 0.3	12.1±2.8	0.2± 0.0		2	0.06
Benzo[a]anthracene	0.4 ± 0.0	255±79	5.1± 0.3	5.6 ± 0.5	6	0.25
Chrysene+Triphenylene	0.3 ± 0.1	438.7±106.1	7.3± 0.2	5.3 ± 0.8§	3	0.02
Benzo[b,j,k]fluoranthene	nd	530.3±126.7	14.1±1.2	10.5 ± 1.4¶	8	0.25
Benzo[e]pyrene	nd	457.9±126.2	10.4± 0.7		7	0.25
Benzo[a]pyrene	nd	481.7±162.8	6.2± 0.7	4.9 ± 0.7	12	0.25
Perylene	1.9 ± 0.3	85.7±37.0	1.5± 0.0		2	0.25
Indeno [1,2,3-cd]pyrene	nd	314.4±47.7	5.2± 0.2	5.2 ± 1.0	3	0.25
Dibenzo[a,h]anthracene	nd	38.3±23.9	0.7± 0.1	(1.1)	13	0.25
Benzo[ghi]perylene	nd	318.4±62.1	5.7± 0.5	4.6 ± 0.5	8	0.25
Coronene	nd	74.7±38.7	1.5± 0.1		5	0.25
Σ16 EPA PAHs	21.5 ± 5.7	3505.2± 874.1	83.5 ± 4.9		6	
Σ34 PAHs	38.4 ± 11.8	4489.9 ± 1145.6	106.5 ± 4.1		6	

† Uncertainty of certified values is given at 95% confidence interval. ‡ n.d. = not detected.
§ Certified value is for chrysene (our measured value includes triphenylene).
¶ Certified value is for benzo (b+k)fluoranthene (our measured value includes benzo(j)fluoranthene).

4.2 Concentrations of PAHs and OPAHs in selected soil samples

The sum of the seven quantified carbonyl–OPAHs was 7, 170 and 15681 ng g^{-1} for Manaus, Mainz and Berlin soils, respectively (Tab. B-1). This mainly reflects the degree of anthropogenic pollution. The Manaus soil is located at a site that is affected little by industrial emissions, whereas the Berlin soil was polluted by the former gasification activity. The Mainz soil is influenced by atmospheric deposition from urban combustion activities (heating, traffic and industry). The concentrations and the composition pattern of three carbonyl-OPAHs in the Berlin soil from a former gasworks site are comparable to those reported from a former gas works site at Husarviken in Stockholm, Sweden where Lundstedt et al. (2006b) found 0.32 ± 0.09, 12 ± 2 and 14 ± 3 µg g^{-1} of 1-indanone, 9-fluorenone, and 9,10-anthraquinone, respectively. The concentration range of carbonyl-OPAHs in the Mainz soil (Tab. 1) falls in the range of 6 to 266 ng g^{-1}, which was measured in soils from children's playgrounds and park areas in Basel, Switzerland (Niederer, 1998). In the latter study of (Niederer, 1998), the concentration of 9,10-anthraquinone (which was the only carbonyl-OPAH also measured in our study) ramged from 11 to 80 ng g^{-1}, which is similar to the 80 ng g^{-1} in the Mainz soil. Our carbonyl-OPAHs are dominated by 9-fluorenone and 9,10-anthraquinone in all samples. These two OPAHs have been identified as major components in soils from former gasworks sites as well as in particulate fractions of ambient air samples in various European cities (Lundstedt et al., 2003; Albinet et al., 2006; Albinet et al., 2007). The sum of the concentrations of eleven hydroxyl/carboxyl-OPAHs followed a similar trend as the carbonyl-OPAHs (i.e Berlin > Mainz > Manaus soils). The ∑11 hydroxyl/carboxyl-OPAHs concentrations were in general lower than ∑7 carbonyl-OPAHs in the Mainz and Berlin soils. Differences in recovery make it difficult to compare these two groups of compounds. The sources of these OPAHs can be direct emission through combustion processes or post-emission abiotic/biotic transformation of parent PAHs. Because OPAHs levels seem to follow the expected trend of degree of anthropogenic pollution, it is more likely that direct emission sources explain the concentration levels and composition patterns of the OPAHs mixtures.

The concentrations of the ∑16 EPA-PAH and total determined PAHs (∑34 PAHs) both decreased in the order, Berlin > Mainz > Manaus soils (Tab. B-2). The ∑21 parent-PAH concentrations of 23 ng g^{-1} of the Manaus soil determined by this method is similar to ∑21 parent PAHs of 16 to 38 ng g^{-1} determined by a different method for this same soil from Manaus, further confirming the accuracy of our method (Krauss et al., 2005). The difference between the Manaus and Mainz soil is attributable to the level of industrial activity which is higher in Germany than in Amazonia, Brazil (Wilcke, 2007; Nam et al., 2009). The Berlin soil was contaminated by a specific point source, whereas the Mainz soil received its PAH load mainly via deposition from the atmosphere of the urban environment in which the Mainz soil is located. The ratios of some OPAHs to that of the corresponding parent-PAH from which they are likely to be derived (metabolite/parent-PAH e.g., 1-indanone/fluorene, 9-fluorenone/fluorene, 9-hydroxyfluorene/fluorene, 1,2-acenaphthenequinone/acenaphthylene, 9,10-anthraquinone/anthracene were > 1 in some soils. The concentrations of OPAHs were similar to those of parent PAHs in the Manaus soil, lower in the Mainz soil and even higher in the Berlin soil. This finding, coupled with the fact that some OPAHs are known to be more toxic than their parent compounds strongly suggests the need to include OPAHs into environmental monitoring efforts (Albinet et al., 2006; Lundstedt et al., 2007).

5 Conclusions

We suggest an analytical protocol to quantify 34 parent/alkyl-PAHs and 18 oxygenated PAHs with satisfactory accuracy and precision, although six target hydroxyl/carboxyl-OPAHs showed low recoveries (2-7%) in a spike experiment. Because of the low detection limits the method can be applied to soils with low levels of contamination (i.e. background soils).

Benzophenone-2,3,4,5,6-D_5 tested as possible processing internal standard for OPAHs showed high stability and recovery (90%), similar to most target carbonyl-OPAHs whereas others showed low or unpredictable behavior. Such variable behavior indicates the need to include more isotope-labelled surrogates when analyzing chemically more reactive substances like OPAHs.

The correlation of OPAH concentrations with those of parent-/alkyl-PAHs suggests that OPAHs are emitted by the same sources as PAHs. Ratios of OPAHs to their parent-PAHs were frequently ≥1, underlining the importance to include them in monitoring programs of PAH contamination. The inclusion of OPAHs will greatly improve risk assessments at contaminated sites, because of their known direct/higher toxicity and ability to spread in the environment. Furthermore, the measurements of OPAH/parent-PAHs ratios will be useful to monitor PAH degradation during soil remediation or natural attenuation.

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8 Supplemental information (Section B)

Table B-3: Type (IUSS Working Group WRB, 2006) and selected properties of soil used in method development, validation and application. Soil texture, Total organic carbon (C_{org}), and pH of ERM®-CC013a were determined by BAM (Berlin, Germany) available in certification reports online (http://www.rm-certificates.bam.de/en/certificates/environment/index.htm).

Site	Soil type	Texture	C_{org}	C/N	pH†	ECEC‡
			g kg^{-1}			$mmol_c$ kg^{-1}
Manaus	Ferralsol	Clay	41	14	3.74	26.2
Mainz	Luvisol	Silty loam	46	16	7.05	334
Berlin (ERM®-CC013a)	Cambisol	Loamy sand	18	24	7.79	not provided§

† pH of Manaus soil was determined in 1 M KCl and others in 0.01 M $CaCl_2$.

‡ ECEC is effective cation-exchange capacity

§ This property was not provided by BAM, Berlin and we did not determine them because the available sample size was too small.

Table B-4: List of OPAHs and fluoranthene-D$_{10}$, purity, supplier, and SIM ions used for their quantification. Compounds are arranged in ascending order of retention times for carbonyl and hydroxyl/carboxyl-OPAHs and numbered accordingly (in brackets). Deuterated internal standards are shown in italics.

OPAHs labels in brackets	Purity (%)	Company	Monitored ion	Qualifier ion
CARBONYL OPAHs				
1-Indanone (1)	99	Sigma Aldrich	132	104
1,4-Naphthoquinone-D$_6$ (2)	99	CDN isotopes	*164*	*108*
1, 4-Naphthoquinone (3)	99	Dr. Ehrenstorfer	158	102
1-Naphthaldehyde (4)	95	Sigma Aldrich	156	128
2-Biphenylcarboxaldehyde (5)	96	Sigma Aldrich	181	152
Benzophenone-2,3,4,5,6-D$_5$ (6)	98	Sigma Aldrich	*187*	*105*
9-Fluorenone (7)	99.5	Dr. Ehrenstorfer	180	152
1, 2-Acenaphthylenequinone (8)	97	Sigma Aldrich	126	182
9, 10-Anthraquinone (9)	99.5	Dr. Ehrenstorfer	208	180
Fluoranthene-D$_{10}$ (10)	99.5	Dr. Ehrenstorfer	*212*	*213*
HYDROXYL/CARBOXYL OPAHs				
1, 2-Dihydroxybenzene (1)	99	Sigma-Aldrich	254	239
2-Hydroxybenzaldehyde (2)	99	Sigma-Aldrich	179	161
2-Hydroxybenzoic acid (3)	99.5	Dr. Ehrenstorfer	267	268
1-Hydroxynaphthalene-D$_7$ (4)	99	CDN isotopes	*223*	*208*
1-Hydroxynaphthalene (5)	98	Sigma Aldrich	216	201
Transcinnamic acid-D$_6$ (6)	98	Sigma Aldrich	*212*	*227*
2-Hydroxynaphthalene (7)	98	Sigma Aldrich	201	216
2-Hydroxybiphenyl (8)	99	Acros	211	242
1, 2, 3, 4-Tetrahydro-2-naphthoic acid (9)	98	Sigma Aldrich	130	248
1-Naphthoic acid (10)	98	Acros	229	244
1-Hydroxyacenaphthene (11)	99	Sigma Aldrich	242	152
2-Naphthoic acid (12)	98	Sigma Aldrich	229	244
5, 6, 7, 8-Tetrahydro-2-naphthoic acid (13)	97	Sigma Aldrich	233	248
2, 5-Dihydroxybenzoic acid (14)	99	Sigma Aldrich	355	370
9-Hydroxyfluorene (15)	96	Sigma Aldrich	165	254
2, 3-Dihydroxynaphthalene (16)	98	Sigma Aldrich	304	305
4, 5-Dihydroxyphenanthrene (17)	-	Sigma Aldrich	354	355
1-Hydroxy-2-naphthoic acid (18)	97	Sigma Aldrich	317	318
Fluoranthene-D$_{10}$ (19)	99.5	Dr. Ehrenstorfer	212	213
9-Hydroxyphenanthrene (20)	99.5	Dr. Ehrenstorfer	266	251
Biphenyl-2´,2´-dicarboxylic acid (21)	97	Sigma Aldrich	269	270
2, 3-Naphthalenedicarboxylic acid (22)	95	Acros	345	360

Table B-5: List of target alkyl/parent-PAHs their deuterium-labelled PAHs, purity, supplier and ions used for their measurements. Target alkyl/parent-PAHs are arranged in ascending order of retention times and numbered accordingly (in parentheses). Each group of PAHs is quantified relative to the labeled deuterated PAH before it.

PAHs and labels in brackets	Purity (%)	Supplier	Monitored ion	Qualifier ion
Naphthalene-D_8 (2)	99.5	Dr. Ehrenstorfer	136	137
1,2,3,4-Tetrahydronaphthalene (1)	96.1	Sigma Aldrich	104	132
Naphthalene (3)	99	Dr. Ehrenstorfer	128	127
2-Methylnaphthalene (4)	99	Dr. Ehrenstorfer	142	141
1-Methylnaphthalene (5)	95	Sigma Aldrich	142	141
1,3-Dimethylnaphthalene (6)	96	Sigma aldrich	156	141
Acenaphthene-D_{10} (8)	99.5	Dr. Ehrenstorfer	164	162
Acenaphthylene (7)	98	Dr. Ehrenstorfer	152	151
Acenaphthene (9)	99.5	Dr. Ehrenstorfer	153	154
2,3,5-Trimethylnaphthalene (10)	97	Acros	170	155
Fluorene (11)	99	Dr. Ehrenstorfer	166	165
2,6-diisopropylnaphthalene (12)	99	Acros	197	212
Phenanthrene-D_{10} (13)	99.5	Dr. Ehrenstorfer	188	189
Phenanthrene (14)	97.5	Dr. Ehrenstorfer	178	179
Anthracene (15)	99.5	Dr. Ehrenstorfer	178	179
2-Methylphenanthrene (16)	99	Ultra Scientific	192	191
1-Methylphenanthrene (17)	99	Ultra Scientific	192	191
3,6-Dimethylphenanthrene (18)	99	Ultra Scientific	206	205
3,5-Dimethylphenanthrene (19)	99	Chiron	206	191
2,6-Dimethylphenanthrene (20)	99	Chiron	206	191
1,7-Dimethylphenanthrene (21)	99.9	Chiron	206	191
Pyrene-D_{10} (23)	98	CIL	212	213
Fluoranthene (22)	99	Dr. Ehrenstorfer	202	200
Pyrene (24)	99	Dr. Ehrenstorfer	202	200
1-Methyl-7-isopropylphenanthrene (25)	95.4	Chiron	219	234
Chrysene-D_{12} (27)	99.5	Dr. Ehrenstorfer	240	241
Benz[a]anthracene (26)	99	Dr. Ehrenstorfer	228	226
Chrysene+triphenylene (28)	99	Dr. Ehrenstorfer	228	226
Perylene-D_{12} (32)	99.5	Dr. Ehrenstorfer	264	265
Benzo[b,j,k]fluoranthenes (29)	99.5	Dr. Ehrenstorfer	252	253
Benzo[e]pyrene (30)	99.5	Dr. Ehrenstorfer	252	253
Benzo[a]pyrene (31)	98	Dr. Ehrenstorfer	252	253
Perylene (33)	99	Dr. Ehrenstorfer	252	253
Benzo[ghi]perylene-D_{12}(36)	98	CIL	288	289
Indeno [1,2,3-cd]pyrene (34)	99.5	Dr. Ehrenstorfer	276	274
Dibenz[a,h]anthracene (35)	98.5	Dr. Ehrenstorfer	278	279
Benzo[ghi]perylene (37)	99.3	Dr. Ehrenstorfer	276	277
Coronene (38)	99.5	Dr. Ehrenstorfer	300	150

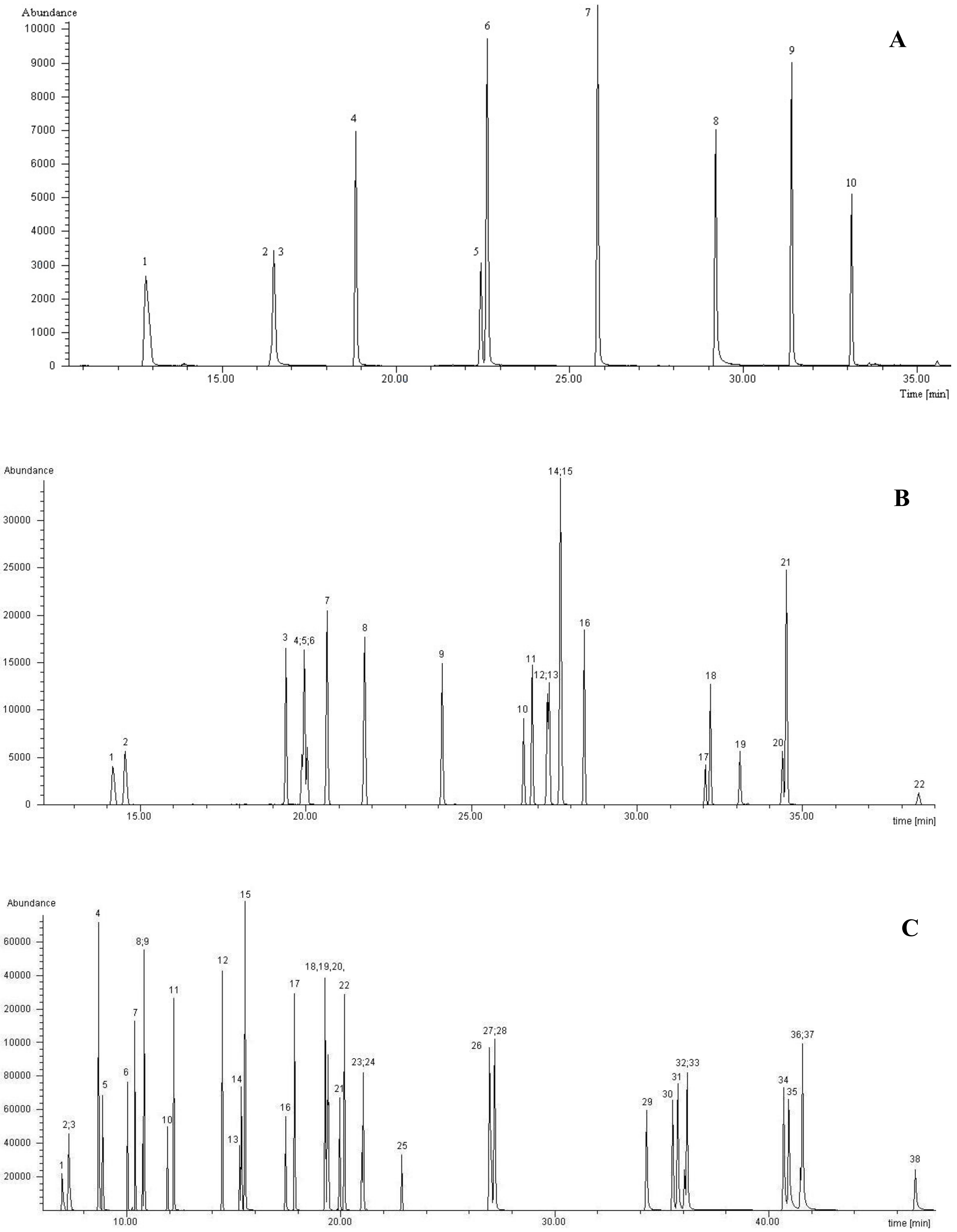

Figure B-2: The GC-MS-SIM chromatogram of the standard mixtures (500 ng/ml) of carbonyl-OPAHs [A], hydroxyl/carboxyl-OPAHs [B], and alkyl/parent-PAHs [C]. FLUA-D10 is included in all three chromatograms at different positions. Numbering for alkyl/parent-PAH and carbonyl and hydroxyl/carboxyl-OPAHs is the same as shown in Tables B-4 and B-5, respectively.

C Polycyclic aromatic hydrocarbons (PAHs) and their oxygen-containing derivatives and metabolites (OPAHs) in soils from the Angren industrial area, Uzbekistan[a]

Benjamin A. Musa Bandowe[1], Nosir Shukurov[2], Michael Kersten[3], Wolfgang Wilcke[4]

[1] Earth System Science Research Center, Geographic Institute, Johannes Gutenberg University Mainz, Johann-Joachim-Becher-Weg 21, 55128 Mainz, Germany

[2] Institute of Geology and Geophysics, Uzbekistan Academy of Sciences, N. Khodjibaev 49 100041, Tashkent, Uzbekistan

[3] Earth System Science Research Center, Institute of Geosciences, Johannes Gutenberg University Mainz, 55099, Mainz, Germany

[4] Geographic Institute, University of Berne, 3012 Berne, Switzerland

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1 Abstract

We measured the concentrations and depth distribution (0-10, 10-20 cm) of 31 PAHs and 12 OPAHs in soils at eleven equidistant sampling points along a 20-km transect in the Angren industrial region (coal mine, power plant, rubber factory, gold mine), Uzbekistan to gain an insight into their concentrations, sources, and fate. Concentrations of all compounds were mostly much higher in the 0-10 cm than in the 10-20 cm layer except in disturbed soil close to the coal mine. Proximity to one of the industrial emitters was the main determinant of PAH and OPAH concentrations. The ∑31PAHs concentrations correlated positively with the ∑7 carbonyl-OPAH ($r = 0.98$, $p < 0.01$), ∑5 hydroxyl-OPAH ($r = 0.72$, $p < 0.05$), and with industrially emitted trace metals in the topsoil, identifying industrial emissions as their common source. Concentrations of several carbonyl-OPAHs were higher than their parent PAHs, but their vertical distribution in soil suggested only little higher mobility of OPAHs than their corresponding parent PAHs..

2 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are persistent and toxic, because of which 16 of them are included in the USEPA list of priority pollutants (Keith and Telliard, 1979). PAH concentrations in soil have been extensively studied in the temperate zone (mainly Europe, North America, and Japan) but little outside the temperate zone such as in the semi-arid regions of Central Asia (Wilcke, 2000, 2007). Central Asia hosts several former Soviet industrial hubs where the environment is likely to be heavily contaminated by a range of pollutants including PAHs and heavy metals. One example is the Angren industrial area near Tashkent in Uzbekistan. Previous studies conducted in this area demonstrated that soil, vegetation, surface, and groundwater are polluted with heavy metals and sulfur and nitrogen compounds, and that this pollution has negatively affected plants, soil nematodes, and soil microbial properties (Talipov et al., 1996; UNECE, 2002; Sanitation Country Profile, 2004; Shukurov et al., 2009).

Both, coal mining and combustion (e.g., coal-based electricity production) occur in the Angren industrial region. Besides the coal combustion activities which emit gaseous and particle-bound PAHs, coal mining itself can emit large quantities of unburnt coal particles which can contain high concentrations of PAHs (Achten and Hofmann, 2009). Generally, concentrations of PAHs in soil decrease exponentially with increasing distance from a point source (Van Brummelen et al., 1996; Wilcke et al., 1996; Bakker et al., 2000). In the atmosphere, the fraction of a given PAH concentration sorbed to particles increases with decreasing volatility (Meharg et al., 1998). Previous studies observed that the transport distance decreases with increasing molecular weight because the proportion associated with fast settling atmospheric particles increases (Yang et al., 1991; Meharg et al., 1998; Bakker et al., 2000; Sharma and Tripathi., 2009). Gaseous PAHs, in contrast, may be distributed over longer distances.

Oxygen-containing PAH derivatives (OPAHs) including microbial metabolites may contain functional groups such as e.g., carbonyl, hydroxyl, carboxyl, anhydrides, and coumarins (Konig et al., 1983; Lundstedt et al., 2007). OPAHs are emitted together with PAHs from combustion activities (primary sources), or are post emission degradation products (secondary sources) of PAH conversion by photooxidation, chemical oxidation, and microbial transformation processes (metabolites) (Cerniglia, 1984; Bamforth and Singleton, 2005; Lundstedt et al., 2007). OPAHs were shown in several studies to occur at elevated concentrations in atmospheric samples (gaseous and particulate) and soil (Moyano and Galceran, 1997; Meyer et al., 1999; Lundstedt et al., 2006; Albinet et al., 2007). Some OPAHs are known to be persistent, ubiquitous, and some are even more toxic than their parent compounds (Lundstedt et al., 2007). There is generally little available information on OPAH concentrations in soil. In particular, the relative contributions of combustion (primary source) and post-emission transformations (secondary sources), including microbial processes, photooxidation, and chemical oxidation to the OPAHs concentrations in soil are unknown.

Usually, PAHs are strongly sorbed to soil organic matter and therefore little leached to greater soil depths (Wilcke et al., 1996; Wilcke, 2000). Nevertheless, PAHs frequently occur in subsoils (Guggenberger et al., 1996; Wilcke et al., 1996; Krauss et al., 2000; Ma et al., 2005). While for the low molecular weight PAHs a (minor) transport in dissolved phase may occur, the transport of high molecular weight PAHs is only possible if facilitated by colloids such as dissolved organic matter (Gauthier et al., 1987; Maxin and Kögel-Knabner, 1995; Chin et al., 1997). In contrast to PAHs, OPAHs should be considerably more mobile because of their higher water solubility as indicated by lower octanol-water partitioning coefficients (Hansch et al., 1995). It has also been directly shown in soil column experiments that OPAHs are more mobile than their parent PAHs (Weigand et al., 2002; Lundstedt et al., 2007). However, we are not aware of any report on the depth distribution of OPAHs in field soils.

The objectives of our study were to (i) determine the spatial distribution of PAH and OPAH concentrations in soils along a linear transect in an industrial semi-arid region in Central Asia, (ii) explore the correlations between PAHs, OPAHs, and heavy metals (taken from the literature) as indication of possible joint sources, and (iii) assess the depth distribution of PAHs and OPAHs in soil. We tested three hypotheses: (i) PAH concentrations are controlled by distance from industrial emission sources and correlated with heavy metal concentrations. (ii) OPAH concentrations are correlated with those of PAHs and heavy metals near emission sources while at greater distance transformation processes (microbial, photooxidation, and chemical oxidation) of PAHs play an increasing role weakening the relationship between parent PAH and OPAH concentrations. (iii) OPAHs are more water soluble than their parent PAHs and therefore more strongly leached into deeper soil layers.

3 Materials and methods

3.1 Description of study location

The study area is located at 114 km south of Tashkent, the capital of the Republic of Uzbekistan, Central Asia. This region is a semi-arid mountain valley located along the Akhangaran River between 41°01’ and 40°58’ N and 70°10’ and 69°57’ E, at 900-950 m a.s.l. The climate is continental with temperature amplitude between -30°C in January and +45°C in July and an annual precipitation between 320-550 mm falling mostly in spring and winter. The study area is covered by grassland. Dominating plant genera include *Astragalus*, *Stipa*, *Medicago*, and *Artemisia*. The prevailing wind direction is western and north-western with thermal inversions providing cyclic circulations of air masses causing pendulum distributions of dust and gas-smoke emission from the industrial complex. Because this area is surrounded by a chain of mountains air circulation is poor. The soils are Haplic Calcisols (IUSS Working Group WRB, 2006) with low organic matter concentrations. A detailed description of the site is given by Shukurov et al. (2009).

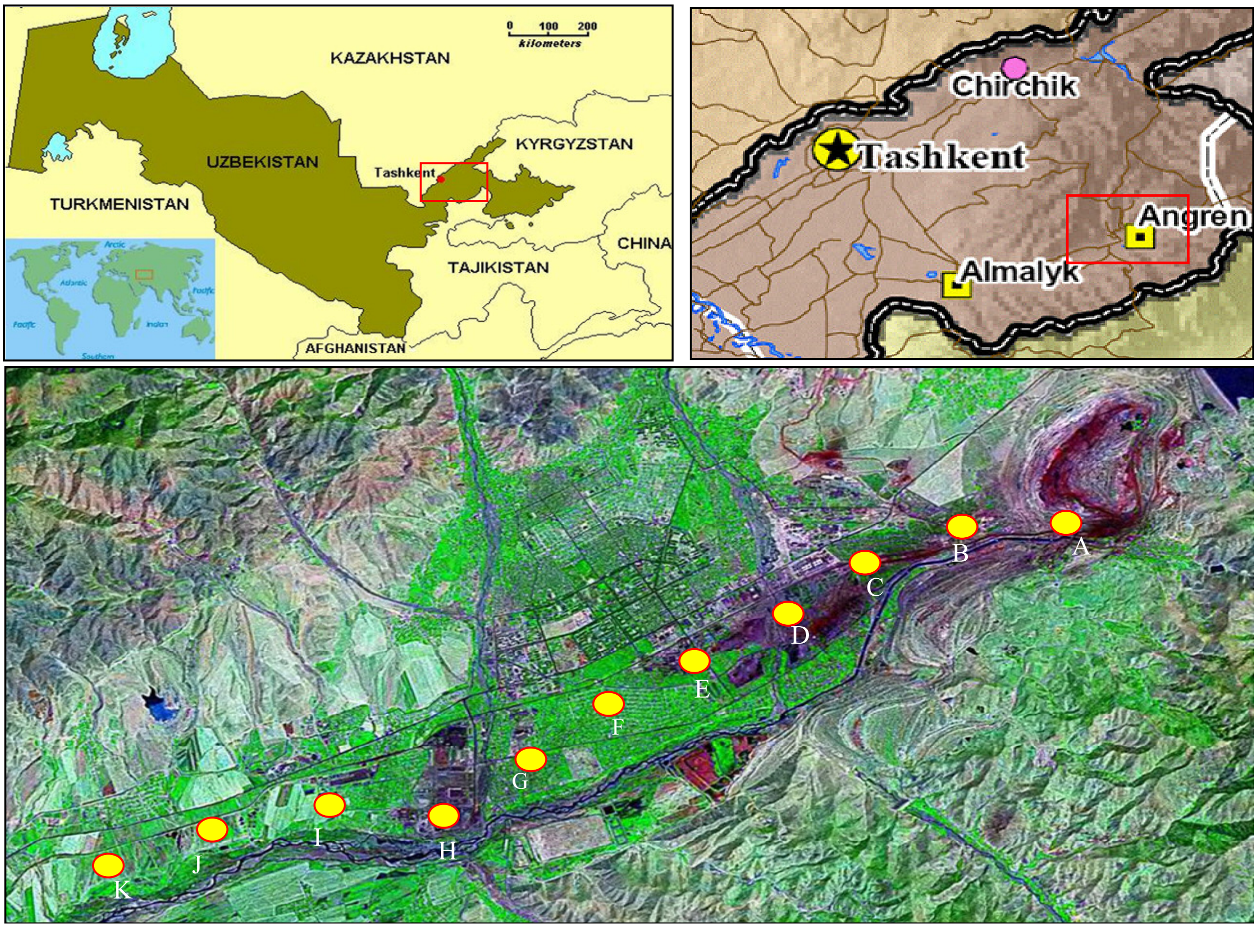


Figure C-1: Location of study area (aerial photo is from Google Earth) with sampling sites A-K described in Table C-1.

3.2 Soil sampling and properties

Soil samples were collected at two depths (0-10 cm and 10-20 cm) from 11 locations along a 20-km transect downwind of the industrial complex. The soils were air-dried, sieved over stainless steel sieves (< 2 mm), and stored at 4°C until analysis. The sampling locations are shown in Figure C-1, and the land use and physico-chemical properties of the soils listed in Table C-1. Soil pH, total organic carbon (C_{org}), and trace metal concentrations were determined as described earlier (Shukurov et al., 2009). Soil pH was measured in H_2O (soil/solution ratio 1:2) with a potentiometric glass electrode. Total organic carbon (C_{org}) was determined by oxidation with K-dichromate with addition of H_2SO_4 without additional heating (Rowell, 1994). Effective cation-exchange capacity (ECEC) was calculated as the sum of the charge equivalents of K, Na, Mg, Al, Ca, and Mn determined by flame AAS (Varian) after extraction with 1 M NH_4NO_3. Soil texture was analyzed using a pipet method after organic matter, lime, and pedogenic iron oxides were destroyed with H_2O_2, HCl, and dithionite/citrate, respectively, and after sand fractions were removed by sieving. In the earlier studies by Shukurov et al. (2009), samples were not taken from sampling sites B, F and J. For the current manuscript we additionally considered these three sampling sites and determined texture, pH, and C_{org} (with an elemental analyzer, Elementar) but not trace metal concentrations.

Table C-1: Sampling locations, land use, and selected physico-chemical properties of soil. Sampling depth 1 is at 0-10 cm and 2 at 10-20 cm depth.

Site/depth	GPS Coordinates	Land use	C_{org} [%]	pH	ECEC[cmol/kg]	Sand [%]	Silt [%]	Clay [%]
A1	41°01´ N/70°10´ E	Border of coal mine (0 km, grassland)	2.2	7.24	13.6	23	60	17
A2			2.0	7.52	10.0	30	54	16
B1	41°01´ N/70°09´ E	Near coal mine (2 km, grassland)	1.1	8.00	9.4	28	64	8
B2			0.7	7.83	9.0	25	61	13
C1	41°00´ N/70° 08´ E	East of Angren power station (4 km, grassland)	2.0	7.78	9.6	37	54	9
C2			2.0	7.95	11.4	40	54	6
D1	41°00´ N/70° 07´ E	West of Angren power station (6 km, grassland)	1.4	8.05	8.0	38	56	6
D2			1.2	8.10	7.9	65	31	3
E1	40°57´ N/70° 05´ E	Near the coal ash depository (8 km, gardening area)	1.9	7.92	9.2	17	71	12
E2			1.9	8.17	9.3	27	60	13
F1	40°57´ N/70° 04´ E	Recreation area (10 km, gardening)	2.3	8.03	10.1	12	71	17
F2			2.0	7.93	9.6	10	75	16
G1	40°58´ N/70° 03´ E	Recreation area/summer camp (12km, grassland)	1.9	7.84	7.0	10	71	19
G2			1.2	7.99	6.6	9	73	18
H1	40°58´ N/70° 02´ E	Near rubber factory (14 km, industrial site)	1.3	7.92	5.2	48	44	7
H2			0.5	8.02	2.5	56	35	9
I1	41°58´ N/70° 00´ E	Farming area (16 km, arable land)	0.3	8.26	9.0	3	76	21
I2			0.2	8.00	8.8	4	73	23
J1	41°49´ N/69° 59´ E	Near the gold refinery (18 km)	1.1	8.09	9.1	30	52	18
J2			0.7	7.83	8.3	40	44	17
K1	40°58´ N/69° 57´ E	Grazing area (20 km)	1.4	7.83	10.1	10	67	23
K2			0.6	7.83	9.5	11	63	26

3.3 PAH and OPAH analyses

3.3.1 Target compounds

In all soil samples, concentrations of 31 PAHs/alkyl-PAHs were determined: naphthalene (NAPH), 2-methylnaphthalene (2-MNAPH), 1-methylnaphthalene (1-MNAPH), 1,3-dimethylnaphthalene (1,3-DMNAPH), acenaphthylene (ACENY), acenaphthene (ACEN), 2,3,5-trimethylnaphthalene (2,3,5-TMNAPH), fluorene (FLUO), phenanthrene (PHEN), anthracene (ANTH), 2-methylphenanthrene (2-MPHEN), 1-methylphenanthrene (1-MPHEN), 3,6-dimethylphenanthrene (3,6-DMPHEN), 3,5-dimethylphenanthrene (3,5-DMPHEN), 2,6-dimethylphenanthrene (2,6-DMPHEN), 1,7-dimethylphenanthrene (1,7-DMPHEN), fluoranthene (FLUA), pyrene (PYR), 1-methyl-7-isopropylphenanthrene (RETENE), benz[a]anthracene [B(A)A], chrysene+triphenylene (CHRY), benzo[b+j+k]fluoranthenes [B(BJK)], benzo[e]pyrene ([B(E)P]), benzo[a]pyrene ([B(A)P]), perylene (PERY), indeno[1,2,3-cd]pyrene (IND), dibenz[a,h]anthracene (DIBE), benzo[ghi]perylene ([B(GHI)]), and coronene (COR); 7 carbonyl-OPAHs: 1-indanone (1-INDA), 1,4-naphthoquinone (1,4-NQ), 1-naphthaldehyde (1-NLD), 2-biphenylcarboxaldehyde (2-BPCD), 9-fluorenone (9-FLO), 1,2-acenaphthylenedione (1,2-ACQ), 9,10-anthraquinone (9,10-ANQ); and 5 hydroxyl-OPAHs: salicylaldehyde (SALYD), 2-naphthol (2-NAPHTHOL), 2-hydroxybiphenyl (2-PP), 1-acenaphthenol (1-ACENOL), 9-fluorenol (9-FLUORENOL) were determined.

Internal standards. A mixture of seven deuterated PAHs (NAPH-D_8, ACEN-D_{10}, PHEN-D_{10}, PYR-D_{10}, CHRY-D_{12}, PERY-D_{12}, and B(GHI)-D_{12} was used as internal standard to quantify parent and alkyl PAHs. Benzophenone-2,3,4,5,6-D_5 was used as internal standard to quantify carbonyl-OPAHs. FLUA-D_{10} solution at 4 µg/ml was used as syringe spike to quantify the hydroxyl-OPAHs and as recovery standard for benzophenone-2,3,4,5,6-D_5. The standards of the target PAHs, alkyl-PAHs, OPAHs, and internal standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany), Ultra Scientific (North Kingston, RI, USA), Chiron (Trondheim, Germany), Acros Organics (New Jersey, USA), and the Sigma-Aldrich group (Steinheim, Germany).

3.3.2 Analytical method

A detailed description of the analytical procedure for the measurement of PAHs and OPAHs is given in Bandowe and Wilcke (2010). Briefly, to about 10 g of soil sample, 100 µl of benzophenone-2,3,4,5,6-D_5 (5 µg/ml) and 50 µl of the mixture of the seven deuterated PAHs NAPH-D_8, ACEN-D_{10}, PHEN-D_{10}, PYR-D_{10}, CHRY-D_{12}, PERY-D_{12}, and B(GHI)-D_{12} (each at 5 µg/ml) were added as internal standards to the accelerated solvent extractor (ASE) cell. Samples were extracted using ASE 200 (Dionex, Sunnyvale, CA, USA) with dichloromethane in a first extraction cycle, and with $CH_3COCH_3/CH_2Cl_2/CF_3COOH$ (1%) [250:125:1 v/v/v] in a second extraction cycle. The two extracts of each sample were combined, dried on Na_2SO_4, and cleaned up/fractionated on silica gel (10% deactivated) columns. PAHs/alkyl-PAHs were eluted with 9 ml hexane/dichloromethane (5:1 v/v), followed by 8 ml dichloromethane and 5 ml acetone to elute the OPAHs. The PAHs/alkyl-PAHs fraction was rotary evaporated to about 0.5 ml and transferred to a 1.5 ml GC vial for GC/MS measurement. The OPAHs fraction was rotary evaporated and spiked with 100 ng FLUA-D_{10} as internal standard for quantification of the hydroxyl-OPAHs and recovery standard for benzophenone-2,3,4,5,6-D_5. This fraction was split and one half measured directly with GC/MS to quantify the carbonyl-OPAHs. To the other half N,O-bis-(trimethylsilyl)-trifluoroacetamide [BSTFA:TMCS (99:1)] was added and heated for 15 min at 60°C to silylate the hydroxyl-OPAHs followed by GC/MS measurement. Target compounds were quantified by the internal standard technique using ten calibration standards prepared from target compound standards each spiked with a constant concentration of internal standard. Hydroxyl-OPAHs standards were derivatized as above before GC/MS measurements. We used an Agilent 6890 N gas chromatograph coupled to an Agilent 5973 B inert mass selective detector in EI and SIM mode for our measurements. All data recording and processing was done with the Agilent MSD ChemStation software package.

3.4 Quality assurance and quality control

All glassware was rinsed with acetone, machine washed, baked at 250°C for 12 h, and rinsed with high purity "picograde" solvents before use. All solvents used for extraction, column chromatography, and standard preparation were high purity "picograde" for residue analysis (Promochem, Wesel, Germany). To check and correct for possible contaminations during the analytical procedure, we processed three blanks made of diatomaceous earth at the beginning, middle, and end of our sample sequence. Most compounds were either not detected in the blanks or were measured in negligible quantities, significantly below those found in the samples. The measured compound concentrations were corrected by subtracting the mean blank concentration. We checked the accuracy of PAH measurements by including the certified reference material ERM$^{®}$-CC013a (BAM, Berlin, Germany) into our analyses. The mean recovery of all PAHs for which certified values were provided was 110%. Since there is no certified reference material for OPAHs, we checked the recovery of benzophenone-2,3,4,5,6-D_5 used as internal standard for carbonyl-OPAHs. The mean recovery of benzophenone-D_5 was 80% (67-88%). The recovery of the hydroxyl-OPAHs determined previously by spike and recovery experiment with this method was 36-70% (Bandowe and Wilcke, 2010). All concentrations are reported without recovery correction.

3.5 Calculations and compound properties

The sum of the concentrations of all parent- and alkyl-PAHs is ∑31 PAHs, of all parent-PAHs ∑21PAHs, of the 16 EPA-PAHs ∑EPA-PAHs, of 1- and 2-methylnaphthalenes ∑C1-NAPH, of 1- and 2-methylphenanthrenes ∑C1-PHEN, and of 3,6-, 3,5-, 2,6-, and 1,7-dimethylphenanthrenes ∑C2-PHEN. The octanol-water partition coefficients (K_{OW}) of PAHs and alkyl-PAHs were taken from literature (Neff et al., 2005; Mackay et al., 2006), and those of OPAHs were taken from Hansch et al. (1995) or estimated from KOWWIN v1.67 EPI Suite^{TM} version 4.0 (USEPA: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm acessed on 5/8/2009). Statistical analysis was performed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). We used the Kolmogorov-Smirnov test to check normal distribution of the data. Significance was set at $P < 0.05$.

4 Results

4.1 PAH and OPAH concentrations

The ∑31 PAHs concentrations in the 0-10 cm topsoil layer ranged from 118 ng/g at site I to 5913 ng/g at site D (Fig. C-2). The 10-20 cm subsoil layer had ∑31 PAHs concentrations in the range from 104 ng/g (I) to 3852 ng/g (A). The ∑31 PAHs concentrations in the topsoil were higher than in the subsoil at all sampling sites except A where the subsoil showed higher ∑31 PAHs concentrations than the topsoil, and C and I where topsoil and subsoil had similar ∑31 PAHs concentrations. The highest ratios of the ∑31 PAHs concentration in topsoil to that in subsoil occurred at the sites close to industrial activities, i.e. B (3, near coal mine), D (5, near power plant), and H (8, near rubber factory).

The mixtures of the 31 parent and alkylated PAHs in topsoil were dominated by NAPH (9-35%, mean: 17%), PHEN (9-18%, mean: 15%), and B(BJK) (5-15%, mean: 10%, Fig. C-3). The contribution of the low molecular weight PAHs NAPH+PHEN to the ∑31PAHs ranged between 18% (site A) and 51% (site H).

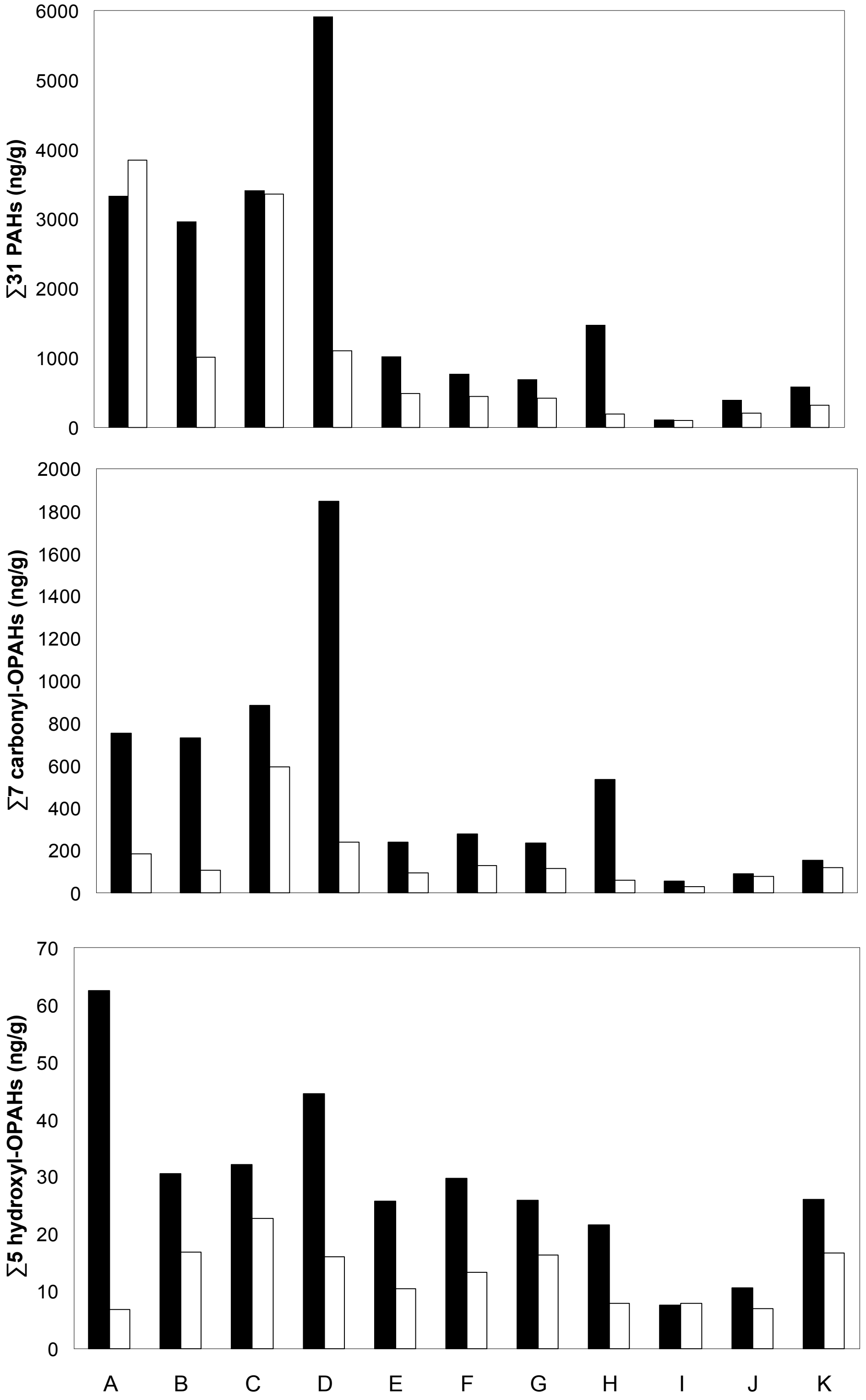


Figure C-2: Concentrations of ∑31PAHs (A), ∑7 carbonyl-OPAHs (B), and ∑5 hydroxyl-PAHs in topsoil (0-10 cm, filled black bars) and subsoil (10-20 cm, open bars) of the 11 study sites.

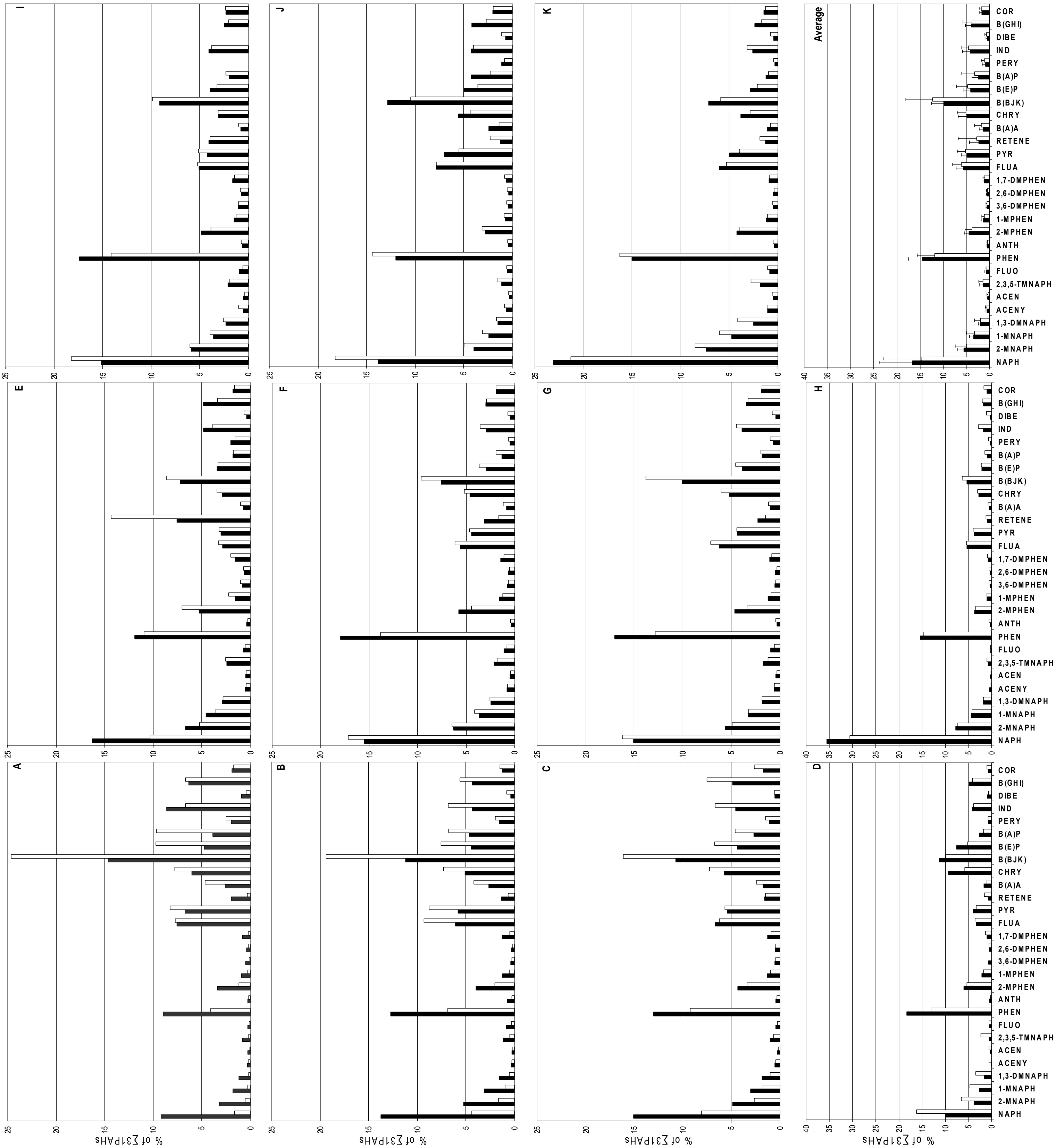


Figure C-3: Composition [%] of parent- and alkylated PAH mixtures in topsoil (0-10 cm, filled black bars) and subsoil (10-20 cm, open bars) of the 11 sampling sites A-K depicted in Fig. C-1.

The contribution of 2- and 3-ring parent-PAHs to ∑ 21PAHs in topsoils was <50% (23-49%) at sites (A, B, C, D, E, G, I, and J), but >50% (50-67%) at sampling sites F, K, and H (Fig. C-4). The smallest contribution of 2- to 3-ring compounds to ∑21PAHs occurred at site A, and the highest at site H (near the rubber factory). There was no systematic change in contribution with distance from one of the local point sources (coal mine, power plant, gold refinery, rubber factory) except a gradual increase from 37% at site D (power plant) to 50% at site F. At most sites, the contribution of 2- to 3-ring parent-PAHs to ∑21PAHs was lower in subsoils compared to their corresponding topsoils except at sites D, J, and K.

The ∑7 carbonyl–OPAHs concentrations showed a similar spatial distribution as those of the PAHs (Fig. C-2). The ∑7 carbonyl–OPAHs concentrations was highest at site D (1848 ng/g) and lowest at site I (54 ng/g, Fig. C-2). The ∑7 carbonyl-OPAHs concentrations were consistently higher in topsoil than in subsoil where they ranged between 29 ng/g (I) and 595 ng/g (C). The ratios of the ∑7 carbonyl-OPAHs concentration in topsoil to that in subsoil were 9 (H), 8 (D), 7 (B), 4.1 (A), and 1.2-2 at all other sites. The carbonyl-OPAHs were dominated by 9-FLO and 9,10-ANQ in all samples. These compounds constituted around 40% (38-47%) each of the Σ7 carbonyl-OPAHs. 1-INDA was also abundant and contributed with another ca. 10% on average (Table C-2).

The ∑5 hydroxyl-OPAHs concentrations in topsoil ranged between 8 ng/g (I) and 63 ng/g (A) (Fig. C-2), and in subsoil between 7 (site A) and 23 ng/g (site C). The topsoil had higher ∑5 hydroxyl-OPAHs concentrations than the subsoil except at site I where topsoil and subsoil had similar ∑5 hydroxyl-OPAHs concentrations. The concentration ratio of the ∑5 hydroxyl-OPAHs in topsoil to that in subsoil were as high as 9 at site A, at all other sites (except I) in the range of 1.4-2.8, and as low as 0.9 at site I. The mixture of hydroxyl-OPAHs was clearly dominated by SALYD, with average contributions to the ∑5 hydroxyl-OPAHs concentrations of 65% in topsoil and 64% in subsoil.

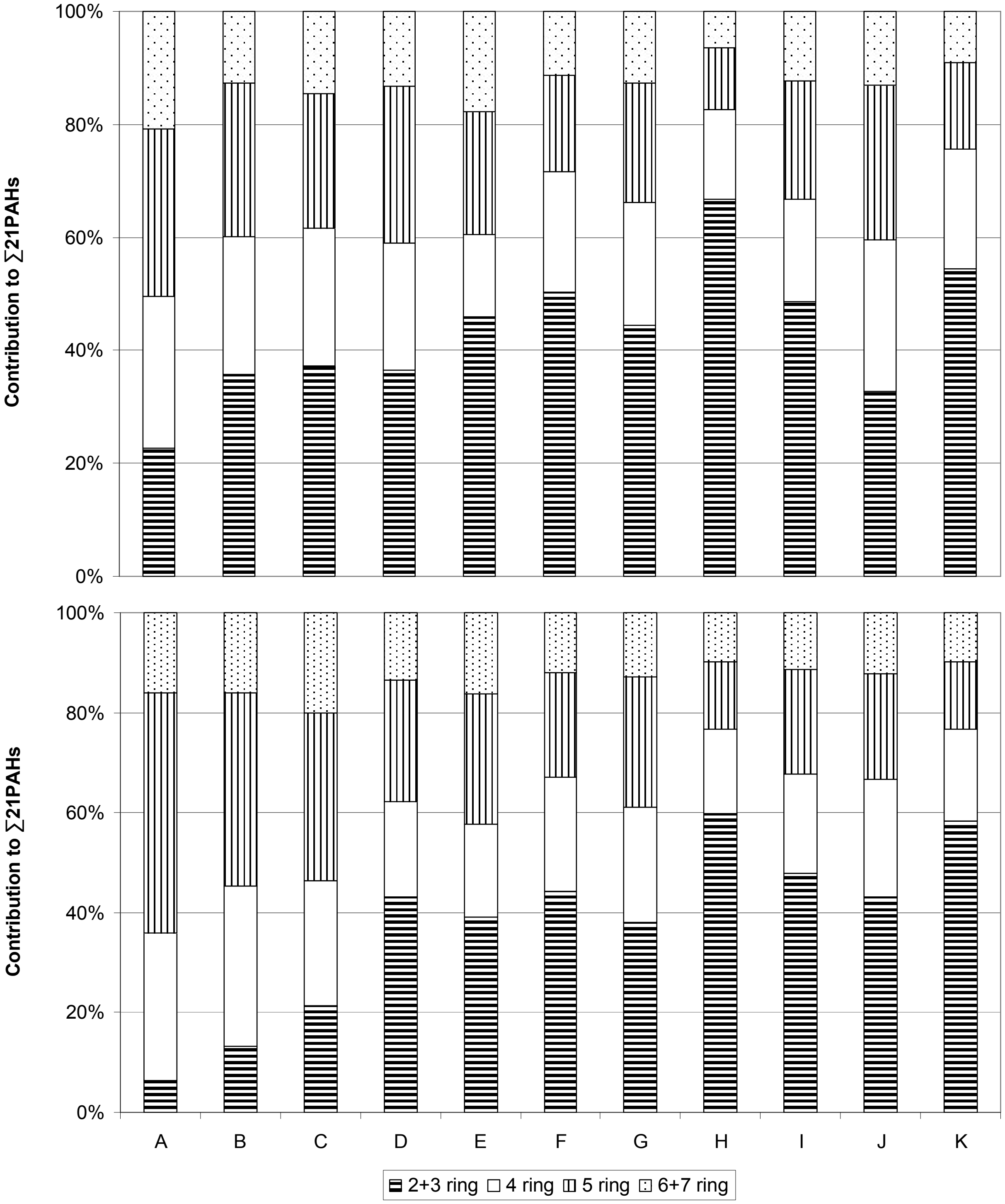


Figure C-4: Percentage contribution of 2-+3-, 4-, 5-, and 6-+7-ring parent-PAHs to the sum of 21 parent-PAHs (∑21PAHs) in topsoils (upper diagram) and subsoils (lower diagram).

Table C-2: Concentration of individual OPAHs and ∑EPA-PAH and ∑21PAHs at the 11 study sites (ng/g). Sampling depth 1 is at 0-10 cm and 2 at 10-20 cm depth.

Site/depth	1-INDA	1,4-NQ	1-NLD	2-BPCD	9-FLO	1,2-ACQ	9,10-ANQ	SALYD	2-NAPHTHOL	2-PP	1-ACENOL	9-FLUORENOL	∑EPA-PAHs	∑21PAHs
A1	32.0	2.0	14	0.7	315.8	9.1	376.0	44.3	7.5	10.7	n.d	n.d	2553.7	2843.4
A2	5.9	0.6	3.1	0.4	62.3	3.7	106.9	6.8	n.d	n.d	n.d	n.d	3182.9	3721.5
B1	6.6	4.0	4.0	n.d	409.6	14	292.6	19.7	3.8	7.1	n.d	n.d	2166.6	2378.5
B2	20.6	0.4	2.4	0.4	33.7	3.5	44.3	6.7	n.d	3.4	4.5	2.3	824.5	936.4
C1	12.3	5.0	18	8.8	466.3	5.6	368.7	20.5	4.0	7.7	n.d	n.d	2476.7	2723.1
C2	7.4	3.6	11	0.4	252.0	5.9	315.2	13.5	4.2	5.0	n.d	n.d	2532.0	2893.9
D1	27.6	6.7	20.6	15.2	810.0	9.6	958.7	29.0	4.1	11.4	n.d	n.d	4234.7	4768.4
D2	22.1	2.3	9.0	0.4	117.4	5.7	82.3	13.0	n.d	3.1	n.d	n.d	721.4	799.0
E1	26.9	1.6	7.1	25	107.3	4.5	67.0	20.1	n.d	5.6	n.d	n.d	601.4	675.0
E2	15.4	0.7	3.4	0.4	41.2	2.6	31.6	7.4	n.d	3.1	n.d	n.d	250.8	282.3
F1	35.0	0.8	7.3	0.4	112.7	3.7	118.5	17.2	3.8	6.3	n.d	2.6	511.3	551.7
F2	11.6	0.7	3.9	0.4	53.3	3.4	53.6	7.2	n.d	3.9	n.d	2.3	312.8	340.8
G1	30.7	0.8	5.6	0.4	104.5	4.2	90.0	17.1	3.6	5.1	n.d	n.d	493.5	538.4
G2	10.2	0.8	3.1	0.4	43.3	2.9	52.9	6.9	3.6	3.6	n.d	2.2	314.9	346.3
H1	2.1	2.8	14	5.4	349.0	6.7	154.9	7.0	7.1	7.6	n.d	n.d	1097.3	1148.1
H2	1.6	n.d	1.9	n.d	34.0	n.d	23.4	4.6	n.d	3.4	n.d	n.d	139.2	147.7
I1	13.5	n.d	2.1	0.4	20.2	3.2	14.7	4.3	n.d	3.3	n.d	n.d	77.8	85.2
I2	2.3	n.d	1.4	n.d	13.8	n.d	11.8	4.6	n.d	3.4	n.d	n.d	70.4	76.3
J1	9.5	0.4	2.5	0.3	36.7	3.2	36.2	10.7	n.d	n.d	n.d	n.d	306.6	339.1
J2	9.4	0.3	2.2	0.4	35.9	3.2	24.9	7.0	n.d	n.d	n.d	n.d	153.4	166.4
K1	25.2	0.7	5.3	0.4	79.6	3.5	39.6	15.8	3.7	4.3	n.d	2.4	417.9	445.3
K2	29.6	0.5	4.1	0.4	42.5	3.5	41.1	7.0	3.6	4.0	n.d	2.2	210.4	222.4

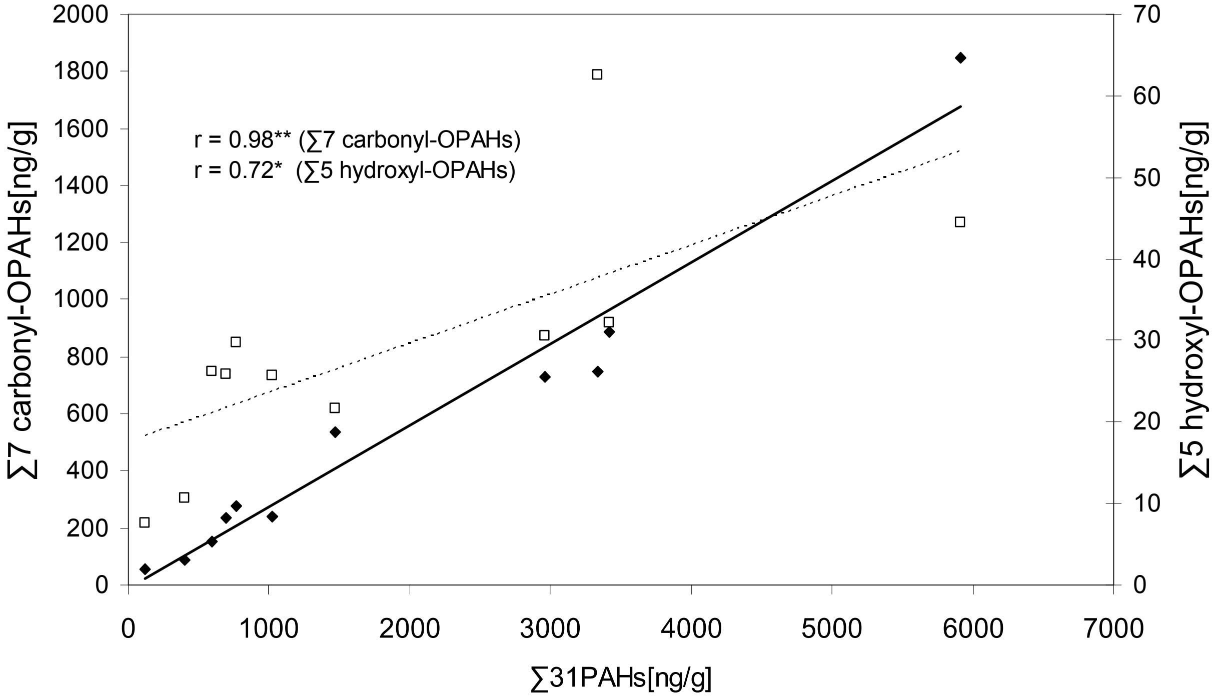
4.2 Correlations among PAH, OPAH, C_{org}, and heavy metal concentrations

Prior to correlation analyses, we tested the data sets for normality. Correlations were only calculated for normally distributed data sets which was the case for most of our data sets. In topsoil, the ∑31PAHs concentrations correlated strongly positively with the ∑7 carbonyl-OPAHs and the ∑5 hydroxyl-OPAHs concentrations (Fig. C-5). The ∑5 hydroxyl-OPAHs concentrations also correlated positively with the ∑7 carbonyl-OPAHs concentrations (Fig. C-5). In subsoil, the ∑31 PAHs concentrations correlated significantly with the ∑7 carbonyl-OPAHs concentrations ($r = 0.72$, $p = 0.01$) but not with the ∑5 hydroxyl-OPAHs concentrations ($r = 0.11$, $p = 0.74$), while the ∑5 hydroxyl-OPAHs were correlated with the ∑7 carbonyl-OPAHs ($r = 0.69$, $p = 0.02$). However the latter correlation disappeared, if site C is removed as an outlier.

Most individual PAHs correlated significantly with individual carbonyl-OPAHs in the topsoil (not shown). Exceptions, however, were PERY, RETENE, 1-INDA, and 2-BPCD. Correlations between individual hydroxyl-OPAHs and individual carbonyl-OPAHs or PAHs were much weaker (not shown). 1-NAPHTHOL correlated significantly with NAPH ($r = 0.62$, $p = 0.04$) while the concentrations of the other hydroxyl-OPAH/parent-PAH pairs did not show significant correlations.

There were no significant correlations between C_{org} and any individual PAH or OPAH in the topsoil except for 1-IND ($r = 0.64$, $p = 0.04$) and SALYD ($r = 0.61$, $p = 0.048$) concentrations.

As some heavy metals are known pollutants emitted by industrial activity in the Angren area, we correlated PAH and OPAH concentrations with those of heavy metals taken from Shukurov et al. (2009) measured at 8 sampling sites in the topsoils. Shukurov et al. (2009) identified Ba, Cu, Pb, Th, U, and Zn concentrations in soil as mainly originating from anthropogenic industrial emissions. There were significant positive correlations of ∑31PAHs and ∑7 carbonyl-OPAHs with Ba, Cu, Pb, Th, U, and Zn concentrations in the topsoil (Tab. C-3).



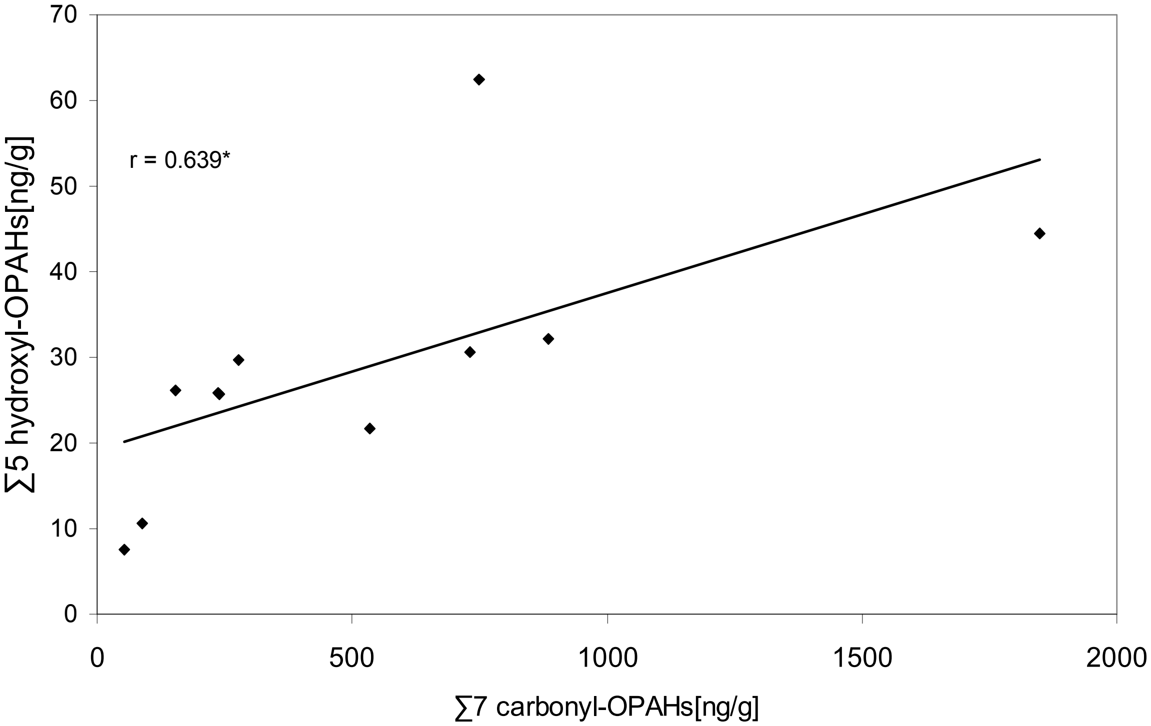


Figure C-5: Relation between ∑7carbonyl-OPAHs (closed boxes), ∑5hydroxyl-OPAHs (open boxes), and ∑31PAHs (upper diagram), and relation between ∑hydroxyl-OPAHs and ∑carbonyl-OPAHs (lower diagram) in topsoil. ** means $P < 0.01$ and * means $P < 0.05$.

Table C-3: Pearson correlation coefficient for ∑31PAHs, ∑7 carbonyl-OPAHs, ∑5 hydroxyl-OPAHs and selected trace metals in top soils (n = 8). Correlations marked in bold are significant at $P \leq 0.05$ (two tailed). Trace metal concentrations were taken from Shukurov et al., (2009).

0-10 cm	∑31PAHs	∑7 carbonyl-OPAHs	∑5 hydroxyl-OPAHs
Cu	0.49	0.50	0.23
Zn	**0.78**	**0.77**	0.35
Ba	**0.78**	**0.82**	0.32
Pb	**0.75**	**0.75**	0.32
Th	**0.70**	**0.76**	0.33
U	**0.81**	**0.82**	0.44

4.3 Concentration ratios of OPAHs to parent-PAHs

Several concentration ratios of OPAHs to their parent-PAHs (1-INDA/FLUO, 9-FLO/FLUO, 1,2-ACQ/ACENY, 1,2-ACQ/ACEN, and 9,10-ANQ/ANTH) were >1 in both topsoils and subsoils, i.e. the soils contained higher concentrations of OPAHs than parent PAHs (Tab. C-4). The ratios of 9-FLO/FLUO and 9,10-ANQ/ANTH were higher than 10, and that of 9-FLO/FLUO even reached >100 at site H near the rubber factory. The ratios 1,4-NQ/NAPH, 1-NLD/1-MNAPH, and 2-BPCD/PHEN were <1. The OPAH/parent PAH ratios were not consistently higher in the subsoil than in the topsoil. We did not see a consistent shift in OPAH/parent PAH ratio with distance except a generally higher ratio in topsoil near emission sources (Tab. C-4).

Table C-4: Ratios of concentration of OPAH metabolite to parent PAH (from which metabolite is derived during metabolism) at different sites.

Site/depth	1-INDA/FLUO	1,4-NQ/NAPH	1-NLD/1-MNAPH	2-BPCD/PHEN	9-FLO/FLUO	1,2-ACQ/ACENY	1,2-ACQ/ACEN	9,10-ANQ/ANTH
A1	**3.40**	0.01	0.24	0.00	**33.51**	0.82	0.95	**44.45**
A2	**1.30**	0.01	0.28	0.00	**13.81**	0.47	**1.21**	**16.72**
B1	0.27	0.01	0.04	0.00	**16.82**	**1.39**	**1.63**	**13.42**
B2	**6.31**	0.01	0.25	0.01	**10.33**	**1.34**	**2.47**	**15.51**
C1	0.85	0.01	0.17	0.02	**32.10**	0.31	0.57	**26.94**
C2	0.87	0.01	0.19	0.00	**29.69**	0.41	1.04	**31.37**
D1	**1.11**	0.01	0.13	0.01	**32.63**	1.01	0.51	**37.20**
D2	**3.51**	0.01	0.17	0.00	**18.64**	0.92	0.95	**32.13**
E1	**3.53**	0.01	0.15	0.20	**14.07**	0.82	0.91	**17.37**
E2	**5.93**	0.01	0.20	0.01	**15.91**	**1.26**	**1.33**	**25.14**
F1	**4.15**	0.01	0.26	0.00	**13.36**	0.59	0.99	**40.51**
F2	**3.29**	0.01	0.21	0.01	**15.11**	1.03	**1.71**	**27.82**
G1	**4.75**	0.01	0.25	0.00	**16.16**	1.02	**1.46**	**41.86**
G2	**4.22**	0.01	0.23	0.01	**17.82**	**1.15**	**2.18**	**30.69**
H1	0.77	0.01	0.21	0.02	**126.31**	**1.08**	**1.29**	**35.45**
H2	**3.12**	0.00	0.24	0.00	**66.20**	0.00	0.00	**23.73**
I1	**11.82**	0.00	0.50	0.02	**17.58**	**5.10**	**5.46**	**20.15**
I2	**3.94**	0.00	0.34	0.00	**23.52**	0.00	0.00	**15.65**
J1	**4.61**	0.01	0.25	0.01	**17.84**	**1.21**	**2.36**	**22.03**
J2	**8.22**	0.01	0.34	0.01	**31.23**	**1.99**	**4.47**	**24.24**
K1	**5.08**	0.01	0.19	0.00	**16.07**	0.57	**1.20**	**17.23**
K2	**8.93**	0.01	0.22	0.01	**12.82**	**0.97**	**1.89**	**26.47**
Average 1	**3.67**	0.01	0.22	0.03	**30.59**	**1.27**	**1.58**	**28.78**
Average 2	**4.51**	0.01	0.24	0.00	**23.19**	0.87	**1.57**	**24.50**

4.4 Depth distribution of PAHs and OPAHs in relation to their hydrophobicity

To assess the mobility of PAHs and OPAHs in soil, we calculated the concentration ratio [CR] defined as the concentration of each compound in the subsoil divided by that in the topsoil at each site (Fig. C-6). The CR for most individual PAHs and OPAHs were variable among different sites but mostly < 1 illustrating that these compounds are accumulated in topsoil. The mean CRs for individual PAHs and individual carbonyl-OPAHs ranged 0.43-0.83 and 0.41-1.02, respectively.

CRs of several higher molecular weight PAHs at sampling sites A and C ranged between 1-2.9 (Fig. C-6). Mean CRs of OPAHs had a slightly higher range (0.41-1.02) than those of the low molecular weight (2-3 ring) parent PAHs; NAPH, 1-MNAPH, ACENY, ACEN, FLUO, PHEN, and ANTH (0.41-0.63, Fig. C-6).

We correlated log CR values with log K_{OW} values of the PAHs and carbonyl-OPAHs at each sampling site to assess factors controlling their vertical distribution (Guggenberger et al., 1996). For PAHs, there were significant positive correlations between log CR values and their log K_{OW} values at sites A, B, C , E, and H ($r = 0.47$-0.86), a significant negative correlation at site D ($r = -0.42$) and no correlation at the remaining sites. Furthermore, there was a significant negative correlation between log CR values of carbonyl-OPAHs and their log K_{OW} values at sampling point D ($r = -0.77$). All other sites did not show correlations. We could not correlate log CR with log K_{OW} of hydroxyl-PAHs because their concentrations were too frequently below the detection limit in either topsoil or subsoil (Tab. C-2).

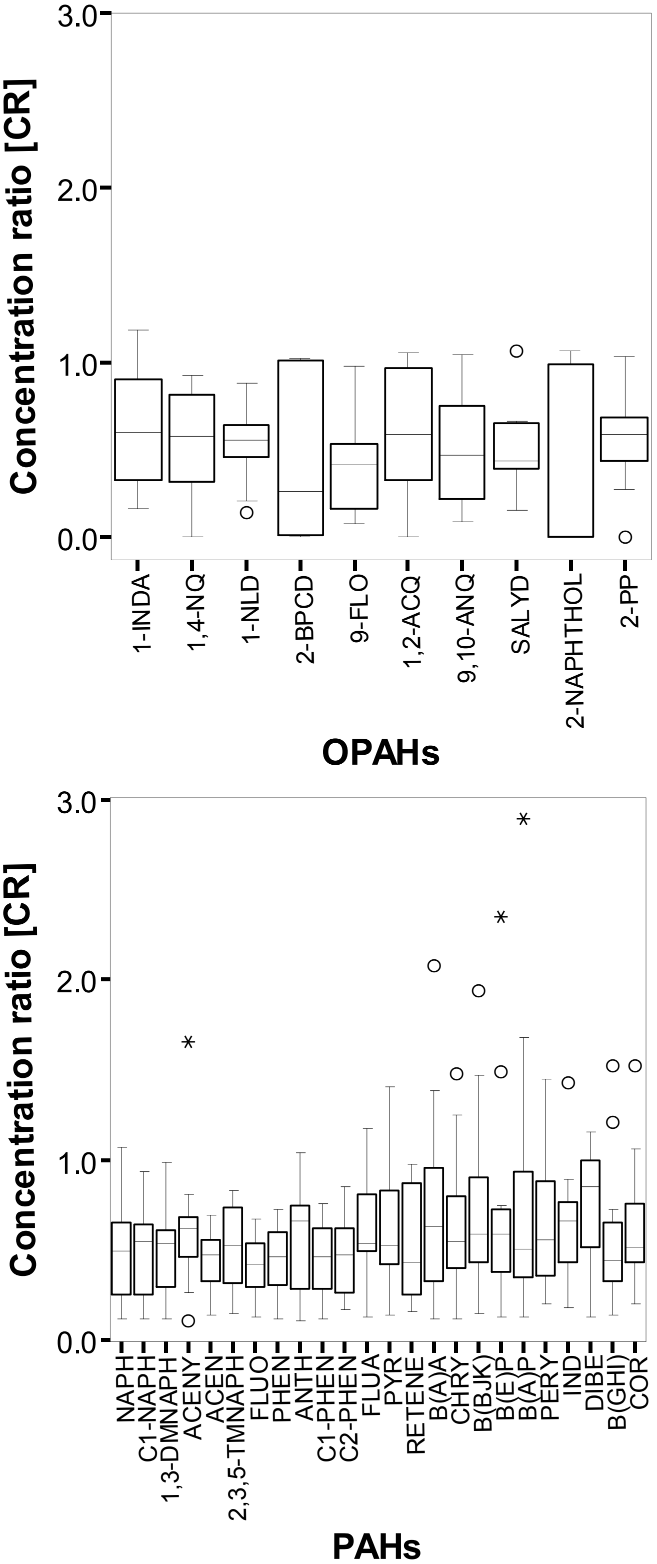


Figure C-6: Box plots of mean concentration ratios [CR] of PAHs and OPAHs of the 11 sampling points. CR is concentration ratio of individual alkyl/parent-PAH or OPAH in the subsoil to that of same compound in topsoil. Boxes define the range, the central line represents the median. Outliers (open circles) and extreme values (stars) are also shown.

5 Discussion

5.1 Spatial distribution of PAH concentrations and relationship with heavy metals

The study sites A-D, located in the immediate proximity to the Angren coal mining area and power plant, were most contaminated by PAHs and OPAHs (Fig. C-2). Industrial combustion of coal for power generation and coal dust emanating from the coal mining activities are the likely main sources of PAHs and carbonyl-OPAHs at these sites. This is in line with other findings that coal dust contains high PAH concentrations (Stout and Emsbo-Mattingly, 2008; Achten and Hofmann, 2009) and combustion is a well known source of PAHs and OPAHs (Oda et al., 1998; Lima et al., 2005; Simoneit et al., 2007). The potential spread of PAHs with coal dust was also observed at a coal mine in India (Jharia coal field) where it is estimated that 9368 kg/day of coal dust is emitted because of mining activities and wind erosion of the exposed area (Ghose, 2007). The soils at all study sites contained both petrogenic PAHs including NAPH and PHEN and their alkyl-derivatives which are typical for coal dust emitted by the open pit coal mining (Stout and Emsbo-Mattingly, 2008; Achten and Hofmann, 2009) and pyrogenic high molecular weight PAHs (Booth and Gribben, 2005; Neff et al., 2005). The PAH sources in soils can therefore be interpreted as mixtures of coal dust deposition and combustion-related emissions with varying mixing ratios. Between sites E and G, PAH and carbonyl-OPAH concentrations decreased with increasing distance from the main coal-related activities. At site H, the rubber factory again increased PAH and carbonyl-OPAH concentrations, while the gold refinery near site J did not seem to release significant amounts of PAHs and carbonyl-OPAHs.

According to the classification of Maliszewska-Kordybach (1996), sites A, B, C, D, and H were heavily polluted (>1000 ng/g ∑EPA-PAHs), E contaminated (600-1000 ng/g), and the remaining sites weakly contaminated (200-600 ng/g) or not contaminated (I). Sites A-H showed comparable PAH concentrations in soil as reported for other PAH-contaminated soils (at industrial sites) in the literature while the soils at sites I-K were in the range of background soils with only diffuse ubiquitous contamination (Wilcke, 2000, Tab. C-5).

PAHs are mostly accumulated in the topsoil because of strong sorption to soil organic matter or other adsorbing materials such as coal or soot (Tab. C-2, Krauss et al., 2000; Cornelissen et al., 2005). Exceptions were sites A, C, and I. At sites A and C the soils might have been disturbed so that a more contaminated material with elevated PAH concentrations reached greater soil depth by burial with other less contaminated soils. We observed that at site A, the uppermost soil layer (0-5 cm) was completely covered with dark-colored coal dust originating from the coal mine while at site C the uppermost soil layer (0-8 cm) was covered with particulates emitted from the stack of the Angren power plants. Other studies from coal-impacted soils also demonstrated that the most polluted layer is sometimes not the topsoil (Pies et al., 2008; Yang et al., 2008). Contaminated subsoil at both locations is dominated by high molecular weight PAHs (Fig. C-3A and C) which is typical of pyrogenic sources. At the agricultural site I, mixing of the top 20-30 cm of the soil by agricultural plowing might explain similar PAH and OPAH concentrations in topsoil and subsoil.

Some studies have suggested higher molecular weight PAHs are deposited near emission sources because of their stronger association with particulates as a consequence of lower vapor pressure), while the more volatile lower molecular weight PAHs are deposited far from emission sources because they occur in the gas phase (Yang et al., 1991). Such a trend appears to be visible between sites D and F (Fig. C-4).

Further indication of the industrial activity in the Angren area as source of PAHs and carbonyl-OPAHs is the close correlation with a number of heavy metals previously identified by Shukurov et al. (2009) as resulting from emissions of the industrial plants (Tab. C-3). This is consistent with the lack of correlation of the concentrations of PAHs and carbonyl-OPAHs with those of C_{org}. Correlations between PAH concentrations and C_{org} can be considered as indication of the partitioning between a homogeneous background concentration in the atmosphere and soil (Wilcke and Amelung, 2000).

Table C-5: PAHs concentrations (ng/g) in soils from other industrial areas in the world (ranges or means ± standard deviations).

Region	Number of PAHs	Concentration [ng/g]	Reference
West Marcedonia, Greece	16	55-359	(Stalikas et al., 1997)
Eordaia basin, Greece	13	300-1600	(Voutsa et al., 2004)
Ziar, Central Slovakia	20	151-2485	(Wilcke et al., 1996)
Eastern Slovakia	21	53-1389	(Wilcke et al., 2003)
Seine River basin, France	14	1670-5650	(Motelay-Massei et al., 2004)
Taragona County, Spain	16	166-1002	(Nadal et al., 2004)
Kohtla-Järve, Estonia	12	12390±9810	(Trapido, 1999)
El Paso, Texas (USA)	16	6-2226	(De La Torre-Roche et al., 2009)
Tangu-Hangu industrialized zone, Tianjin, China	16	2430±1780	(Zuo et al., 2007)
Agra (semi-arid region), India	14	13720±11900	(Masih and Taneja., 2006)
Balaju industrial district, Kathmandu, Nepal	20	184-6899	(Aichner et al., 2007)
Mosel and Saar river bank soils, Germany	45	12000-50000 ng/g	(Pies et al., 2008)
Angren industrial region, Uzbekistan	31	118-5913 (0-10 cm) 104-3852 (10-20 cm)	This study

5.2 Spatial variation of the relationship of OPAHs with PAHs and heavy metals

The ∑7 carbonyl-OPAHs concentrations in the contaminated Angren soils were higher than the 170 ng/g observed in a botanical garden topsoil of the German city of Mainz but lower than in soil at a former gasworks site in Berlin, Germany (15681 ng/g, Bandowe and Wilcke, 2010), and other contaminated soils including wood preservation sites in Sweden (∑3 carbonyl-OPAHs concentrations of 2870-134000 ng/g, Lundstedt et al., 2007), or creosote contaminated sites from northern Germany (6100-21800 ng/g for ∑2 carbonyl-OPAHs; Meyer et al., 1999). The compounds 9-FLO and 9,10-ANQ dominating in Angren soils were also identified as major components of carbonyl-OPAHs mixtures in both particulate fractions of atmospheric aerosol samples and in impacted soils (Lundstedt et al., 2003; Albinet et al., 2006, 2007, 2008; Liu et al., 2006).

The ∑5 hydroxyl-OPAHs concentrations were in the range or even lower than those in little contaminated topsoils in Germany and Brazil (9-26 ng/g), and clearly lower than in soil at a former gasworks site from Germany (383 ng/g, Fig. C-2, Bandowe and Wilcke, 2010). The dominating hydroxyl-OPAH, SALYD, is one of the end products of all metabolic PAH degradation paths (Bamforth and Singleton, 2005). It can, however, also be produced during the biodegradation of other organic matter components in soil.

The close correlations between the ∑31PAHs concentrations and the ∑7 carbonyl-OPAHs and ∑5 hydroxyl-OPAHs (Fig. C-5), and the close correlations among the individual PAHs and carbonyl-OPAHs except for PERY, RETENE, 1-INDA, and 2-BPCD concentrations in the topsoil and partly also in the subsoil, suggests that PAHs and carbonyl-OPAHs are jointly produced by combustion processes and coal mining activities and deposited together from the atmosphere to the study soils. The contribution of post-emission physico-chemical/microbial transformation processes might be limited because of the short travel distances and high soil contamination in the study area. This was already previously shown to negatively affect several indicators of biological activity in soil (Shukurov et al., 2009). However, the lack of correlations of PERY, RETENE, 1-INDA, and 2-BPCD with other PAHs/OPAHs indicates that these compounds have different sources. For PERY it is known that it can be produced biologically (Venkatesan, 1988), and RETENE was found in needles of coniferous trees (Sims and Overcash, 1983). 2-BPCD is a product of photooxidation of PHEN in water, a process enhanced by hydrogen peroxide and fulvic substances (Wang et al., 1995) and 1-INDA a microbial metabolic product of FLUO (Casellas et al., 1997).

For some OPAHs/corresponding parent PAHs, the concentration ratios were greater than 1, showing that the OPAHs were present in higher concentrations than their corresponding parent

PAHs (Tab. C-4). This was particularly evident for 9-FLO/FLUO and 9,10-ANQ/ANTH, and. is in line with previous findings (Lundstedt et al., 2007; and Bandowe and Wilcke, 2010). We attribute this to differences in vapor pressures between carbonyl-OPAHs and their parent PAHs. OPAHs have lower vapor pressures than their parent PAHs. Therefore, a larger part of the emitted carbonyl-OPAHs will be particle-associated and hence deposited nearer to the emission source than their parent PAHs (Yang et al., 1991; Allen et al., 1997; Albinet et al., 2006, 2007, 2008). Because of the high abundance of some OPAHs relative to their parent PAHs, it is necessary that OPAHs are included in monitoring programs given the high toxicity of some OPAHs compared to their parent PAHs.

Ratios of OPAHs/corresponding parent PAHs can be useful in several ways. For example, it is suggested that ratios of hydroxylated quinolines to parent-quinolines in tar oil contaminated sites might be useful indicators of biological natural attenuation (Reineke et al., 2007). McKinney et al. (1999) suggested that the 9,10-ANQ/ANTH ratio could be used for source apportionment in sediments because sediments affected by point sources will have ratios <1. On the other hand, those affected by long-range transported depositions will have ratios >1 because of the advanced transformation of ANTH to 9,10-ANQ by photochemical reactions during atmospheric transport. However, at our study site, we did not observe a consistent direct relationship between distance from the emission source (i.e., the industrial works) and the 9,10-ANQ/ANTH or any other tested ratio. We attribute this to the short considered distance which does not allow for much transformation in the atmosphere and the joint continuous emission of OPAHs and PAHs. There were also no indications that with decreasing contamination the carbonyl-OPAH/corresponding parent-PAH ratios increased because of enhanced microbial degradation. On the contrary, the ratio was generally high at locations with higher PAH contamination (e.g. 9-FLO/FLUO at site H). This again is probably related to the dominance of carbonyl-OPAH deposition from the atmosphere over microbial production.

The significant positive correlation between ∑7 carbonyl-OPAHs and trace metals identified to be produced from industrial emissions further indicate the common source of PAH, carbonyl-OPAH and these trace metals (Tab. C-3). The lack of significant correlation between ∑5 hydroxyl-OPAHs and these trace metals might be attributable to the fact that the concentrations of several hydroxyl-OPAHs were below the detection limit or that these hydroxyl-OPAHs might have additional sources such as degradation of other organic compounds in soil resulting in SALYD.

5.3 Mobility of OPAHs as compared to PAHs

Mean concentration ratios of (CR, i.e subsoil/topsoil concentration ratios) of OPAHs were sometimes higher than those of PAHs with similar number of fused rings (Fig. C-6). Our results therefore suggest that in the sampled soils, OPAHs were only slightly (but not consistently) more mobile than their parent PAHs. The solubility differences between OPAHs and parent PAHs as well as previously reported column leaching experiments in the laboratory suggest a much bigger difference in mobility between OPAHs and corresponding parent PAHs than observed in our study (Weigand et al., 2002; Lundstedt et al., 2007). Log CR values of carbonyl-OPAHs were only significantly negatively correlated with log K_{OW} values at sampling site D indicating that leaching of parent PAHs and and carbonyl-OPAHs as dissolved solute occurred at this site. The small differences in CR between OPAHs and their parent PAHs, and the lack of negative correlation between log CR values of carbonyl-OPAHs/parent PAHs and their log K_{OW} values (except at site D) might in part be related to the prevalent semi-arid conditions providing little water for leaching. Small leaching rates also provided little opportunity for differential leaching of PAHs and OPAHs. Other reasons for these observations may be that not only the water solubility controls the transport of OPAHs in soil, but a range of interactions with the soil solid phase including hydrophobic interactions, anion sorption, ligand exchange, and complexation with Al and Fe oxides (Weigand et al., 2002). It has also been observed previously that the K_{OW} alone is unable to reliably predict the adsorption (and hence leaching) behavior of naphthalene and its oxygen-containing derivatives in soil, and that the electrostatic potential played a major role (Novoszad et al., 2005). Higher degradation rates of the more water soluble OPAHs, containing more labile and reactive functional groups compared to their parent PAHs might additionally affect the vertical distribution of OPAHs in relation to parent PAHs. It is e.g., possible that the faster OPAH than PAH degradation in the biologically more active topsoil increase CR values of OPAHs relative to those of parent PAHs without a contribution of leaching which would even cause an overestimation of the small OPAH leaching rates at our study sites. Furthermore, some of our study sites are likely disturbed as indicated by partly higher PAH concentrations in the subsoil than in the topsoil (Fig. C-2).

At sampling site D, the significant negative correlation between log CR values of PAHs and their log K_{OW} is consistent with other studies. This indicates that leaching as truly dissolved solute is the main mechanism of transport at this site leading to an enrichment of the less hydrophobic compounds in the subsoil layer. Positive relationships between log CR values of PAHs and their log K_{OW} values at the sites near the industrial works, and particularly near the open-pit coal mine were in contrast to findings reported for less contaminated and undisturbed sites (Guggenberger et al., 1996; Wilcke et al., 1996; Krauss et al., 2000). This might be attributable to an enrichment of petrogenic low molecular weight PAHs via deposition of coal dust which decreases the log CR values of the low molecular weight PAHs, or burial of higher molecular weight PAHs in the subsoil.

6 Conclusions

Our results support the hypothesis that PAH concentrations in soils are mainly controlled by distance to industrial activities with exception of the gold refinery that does not release significant amounts of PAHs.

The similarity in the spatial distribution of PAHs, carbonyl-OPAHs, and heavy metals suggests that these pollutants all result from combustion processes and emissions of coal dust and reached all study soils via deposition from the atmosphere. Exceptions were PERY, RETENE, 1-INDA, and 2-BPCD, for which biological and photochemical oxidation sources are more likely.

The mobility of OPAHs in the study soils was only marginally higher than that of low molecular weight PAHs with similar K_{OW} which is likely attributable to the more complex interactions of OPAHs than of PAHs with soil. The subsoil/topsoil concentration ratios of PAHs and carbonyl-OPAHs were negatively correlated with K_{OW} values at the site of highest PAH and carbonyl-OPAH concentrations near the power plant (site D). This indicates that leaching was an important transport mechanism at this site. Positive correlations of subsoil/topsoilconcentration ratio of PAHs with K_{OW} values at several sites are attributable to different reasons including enrichment of low molecular weight PAHs in surface soil via deposition of coal dust, and burial of higher molecular weight PAHs in deeper soil.

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D Oxygen-containing polycyclic aromatic hydrocarbons in urban soils of Bratislava, Slovakia: patterns, relation to PAHs and vertical distribution[a]

Benjamin A. Musa Bandowe[1], Jaroslava Sobocka[2], Wolfgang Wilcke[3]

[1] Earth System Science Research Center, Geographic Institute, Johannes Gutenberg University Mainz, Johann-Joachim-Becher-Weg 21, 55128, Mainz, Germany

[2] Soil Science and Conservation Research Institute, 82713 Bratislava, Slovakia

[3] Geographic Institute University of Berne, Hallerstrasse 12, 3012 Berne, Switzerland

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1 Abstract

We determined concentrations, sources, and vertical distribution of OPAHs and alkyl and parent-PAHs in urban soils. The ∑14 OPAHs concentrations in surface soil horzons ranged 88-2692 ng g^{-1} and those of ∑34 alkyl/parent-PAHs 842-244870 ng g^{-1}. The concentrations of the ∑9 carbonyl-OPAHs ($r=0.92$, $p=0.0001$) and the ∑5 hydroxyl-OPAHs ($r=0.73$, $p=0.01$) correlated significantly with ∑34 PAHs concentrations indicating that combustion was the joint source. The OPAHs were quantitatively dominated by 9-fluorenone, 9,10-anthraquinone, 1-indanone and benzo[a]anthracene-7,12-dione. At several sites, individual carbonyl-OPAHs had higher concentrations than parent PAHs. The concentration ratios of several OPAHs to their parent-PAHs and contribution of the more soluble OPAHs (1-indanone and 9-fluorenone) to ∑14 OPAHs concentrations increased with soil depth suggesting that OPAHs were faster vertically transported in the study soils by leaching than PAHs which was supported by the correlation of subsoil:surface soil ratios of OPAH concentrations at several sites with K_{OW}.

2 Introduction

Oxygen-containing PAH derivatives and metabolites (OPAHs) originate – like their parent compounds, the polycyclic aromatic hydrocarbons (PAHs) – from combustion of fossil fuels. Soils and water bodies may be contaminated by direct introduction of OPAH-containing materials or deposition of OPAHs from the atmosphere in which OPAHs released from distributed sources are transported (Baek et al., 1991; Lundstedt et al., 2007). Alternatively, OPAHs are also produced by post-emission photooxidation, chemical oxidation or microbial transformation of PAH (Cerniglia, 1984; Nikolaou et al., 1984; Lundstedt et al., 2007). While it is estimated that 90% of the PAHs released in the UK is finally stored in soils (Wild et al., 1995), the role of soil in the environmental distribution of OPAHs is unknown.

Some OPAHs are more persistent than their parent-PAHs and considered to be more toxic than parent-PAHs because they are direct acting mutagens compared to PAHs which require enzyme activation to manifest their toxicity. Furthermore, OPAHs also cause oxidative stress (Bolton et al., 2000; Xue et al., 2005; Lemieux et al., 2008). The OPAH-containing extracts of environmental samples exhibit higher toxicity to microbes and human cells than PAH-containing extracts (Alsberg et al., 1985; Xia et al., 2004).

In contrast to the ubiquitous PAHs, which are frequently monitored priority pollutants (Keith et al., 1979; Menzie et al., 1992; Wilcke, 2000) only little information is available concerning the concentrations of OPAHs in urban soils (Niederer, 1998). Concentrations of PAHs in urban soils are frequently higher than in rural areas and controlled by population density, proximity to point sources (roads, industries, and waste deposits), land use, and vegetation cover (Wilcke, 2000). Air monitoring programs in urban areas demonstrated that the atmosphere contains significant OPAH concentrations which are related to urban anthropogenic activities such as heating, vehicular traffic, and industrial activity (Schnelle-Kreis et al., 2005; Liu et al., 2006; Albinet et al., 2007). Consequently, also elevated OPAH concentrations can be expected in urban soils. In soils of strongly contaminated industrial areas with high PAH concentrations (former gasworks site, wood preservation sites, power plants), OPAH concentrations were correlated with PAH concentrations and in several cases individual OPAH concentrations were higher than those of the corresponding parent-PAHs (Meyer et al., 1999; Lundstedt et al., 2007). The contribution of in-situ formation of OPAHs in urban soils by abiotic or biotic oxidation of parent-PAHs, however, is unknown.

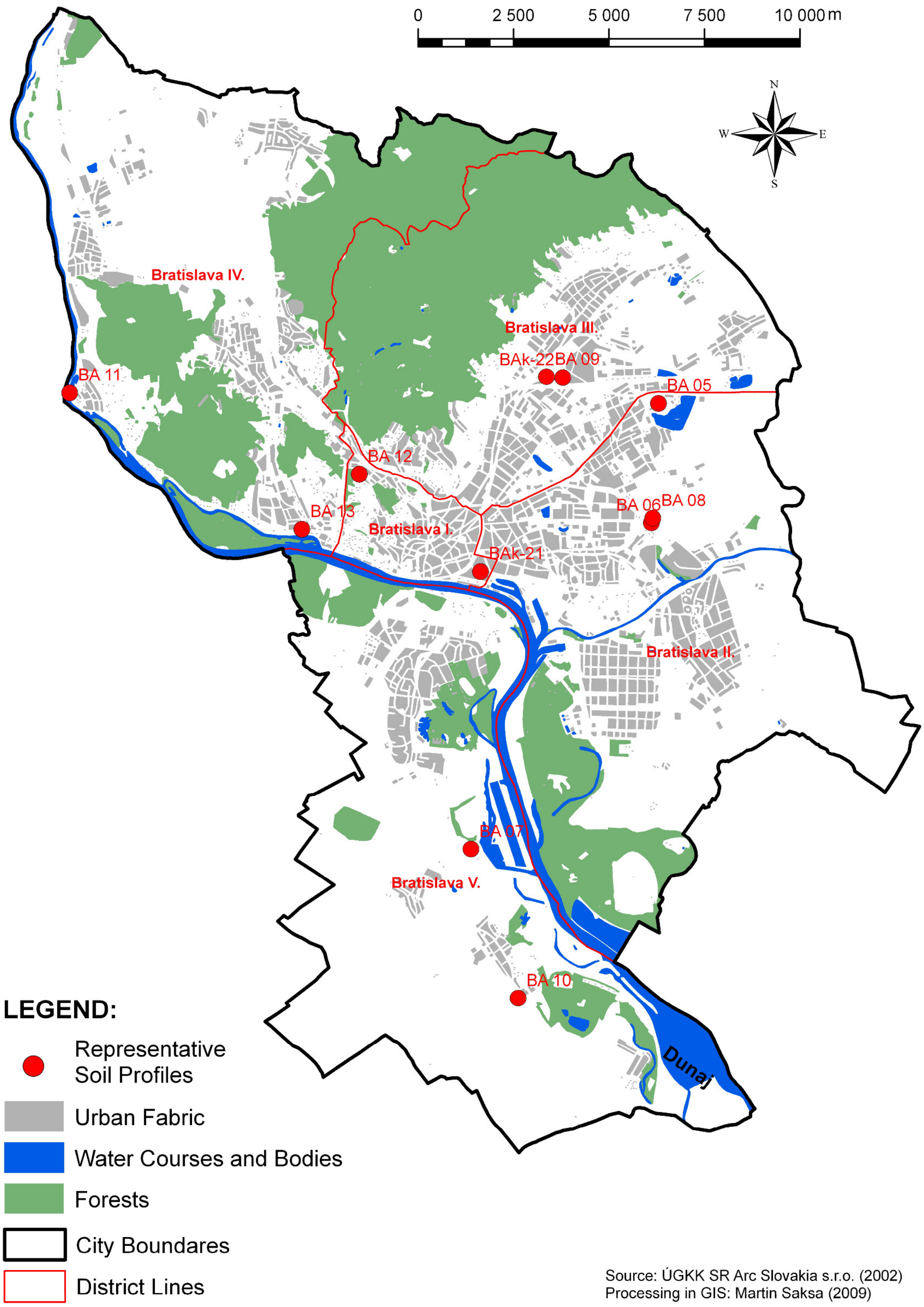
Deposited PAHs partition mainly into soil organic matter, particularly in aromatic moieties such as black carbon because of their hydrophobic nature and are therefore little leached into deeper layers of soil (Chiou et al., 1998; Cornelissen et al., 2005). The vertical migration of PAHs in mineral soil depends on the extent of sorption to soil organic matter described by the water-organic carbon partitioning coefficient (K_{OC}) which is correlated with the octanol-water partitioning coefficient (K_{OW}) (Means et al., 1980; Karickhoff, 1981). Although PAHs accumulate in the organic matter-rich topsoils, some PAHs are found in the subsoil as a result of leaching as truly dissolved solute, in association with dissolved organic matter and colloids or bioturbation (McCarthy et al., 1989; Maxin et al., 1995; Guggenberger et al., 1996; Cousins et al., 1999). The sorption of OPAHs, however, especially those with hydroxyl/carboxyl functional groups is more complicated than that of parent-PAHs because of more possible interactions with soil organic matter (Novoszad et al., 2005). Laboratory experiments indicated that OPAHs may be more mobile in soil than parent-PAHs because of their higher water solubility (Weigand et al., 2002; Lundstedt et al., 2007). Consequently, at coal tar-contaminated sites groundwater is substantially contaminated with OPAHs (Schlanges et al., 2008).

Our objectives were to (1) determine the state of contamination of the soils of Bratislava, the capital of Slovakia with 450,000 inhabitants (population density: 1200 inhabitants km^{-2}) located in the Danubian lowlands at the border with Austria and Hungary, with OPAHs and PAHs to enlarge the up to now small data basis of OPAH concentrations in soil, (2) identify the sources of OPAHs (i.e., combustion vs. post-emission transformation), (3) assess the importance of OPAHs in soil relative to the corresponding parent-PAH concentrations, and (4) investigate into the vertical transport of OPAHs in soils with the help of subsoil/topsoil concentration ratios and the assessment of the change in composition of the OPAH mixtures with soil depth.

3 Materials and methods

3.1 Soil sampling and soil properties

Soil samples were collected from the different districts of Bratislava, Slovakia. Whole soil profiles (up to 5 horizons) were sampled at nine locations; at two further locations (BAk-21 and BAk-22) only surface soils (A horizons) were sampled in a horizon-representative way from hand-dug soil pits (Fig. D-1). The samples were air-dried, sieved to <2 mm and stored at 4°C until analysis. Selected soil properties including texture, pH, and organic carbon concentration (C_{org}) were determined according to methods reported in earlier publications (Sobocka, 2007). Table 1 summarizes coordinates, sequence and thickness of horizons, and current land use, while Table 2 shows soil properties.



Source: ÚGKK SR Arc Slovakia s.r.o. (2002)
Processing in GIS: Martin Saksa (2009)

Figure D-1: Location of sampling sites in Bratislava.

Table D-1: Sampling locations, horizons and land use of Bratislava soils.

Sampling site	Coordinates	Soil horizon designation according to MSCS 2000	Soil horizon designation according to FAO 2006	Horizon depth [cm]	Land use
BA-05 (Zlate Piesky)	N48 11. 168 E17 10.933	Akpc	Akp	0-23	Abandoned area near Tesco
		Amc	Ak	23-60	former arable land
		A/CGoc	A/Ckr	70-96	
		CGoc	Ckr	> 96	
BA-06 (Vrakuna)	N48 09.517 E17 11.073	Aoc	Ak	0-20	Former farmland (grassland)
		C1c	Ck1	33-50	near chemical waste dump
		C2c	Ck2	50-72	
BA-07 (Rusovce)	N48 03. 832 E17 08. 852	Aoc	Akp	0-25	Agricultural land (meadow)
		Cc	Ck	50-120	near highway
BA-08 (Vrakuna)	N48 09.542 E17 11.084	Adic	Aku	0-2	Chemical waste dump
		C1c	Cku1	2-18	(abandoned area)
		C2c	Cku2	18-66	
		C3c	Cku3	66-94	
		C4c	Cku4	> 94	
BA-09 (Zabi Majer)	N48 11. 373 E17 09.137	Akmc	Akp	0-38	Garden allotment near chemical
		A/C	A/Cb	38-54	plant (buried by organo-mineral material)
		CGro	Crb	70-96	
BA-10 (Cunovo)	N48 10. 485 E16 58.585	Akpc	Akp	0-30	Agricultural land near Bratislava-Vienna highway (50 m)
		Amc	Ak	30-75	
		A/CGoc	A/Ckr	75-102	
		CGoc	Ckr	> 102	
BA-11(Devin)	N48 10. 485 E16 58.585	Adic	Aku	0-8	Historical and recreation area
		D1	2Cu	19-50	anti-flooding embankment at confluence of Morava and Danube river
BA-12 (Lovinsky)	N48 09. 774 E17 04.842	Akmc	Akp	0-42	Urban garden in villa housing estate
		A/Cc	A/Ck	42-70	(currently sealed for construction activities)
		Cc	Ck	> 70	
BA-13 (Riviera)	N48 08. 868 E17 03.752	Akmc	Akp	0-48	Garden in villa housing at village of Karlova Ves
		A/Cc	A/Ck	48-90	(currently sealed for construction activities)
		A/Cc1	A/Ck1	90-120	
		Cc	Ck1	> 120	
BAk-21 (Apollo factory)	N48 08. 582 E17 03.650	Ao	Au	0-5	Former Apollo chemical factory
BAk-22 (Zabi Majer)	N48 11. 448 E17 03.021	Ao	Au	0-5	Garden allotment near Zabi Majer

Table D-2: Physico-chemical soil properties and total concentrations (ng g^{-1}) of PAHs, carbonyl-OPAHs and hydroxyl-OPAHs in Bratislava soils.

Sampling point	Horizon	pH ($CaCl_2$)	C_{org} [mg g^{-1}]	Sand [%]	Silt [%]	Clay [%]	∑EPA-PAH	∑34PAHs	∑9 Carbonyl-OPAHs	∑5 Hydroxyl-OPAHs
BA-05	Akpc	7.50	23	61	15	24	834	1265	173.6	2.4
	Amc	7.66	14	34	43	23	438	664	81.8	3.4
	A/CGoc	7.98	9.2	40	42	18	147	256	96.9	1.7
	CGoc	7.96	3.7	40	44	16	66	116	51.8	2.2
BA-06	Aoc	7.87	9.6	38	52	10	1325	2253	90.4	1.3
	C1c	8.04	5.6	54	38	8	237	350	68.3	2.5
	C2c	8.32	4.1	88	10	3	103	161	61.9	0.4
BA-07	Aoc	7.50	17	37	49	14	569	842	144.3	6.6
	Cc	7.87	8.8	32	51	16	327	516	84.4	3.3
BA-08	Adic	7.17	19	67	22	11	232047	244870	2641.2	50.5
	C1c	7.17	12	57	27	16	9086	10892	372.5	3.1
	C2c	7.59	8.0	63	23	13	11080	13430	433.8	5.6
	C3c	7.62	4.7	68	18	14	2261	2765	153.5	4.8
	C4c	7.79	6.4	71	16	12	4022	5063	294.4	4.7
BA-09	Akmc	7.04	28	50	32	18	1962	2829	200.6	7.4
	A/C	6.74	15	39	37	24	1121	1615	190.0	3.5
	CGro	6.51	4.8	42	39	20	1014	5831	104.1	2.0
BA-10	Akpc	7.65	18	41	42	17	834	1187	84.9	2.7
	Amc	7.98	13	30	50	20	280	450	119.7	14.5
	A/CGoc	8.03	11	27	53	20	201	344	63.1	0.2
	CGoc	8.16	5.7	31	56	13	147	249	72.6	2.0
BA-11	Adic	7.26	53	43	44	14	2460	3414	422.2	4.0
	D1	7.33	12	75	18	8	363	533	144.3	3.8
BA-12	Akmc	7.13	18	59	27	12	2289	2911	318.2	5.1
	A/Cc	7.50	6.2	61	28	11	1394	1908	98.0	1.0
	Cc	8.12	0.5	64	3	5	33	82	33.8	0.0
BA-13	Akmc	7.60	15	45	41	14	7718	9842	501.8	7.1
	A/Cc	7.79	11	40	43	16	647	942	114.4	2.5
	A/Cc1	7.69	5.1	35	46	19	196	288	85.3	4.6
	Cc	8.32	1.6	56	37	8	53	93	64.0	1.1
BAk-21	Surface soil	6.54	13				3911	4830	340.9	22.4
BAk-22	Surface soil	6.72	30	31.03	50	18.86	3466	4393	422.5	18.5

3.2 OPAH and PAH analysis

We determined the concentrations of 14 OPAHs (nine carbonyl- and five hydroxyl-OPAHs) and 34 parent/alkyl-PAHs. The nine carbonyl-OPAHs included 1-indanone (1-INDA), 1,4-naphthoquinone (1,4-NQ), 1-naphthaldehyde (1-NLD), 2-biphenylcarboxaldehyde (2-BPCD), 9-fluorenone (9-FLO), 1,2-acenaphthenequinone (1,2-ACQ), 9,10-anthraquinone (9,10-ANQ), 1,8-naphthalic anhydride (1,8-NAA), benzo[a]anthracene-7,12-dione [7,12-B(A)A] and the five hydroxyl-OPAHs: 2-hydroxybenzaldehyde (SALYD), 2-hydroxynaphthalene (2-NAPHTHOL), 2-hydroxybiphenyl (2-PP), 1-hydroxyacenaphthene (1-ACENOL), 9-hydroxyfluorene (9-FLUORENOL).

The 34 parent/alkyl-PAHs included naphthalene (NAPH), 2-methylnaphthalene (2-MNAPH), 1-methylnaphthalene (1-MNAPH), biphenyl (BP), 1,3-dimethylnaphthalene (1,3-DMNAPH), acenaphthylene (ACENY), acenaphthene (ACEN), 2,3,5-trimethylnaphthalene (2,3,5-TMNAPH), fluorene (FLUO), 2,6-diisopropylnaphthalene (2,6-DIISOPNAPH), phenanthrene (PHEN), anthracene (ANTH), 2-methylphenanthrene (2-MPHEN), 1-methylphenanthrene (1-MPHEN), 3,6-dimethylphenanthrene (3,6-DMPHEN), 3,5-dimethylphenanthrene (3,5-DMPHEN), 2,6-dimethylphenanthrene (2,6-DMPHEN), 1,7-dimethylphenanthrene (1,7-DMPHEN), fluoranthene (FLUA), pyrene (PYR), 1-methyl-7-isopropylphenanthrene (RETENE), benz[a]anthracene [B(A)A], chrysene+triphenylene (CHRY), benzo[b+j+k]fluoranthenes [B(BJK)], benzo[e]pyrene ([B(E)P]), benzo[a]pyrene ([B(A)P]), perylene (PERY), indeno[1,2,3-cd]pyrene (IND), dibenz[a,h]anthracene (DIBE), benzo[ghi]perylene ([B(GHI)]), and coronene (COR).

Twenty g of soil were extracted by pressurized liquid extraction, followed by silica gel clean-up/fractionation into OPAH and PAH fractions. The target compounds were separated and quantified with an Agilent 6890 N gas chromatograph coupled to an Agilent 5973 B inert mass spectrometer in the electron ionization mode. Seven deuterated-PAHs (NAPH-D_8, ACEN-D_{10}, PHEN-D_{10}, PYR-D_{10}, CHRY-D_{12}, PERY-D_{12}, B(GHI)-D_{12}) and benzophenone-D_5 were added to soils before extraction as internal standards for quantification of PAHs and carbonyl-OPAHs, respectively. The OPAHs were split into two fractions after fluoranthene-D_{10} (as syringe spike for hydroxyl-OPAHs) had been added. One part was used to quantify carbonyl-OPAHs using benzophenone-D_5 spiked to soils before extraction as internal standard. To the second part of the OPAH fraction, 150 µl of BSTFA:TMCS (99:1) was added to derivatize the hydroxyl-OPAHs before GC/MS measurements. The hydroxyl-OPAHs were quantified with the help of fluoranthene-D_{10} as syringe spike. Sample extraction and instrumental analysis followed the earlier reported protocol (Bandowe and Wilcke, 2010) except the GC oven temperature conditions for the carbonyl-OPAHs which were slightly modified to reduce the retention of little volatile compounds like 1,8-NAA and 7,12-B(A)A that were not included in earlier measurements (Bandowe and Wilcke, 2010). The GC oven temperature program used for the OPAHs included an initial temperature of 80°C held for 6 min, increased to 145°C at a rate of 5°C min^{-1}, held for 3 min, further increased to 200°C at a rate of 5°C min^{-1}, held for 7 min and finally increased to 300°C at a rate of 15°C $min^{-1}$and held for 1 min.

3.3 Quality assurance and quality control

The used solvents were all high purity "picograde" quality for residue analysis (Promochem, Wesel, Germany). Glassware was thoroughly cleaned by rinsing with technical grade acetone, machine washed, baked for 12 h at 250°C and rinsed with high purity solvent before use. To correct for contamination during sample preparation, five blanks made of diatomaceous earth were processed simultaneously with our samples at the beginning and after every 15 samples. The measured concentrations of the target compounds were corrected by subtracting the mean concentrations of these compounds in the blanks. For each sampled soil profile the analysis was performed in duplicate for at least one horizon. The relative standard deviations (RSDs) for duplicate analysis were 0.4-25%, 1-10%, and 2-57% for the sum of PAHs, the sum of carbonyl-OPAHs, and the sum of hydroxyl-OPAHs, respectively. The accuracy of our measurement of PAH concentrations was checked by analyzing the certified European reference material ERM®-CC013a-Polycyclic aromatic hydrocarbons in soil (BAM, Berlin, Germany). Mean recovery of the sum of PAHs, for which certified and indicator values were provided, was 93% (86-100%, n = 3). The mean recovery of benzophenone-D_5 was 67% (50-95%) and the mean recovery of hydroxyl-OPAHs determined in an earlier recovery experiment was (36-70%, Bandowe and Wilcke, in press).

3.4 Calculations and compound properties

The sum of the concentrations of all parent- and alkyl-PAHs is ∑34 PAHs, all parent-PAHs ∑21PAHs, the 16 EPA-PAHs ∑EPA-PAHs, the combustion-derived PAHs [FLUA, PYR, B(A)A, CHRY, B(BJK), B(E)P, B(A)P, IND and B(GHI)] ∑COMB-PAH (Hwang et al., 2003), 1- and 2-methylnaphthalenes ∑C1-NAPH, 1- and 2-methylphenanthrenes ∑C1-PHEN and 3,6-, 3,5-, 2,6- and 1,7-dimethylphenanthrenes ∑C2-PHEN. Furthermore, we calculated concentration ratios of OPAHs to parent-PAHs (i.e. PAH from which the OPAH is likely derived either by microbial or inorganic chemical transformation) for 1,4-NQ and 2-NAPHTHOL relative to NAPH, 1-NLD relative to 1-MNAPH, 2-BPCD relative to PHEN, 1-INDA, 9-FLO, 9-FLUORENOL relative to FLUO, 1,8-NAA relative to ACENY, 1,2-ACQ, 1-ACENOL relative to ACENY/ACEN, 9,10-ANQ relative to ANTH, 2-PP relative to BP and 7,12-B(A)A relative to B(A)A. The octanol-water partition coefficients (K_{OW}) of PAHs and alkyl-PAHs were taken from Neff et al. (2005) and Mackay et al. (2006), and those of OPAHs were taken from Hansch et al. (1995) or estimated from KOWWIN v1.67 EPI Suite^{TM} version 4.0 (USEPA: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm, verified on 05/08/2009). Statistical analysis was performed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Normal distribution of data was checked by the Kolmogorov-Smirnov test. Data was log-transformed to improve normal distribution before correlation analysis.

4 Results

4.1 Concentrations of OPAHs and PAHs in topsoils

The ∑carbonyl-OPAH concentrations in surface soil horizons ranged from 85 ng g^{-1} (BA 10) to 2641 ng g^{-1} (BA-08, Table D-2). Soils at a former chemical waste dump (BA-08) had the highest carbonyl-OPAH content while arable lands (BA-10, BA-05, BA-07) had the lowest carbonyl-OPAHs. At all sampling points except BA-10, the highest concentration of ∑carbonyl-OPAHs was in the surface soil horizon. The mixtures of carbonyl-OPAHs were dominated by 1-INDA (3-31%, mean: 19%), 9-FLO (8-27 %, 20%) and 9,10-ANQ (17-40 %, 25%) in most surface soil horizons (Fig. D-2). However, the surface soil horizons of the highly contaminated sampling sites BA-08 , BAk-22, BA-11 and BAk-22 were dominated by 7,12-B(A)A (a four-ring carbonyl-OPAH) with contributions of 56%, 30% and 25%, respectively, to the sum of carbonyl-OPAH concentrations.

The ∑hydroxyl-OPAH concentrations in surface soil horizons ranged from 1.3 ng g^{-1} (BA 06) to 50 ng g^{-1} (BA-08, Tab. D-2). The majority of the soils had their highest hydroxyl-OPAH concentrations in the surface soil horizons decreasing in deeper horizons but the depth distribution of hydroxyl-OPAHs was less distinct than that of carbonyl-OPAHs. The hydroxyl-OPAH mixtures were in all horizons dominated by SALYD (32-100%, 83%), with smaller contributions from 1-ACENOL (0-59%, 7%) and 2-PP (0-26%, 5%) to the sum of hydroxyl-OPAH concentrations.

The ∑34 PAH concentrations in surface soil horizons ranged from 842 ng g^{-1} (BA-07) to 244869 ng g^{-1} (BA-08). The ∑EPA-PAH concentrations ranged from 569 ng g^{-1} (BA-07) to 23047 ng g^{-1} (BA-08). The highest ∑34PAH concentration occurred in the surface soil horizons except at site BA-09 (Tab. D-2). The PAH mixtures in surface soil horizons were, on average, dominated by PYR (3-32%, 10%), NAPH (0.2-22%, 9%), PHEN (0.5-15%, 9%) and other higher molecular weight PAHs (FLUA, B(BJK) and B(A)P). The two most dominant PAHs at sites BA-05, BA-07, BA-09, BA-10, BA-11 were NAPH and PHEN while BA-08, BAk-21, BAk-22 were dominated by PYR and FLUA and BA-06 and BA-13 by benzopyrenes and B(BJK) (Fig. D-3). The surface soil horizons at sites BA-05 (34%), BA-07 (36%), BA-09 (40%) and BA-10 (48%) showed similar contributions of ∑COMB-PAH concentrations to the ∑34PAHs concentrations (all <50%), while the surface soil horizons at sites BA-11 (52%), BA-12 (62%), BAk-21 (70%), BA-06 (72%) and BAk-22 (72%) and BA-08 (94%) contained >50% of combustion-derived PAHs.

Based on the OPAH and PAH concentrations, the most contaminated areas are the chemical waste dump (BA-08), a house garden currently sealed for construction activities (BA-13), areas near the former Apollo factory (BAk-21), a garden allotment in Zabi Majer (BAk-22, near a chemical company) and the anti-flooding embankment (BA-11). Current and former agriculturally used soils, even when close to a factory, chemical waste dump or highway, had lower PAH and OPAH concentrations (BA-06, BA-07, BA-10).

The ∑carbonyl-OPAHs concentrations correlated significantly with those of the ∑hydroxyl-OPAHs ($r = 0.81$, $p = 0.002$), ∑34PAHs ($r = 0.92$, $p<0.0001$) and ∑COMB-PAHs ($r=0.90$, $p<0.0001$). The concentrations of most individual carbonyl-OPAHs correlated significantly with each other and their parent-PAHs, ∑COMB-PAH and ∑34PAHs, except 1-INDA and 2-BPCD. The concentrations of individual hydroxyl-OPAHs did not correlate significantly with their parent-PAHs, i.e. 2-NAPHTHOL, 2-PP, 1-ACENOL and 9-FLUORENOL with NAPH, BP, ACEN/ACENY and FLUO, respectively. The 2-PP concentrations correlated significantly with those of ∑COMB-PAHs ($r = 0.85$, $p = 0.03$) and ∑34PAHs ($r = 0.83$, $p = 0.04$). There were several significant correlations among individual OPAH and PAH concentrations (not shown).

The concentrations of 1-INDA ($r = 0.68$, $p = 0.02$), NAPH ($r = 0.74$, $p = 0.009$) and ∑C1-NAPH ($r = 0.82$, $p = 0.002$) and BP ($r = 0.74$, $p = 0.01$) correlated significantly with organic C (C_{org}) concentrations in surface soil horizons. No other individual or sum of PAH and OPAH concentration correlated with C_{org}. The concentrations of SALYD ($r = -0.64$, $p = 0.03$), 1,8-NAA ($r = -0.60$, $p = 0.05$) and ANTH ($r = -0.76$, $p = 0.006$) correlated significantly and negatively with soil pH. There was no significant correlation between sand, silt or clay concentrations and those of any OPAH or PAH. The OPAH/parent-PAH concentration ratios of carbonyl-OPAHs were >1 in several surface soil horizons except 1,4-NQ/NAPH, 1-NLD/1-MNAPH, 2-BPCD/PHEN (Tab. D-3). The hydroxyl-OPAHs/parent-PAH concentration ratios were all clearly <1.

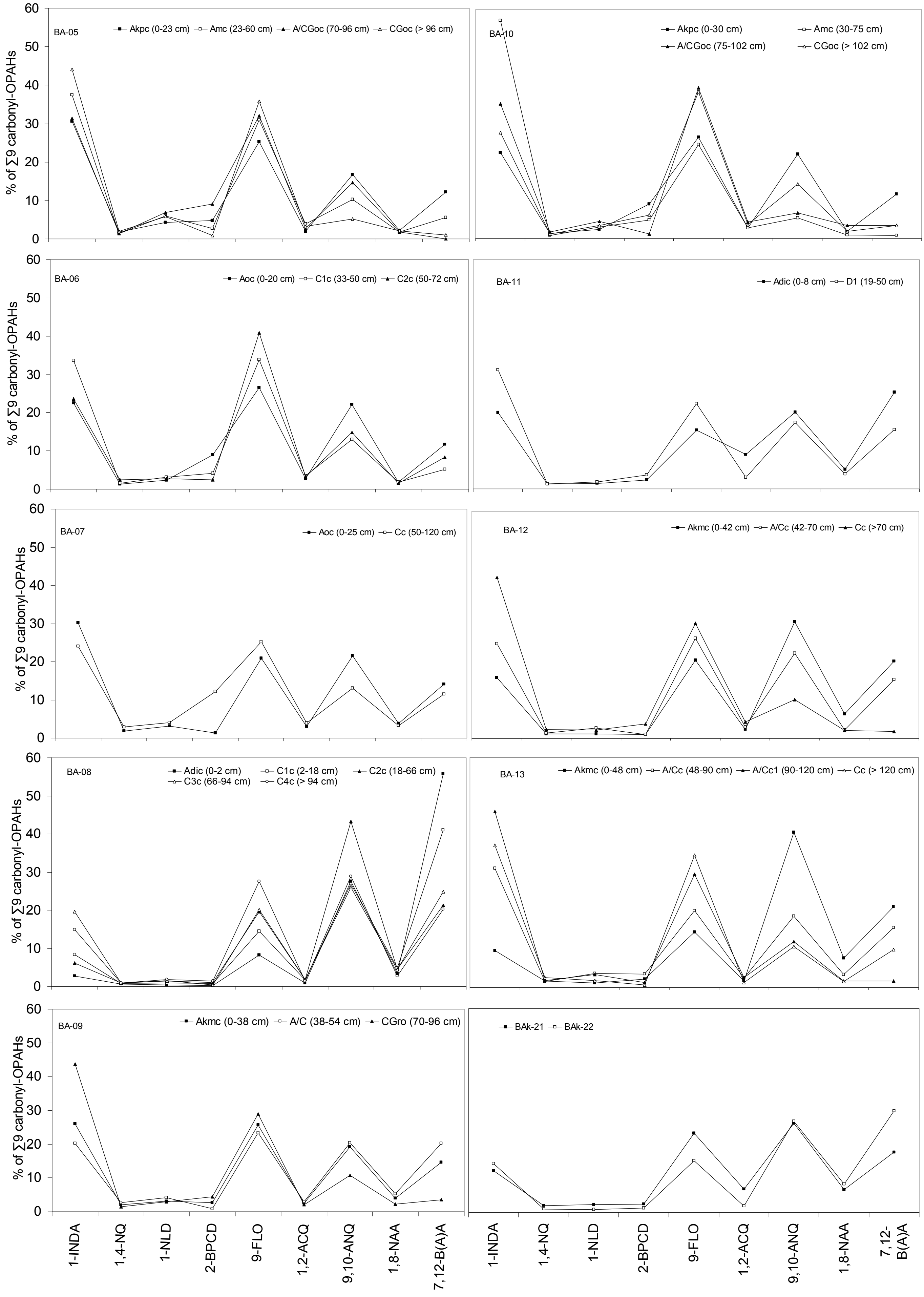


Figure D-2: Composition of the carbonyl-OPAH mixtures in soils of Bratislava.

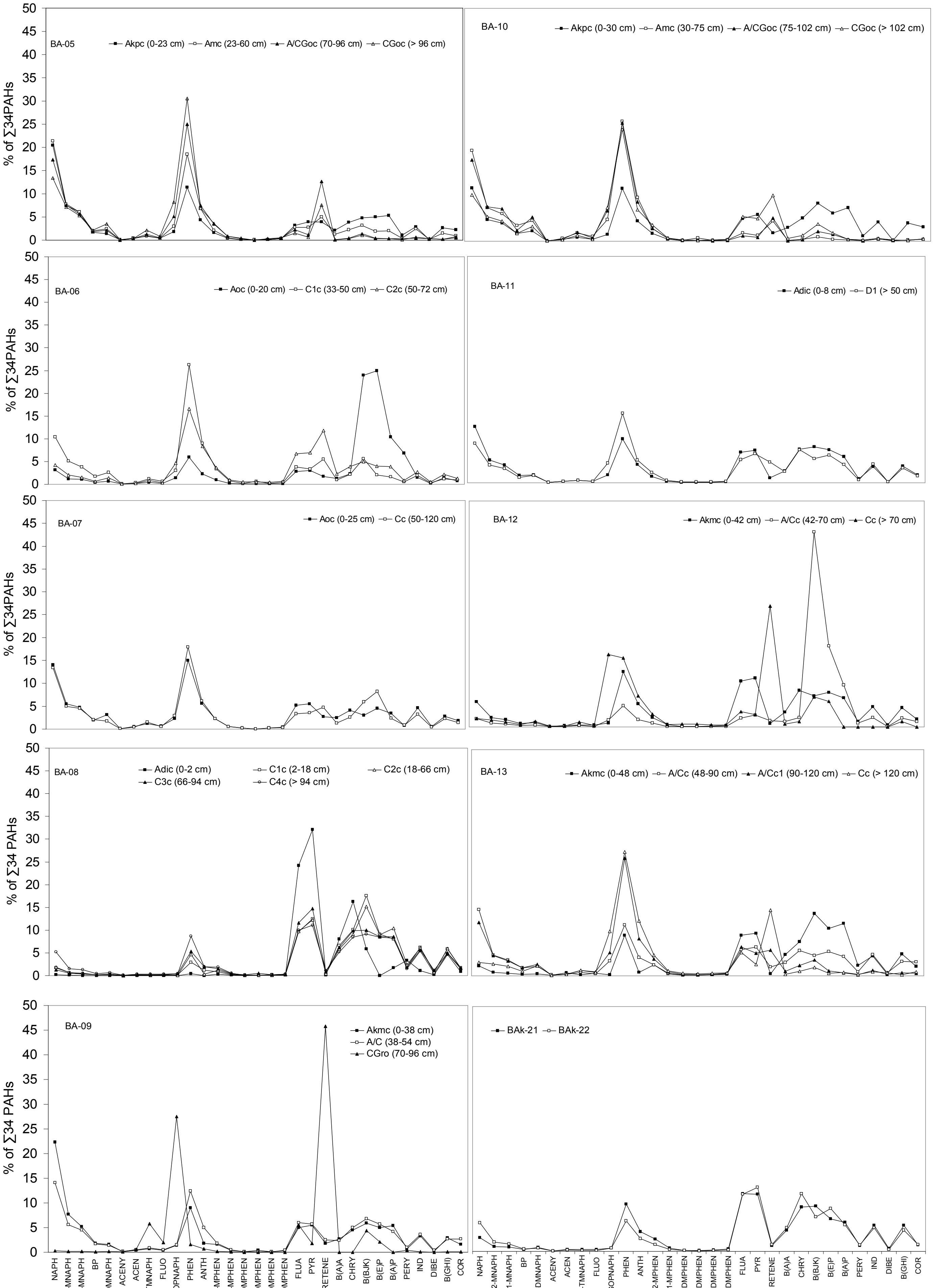


Figure D-3: Composition of alkyl/parent-PAH mixtures in soils of Bratislava.

Table D-3: Concentrations ratios of OPAH to their parent-PAH in soils from Bratislava. Labels ending with “t” refers to surface soil horizon and “s” refers to subsoil horizon. Subsoil horizon is given as mean and range (in brackets) of all subsoil horizon at a particular sampling site.

Sampling point	1-INDA/FLUO	1,4-NQ/NAPH	1-NLD/1-MNAPH	2-BPCD/PHEN	9-FLO/FLUO	1,2-ACQ/ACENY	1,2-ACQ/ACEN
BA-05t	10.3	0.01	0.10	0.06	8.5	3.5	0.6
BA-05s	19.3(10-24)	0.03(0.0-0.1)	0.4(0.1-0.5)	0.06(0.0-0.1)	17.4(8-24)	17.1(4-24)	3.2(1.6-4.8)
BA-06t	4.4	0.02	0.09	0.06	5.2	1.9	0.6
BA-06s	19.1(11-27)	0.13(0.0-0.2)	0.43(0.2-0.7)	0.04(0-0.1)	29.1(11-47	10.5(8.4-12.6)	3.7(1.9-5.4)
BA-07t	7.7	0.0	0.1	0.0	5.3	6.4	1.0
BA-07s	7.4	0.0	0.2	0.1	7.7	7.1	1.4
BA-08t	3.6	0.0	0.1	0.0	10.9	1.8	1.3
BA-08s	2.99(0.7-5.6)	0.02(0.01-0.04)	0.12(0.1-0.2)	0.01(0.0-0.01)	4.6(2.4-5.8)	1.3(0.7-2.3)	0.5(0.3-0.8)
BA-09t	4.9	0.01	0.04	0.02	4.8	1.9	0.4
BA-09s	2.8(0.4-5.2)	0.05(0-0.1)	0.19(0.1-0.3)	0.03(0.01-0.05)	3.1(0.3-6.0)	1.1(0.4-1.8)	0.4(0.1-0.7)
BA-10t	4.1	0.01	0.04	0.06	4.9	1.4	0.5
BA-10s	13.9(9.1-18.0)	0.02(0.01-0.03)	0.16(0.1-0.2)	0.04(0.0-0.1)	13.8(6.3-25)	12.7(10.1-14.1)	2.0(1.2-2.9)
BA-11t	7.0	0.01	0.05	0.03	5.4	11.9	3.0
BA-11s	27.4	0.04	0.2	0.06	19.6	6.9	2.4
BA-12t	3.9	0.02	0.08	0.01	5.0	2.3	0.9
BA-12s	36.4(8.9.6)	0.3(0.0-0.5)	0.5(0.2-0.8)	0.06(0.0-0.1)	27.6(9.4-46)	5.6(1.4-9.8)	4.6(1.1-8.0)
BA-13t	0.9	0.04	0.10	0.01	1.3	1.4	0.1
BA-13s	19.7(8.6-34.2)	0.2(0.01-0.6)	0.3(0.1-0.6)	0.02(0.02-0.04)	15.9(6.0-32)	6.28(1.7-9.9)	1.97(0.9-2.9)
BAk-21t	2.1	0.05	0.2	0.02	4.0	4.0	1.2
BAk-22t	5.7	0.02	0.06	0.02	6.0	2.2	0.7

Sampling point	9,10-ANQ/ANTH	1,8-NAA/ACENY	7,12-B(A)A/B(A)A	2-NAPHTHOL/NAPH	2-PP/BP	1-ACENOL/ACEN	9-FLUORENOL/FLUO
BA-05t	0.5	4.1	0.8	0.0	0.0	0.0	0.0
BA-05s	0.4(0.2-0.7)	10.9(1.9-15.5)	1.2(0.0-2.9)	0.0	0.00	0.00	0.02(0.0-0.1)
BA-06t	0.40	1.27	0.35	0.0	0.04	0.00	0.03
BA-06s	0.5(0.3-0.7)	5.3(4.5-6.0	1.2(1.0-1.4)	0.0	0.00	0.00	0.00
BA-07t	0.7	8.0	1.0	0.0	0.0	0.0	0.0
BA-07s	0.4	5.9	1.5	0.0	0.0	0.0	0.0
BA-08t	6.8	6.2	0.08	0.01	0.07	0.2	0.1
BA-08s	1.6(0.7-3.9)	3.3(1.3-5.4)	0.2(0.1-0.2)	0.0	0.00	0.01(0-0.01)	0.00
BA-09t	0.76	3.04	0.38	0.0	0.01	0.00	0.00
BA-09s	0.4(0.3-0.5)	1.9(0.5-3.3)	1.9(1.0-2.9)	0.0	0.00	0.00	0.00
BA-10t	0.37	0.91	0.28	0.0	0.00	0.00	0.00
BA-10s	0.3(0.1-0.6)	6.9(5.0-11)	2.7(1.1-5.3)	0.0	0.00	0.00	0.04(0-0.12)
BA-11t	0.6	6.7	1.2	0.0	0.00	0.00	0.00
BA-11s	0.9	9.0	1.7	0.0	0.00	0.00	0.00
BA-12t	0.7	6.4	0.7	0.0	0.02	0.03	0.00
BA-12s	0.7(0.6-0.7)	2.8(1.0-4.6)	0.9(0.7-1.1)	0.0	0.00	0.00	0.00
BA-13t	2.85	6.56	0.23	0.0	0.00	0.01	0.00
BA-13s	0.5(0.4-0.6)	6.4(2.3-12.3)	6.5(2.3-12.3)	0.0	0.00	0.00	0.01(0-0.04)
BAk-21t	0.46	3.90	0.29	0.02	0.11	0.00	0.02
BAk-22t	1.00	9.67	0.59	0.00	0.02	0.96	0.00

4.2 Depth distribution of OPAHs and PAHs

The ∑carbonyl-OPAH concentrations were highest in the surface soil horizons and decreased systematically with depth except at site BA-10 where the Amc horizon at 0.30-0.75 m depth had the highest concentration. The ratio of the ∑carbonyl-OPAH concentrations in surface soil to the uppermost subsoil horizon ranged from 0.7 (BA-09) to 7 (BA-08, Tab. D-2). The contribution of the more soluble 1-INDA and 9-FLO to the ∑carbonyl-OPAH concentrations increased markedly with depth at the expense of the more hydrophobic 9,10-ANQ and 7,12-B(A)A (Fig. D-2). The concentration ratios of OPAH/parent-PAH increased with increasing depth (Tab. D-3). At site BA-05, the concentration ratio of 1-INDA/FLUO increased from 10 in the surface soil to 24 in the deepest subsoil horizon (BA-05/4). Similar trends of these ratios occurred for several other carbonyl-OPAH/parent-PAHs (Tab. D-3).

At the majority of the sampling sites, subsoil horizons had lower ∑hydroxyl-OPAH concentrations than surface soil horizons (Tab. D-2). However, at several sites concentrations hydroxyl-OPAH concentrations were higher in subsoil than surface soil horizons. The concentration ratios of ∑hydroxyl-OPAHs in surface soil to subsoil horizons ranged from 0.2 (BA-10) to 16 (BA-08). This was mainly attributable to the quantitative dominance of SALYD.

The ∑34PAHs concentrations were highest in the surface soil horizons and decreased systematically in the subsoil at all sampling sites except BA-09 where the deepest horizon, the CGro horizon at 0.70-0.96 m depth had a higher concentration than the surface soil horizon. The concentration ratio of ∑34 PAH in the surface soil to the uppermost subsoil horizon ranged from 1.6 (BA-07) to 22 (BA-08, Tab. D-2). The composition of the PAH mixtures was similar in all horizons (Fig. D-3). However, at several sampling sites (e.g., BA-06 and BA-10, Fig. D-3), the contribution of the lower molecular weight PAHs increased with depth at the expense of that of the higher molecular weight PAHs.

To assess whether the depth distribution of OPAHs and PAHs was the result of leaching (as truly dissolved solute) we correlated log concentration ratios (CR) of all compounds with their log K_{OW} values at each sampling site. The CR is the concentration of a compound in the uppermost subsoil horizon to that in the surface horizon. Log CR and log K_{OW} were significantly negative correlated for carbonyl-OPAHs at sites BA-06 ($r = -0.72$, $p = 0.03$) and BA-10 ($r = -0.61$, $p = 0.04$) and marginally significantly at site BA-05 ($r = 0.63$, $p = 0.07$). We also found a significant negative correlation between log CR and log K_{OW} for PAHs at sites BA-05 ($r = -0.39$, $p = 0.05$), BA-06 ($r = -0.49$, $p = 0.01$), BA-08 ($r = -0.43$, $p = 0.03$) and BA-10 ($r = -0.571$, $p = 0.002$).

5 Discussion

5.1 State of contamination of the urban soils of Bratislava with OPAHs and PAHs

The concentrations of all OPAHs in the soils of Bratislava are similar to those in Mainz and Basel (if the soil of the chemical waste dump, BA-08, is excluded) but higher than in a rural region near Manaus, Brazil (Tab. D-4). The concentration of OPAHs in soil of the chemical waste dump (BA-08) is similar to those in soil of the Angren industrial region (coal mining, energy production, metallurgical industry) of Uzbekistan (Tabs. D-2 and D-4). The OPAH concentrations in Bratislava soils were, however, lower than at highly contaminated sites in Sweden and Germany and in roadside soil in the Czech Republic (Tab. D-4). In the Bratislava soils, the concentrations of hydroxyl-OPAHs were generally lower than those of the carbonyl-OPAHs, which is consistent with other earlier studies of atmospheric samples and soils (Moyano et al., 1997; Eriksson et al., 2000).

The PAH concentrations in Bratislava soils are similar as in other cities in the USA and Europe (Tab. D-4), except the soil at BA-08 near the waste dump which is particularly PAH-contaminated similar to soil at former gaswork sites like described by Krauss et al. (2003) for a site in Bayreuth, Germany. Using the classification of Maliszewska-Kordybach (1996) the surface soils from sites, BA-06, BA-08, BA-09, BA-11, BA-12, BA-13, BAk-21, BAk-22 are highly contaminated (>1000 ng g^{-1} ∑EPA-PAHs), BA-05 and BA-10 contaminated (600-1000 ng g^{-1}), and BA-07 weakly contaminated (200-600 ng g^{-1}).

The contaminated and weakly contaminated soils were all formerly or currently under agricultural use (Tab. D-1). Therefore, direct deposition of OPAH- and PAH-containing materials was negligible and furthermore regular ploughing resulted in the dilution of OPAH and PAH concentrations because of the mixing of little contaminated subsoil and more strongly contaminated topsoil.

Table D-4: Comparison of OPAH and PAH concentrations in surface soil horizons of Bratislava with other cities.

Region	Land use	Number of OPAHs	Concentration [ng g^{-1}]	Reference
Northeast, France	Coking plant sites	8	113120-543360	Biache et al. (2008)
North Region, Germany	Wood preservation	6	57600	Meyer et al. (1999)
North Region, Germany	Military site	6	39100	Meyer et al. (1999)
Berlin, Germany	Former gasworks site	18	16208.00	Bandowe and Wilcke (2010)
Mainz, German	Urban garden	18	206.00	Bandowe and Wilcke (2010)
Manaus, Brazil	Rural	18	23	Bandowe and Wilcke (2010)
Tyn-Lipnik, Czech Republic	Urban sites	3	35960	Zdrahal et al. (2000)
Basel, Switzerland	Urban sites	4	29-440	Niederer (1998)
Angren, Uzbekistan	Industrial area	12	62-1893	Bandowe et al. (in press)
Husarviken, Sweden	Gasworks site	16	426660	Lundstedt et al. (2007)
Lulea, Sweden	Coking plant sites	16	106760	Lundstedt et al. (2007)
Holmsund, Sweden	Wood preservation	16	252880	Lundstedt et al. (2007)
Boden, Sweden	Wood preservation	16	9338	Lundstedt et al. (2007)
Bratislava	Urban sites	14	88-2692	This study.
Bratislava[a]	Urban sites	14	88-509	This study.
		Number of PAHs		Reference
Detroit, USA	Urban sites	17	7843	Wang et al. (2008)
New Orleans, USA	Urban sites	17	5100	Wang et al. (2008)
El Paso, Texas, USA	Urban sites, industrial	16	0.1-2226	De La Torre-Roche et al. (2009)
Seine River basin, France	Urban sites, industrial	14	450-5650	Motelay-Massei et al. (2004)
Taragona County, Spain	Urban and industrial	16	166-1002	Nadal et al. (2004)
Tallin, Estonia	Urban sites	13	2200-9015	Trapido (1999)
Helsinki, Finland	Urban sites	16	87-418241	Saltiene et al. (2002)
Vilnius, Lithuania	Urban sites	16	397-1064	Saltiene et al. (2002)
Chicago, USA	Urban Sites	16	4520-62038	Saltiene et al. (2002)
London, UK	Urban sites	16	4728-57056	Saltiene et al. (2002)
Belgrade, Serbia	Urban Site	16	283±548	Saltiene et al. (2002)
Birmingham, UK	Urban sites	16	2020	Smith et al. (1995)
Glasgow, UK	Urban sites	15	1487-51822	Morillo et al. (2007)
Ljubljana (Slovenia)	Urban sites	15	218-4488	Morillo et al. (2007)
Torino (Italy)	Urban sites	15	148-23500	Morillo et al. (2007)
Bayreuth, Germany	Urban sites	20	280-186000	Krauss et al. (2003)
Hamburg, Germany	Urban sites	19	100-44300	Gras et al. (2000)
Basel, Switzerland	Urban sites	16	11100±11900	Niederer (1995)
Bratislava, this study	Urban Sites	34	842-244870	
Bratislava, this study*	Urban Sites	34	842-9842	

[a] excluding most polluted site BA-08.

5.2 Sources of OPAHs and PAHs

The carbonyl-OPAH mixtures in topsoils were dominated by 1-INDA, 9-FLO and 9,10-ANQ and the hydroxyl-OPAH mixtures by SALYD which is consistent with previous studies of soil and atmospheric samples(Lundstedt et al., 2003; Albinet et al., 2007; Bandowe and Wilcke, 2010). However, the higher contribution of 7,12-B(A)A to the ∑carbonyl-OPAH concentrations in some of the most polluted soils (BA-08, BA-11, BAk-22) with high contributions of ∑COMB-PAH to the ∑34 PAH concentrations and the close correlation of 7,12-B(A)A concentrations with those of ∑COMB-PAH suggest that high molecular weight carbonyl-OPAHs were emitted together with combustion-derived PAHs. The lack of correlation of 1-INDA and 2-BPCD concentrations with their parent-PAH and combustion derived-PAH concentrations suggests that 1-INDA and 2-BPCD reached the study soils from a diffuse atmospheric source. This is further backed by the fact that 1-INDA mainly dominated the OPAH mixtures in little polluted soils while more polluted soils were dominated by high ΣCOMB-PAH and 7,12-B(A)A (e.g., BA-08) . We hypothesize that soils affected by combustion-related point sources of pollution will have higher contribution of higher molecular weight OPAHs (≥ 4 aromatic rings) like 7,12-B(A)A because the higher molecular weight OPAHs are predominantly associated with the particulate phase and will therefore settle closer to their emission source, while lower molecular weight OPAHs like 1-INDA will predominantly occur in the gas phase and can thus be transported further away from the emission source (Yang et al., 1991; Albinet et al., 2007).

The significant correlation of the 1-INDA, NAPH, methyl-naphthalenes and 2-BPCD concentrations with those of C_{org} indicate that these compounds are partitioned from the atmosphere, which has a homogeneous concentration of these compounds in the urban area of Bratislava, to soil organic matter. This assumption is also backed by the lack of correlation of the volatile 1-INDA and 2-BPCD with parent-PAHs which we interpreted as result of the further distribution of these compounds from the emission source. All other compounds may either be released to the atmosphere in particulate phase (or condense to particles after emission) or reach the soil from specific point sources (Wilcke et al., 2000). The negative correlation of SALYD and 1,8-NAA concentrations with soil pH might indicate that these compounds are at least partly produced by microbial turnover which is reduced at low pH. The negative correlation of ANTH with soil pH, in contrast, might be attributable to the higher hydrophobicity of soil organic matter at low pH (Marschner, 1999; Wenzel et al., 2002; Bucheli et al., 2004).

5.3 Importance of OPAHs relative to parent-PAHs

Some carbonyl-OPAHs (1-INDA, 9-FLO, 9,10-ANQ) had higher concentrations than the parent-PAHs from which they are derived (Tab. D-3). This may be related by higher concentrations of OPAHs than parent-PAHs in the emissions or fast transformation of parent-PAHs to OPAHs in the atmosphere. Alternatively, OPAHs might be faster deposited than their parent-PAHs because of the reduced volatility and thus enhanced association with atmospheric particles which settle faster than gases. Given their partly even higher toxicity than the parent-PAHs, more attention needs to be paid to OPAHs in monitoring and remediation efforts in future. In contrast, the hydroxyl-OPAHs only occurred in much lower concentrations than the parent-PAHs. Furthermore, there might even be other sources for some OPAHs than PAHs, particularly for SALYD which is a metabolite of the degradation of any aromatic compound in soil

5.4 Vertical distribution of OPAHs and PAHs

The concentrations of OPAHs and PAHs were generally higher in surface soil than in subsoil horizons because of the strong sorption to soil organic matter which is mainly accumulated in the topsoil (Tab. D-2, Guggenberger et al., 1996; Migaszewski et al., 2002). The concentration ratios of OPAHs in surface soil to the uppermost subsoil horizons were generally lower than those of PAHs which we attribute to the higher water solubility of the OPAHs and thus more pronounced vertical transport. This is supported by the finding that the contribution of the most soluble OPAHs, 1-INDA and 9-FLO to the Σcarbonyl-OPAH concentrations increased with increasing soil depth while that of the less soluble 9,10-ANQ und 7,12-B(A)A decreased (Figure D-2). Furthermore, the OPAH to parent-PAH ratios increased for several compound pairs with increasing soil depth (Tab. D-3). The correlations of logCR of the carbonyl-OPAHs with $\log K_{OW}$ at sites BA-06, BA-10, and BA-05 (marginally significant) suggests that the carbonyl-OPAHs were leached in dissolved form. Consequently, there is a larger risk that OPAHs reach the groundwater than PAHs. At coal tar-contaminated sites it was already shown that OPAHs occur in groundwater (Schlanges et al., 2008).

6 Conclusions

The contamination of Bratislava soils with OPAHs and PAHs is similar as in other urban areas in Europe and North America. Carbonyl-OPAH concentrations were higher than hydroxyl-OPAH concentrations. Our results indicate that a large part of the OPAHs in urban soils of Bratislava was emitted together with PAHs as shown by the close correlation of OPAH and PAH concentrations and by the partitioning of some OPAHs from the atmosphere to soil organic matter. Post-emission transformations seemed to play a minor role for the formation of OPAHs although there were indications that SALYD, 1-NLD and 1,8-NAA had at least partly inorganic chemical or biological sources. Several carbonyl-OPAHs had higher concentrations than their parent-PAHs calling for their inclusion into monitoring programs and environmental regulation. This was not the case for hydroxyl-OPAHs which only occurred in minor concentrations in the studied soils. The depth distribution of OPAHs and the change in OPAH/parent-PAHs ratios with increasing soil depth suggest that OPAHs can partly be faster transported than their parent-PAHs and thus pose a bigger risk for groundwater than PAHs.

7 Acknowledgement

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E Appendix

Published papers

Paper E-1**:** Naphthalene production by microorganisms associated with termites: evidence from a microcosm experiment (2009). **Soil Biology and Biochemistry 41, 630-639, doi: 10.1016/ j.soilbio.2008. 12.029.**

Paper E-2: Method optimization to measure polybrominated diphenyl ethers (PBDEs) in soils of Bratislava, Slovakia (2010). **Environmental Pollution 158, 2208-2217, doi: 10.1016/j.envpol.2010. 02.021.**

Tables

Table E-3a: Structure and properties of OPAHs under standard conditions (25°C, 101.3 kPa). Data were taken from the PhysiProp data base or estimated using estimation tools from EPI-SuiteTM version 4.0. (USEPA: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm, accessed on 5/8/2009). Bold numbers are experimental data and others are estimated data.

Table E-3b: Physical properties of alkyl/parent-PAHs. Octanol-water partition coefficients (K_{OW}) are from Neff et al. (2005) and Mackay et al. (2006), solubility and vapor pressure values were taken from the PhysiProp data base or estimated using estimation tools from EPI-SuiteTM version 4.0. (USEPA:http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm, accessed on 5/8/2009). Bold numbers are experimental data and others are estimated data.

Table E-3c: Concentrations [ng g^{-1}] of OPAHs and alkyl/parent-PAHs in soils from Angren industrial area, Uzbekistan. See Fig. C-1 and Tab. C-1 for description of sampling sites/location.

Table E-3d: Concentrations [ng g^{-1}] of OPAHs and alkyl/parent-PAHs in soils from Bratislava, Slovakia. See Fig. D-1 and Tab. D-1 for description of sampling sites/location.

E-1 Naphthalene production by microorganisms associated with termites: evidence from a microcosm experiment[a]

Benjamin A. Musa Bandowe[1], Daniel Rückamp[2], Marcos Bragança[3], Volker Laabs[2], Wulf Amelung[2], Christopher Martius[4], Wolfgang Wilcke[1]

[1] Professorship of Soil Geography/Soil Science, Geographic Institute, Johannes Gutenberg University Mainz, Johann-Joachim-Becher-Weg 21, 55128 Mainz, Germany

[2] Institute of Crop Science and Resource Conservation (INRES), Soil Science and Soil Ecology, University of Bonn, Nussallee 13, 53115 Bonn, Germany

[3] Federal University of Tocantins, Department of Biological Science, 77500-00, Porto Nacional, TO, Brazil

[4] Center for Development Research (ZEF), University of Bonn, Walter-Flex-Strasse 3, 53113 Bonn, Germany (currently: ICARDA Program for Sustainable Agriculture in Central Asia and Caucasus, Tashkent, Uzbekistan)

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1 Abstract

There have been several reports published which suggest that it is possible the polycyclic aromatic hydrocarbons (PAHs) naphthalene (NAPH), phenanthrene (PHEN) and perylene (PERY) in tropical environments have a biological source. This source might be related to the activity of termites or their associated microorganisms. We aimed to provide direct evidence for the biological production of NAPH, PHEN and PERY by conducting microcosm experiments in the State of Tocantins, Brazil, in which termite nests (with or without termites) were placed in an enclosed environment in which we controlled all PAH fluxes and monitored changes of PAH stocks. The experiments were carried out with termites from a tropical floodplain forest environment at the Estação Canguçu (Ilha do Bananal) in the State of Tocantins, Brazil. We set up the following treatments: live nest of *Nasutitermes cf. minor* using PAH-poor wood as food (LNW), live nest of *Nasutitermes cf. minor* using PAH-poor corn as food (LNC), termite nest without live termites called dead nest (DNC) and dead nest with additional treatment by a combined fungicide/bactericide (FDN) in several replicates. In LNW, LNC, and DNC, there were mean increases of 43, 21, and 46% in NAPH stock after 20 d while the stocks of the 20 other PAHs studied did not change or even decreased. In contrast, FDN lost 20% of the NAPH stock after 20 d of the microcosm experiment because of dissipation and lack of microbial synthesis of new NAPH. In LNW and LNC, low-molecular weight PAHs (acenaphthylene to chrysene) were significantly lost at a mean percentage which was strongly correlated with the octanol-water partitioning coefficient (K_{ow}, $r = 0.78$). This was not the case in DNC and FDN. There were no indications that in the studied termite nests PHEN and PERY were produced. Our microcosm experiments suggest that NAPH can be produced by fungi and bacteria in termite mounds while all other low-molecular weight PAHs are degraded in microcosms with live termite nests. PAH degradation seems to be enhanced by the combined activity of termites and microorganisms.

2 Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute a group of organic compounds containing at least two fused benzene rings in various arrangements (Blumer, 1976). PAHs are known to be hazardous (Menzie et al., 1992) and 16 of them are included in the US EPA class of priority pollutants (Keith and Telliard, 1979). The sources of PAHs in the environment can be anthropogenic or natural. Significant increases in PAH concentrations in dated sediment cores and archived soils since the onset of industrialization demonstrated that in the temperate industrialized world the main source of PAH contamination is a result of human activities, mainly combustion of fossil fuels (Jones et al., 1989; Fernandez et al., 2000; Elmquist et al., 2007).

Data on PAH patterns in the tropics are scarce (Connell et al., 1999; Wilcke 2000, 2007) even though such data are needed to understand the global PAH dynamics, because PAHs emitted into tropical environments may be globally distributed (Wania and Mackay, 1996). Recent studies in tropical environments indicate that there are differences in both the concentrations and the composition patterns of PAHs between tropical soils and soils of the industrialized part of the temperate zone. Tropical environments have lower PAH concentrations and are dominated by naphthalene (NAPH), phenanthrene (PHEN) and perylene (PERY) while industrialized temperate environments have higher PAH burdens and are dominated by high molecular weight PAHs (Wilcke 2000, 2007).

The high concentrations of NAPH, PHEN and PERY in tropical environments have been suggested to result from biological sources (Wilcke, 2000). An alternative explanation for the dominance of low-molecular weight PAHs (NAPH and PHEN) in tropical soils is that this may reflect higher source contributions from biomass burning in the tropics which emit more lower molecular weight PAHs, as well as preferential loss of lower molecular weight PAHs from temperate soils because of the higher age of their PAH loads (Dalya et al., 2007). An exact source assignment is still lacking. There are, however, increasing indications that biological production of NAPH is possible. These include reports of NAPH production by flowers of members of the plant genus *Magnolia* from Japan, China and North America (Azuma et al., 1996) and the plant family *Annonaceae* from the Amazonian rain forest (Jurgens et al., 2000) and by *Muscodor vitigenus,* a fungus from the Amazonian region of Peru (Daisy et al., 2002). Significant NAPH concentrations have also been detected in *Coptotermes formosanus* termite nests (Chen et al., 1998a, 1998b), while NAPH, PHEN and PERY dominate in nests of other termite genera and plants sampled from Brazil (Wilcke et al., 2000, 2003, 2004). Dalya et al.

(2007) reported high concentrations of PAH in air samples collected from the surroundings of termite nests. Perylene is known to be produced under anaerobic conditions by microorganisms in soils and sediments (Venkatesan, 1988; Guggenberger et al., 1996). These pieces of evidence led to the hypothesis that the partly high concentrations of NAPH, PHEN and PERY in tropical environmental media may be related to biological production by termites or microorganisms (i.e., fungi and bacteria) associated with termites (Wilcke, 2007).

Naphthalene is believed to play a role in the chemical defence against biological enemies (Wiltz et al., 1998; Wright et al., 2000). It may be produced by metabolic processes in termites or by associated microorganisms which inhabit, e.g., the termite guts (Varma et al., 1994). Direct evidence of the production of NAPH, PHEN or PERY by termites or associated microorganisms can be obtained with the help of microcosm experiments, in which termites are held in a closed environment under a PAH-free atmosphere. Microcosm experiments in which termite groups are fed with different food resources under controlled conditions have already been successfully used in ecological and ecotoxicological studies (Morgan and Knacker, 1994; Knacker and Römbke, 1997; Sheppard, 1997). Microcosm experiments with termites are furthermore a standard method to determine food consumption rates (Martius, 1997), food selection (Bustamante and Martius, 1998), and effects of pesticides (Su et al., 1987).

The aim of our study is to provide for the first time direct evidence of a potential production of NAPH, PHEN and PERY in termite nests. For this purpose, we ran microcosm experiments in Brazil in which we held termites under controlled environmental conditions. To separate the role in the potential PAH production of the termites themselves from that of the microorganisms in the guts of the termites and microorganisms just inhabiting the termite mounds in the potential PAH production we also set up microcosms with dead nests which were either kept untreated in the microcosm and thus contained an intact microflora or treated with biocide to markedly reduce the activity of microorganisms.

3 Materials and methods

3.1 Study site

Termite nests used for the microcosm experiments were collected from the periodically flooded forest at the Estação Canguçu (Ilha do Bananal), Federal State of Tocantins, Brazil. The termites belong to the species *Nasutitermes cf. minor* which are potential wood feeders (Martius, 1994).

Soil material (loamy clay, 5 g organic C kg^{-1}, 12% water content, subsoil usually used for pottery) and corn (*Zea mays* L.) used for the microcosm experiment were collected from Taquaruçu, a small town near the city of Palmas, Federal State of Tocantins, Brazil. The soft wood samples, *Tilia platyphylla* C. A. Mey. were collected in Germany, because there are some indications that tropical trees may contain NAPH and PHEN (Wilcke et al. 2000; Krauss et al. 2005). Microcosm experiments were conducted in the laboratories of the Federal University of Tocantins in Palmas, Federal State of Tocantins, Brazil to keep transport distances as short as possible and to avoid strong changes of climatic conditions.

3.2 Microcosm experiments

The basic design of the microcosms consisted of a rectangular stainless-steel box with a height of 35 cm, a width of 35 cm, and a depth of 25 cm and a front plate made of transparent glass with an inlet and an outlet tube. The nests were taken from the forest and placed into the stainless-steel boxes on about 2000 g of the loamy clay material (to better control the humidity in the microcosms). Before being placed in the microcosms, the nests were temporarily left in the forest until the outer wall, which was slightly damaged during the collection procedure was repaired by the termites. The food source of termites in rain forest is wood or soil or a combination of it depending on the species (Martius, 1994). We supplied wood (*T. platyphylla*) or corn (*Z. mays*) to serve as food for the termites. After termite nests, soil, wood/or corn had been placed in the rectangular boxes, a transparent glass plate was used to seal the front of the boxes and glued air-tight with silicon except for the two channels left as inlet and outlet for air. The inlet was connected via a tube to a pump for air supply to the microcosm and the outlet was connected to an outflow tube serving as an exhaust for outflowing air. In front of the inlet tube, XAD-2 cartridges were placed to clean the inbound air of PAHs, while at the outlet similar XAD-2 cartridges were placed to trap air-borne PAHs released in the microcosm. The XAD-2 cartridges were selected based on preliminary experiments in which activated charcoal was shown to be unsuitable. Gaseous PAHs are commonly sampled with XAD-2 resins (Liu et al., 2001; Krauss et al., 2005).

We set up six treatments as shown in Table E1-1, two of which were without termites and thus served as controls. For the two treatments with live termites, the nests (104-927 g dry mass) were placed on a soil layer (1843-2690 g dry mass) and supplied with corn (149-165 g dry mass) (LNC) or *T. platyphylla* wood (172-206 g dry mass) (LNW). For the two controls without live termites (DNC, FDN) the nests were separated into various parts and the termites manually removed completely. After removal of termites, the parts were combined again to minimize the surface area. One set of the dead nest was additionally treated with a combined bactericide and fungicide sold with the market name “Lysoform spray” (Milana Industrial e Commercial, Itaquaquecetuba, Federal State of São Paulo, Brazil) with the active ingredient being alkyl dimethyl benzyl ammonium chloride (0.172%). We applied ca. 150 mg of the active ingredient per kg of microcosm content (i.e. soil+nest+food) and thus more than needed for a fungistatic effect (Tubajika, 2006). This set of fungicide/bactericide-treated dead nests is abbreviated as FDN. Two other treatments were without termite nest or termites and were called blank treatments. In the first treatment called blank corn (BC) 93 g of corn was placed on a layer of 1855 g of soil and in the second 180 g of *Tilia* wood was placed on a layer of 2158 g of soil to form blank wood (BW). The blank treatments were set up to control whether PAHs are produced from termite food and soil alone or are concentrated because of microbial food or soil organic matter decomposition.

Table E1-1: Overview of the microcosm treatments. “Live nest” denotes a termite mound inhabited by termites, “Dead nest” is a termite mound not inhabited by termites.

Food	Treatment	Replicates	Mean dry mass (without cage)[g]
Corn	Live nest with corn (LNC)	3	2676
Tilia wood	Live nest with wood (LNW)	3	2966
Corn	Dead nest with corn (DNC)	2	3028
Corn	Fungicide/bactericide-treated dead nest (FDN)	2	2944
Corn	Blank corn-without nest (BC)	1	1949
Tilia wood	Blank wood-without nest (BW)	1	2339

The air flux through the cartridges was adjusted to 1 l min^{-1} for 475 hours (ca. 20 d). A period of 20 d was chosen because preliminary experiments had shown that 20 d is sufficient to accumulate enough NAPH for the analysis and the termites can survive a 20 d period in a microcosm. After 20 d, the termite nest, corn, wood, and XAD-2 cartridges from each microcosm were collected separately, wrapped in aluminum foil, shipped to Germany and kept frozen until analysis.

3.3 Analyses

Organic carbon (C_{org}) and total nitrogen (N) were determined in dried and ball-milled samples of soil, wood, corn, and termite nest using a Fisons NA 2000 elemental analyzer (Fisons Instruments, Rodano, Milan, Italy).

Nests, soil, and wood were analyzed for both parent PAHs and their alkylated derivatives (Tab. E1-2), while the XAD-2 collected from the outlet of the microcosm was analyzed for only the 21 parent PAHs. XAD-2 was transferred from the cartridges into conical flasks spiked with a mixture of seven deuterated PAHs as internal standards (NAPH-D_8, ACEN-D_{10}, PHEN-D_{10}, PYR-D_{10}, CHRY-D_{12}, PERY-D_{12}, and B(GHI)-D_{12} and extracted overnight with hexane. Extracts were dried over Na_2SO_4 and rotary evaporated to about 0.5 ml after addition of drops of toluene as a keeper and transferred into autosampler vials for GC measurement. For the wood, soil and termite nest samples a portion (5 g for wood, 10-15 g soil/termite nest) was mixed with diatomeous earth placed in a 33 ml accelerated solvent extractor extraction cell and spiked with the same mixture of seven deuterated PAHs as above as internal standards. Samples were extracted with hexane:acetone (2:1 v/v) in an accelerated solvent extractor (ASE 200, Dionex, and Sunnyvale, CA, USA). During extraction, cells were filled with solvent, pressurized to 14 MPa, and heated to 120°C for 6 min. Pressure and heat were held for 5 min (static extraction) and then rinsed with cold solvent (60% of cell volume) and purged for 90 s with nitrogen. The extraction cycle was repeated once with fresh solvent. The two extracts from each sample were then combined, dried over Na_2SO_4 and rotary evaporated to about 1 ml. The extracts were cleaned up with 8-ml glass columns filled with 2 g of 5% water-deactivated silica gel on top of 2 g of 5% water-deactivated aluminum oxide. The extract was transferred onto this column and sequentially eluted with 15 ml of hexane, 5 ml of hexane/dichloromethane (9:1 v/v) and 20 ml of hexane/dichloromethane (4:1 v/v). For wood samples which contained high matrix loads, the sample was purified further using columns filled with 1 g HR-P resin (polystyrene-divinylbenzene co-polymer, Macherey-Nagel, Düren, Germany). The combined eluates were evaporated to about 0.5 ml after adding 3 drops of toluene as keeper and were transferred into autosampler vials for GC measurement. Further details of the used method can be found in Wilcke et al. (1999, 2003). The accuracy of the method was tested by analyzing the European Reference Material "ERM®-CC013a-Polycyclic Aromatic Hydrocarbons in Soil" (Federal Institute for Materials Research and Testing (BAM), Berlin Germany). Extracts were analyzed with an Agilent 6890 N gas chromatograph coupled to an Agilent 5975 B inert mass selective detector equipped with a changeable EI/CI ion source, autosampler and a split/splitless injector. All reported data were acquired using the EI ionization mode at 70 eV. The instrumental conditions for the analysis were: injection port temperature 280°C, transfer line 310°C, and the ion source at 230°C. One µl of analyte was routinely injected into the gas chromatograph in the splitless mode using the autosampler. Separation of compounds was achieved on a HP-5MS fused silica capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness, J&W Scientific, Folsom, USA). Helium was used as carrier gas with a constant flow rate of 1.2 ml min^{-1}. The oven temperature was initially set at 80°C, held for 4 min, increased to 160°C at a rate of 14°C min^{-1}, held for 1.5 min, then increased to 225°C at a rate of 5°C min^{-1}, held for 5 min, and finally increased to 300°C at a rate of 5°C min^{-1} and held for 11 min. The mass spectrometer was operated in the single ion monitoring mode for quantification. For each compound, two ions of which the m/z ratios are listed in Table E1-2 were monitored. The first ion was used for quantifying and the second one as a qualifier. A compound was only accepted as identified in a sample/blank if the ratio of the two ions monitored was within ±20% of that measured in standards and a retention time difference of less than ±6 s was observed.

Table E1-2: Names of PAHs measured, their abbreviations used in test, and ions monitored during GC-MSD measurements (m/z). The deuterated PAHs in italics are used as internal standards for the group of non-deuterated compounds under it.

PAHs	Abbreviation	Target ions	Qualifier ion
Naphthalene-D8	*NAPH-D8*	136	137
Naphthalene	NAPH	128	127
2-Methylnaphthalene	2-MNAPH	142	141
1-Methylnaphthalene	1-MNAPH	142	141
1,3-Dimethylnaphthalene	1,3-DMNAPH	156	141
Acenaphthene-D10	*ACEN-D10*	164	162
Acenaphthylene	ACENY	152	151
Acenaphthene	ACEN	153	154
2,3,5-Trimethylnaphthalene	2,3,5-TMNAPH	170	155
Fluorene	FLUO	166	165
Phenanthrene-D10	*PHEN-D10*	188	189
Phenanthrene	PHEN	178	179
Anthracene	ANTH	178	179
2-Methylphenanthrene	2-MPHEN	192	191
1-Methylphenanthrene	1-MPHEN	192	191
3,6-Dimethylphenanthrene	3,6-DMPHEN	206	205
3,5-Dimethylphenanthrene	3,5-DMPHEN	206	191
2,6-Dimethylphenanthrene	2,6-DMPHEN	206	191
1,7-Dimethylphenanthrene	1,7-DMPHEN	206	191
Pyrene-D10	*PYR-D10*	212	213
Fluoranthene	FLUA	202	200
Pyrene	PYR	202	200
1-Methyl-7-isopropylphenanthrene	RETENE	219	234
Chrysene-D12	*CHRY-D12*	240	241
Benz[a]anthracene	[B(A)A]	228	226
Chrysene+triphenylene	CHRY	228	226
Perylene-D12	*PERY-D12*	264	265
Benzo[b,j,k]fluoranthenes	B(BJK)	252	253
Benzo[e]pyrene	B(E)P	252	253
Benzo[a]pyrene	B(A)P	252	253
Perylene	PERY	252	253
Benzo[ghi]perylene-D12	*[B(GHI)]-D12*	288	289
Indeno[1,2,3-cd]pyrene	IND	276	274
Dibenz[a,h]anthracene	DIBE	278	279
Benzo[ghi]perylene	B(GHI)	276	277
Coronene	COR	300	150

3.4 Calculations and statistical analyses

The sum of concentration of all 21 parent PAHs is Σ21PAHs concentration, the sum of the concentrations of all parent PAHs excluding COR is Σ20PAHs concentration, the sum of the concentration of all parent PAHs excluding NAPH, PHEN and PERY is Σ18PAHs concentration, the sum of the concentrations of 1-MNAPH, 2-MNAPH, 1,3-DMNAPH, 2,3,5-TMNAPH is Σalkyl-NAPH concentration, and the sum of the concentrations of 1-MPHEN, 2-MPHEN, 3,6-DMPHEN, 3,5-DMPHEN, 2,6-DMPHEN, 1,7-DMPHEN and RETENE is Σalkyl-PHEN concentration.

Mean dissipation of PAHs during the microcosm experiment was correlated with the octanol-water partition coefficient (K_{OW}) using the least-squares method. The K_{OW} values at 1.013 kPa and 25°C for selected PAHs used in this paper were taken from Mackay et al. (1992). The K_{OW} value for CHRY was used for the mixture of chrysene and triphenylene and that of benzo(b)fluoranthene was used for mixture of benzo[b,j,k]fluoranthenes since these mixtures coeluted on our GC and were therefore quantified together.

The budget (B) in ng was calculated using Equation 1:

$$B = [(C_f - C_i) * M]_{soil} + [(C_f - C_i) * M]_{corn/wood} + [(C_f - C_i) * M]_{nest} + C_{XAD\text{-}2} * 28.5 \quad [1]$$

where C_f [ng g^{-1}] is the concentration of an individual PAH after 20 d of microcosm experiment, C_i [ng g^{-1}] is the initial concentration of the same PAH before the start of the microcosm experiment, $C_{XAD\text{-}2}$ [ng m^{-3}] is the concentration of PAH enriched on XAD-2 resin at the outlet of the microcosm after 20 d of experiment, during which 28.5 m^3 air was pumped through the microcosm, and M is the dry mass of soil, corn, wood or termite nest.

Statistical analysis were performed using STATISTICA for Windows 5.1 (StatSoft, Hamburg, Germany). Significant differences in mean PAH, C and N budgets among the different treatments was tested using one-way ANOVA followed by Tukey´s honest significant difference (HSD) test for unequal N (Spjotvoll/Stoline test). Significant differences in PAH stocks, C concentrations and C/N ratios at the beginning and end of a microcosm experiment for a combination of all treatments with live termites (LNC and LNW) were further tested with a one-sided paired-differences test (Hartung, 1989, p. 534). Significance was generally set at $p < 0.05$.

4 Results

4.1 PAH concentrations in reference material, soil, wood, corn, air traps and termite nests

4.1.1 European reference material "ERM-CC013a-PAHs in soil"

The measured PAH concentrations and the associated standard deviations are shown together with the certified reference values in Table E1-3. We measured, on average of all compounds for which certified reference values were provided, 99% of the certified concentrations.

4.1.2 Soil

The Σ21PAHs concentration in soil before the start of the microcosm experiment was 11 ng g^{-1} (Tab. E1-4). The parent-PAH pattern in soils (i.e. the composition of the mixture of non-substituted PAHs) before the experiment was dominated by PERY (22% of the Σ21PAHs concentration) followed by PHEN (14%) and NAPH (10%). Individual alkyl-NAPH concentrations were lower than that of NAPH and individual alkyl-PHEN concentrations were lower than that of PHEN indicating that the parent compounds were more abundant than any alkyl derivative. Organic C concentrations were typical for subsoil and C/N ratios were wide compared to a usually found value around 10. For all treatments and blanks, there were no significant changes in concentrations and pattern of PAHs in soil before and after the end of the microcosm experiment. Although the organic C concentrations consistently increased after the experiment, the C/N ratio remained within the range of 16-17 before and after the experiment and for all treatments.

Table E1-3: Mean PAH concentrations (mg kg^{-1}) of three replicates and their standard deviation (SD) measured with our method and certified concentrations (mg kg^{-1}) from European Reference Material "ERM®-CC013a-Polycyclic Aromatic Hydrocarbons in Soil" (Federal Institute for Materials Research and Testing (BAM), Berlin Germany). Reference values in parentheses are non certified indicative values without uncertainty statement.

PAHs	Measured concentration	SD	Reference value	Uncertainty[1]
NAPH	3.2	0.19	2.4	0.5
2-MNAPH	1.05	0.06		
1-MNAPH	1.2	0.06		
1,3-DMNAPH	1.2	0.10		
ACENY	1.1	0.07	(0.77)	
ACEN	0.70	0.07	(0.75)	
2,3,5-TMNAPH	0.46	0.04		
FLUO	1.4	0.10	1.14	0.11
PHEN	13	0.43	12.0	0.6
ANTH	0.96	0.03	1.41	0.22
2-MPHEN	2.8	0.16		
1-MPHEN	0.80	0.06		
3,6-DMPHEN	0.00	0.00		
3,5-DMPHEN	0.00	0.00		
2,6-DMPHEN	0.18	0.32		
1,7-DMPHEN	0.77	0.03		
FLUA	10	0.78	12.9	0.7
PYR	9.2	0.69	9.6	0.3
RETENE	0.67	0.03		
[B(a)A]	4.0	0.31	5.6	0.5
CHRY	6.1	0.43	5.3	0.8
[B(BJK)]	6.3	2.1		
[B(E)P]	4.0	0.48		
[B(A)P]	3.1	0.53	4.9	0.7
PERY	1.1	0.12		
IND	4.3	0.32	5.2	1.0
DIBE	2.0	0.18	(1.1)	
[B(GHI)]	3.2	0.17	4.6	0.5
COR	1.7	0.06		

[a] Uncertainty of reference values is given at 95 % confidence interval.

[b] Includes only chrysene (but not triphenylene).

[c] Includes only benzo(b)fluoranthene and benzo(k)fluoranthene

4.1.3 Wood

The initial Σ21PAHs concentration in *T. platyphylla* wood was 127 ng g^{-1} (Tab. E1-4). The initial parent-PAH pattern was dominated by PHEN (51%) and Σ18PAHs (49%) while NAPH and PERY were not detected. There were considerable Σalkyl-NAPH concentrations in wood although NAPH was not detected. The Σalkyl-PHEN concentration was 20% of that of PHEN. Wood had the highest C concentration and the widest C/N ratio of all used materials. After 20 d in the microcosm, there was a 50% loss in Σalkyl-NAPH, PHEN, Σalkyl-PHEN, and the remaining Σ18PAH concentrations in wood from LNW compared to the starting concentration. Similar percentage losses in Σalkyl-NAPH, PHEN and Σalkyl-PHEN concentrations were recorded in BW. The C/N ratio decreased by 10% compared to the initial C/N.

4.1.4 Corn

Initially, the Σ21PAHs concentration in corn was 6.6 ng g^{-1} and the parent-PAH pattern was dominated by PHEN (36%), followed by NAPH (21%, Tab. E1-4). PERY was not detected in corn at the start of the experiment. The initial Σalkyl-NAPH and Σalkyl-PHEN concentrations were similar to that of Σ21PAHs. The initial organic C concentration of corn was slightly lower than that of wood but the C/N ratio was by a factor of 10 narrower. After 20 d of microcosm experiment the average concentration of NAPH in corn increased but those of PHEN and Σ18PAHs decreased in all treatments. These changes, however, had large standard deviations. The losses of Σalkyl-NAPH, PHEN and Σalkyl-PHEN concentrations in corn (up to 50% reductions) were similar in magnitude to those in the wood samples. PERY was detected in LNC at the end of the experiment. DNC and LNC experienced on average 27% and 13% decreases in C/N ratios of corn, respectively, while FDN experienced a 23% increase. BC had a similar C/N ratio before and after the microcosm experiment.

4.1.5 Air traps

Substantial quantities of NAPH, but in comparison much less PHEN or any of the remaining PAHs, originating from the microcosm were trapped in XAD-2 cartridges at the outlet of all treatments. In BC a similar concentration of NAPH was trapped on the XAD-2 as in the other treatments except for BW while in BW most NAPH of all treatments was trapped (40 ng m^{-3}).

4.1.6 Termite nests

The Σ21PAHs concentrations in termite nests were similar in all nests with a range of 11-23 μg kg^{-1} (Tab. E1-5). The Σ18PAHs concentrations ranged between 2.0-4.3 μg kg^{-1}. The parent-PAH pattern was in all cases dominated by NAPH or PERY. The nests also contained considerable concentrations of Σalkyl-NAPH and Σalkyl-PHEN although no individual alkyl-NAPH and alkyl-PHEN had higher concentrations than the respective parent compounds (NAPH and PHEN). Organic C concentrations were similar to those in wood, but C/N ratios were narrower by a factor of 10 and similar to those of corn. Changes in NAPH concentrations in the nests after 20 d of microcosm experiment contrasted with changes in PHEN and PERY concentrations. In DNC, LNW and LNC, there were average increases in NAPH concentrations of 36%, 51% and 8.5%, respectively, in the nests. However, in FDN, the mean NAPH concentration decreased by 60%. The PHEN concentrations remained unchanged in DNC, decreased by 23% and 33% in LNW and LNC, respectively, but increased by 43% in FDN. The PERY concentrations decreased by 86%, 90% and 33% in DNC, LNW and LNC, respectively, but in contrast increased by 181% in FDN. The Σ18PAHs concentrations decreased in DNC, LNW and LNC but increased in FDN. The decrease in Σ18PAHs concentrations of the six live nests (LNW and LNC) during the microcosm experiment was significant (paired-differences test). Organic C concentrations and C/N ratios did not change consistently and the mean differences before and after the microcosm experiment were not significant.

Table E1-4: Mean PAH concentrations in soil (ng g^{-1}), wood (ng g^{-1}), corn (ng g^{-1}), outlet air (ng m^{-3}), organic C (C_{org}, g kg^{-1}), nitrogen (N, g kg^{-1}) concentration with standard deviation (in brackets) before and after 20 d of microcosm experiment and C/N ratios of soil, wood and corn. Abbreviations with “s”, “w”, “c” and “a” before them stand for soil, wood, corn and air (XAD-2) respectively. BE is the concentration in sample before experiment.

	NAPH	Σ alkyl-NAPH	PHEN	Σ alkyl-PHEN	PERY	Σ 18 PAHs	OC	N	C/N
Soil									
s-BE	1.1	1.7	1.6	1.1	2.5	6.1	5.0	0.3	17
s-DNC	1.5 (1.2)	1.8 (1.0)	1.0 (0.3)	1.6 (0.1)	2.9 (1.2)	5.9 (3.7)	8.5 (0.7)	0.6 (0.1)	16 (0.7)
s-FDN	1.3 (0.0)	2.3 (0.1)	2.8 (0.3)	4.7 (1.9)	1.0 (0.6)	7.8 (2.8)	11 (2.8)	0.7 (0.1)	17 (2.5)
s-LNW	1.0 (0.2)	1.1 (0.6)	1.2 (0.3)	0.5 (0.2)	1.7 (0.6)	6.0 (2.9)	8.1 (1.0)	0.5 (0.1)	16 (1.2)
s-LNC	1.2 (0.3)	1.0 (0.2)	1.0 (0.2)	0.7 (0.1)	2.8 (1.9)	4.6 (2.1)	8.3 (0.6)	0.5 (0.1)	16 (0.6)
s-BW	0.9	1.4	1.5	0.7	0.5	3.7	10	0.6	17
s-BC	1.5	1.2	1.1	1.8	2.0	5.6	8.0	0.5	16
Wood									
w-BE	n.d.	60	66	20	n.d.	61	474	1.3	354
w-LNW	n.d.	25 (2.9)	28 (7.3)	8.5 (1.3)	0.1 (0.2)	37 (20.2)	479 (3.8)	1.5 (0.0)	318 (8.6)
w-BW	n.d.	34	31	9.2	4.9	80	476	1.4	338
Corn									
c-BE	1.4	7.2	2.4	5.6	n.d.	2.8	431	15	30
c-DNC	1.7 (0.6)	3.3 (1.1)	1.2 (1.2)	3.2 (4.4)	n.d.	4.4 (2.0)	421 (6.4)	19 (0.2)	22 (0.6)
c-FDN	2.4 (1.2)	4.1 (0.5)	0.8 (0.2)	4.9 (2.7)	n.d.	3.3 (2.0)	428 (0.7)	12 (0.1)	37 (0.2)
c-LNC	2.0 (1.3)	2.9 (1.3)	1.4 (0.9)	2.5 (1.2)	4.6 (3.7)	7.0 (1.7)	423 (2.0)	17 (0.2)	26 (0.2)
c-BC	1.9	3.2	0.8	1.2	0.00	1.9	429	15	30
Air (outlet)									
a-DNC	13 (2.3)		n.d.		n.d.	1.2 (0.4)			
a-FDN[a]	11		5.0		n.d.	4.0			
a-LNW	19 (17.2)		0.7 (1.3)		n.d.	0.5 (0.5)			
a-LNC	13 (6.5)		1.3 (1.2)		n.d.	1.7 (2.1)			
a-BW	40		0.00		n.d.	3.6			
a-BC	12		0.00		n.d.	3.8			

[a] No standard deviation because one replicate of the XAD-2 cartridges was lost. The value of the remaining cartridge was used for the budget.

Table E1-5: Mean PAH (µg kg^{-1}), organic C (C_{org}, g kg^{-1}) and total nitrogen concentrations (N, g kg^{-1}) and C/N ratios in termite nests before and after 20 d of microcosm experiment. n-DNC, n-FDN, n-LNW and n-LNC represent the nests used in the microcosm for the treatments dead nest with corn, fungicide/bactericide-treated dead nest, live nest with wood and live nest with corn, respectively.

	n-DNC		n-FDN		n-LNW		n-LNC	
	before	after	before	after	before	after	before	after
NAPH	4.7 (0.5)	6.4 (0.3)	5.8 (0.5)	2.3 (2.2)	5.7 (3.2)	8.6 (2.0)	5.9 (0.8)	6.4 (3.2)
Σalkyl-NAPH	7.8 (0.3)	11.2 (3.0)	11.6 (1.6)	10.8 (2.4)	10.2 (4.4)	13.7 (3.5)	13.8 (5.4)	15.2 (3.4)
PHEN	1.1 (0.3)	1.1 (0.2)	0.7 (0.4)	1.0 (0.2)	1.3 (0.5)	1.0 (0.1)	1.8 (0.6)	1.2 (0.5)
Σalkyl-PHEN	1.5 (0.3)	1.9 (0.7)	1.2 (0.3)	1.7 (0.8)	1.9 (0.8)	1.2 (0.5)	5.2 (2.4)	2.7 (1.1)
PERY	9.1 (11.6)	1.3 (0.3)	2.6 (0.7)	7.3 (6.2)	11.5 (17.6)	1.1 (1.0)	2.1 (2.6)	1.4 (1.3)
Σ 18 PAHs	3.3 (2.7)	2.1 (0.2)	2.3 (0.5)	3.5 (0.8)	4.3 (2.4)	2.0 (0.2)	3.8 (0.7)	2.3 (1.0)
OC	387	468	474	383	444	464	428	460
N	12	12	10	11	11	11	13	14
C/N	32	40	47	35	40	44	33	34

4.2 Total stocks of PAHs

The average dry mass of the microcosm including soil, nest, corn or wood of all treatments (without BC and BW) ranged from 2676 to 3028 g (Tab E1-1). The initial and final stocks of NAPH, PHEN, PERY and Σ18PAHs for all treatments are shown in Figure E1-1 (A-E) and the mean changes in Table E1-6.

Mean NAPH stocks increased in DNC, LNW and LNC by 46%, 43% and 21%, respectively. In FDN, however, the NAPH stock decreased by 20% while the stocks of many lower molecular weight PAHs increased (Fig. E1-1B). These changes in mean NAPH stocks between the different treatments were not significant because of the large variations in the size (but not in the direction) of the changes among the individual replicate microcosms. When we pooled the LNW and LNC treatments (all treatments with live termites) and compared initial with final stocks (paired-differences test), the difference was marginally significant ($p < 0.1$, n=6). After removal of the only outlier of the six microcosms with live termite nests showing decreasing NAPH stock (Fig. E1-2B) the difference was significant at $p<0.05$ (n = 5). In contrast, the stocks of most other lower molecular weight PAHs (3-4 rings, up to CHRY) decreased in DNC, LNW and LNC but increased in FDN (except ACENY and

ANTH) while stocks of the higher molecular PAHs remained unchanged. For several compounds, these changes were significant (Tab. E1-6). The direction of the changes in total stocks of NAPH in the microcosms paralleled that of the changes in NAPH stocks in the termite nests alone (Fig. E1-2).

In microcosms with live termites (LNC and LNW, n = 6), the mean percentage decrease of all PAHs except for NAPH correlated closely with the K_{OW} (Fig. E1-3). NAPH did not fit into this relationship. A similar relationship between percentage change and K_{ow} was not observed in the treatments with dead nests (DNC and FDN) where there was no correlation between mean changes in PAH concentrations and K_{OW} (not shown). Instead, NAPH was lost while all other lower molecular weight PAHs (except ACENY and ANTH) were accumulated in FDN. In DNC, NAPH was accumulated while most other PAHs decreased marginally (Tab. E1-6).

Table E1-6: Mean PAH (µg), organic C (C_{org}, g), nitrogen (N, g) budgets, standard deviation (in brackets) and C/N ratio in different microcosm experiments after 20 d. DNC is dead nest with corn, FDN is fungicide/bactericide-treated dead nest, LNW is live nest with wood and LNC is live nest with corn. Different lower case letters indicate significant differences in means of the various treatments according to the Tukey's Honest Significant Difference test for uneven N (Spjotvoll/Stoline test, $P < 0.05$).

	DNC	FDN	LNW	LNC
NAPH	2.4 (1.9)	-1.2 (1.1)	1.9 (1.6)	0.6 (2.5)
ACENY	-0.2 (0.0)	-0.5 (0.1)	-0.3 (0.2)	-0.3 (0.2)
ACEN	0.0 (0.0)a	0.1 (0.0)a	-0.5 (0.1)b	-0.1 (0.2)a
FLUO	-0.1 (0.2)a	0.4 (0.4)a	-1.8 (0.6)b	-0.2 (0.0)a
PHEN	-1.4 (1.1)ab	2.7 (1.1)a	-8.5 (1.4)c	-2.0 (0.8)b
ANTH	-0.3 (0.0)	-0.1 (0.2)	1.8 (3.5)	-0.3 (0.2)
FLUA	-0.8 (0.5)ab	1.9 (1.8)a	-3.4 (0.6)b	-0.9 (0.6)ab
PYR	-0.4 (0.0)ab	1.6 (1.3)a	-3.1 (0.4)c	-1.1 (0.5)bc
B(A)A	-0.1 (0.2)	0.4 (0.3)	-0.3 (0.2)	-0.2 (0.1)
CHRY	-0.6 (0.8)	1.2 (1.2)	-1.5 (0.7)	-1.1 (0.4)
B(BJK)	-1.5 (0.0)	-1.5 (0.0)	-0.2 (0.7)	-0.1 (1.2)
B(E)P	-1.4 (0.1)	-1.4 (0.1)	0.9 (1.9)	-0.7 (1.0)
B(A)P	0.1 (0.1)	0.9 (1.3)	0.6 (0.2)	0.1 (0.1)
PERY	-6.9 (8.7)	0.1 (3.7)	-8.2 (8.3)	0.6 (3.5)
IND	2.2 (3.1)	1.3 (0.4)	1.0 (1.1)	0.6 (0.5)
DIBE	0.0 (0.0)	0.0 (0.0)	0.5 (0.4)	0.0 (0.0)
B(GHI)	1.3 (2.0)	0.4 (0.7)	0.0 (0.2)	0.0 (0.2)
COR	0.0 (0.0)	0.0 (0.0)	-0.1 (0.2)	0.0 (0.0)
Σ 21 PAHs	-7.6 (6.2)	6.3 (12.6)	-21 (13.9)	-5.0 (2.1)
OC	442 (612)	55 (251)	-78 (153)	19 (335)
N	12 (16.2)	-1.6 (1.6)	-1.9 (2.2)	0.7 (9.9)
C/N before	30	33	39	37
C/N after	34	42	37	34

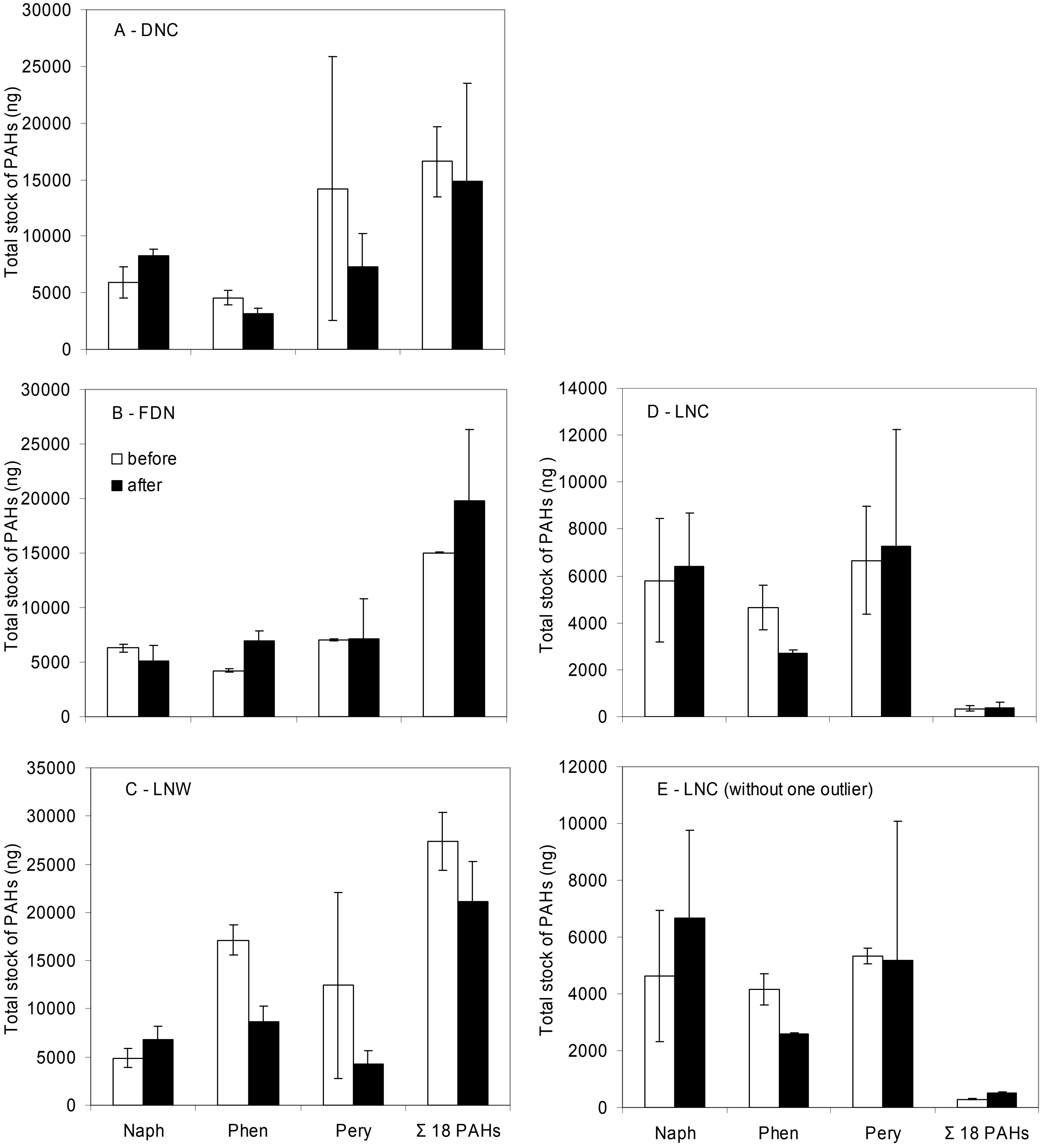

Figure E1-1: Mean stock (ng) of NAPH, PHEN, PERY and ∑18PAHs in microcosms before (white bars) and after (black bars) 20 d of microcosm experiment for (A) dead nest with corn (DNC), (B) fungicide/bactericide-treated dead nest (FDN), (C) live nest with wood (LNW), (D) live nest with corn (LNC) and (E) live nest with corn without outlier. Error bars indicate standard deviations.

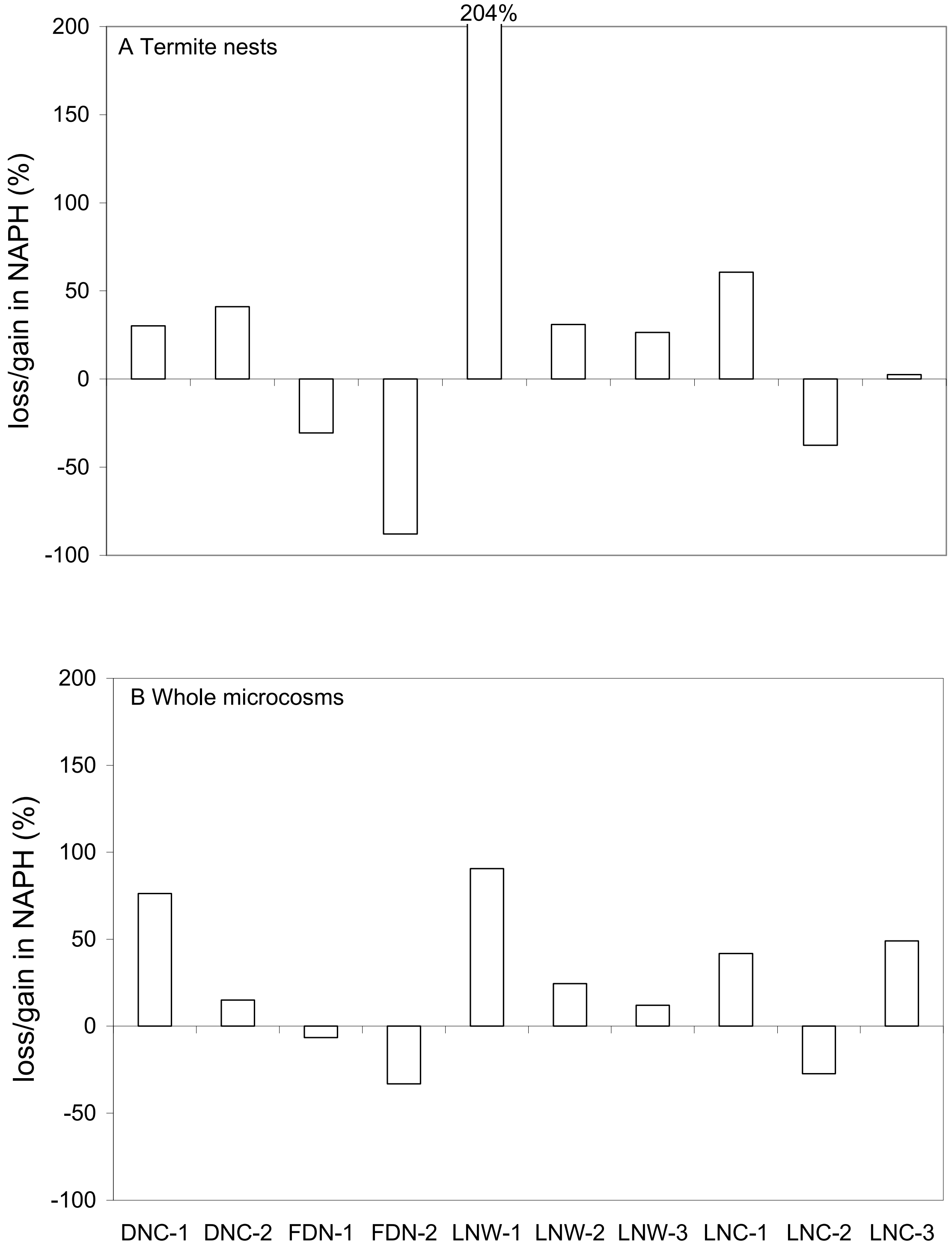

Figure E1-2: Percentage gain/loss of NAPH stock in (A) termite nests and (B) whole microcosms. DNC is dead nest with corn, FDN is fungicide/bactericide-treated dead nest, LNW is live nest with wood, and LNC is live nest with corn. Replicates are numbered.

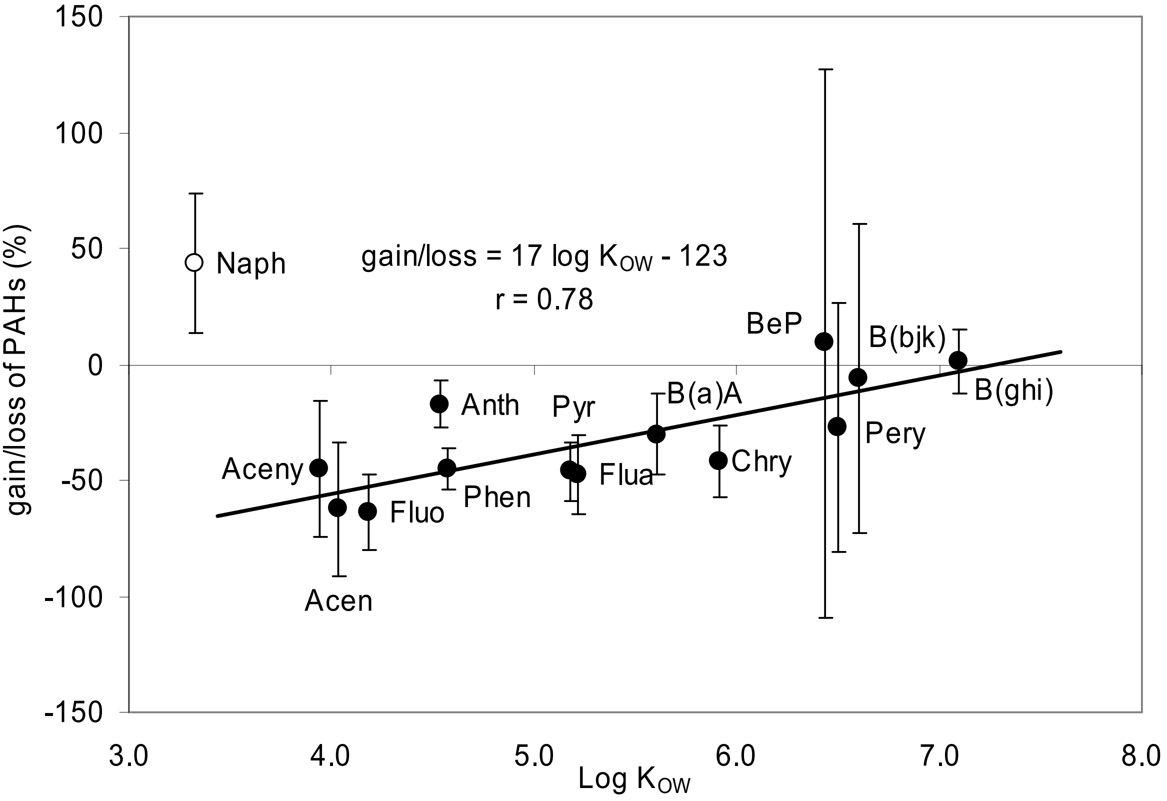

Figure E1-3: Relationship between log K_{ow} and percentage gain/loss of selected PAHs in pooled data of microcosms with living termites [live nest with wood (LNW) and live nest with corn (LNC)] without one outlier. NAPH has been omitted from the regression line.

5 Discussion

In spite of much indication of biological production of PAHs like NAPH, PHEN, and PERY in the tropics, in particular in association with termite activity, direct evidence of biological PAH production is still lacking. The knowledge of PAH sources is important to assess the state of pollution of tropical environments with PAHs. Therefore, our objective was to provide direct evidence for PAH production in termite nests with the help of microcosm experiments in Brazil. We not only evaluated changes in total stocks of the microcosms but also explored the changes in PAH concentrations of the different constituents of the microcosms (i.e., soil, food, nest). We furthermore tried to find out whether the biological PAH production can be attributed to the termites or to associated microorganisms by including a fungicidal/bacteriocidal treatment.

5.1 PAH concentrations in soil, wood, corn, air traps and termite nests

5.1.1 Soil

The accuracy of our analytical method for quantifying the PAHs is demonstrated by the close match between measured values with our method and the reference values (Tab. E1-3). The measured initial concentration of 21 parent PAHs (Σ21PAHs, Tab. E1-4) was at the lower end of the range of 7-397 ng g^{-1}(for the Σ16 EPA-PAHs) reported for tropical mineral soils (Wilcke, 2000). Similar low Σ21PAHs concentrations of 16-38 ng g^{-1} have been reported in studies of tropical forest soils near Manaus, Brazil (Krauss et al., 2005) and soils from Costa Rica (1.1-36 ng g^{-1} for the Σ16 EPA-PAHs, (Dalya et al., 2007). Such low PAH concentrations and patterns dominated by NAPH, PHEN and PERY are typical of tropical background conditions and can also be explained by the fact that the sample came from subsoil (Wilcke, 2007). The low individual alkyl-NAPH and individual alkyl-PHEN portions compared to their parent PAHs (NAPH and PHEN) and the fact that the PAH pattern had low concentrations of high molecular weight PAHs (HMPAH) indicates that neither petrogenic nor combustion sources can explain the PAH pattern in these soils (Booth and Gribben, 2005; Neff et al., 2005). This provides evidence that the observed PAH pattern might be mainly attributable to biological sources. There was some organic matter accumulation probably because of incorporation of food and nest materials into the soil but no substantial organic matter degradation in the soil during the microcosm experiment as indicated by the fact that C/N remained unchanged. As changes in soil PAH concentrations were minor, inconsistent, and similar among all treatments and blanks we conclude that the observed differences can be mainly attributed to sample heterogeneity. If there was production or degradation of NAPH, PHEN or PERY during the microcosm experiment, then this did not take place in the soils.

5.1.2 Wood

We are not aware of reported PAH concentrations in wood from non-tropical sources. NAPH concentrations in vegetation from temperate ecosystems are usually low and even sometimes lower than in tropical ecosystems (which are dominated by NAPH) with patterns frequently dominated by PHEN (Tremolada et al., 1996; Alfani et al., 2001; Hwang et al., 2003; Krauss et al., 2005) which is consistent with our results. We interprete the 50% loss in Σalkyl-NAPH, PHEN, Σalkyl-PHEN, and the remaining Σ18PAH concentrations in wood from LNW and BW and the associated small (10%) decrease in C/N ratio compared to the starting concentration as indication that organic matter degradation was accompanied by decreases in PAH concentrations of wood (Tab. E1-4). Loss mechanisms of PAH in the environment include volatilization, abiotic loss (photooxidation, chemical oxidation), irreversible sorption to soil particles, leaching, biotic losses (microbial degradation) and bioaccumulation (Park et al., 1990; Cerniglia, 1992; Wild and Jones, 1993). All of the above factors may explain the decreases in concentrations of the PAHs in wood after 20 d in the microcosm. Volatilization might have been the most important loss pathway for alkyl-NAPH, PHEN and alkyl-PHEN because of the high volatility of these compounds. Loss of NAPH and 1-MNAPH by volatilization from PAH-spiked soil is reported to reach 30 and 20% within 48 hours, respectively, while volatilization losses were negligible for higher ring number PAHs in the same study (Park et al., 1990).

5.1.3 Corn

Corn from Brazil had considerable NAPH concentrations, in contrast to the *T. platyphylla* wood from Germany (Tab. E1-4). The reason for the high NAPH concentration in corn from Brazil might again be biological production or the higher biomass burning activity in the Tropics than in the temperate zone. PAHs released by biomass burning, however, would mainly sorb to the outer parts of the corn plant but not to the grain protected by cover leaves ("husk"). Although the husks were also fed, they only contributed a minor part to the total mass of corn feed. Furthermore, biomass burning can only explain the high initial NAPH concentration but not the increase in NAPH concentrations of corn after 20 d in the microcosm. The differences in NAPH concentrations among the treatments at the end of the microcosm experiment might be attributable to initially heterogeneous NAPH concentrations in the used corn, redistribution of NAPH from other constituents of the microcosms or biological production. The decreases in C/N ratio of DNC (-26%) and LNC (-13%) can be explained by organic matter degradation. The FDN treatment experienced an average increase in C/N ratio of 30% which might indicate N loss while organic matter degradation was reduced by the biocide application. As the blank corn (BC) had a similar C/N ratio as the corn at the start of the experiment, N loss as explanation for the high C/N in FDN, however, is unlikely. Therefore differences in C/N ratios among BC, FDN and corn at the start of the experiment might again be the result of sample heterogeneity of the used corn.

5.1.4 Air traps

The NAPH concentrations were similar in all air traps except for BW showing a higher NAPH concentration in the air trap although no NAPH was detected in the wood (Tab. E1-4). However, BW was not replicated and this result should therefore not be overinterpreted.

Furthermore, the air traps contributed little to the total NAPH budgets. NAPH was the most abundant PAH in the air traps followed by PHEN and only low concentrations of other PAHs were detected. This can be attributed to volatilization particularly of NAPH initially stored in the constituents of the microcosms or produced in the microcosms (Park et al., 1990).

5.1.5 Termite nests

The average Σ21PAHs concentrations in termite nests (Tab. E-5) were at the lower end of those reported in previous studies from the whole Amazon basin (NAPH: 33-3645; PHEN: 5.6-154; PERY: 2.0-825; Σ20PAHs: 66-4208 $\mu g\ kg^{-1}$, Wilcke et al., 2003) but similar to the concentrations reported from a single site in the Amazon basin (Manaus, NAPH: 38-160; PHEN: n.d.-15; PERY: 1.2-193; Σ18PAHs:1.2-193 $\mu g\ kg^{-1}$, Krauss et al., 2005). The parent-PAH patterns dominated by NAPH or PERY are similar to those reported by Krauss et al. (2005) from Manaus in which NAPH and PERY were in most cases the dominant PAHs.

Even though changes in mean NAPH, PHEN and PERY concentrations in the nests for the different treatments after 20 d of microcosm experiment were not significant, there are two indications of biological NAPH production in the nests. First, NAPH concentrations increased in seven of the eight DNC, LNW and LNC nests (with one nest of the LNC treatment being an exception, cf. Fig. E-2a), but decreased in FDN nests (Tab E1-5). These points to NAPH production by the microorganisms living in the termite nests irrespective of the presence or absence of live termites. The produced NAPH is partly enriched in the termite nests. Second, PHEN and PERY showed opposite fate trends to NAPH in the nests of all microcosms. This result is unexpected because the physico-chemical properties (such as K_{OW} values and water solubility) of PHEN (a 3-ring compound) are more similar to those of NAPH than of PERY. The K_{OW} and water solubility are often cited as the most important compound properties determining the environmental fate of PAHs (Wild et al., 1991a; Cerniglia, 1992), therefore, NAPH should show a similar response to the microcosm experiment as PHEN unless it is produced.

Although all treatments were replicated, the individual replicates differed considerably in mass of termite nest, number of involved termites, and likely also in the size of the associated microorganism communities, which explains a large heterogeneity of the size of responses of NAPH concentrations in the nests to the microcosm experiments. Therefore, it cannot be expected that the constituents of the individual replicate microcosms show the quantitatively same change in PAH concentrations which – together with the analytical error – explains the high standard deviations of the changes in PAH concentrations in the various constituents of the microcosms and the whole microcosms during the 20-d experiment (Tabs. E1-4 and E1-5).

5.2 Total stocks of PAHs in the microcosms

The fact that NAPH stocks increased in the microcosms with dead (but not biocide-treated) and live termite nests (LNW and LNC) is evidence for the biological production of NAPH in DNC, LNW, and LNC. These changes in NAPH stocks were driven by changes in the termite nests alone (Fig. E1-2). However, the size of the NAPH production varies widely and in one (of six) microcosms with live nests even no NAPH production seemed to have occurred. The finding that NAPH production occurred in the microcosms with dead and live nests (DNC, LNW and LNC) but not in microcosms with fungicide/bactericide-treated dead nests (FDN) suggests that fungi and/or bacteria living in the termite nests or even in the termite guts but not the termites themselves produce NAPH. If the activity of fungi/bacteria was reduced as was the case in FDN, there was no new NAPH produced in the microcosm and therefore loss mechanisms dominated. This result is corroborated by the finding of Daisy et al. (2002) that the fungus *Muscodor vitigenus* produced NAPH.

The close correlation of the mean percentage decrease of all PAHs except for NAPH with the log K_{OW}, which is a measure for the molecular weight and polarity, and thus also the susceptibility to degradation and volatilization of the individual PAHs, is a further indication of biological NAPH production in the microcosms (Fig. E1-3). As the most easily degraded PAH, NAPH should fit into the regression line of mean percentage decrease on K_{OW}, if it was not produced.

The finding of a relationship between polarity and thus degradability of PAHs and mean percentage decrease of PAHs except NAPH in the microcosms with live nests (LNC and LNW) but not in those with dead nests (DNC and FDN) suggests that the dissipation of PAHs is enhanced if termites are present. The PAH dissipation may mainly be a degradation, e.g., in the guts of termites which are densely inhabited by microorganisms (Varma et al., 1994). All PAHs, especially the lower molecular weight ones, are known to be degraded by bacteria, fungi and algae (Cerniglia, 1992; Bamforth and Singleton, 2005). It is furthermore probable that bacteria/fungi which inhabit the gut of termites are released into the termite nest during nest construction (which involves mastication of wood and soil mixed with saliva) and that this influences the PAH stocks in nests either by degradation or production. It is already known that the microbial communities associated with termites are active in degradation of lignocellulose (Varma et al., 1994; Ohkuma, 2003). After 20 d of microcosm experiment with live nests, the percentage loss of most of the low-molecular weight PAHs (up to CHRY, Fig. 2) was between 17 and 64 % of their initial concentration (except NAPH). A rough estimate of the half-lives (i.e., the time needed to degrade 50% of the initial concentration) of the studied low-molecular weight PAHs from data shown in Figure E1-3 using a simple exponential decay model predicts half-life values in the range of months in our microcosms. These half-lives are at the lower end of literature values where half-lives vary widely from days to years (Park et al., 1990; Wild et al., 1991a,b; Wild and Jones,1993). The shortest half-lives are reported for laboratory experiments with artificially applied PAHs which may be more susceptible to biotic and abiotic degradations (Wild et al., 1991b). The half-lives of PAHs in our microcosm experiments with live termite nests are far shorter than reported for aged PAHs in soil in most other studies. Our data therefore suggest an enhancement of PAH degradation by the joint effect of termite and microorganisms associated with termites.

6 Conclusions

Our microcosm experiments indicate that in microcosms with dead (but not fungicide/bactericide-treated) and live termite nests NAPH is produced within 20 d. This is illustrated by the increase in NAPH stocks in seven out of eight microcosms with intact microflora and by the finding that NAPH accumulates while concentrations of all other low-molecular weight PAHs decrease. The NAPH accumulation was driven by concentration changes of NAPH in termite nests which paralleled those of the total microcosms. However, NAPH production is highly variable and there was even one nest with live termites, in which no NAPH was produced. The finding that no NAPH was produced in fungicide/bactericide-treated dead nests indicates that NAPH did not originate from the termites themselves but from associated microorganisms.

Low-molecular weight PAHs (up to CHRY but except NAPH) dissipated in microcosms with live termite nests during the 20 d of our microcosm experiment. The percentage loss was correlated with the polarity of the individual PAHs (as expressed by the K_{ow} values). Interestingly, no such correlation of K_{ow} with net dissipation was observed during the 20 d of the experiment in the treatments with dead nests (even if not fungicide/bactericide-treated). This suggests that the interaction of termites and fungi/bacteria enhances PAH dissipation. The latter requires further and broader research also including other taxa and pollutants. However, if it is later confirmed that insect/microorganism interaction accelerates the degradation of persistent organic pollutants this would have important application in remediation strategies of sites contaminated with PAHs or other persistent organic pollutants.

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E-2 Method optimization to measure polybrominated diphenyl ether (PBDE) in soils of Bratislava, Slovakia[a]

Ute Rita Thorenz[1], **Benjamin A. Musa Bandowe[1]**, Jaroslava Sobocka[2], Wolfgang Wilcke[3]

[1] Earth System Science Research Center, Geographic Institute, Johannes Gutenberg University, Mainz, Johann-Joachim-Becher-Weg 21, 55128, Mainz, Germany

[2] Soil Science and Conservation Research Institute, 82713, Bratislava, Slovakia

[3] Geographic Institute, University of Berne, Hallerstrasse 12, 3012, Berne, Switzerland

[a] Environmental Pollution 158, 2208-2217 (2010)

1 Abstract

We modified an analytical method to determine polybrominated diphenyl ethers (PBDEs) in urban soils of Bratislava (Slovakia). Gel permeation chromatography (GPC) introduced as a clean-up step for soil extracts substantially reduced matrix enhancements when PBDEs were measured with gas chromatography-negative chemical ionization-mass spectrometry (GC-NCI-MS). The resulting method proved to be accurate, precise, and showed low detection limits. The sum of 15 PBDE concentrations in surface horizons of Bratislava soils ranged from 87 to 627 pg g-1. PBDE concentrations were mostly higher in surface than deeper horizons probably because of atmospheric deposition and lack of substantial vertical transport. Lower brominated PBDEs undergo more soil-atmosphere exchanges or are more scavenged and transferred with litter fall to the soil organic matter than higher brominated ones as suggested by the correlation between lower brominated PBDEs and soil organic C (Corg) concentrations.

2 Introduction

Polybrominated diphenyl ethers (PBDEs) are frequently used as flame retardants, because they prevent ignition or slow down the initial phase of the fire. They are additively incorporated into polymers used for a variety of products. There are three major commercial formulations: penta-mix, octa-mix and deca-mix, named according to the average bromine concentrations. The worldwide use of all mixes was in 2001 more than 67,000 metric tons (Law et al., 2006). PBDEs, released from the polymer products, can enter the environment during manufacture, product use and disposal.

As result of their ubiquitous use, PBDEs have become widespread in the environment. Concern about the toxicity of PBDEs which may have carcinogenic and thyroid disturbing effects (Knoth et al., 2007; Naert et al., 2007) caused the European Union to ban penta and octa mixes on 1 July 2006 based on the Restriction of Hazardous Substances (RoHS, EU, 2003) ordinance and the deca mixes on 1 July 2008 (Talsness, 2008). Similar to other persistent organic pollutants (POPs), PBDEs bioaccumulate and they were detected in biotic and abiotic media (de Wit 2002; Hale et al., 2003; de Wit et al., 2006; Hale et al., 2006; Law et al., 2006). Because of the persistence of PBDEs, it can be expected that they will exist in the environment for a long time after stopping their use. It is also likely that PBDEs will continue to be released from PBDE-containing materials currently being used but manufactured before their ban came into force.

PBDEs were detected in the atmosphere in gaseous form and sorbed to particles (e.g. Sjodin et al., 2001; Hoh and Hites, 2005; Cetin and Odabasi, 2007) and in sediments (e.g. Eljarrat et al., 2005; Klamer et al., 2005; Zhu and Hites, 2005). Soils are the least studied compartment (Vonderheide et al., 2008) although soils are assumed to be the dominant environmental sink (Palm et al., 2002; Moeckel et al., 2008). Available studies concern rural soils, industrial soils (often located in Asia), and soils fertilized with sewage sludge (Hassanin et al., 2004; Harrad and Hunter, 2006; Müller et al., 2006; Zou et al., 2007; Eljarrat et al., 2008; Yun et al., 2008; Luo et al., 2009; Zhao et al., 2009).

The reason for the low number of studies reporting PBDE concentrations in soil may be related to the fact that the PBDE analysis in soils requires sophisticated analytical methods, since the soil matrix is complex and PBDE concentrations comparatively low. Established analytical methods for PBDEs include exhaustive extraction using soxhlet, microwave-assisted extraction [MAE], pressurized liquid extraction [PLE]), clean-up (solid phase extraction [SPE], gel permeation chromatography [GPC]) and chromatographic separation followed by mass spectrometric determination (Covaci et al., 2003; Gomara et al., 2006;

Covaci et al, 2007). Analyses of PBDEs in mass spectrometry are usually based on electron ionization (EI) or negative chemical ionization (NCI) by which charged fragments of the target compounds are produced. EI usually produces highly specific ions facilitating compound identification while the strength of NCI is its high sensitivity allowing for the quantification of trace amounts of PBDEs (Hites, 2008). However, the quantification in NCI is associated with a high risk of matrix interferences by other compounds coeluting with PBDEs and resulting in fragments with mass/charge (m/z) ratios of 79 and 81 like particularly other brominated compounds such as the polybrominated biphenyls, since only the unspecific bromide ions are used for quantification (Korytar et al., 2005). Thus, to optimize an analytical method for PBDEs in soil, analytical sensitivity and matrix interferences need to be balanced.

The investigation of entry pathways and processes in soil, like sorption to different soil compounds, are essential requirements for understanding the overall fate of PBDEs in the environment. As for other POPs, deposition from the atmosphere is the main pathway for PBDEs to enter the soil system (Müller et al., 2006; Moeckel et al., 2008). The transport in soil depends on sorption-desorption processes. The major sorbent is, like for other POPs, soil organic matter (SOM) (Chiou et al., 1983). The strength of POP sorption to SOM is frequently described by the partitioning between SOM and the aqueous phase using the C-normalized partitioning coefficient K_{OC} related to the octanol-water partitioning coefficient K_{OW}, which are available in the literature (Palm et al., 2002; Braekevelt et al., 2003) or may be determined in the laboratory. The high K_{OW} of tri to octa brominated PBDEs of 10^5-10^8 (Li et al., 2008b), suggests that these compounds are strongly sorbed to SOM. The strong sorption of PBDEs explains their small vertical mobility and their resulting accumulation in surface soil horizons (Hassanin et al., 2004). POPs emitted into the atmosphere are either directly deposited to soil in wet or dry form or scavenged by vegetation and deposited with falling litter. It has been suggested that in background soils the correlation between SOM, frequently approximated by concentrations of organic C (C_{org}) and concentrations of POPs that occur in gaseous form in the atmosphere can be interpreted as indicative of a near-equilibrium state of air-soil partitioning (Wilcke and Amelung, 2000; Meijer et al., 2003; Hassanin et al., 2004). However, in vegetation-covered soils PBDEs will be additionally transferred to soil by falling leaves or needles (filter effect of the vegetation canopy), and a correlation between SOM and POP concentrations is the consequence of rapid incorporation of the litter-derived POPs into SOM (Horstmann and McLachlan, 1998).

We had two objectives. (1) We aimed to optimize an analytical method used for polychlorinated biphenyls (PCBs) to determine PBDEs in soils with a reliable precision and accuracy. We placed a particular focus on the comparison of the performance of EI compared to NCI and on the use of GPC for reducing matrix effects in GC-MS analyses. (2) We applied the developed method to urban soils of Bratislava (Slovakia). This part focused on the concentrations, patterns, depth distributions, and relationships between soil C_{org} and PBDE concentrations to elucidate the fate of PBDEs in urban soils. The study soils were sampled and well characterized as part of a project to assess environmental hazards resulting from urban soils (Sobocka, 2007). The measurement of PBDEs in Bratislava soils is important because the capital of Slovakia has a large population of 450,000, and a high population density of 1200 inhabitants per km^2 which might be at risk because of soil contamination (Ministry of the Environment of the Slovak Republic, 2005; Krčmová et al., 2009). Previous reports already indicated that the population of Bratislava is affected by the pollution of their environment with organic contaminants (Reichrtová et al., 1999. Furthermore, little information exists on the concentration of PBDEs in soils and air of eastern Europe (Jeward et al., 2004; Kumar et al., 2009). The city of Bratislava borders two neighbor countries (Austria and Hungary), hence pollution of urban soils of Bratislava has a transboundary dimension. Finally, Bratislava is located on the Danube River and thus soil pollution might finally even affect the marine environment in the Danube estuary of the Black Sea.

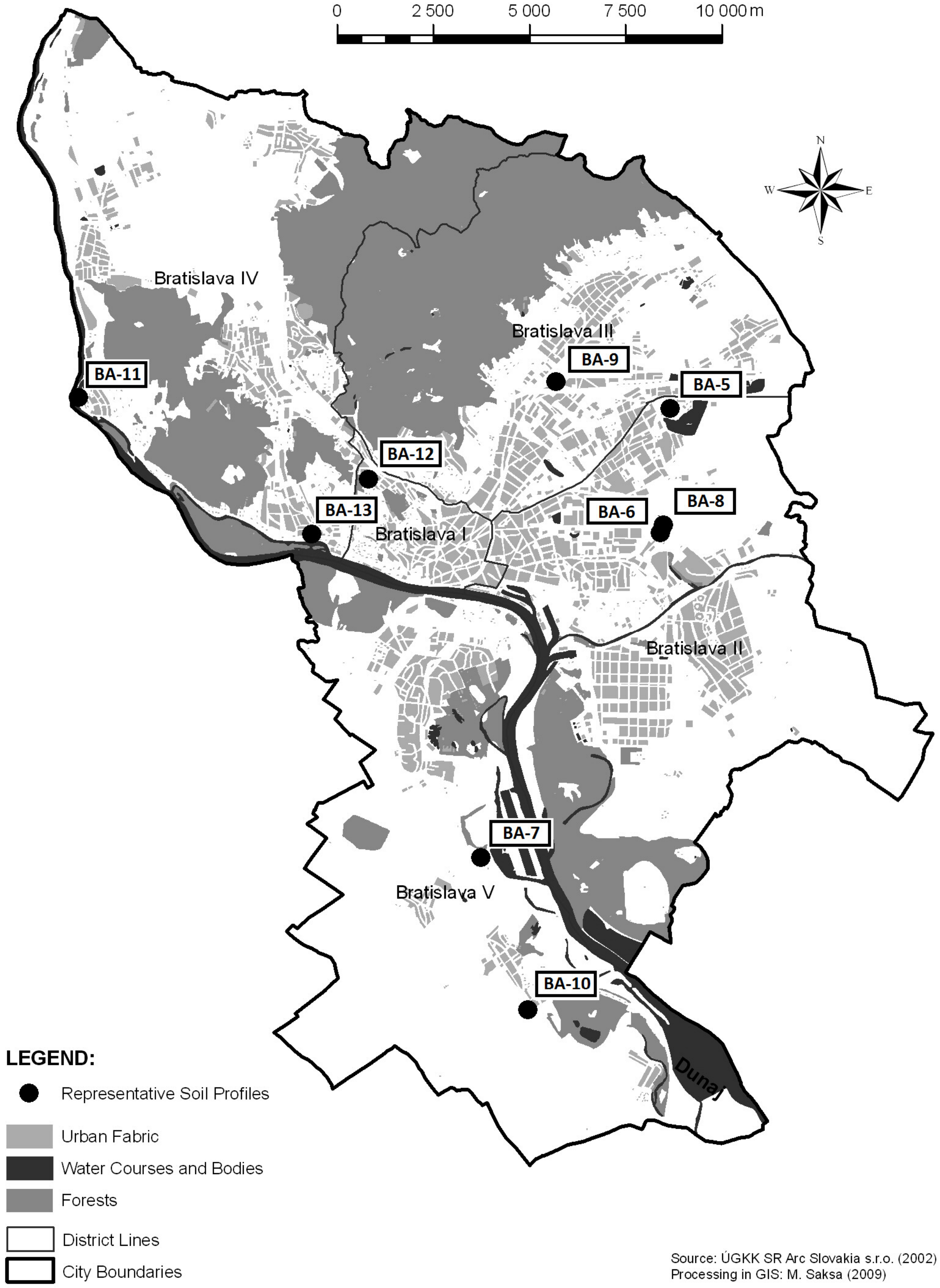
3 Materials and Methods

3.1 Soils

To optimize the analytical method we used two soil samples with contrasting properties. One sample was collected from the Botanical Garden of the University of Mainz, Germany and the other sample from the Terra firme region 30 km north of Manaus, Amazonas, Brazil (Krauss et al. 2005). In both cases we sampled the 0-10 cm layer of the mineral soil. The Mainz soil was a Luvisol and the Manaus soil was a Ferralsol (IUSS Working Group WRB, 2006). The area where the Mainz soil was sampled is located within the University of Mainz campus close to residential and industrial sites while the Manaus soil represented a little polluted rural background area based on the PAH concentrations detected by Krauss et al. (2005). We considered these two samples as typical of temperate soils with a dominance of three-layer clay minerals and tropical soils with a dominance of two-layer clay minerals and Fe and Al oxides thus covering a broad range of different mineral soil properties while the organic matter concentration was similarly low. The final optimized PBDE analysis method was then applied to nine urban soils from Bratislava, where we sampled up to five horizons per soil (Fig. E2-1, Tab. E2-1). Sample locations, current land use, and sampling depths are shown in Table E2-1.

Aliquots of the Mainz and Manaus samples were dried at 45°C in an oven. For PBDE analyses aliquots of the Mainz, Manaus and Bratislava samples were dried at room temperature (ca. 20°C) in a storage room. All samples were dry sieved to <2 mm and stored in aluminum containers at 4°C until extraction.

The C_{org} concentrations of the soils were determined using a CHNOS-Autoanalyzer (Elementar Vario EL III, Elementar Analysensysteme, Hanau, Germany). Texture and pH were measured using established methods (Wilcke et al., 1999). The Manaus soil had a pH in 1 mol l^{-1} KCl of 3.9, a C_{org} concentration of 41 mg g^{-1} and contained 46% sand, 6% silt and 48% clay. The Mainz soil had a pH in 0.01 mol l^{-1} $CaCl_2$ of 7.1, a C_{org} concentration of 46 mg g^{-1} and contained 33 % sand, 52% silt and 16% clay. The properties of the Bratislava soils are summarized in Table E2-1. More information about the study soils including their heavy metal concentrations are offered in Sobocka (2007).



Source: ÚGKK SR Arc Slovakia s.r.o. (2002)
Processing in GIS: M. Saksa (2009)

Figure E2-1: Location of the study sites in the city of Bratislava, Slovakia.

Table E2-1: Sampling locations in Bratislava, land use, sampling depths, and selected soil properties.

Site	Land use	Coordinates	Depths [cm]	pH ($CaCl_2$)	C_{org} [mg g^{-1}]	Sand [%]	Silt [%]	Clay [%]
BA-05	Abandoned site near TESCO supermarket (former arable land)	N4811.168 E17 10.933	0 – 23	7.5	23	61	15	24
			23 – 60	7.7	14	34	43	23
			70 – 96	8.0	9.2	40	42	18
			>96	8.0	3.7	40	44	16
BA-06	Former farmland near the chemical waste dump	N48 09.517 E17 11.073	0 – 20	7.9	9.6	38	52	10
			33 – 50	8.0	5.6	54	38	8
			50 – 72	8.3	4.1	88	9	3
BA-07	Agricultural land (meadow) near Bratislava-Vienna highway (10 m) and the Danube river (70 m)	N48 03.832 E17 08.852	0 – 25	7.5	17	37	49	14
			50 – 120	7.9	8.8	32	52	16
BA-08	Chemical waste dump (abandoned area)	N4809.542 E17 11.084	0 – 2	7.2	19	67	22	11
			2 – 18	7.2	12	57	27	16
			18 – 66	7.6	8	63	23	13
			66 – 94	7.6	4.7	68	18	14
			>94	7.8	6.4	71	16	12
BA-09	Garden allotment near chemical plant (200m)	N48 11.373 E17 09.137	0 – 38	7.0	28	50	32	18
			38 – 54	6.7	15	39	37	24
			70 – 96	6.5	4.8	42	39	20
BA-10	Arable land near the Bratislava-Vienna highway (50 m)	N4802.593 E17 09.290	0 – 30	7.7	18	41	42	17
			30 – 75	8.0	13	30	50	20
			75 – 102	8.0	11	27	53	20
			>102	8.2	5.7	31	56	13
BA-11	Anti-flooding embankment at the confluence of Morava and Danube rivers	N4810.485 E16 58.585	0 – 8	7.3	53	43	44	14
			19 – 50	7.3	12	75	18	8

3.2 Chemicals

A mixture of 15 commonly occurring PBDE congeners (BDE-3, BDE-17, BDE-28, BDE-71, BDE-47, BDE-66, BDE-100, BDE-99, BDE-85, BDE-154, BDE-153, BDE-138, BDE-183, BDE-190, and BDE-209) was obtained from AccuStandards (New Haven, USA). The single standard of BDE-209 was purchased from Dr.Ehrenstorfer (Augsburg, Germany). A mixture of nine ^{13}C-labeled PBDEs used as internal standard for the EI method was purchased from Cambridge Isotope Laboratories (Andover, USA) and [$^{13}C_{12}$]-2,2'3,4,5,5'-Hexachlorodiphenylether (CDE-141) used as internal standard for the NCI method and 1,3,4-metheno-2h-cyclobutyl(cd)pentane (mirex) used as recovery standard were from Dr. Ehrenstorfer (Augsburg, Germany). Dichloromethane, hexane and toluene were purchased in picograde quality from Promochem (Wesel, Germany). Neutral Silica gel (70-230 mesh) was purchased from Carl Roth (Karlsruhe, Germany) and neutral aluminum oxide (150 mesh) was obtained from Sigma-Aldrich (Steinheim, Germany). Both were deactivated after heating at 300°C overnight, by adding 5 weight percent of water and shaking mixing for 1 h. Bio-Beads S-X3 were obtained from Bio-Rad Laboratories (Hercules, USA).

3.3 Sample extraction and clean-up

Soil samples were extracted with an ASE 200 (Dionex, Sunnyvale, USA) using dichloromethane as extraction solvent. Samples (20 g of soil) were mixed with 3-4 g of diatomaceous earth (which serves as a drying agent and bulk material) and 1 g of Cu for S removal. Internal standard (2.1 ng) was added prior to extraction. The extraction method is a further development of an extraction method used for other POPs (Krauss and Wilcke, 2003). The 33 ml extraction cells were filled with dichloromethane, pressurized at 14 MPa and heated to 120°C for 6 min. Static extraction lasted 5 min; the cells were rinsed with cold solvent (60% cell volume) and purged with N_2 for 90 s. Two extraction cycles were performed for each sample. The extracts were then dried over Na_2SO_4 and evaporated with a rotary evaporator (55 MPa, 30°C) to about 500 µl.

For method optimization, a GPC step was added to the basic clean-up procedure using only SPE columns. As suggested by Saito et al. 2004 we prepared the GPC column with 35 g of Bio-Beads S-X3. Elution solvent was hexane: dichloromethane 1:1 (v/v). Extracts were injected manually and fractions were also manually collected as follows. The first 90 ml fraction was discarded, the following 70 ml, containing PBDEs, were collected and afterwards the column was rinsed with another 50 ml between two samples. After evaporating the extracts to about 500 µl a further clean-up with SPE columns followed. The columns were filled manually with 2 g of 5% deactivated, neutral aluminum oxide (upper part) and 2 g of 5% deactivated silica gel (lower part). Elution of the columns followed the method of Krauss and Wilcke (2003) for PAHs, PCBs and PCNs, i.e. 15 ml hexane, 5 ml hexane:dichloromethane 9:1 (v/v) and 20 ml hexane:dichloromethane 4:1 (v/v). After evaporating the samples to about 500µl, 2.1 ng of the recovery standard were added to check the recovery of internal standard.

3.4 Instrumental analysis

Compound separation and quantification was performed with an Agilent 6890 gas chromatograph equipped with an inert mass spectrometric detector HP 5973N with an ion source that can be switched from EI to NCI. BDE congeners 3, 17, 28, 71, 47, 66, 100, 99, 85, 154, 153, 138, 183 and 190 were analyzed on an HP-5MS capillary column (30 m x 0.25 mm x 0.25 µm). Extracts (2 µl) were injected to the column in splitless mode at 270°C. The flow rate of He was 2 ml min^{-1} and the temperature program followed the method of Yun et al. (2008). The temperature program started at 100°C, then increased at 20°C min^{-1} to 200°C and at 10°C min^{-1} to 220°C (held for 2 min) and finally at 4°C min^{-1} to 280°C. BDE-209 was analyzed on an HP-5MS column (15m x 0.25mm x 0.25µm). Extracts (1 µl) were injected to the short column in splitless mode at 280°C. The flow rate was 1.5 ml min^{-1} and the temperature program followed the method of Gomara et al. (2006). The start temperature of 110°C was maintained for 1.5 min, then increased at 5°C min^{-1} to 275°C, then at 40°C min^{-1} to 300°C (held for 10 min), and finally at 10°C min^{-1} to 310°C (held for 6 min). Compounds were analyzed in both, the EI and NCI modes (except for BDE-209, which was only analyzed with NCI). The EI mode was adjusted to ionization energy of 70 eV, an ion source temperature of 230°C, and a quadrupole temperature of 150°C. The NCI mode was adjusted to a flow rate of methane reagent gas of 40%, an energy of primary electron beam of 235 eV, an ion source temperature and pressure of 150°C and $2.44\text{x}10^{-7}$ Pa, respectively, and a quadrupole temperature of 150°C. The ions used as qualifier and quantifier in EI were the positively charged molecule ion and the two fold debrominated positively charged molecule ion, respectively. In NCI, Br^- was used to quantify the tri to hepta brominated compounds whereby Br^- with m/z = 79 served as quantifier and Br^- with m/z = 81 as qualifier. BDE-209 was quantified via $C_6Br_5O^-$, (m/z = 487 as quantifier and 489 as qualifier, respectively), similar to the method used in the study of Hites (2008). Target compound concentrations were quantified with the help of ^{13}C-labeled PBDEs as internal standards in EI and with ^{13}C-labeled CDE-141 in NCI. The calibration solutions prepared for the tri to hepta brominated compounds were: 1, 10, 25, 50 and 100 ng ml^{-1} in EI and 0.1, 1, 10, 25, 50 and 100 ng ml^{-1} in NCI. For the deca brominated congener we calibrated with 1, 10, 50, 100 and 200 ng ml^{-1}. As confirmation criterion for the detection and quantification of the PBDEs, we used the chromatographic peak for a given analyte and accepted an error of ±6 s in retention time and of ±15% of the theoretical value determined with the standard solution in the ratio of qualifier to quantifier ion. The Bratislava soils were only analyzed with the final optimized method (i.e. PLE-GPC-SPE-GC-NCI-MS).

3.5 Method development

We started with the PLE-SPE-GC-EI-MS method of Krauss and Wilcke (2003, termed "basic method") and first compared the performance of EI versus NCI and thereafter introduced an additional GPC clean-up step before SPE (termed "optimized method") and again tested the performance of EI versus NCI. Because no reference soil with certified PBDE concentrations was available, we determined accuracy, precision and limits of detection (LOD) of the different method versions with spike and recovery experiments on the soils from Manaus and Mainz and by running blank analyses using diatomaceous earth as blank material. Two to three replicates of each soil (20 g) were spiked with 10 ng of the tri to hepta brominated PBDEs and 50 ng of the deca brominated BDE-209. LODs of the basic and optimized methods were determined as three times the background level of the blanks, which was obtained by integrating the baseline noise at the corresponding retention time of each PBDE congener. For the optimized method (PLE-GPC-SPE-GC-NCI-MS), we determined the ranges of spiked PBDEs that produced linear responses of the mass detector separately for the Manaus and Mainz soils. The soil samples were spiked with 2-100 ng of the tri to hepta brominated congeners and 10-500 ng of the deca brominated congener. Results of the different method versions were compared with each other using the Wilcoxon rank-sum test with SPSS 15.0 for Windows.

4 Results and Discussion

4.1 Method validation

Table E2-2 shows the recovery results of the basic and the optimized methods using EI and NCI, respectively, for the soils from Manaus and Mainz. In the method blanks no PBDEs were detected, except for BDE-47. We therefore corrected the BDE-47 concentrations in soil by subtracting the blank value. The recoveries of spiked PBDEs from both soils ranged for the basic method from 49 to 119% in EI and from 87 to 284% in NCI. The precision expressed as the relative standard deviation (RSD) was better than 20% for both ionization techniques. The seemingly high recoveries in the basic method using NCI can be explained by a high matrix enhancement. NCI is more sensitive to matrix-related interferences in mass spectrometry than EI because bromine ions are used for quantification and thus any brominated compound co-eluted from the SPE columns interferes with the determination besides any other organic compound producing fragments with m/z ratios of 79 or 81 (Korytar et al., 2005). The increase in recovery for later eluting compounds can be explained by the missing matrix compensation of the internal standard because in NCI only one internal standard was used, the ^{13}C-labeled CDE-141, which elutes early. In EI, we used nine internal standards which compensate matrix effects for the whole elution period.

The recoveries of the optimized method for both soils with the additional GPC clean-up step ranged from 72 to 112% in EI and from 74 to 126 % in NCI and the precision of the analytical procedure was still better than 20% (RSD) for all compounds in both ionization modes. The PBDE recoveries in the basic method differed between the soils of Manaus and Mainz with their different chemical composition. Thus, the results achieved with the basic method are sensitive to different matrices. The partly too high recoveries in NCI on the one hand and the different results for the different soils on the other hand clearly indicate the need of a better separation of the target compounds from the matrix.

With the optimized clean-up method recoveries closer to 100% (improved accuracy) were achieved for the two soils and ionization techniques. The differences in mean recoveries of the sum of PBDEs between the basic and the optimized clean-up were significant for the Mainz soil in the EI mode and for both tested soils in the NCI mode (Wilcoxon test, $P \leq 0.01$). The improvement in recoveries illustrates that the introduction of the GPC step minimized matrix effects of the soil, particularly in the NCI mode. Since the negative ionization is more sensitive for PBDEs, NCI becomes the ionization technique of choice only if combined with a clean-up like GPC. The recoveries of our optimized method are comparable with those of the studies of Hassanin et al. (2004), Sanchez-Brunete et al. (2006) and Zou et al. (2007).

The recovery of the internal standards in EI (^{13}C-labeled PBDEs) was 117±9% for the soil from Manaus and 74±15% for the soil from Mainz. In NCI, the recovery of the internal standard (^{13}C-labeled CDE-141) was 74±15% for the soil from Manaus and 95±4% for the soil from Mainz. The systematically different recoveries of spiked PBDEs from the Manaus and Mainz soils demonstrate the need to test analytical methods specifically for soils that differ strongly in mineralogical composition like temperate and tropical soils usually do (Laabs et al., 1999; Amelung et al., 2007). From the good performance of our optimized method in extracting soils with markedly different mineralogical composition we conclude that our method is applicable to a wide range of mineral soils with varying properties. However, a further improvement is likely necessary if particularly organic matter-rich soil horizons, such as the organic layer of forest soils (O horizons) or peats (H horizons) that may contain more than 80% organic matter are to be analyzed, because of the high number of possibly interfering compounds.

The losses of internal standards spiked to the samples prior to extraction were small and comparable with those observed in other published studies of PBDE analyses in soil. Using EI, Yun et al. (2008) reported for ^{13}C-labeled PBDEs recoveries of 88±20%. Using NCI, Dodder at al. (2002) reported for ^{13}C-labeled CDE-156, which is an isomer of CDE-141, recoveries between 70 and 120% of internal standard spiked to fish samples prior to extraction. Fish is not fully comparable to soil, but we are not aware of any study in soils/sediments using labeled CDEs as internal standard for comparison. The recoveries of internal standard showed no difference between the basic and the optimized methods, illustrating that there are no significant losses of target compounds during the GPC step.

Table E2-3 summarizes the detection limits for the basic and optimized methods. The optimized method had lower detection limits for the studied PBDE congeners ranging from 11 to 231 pg g^{-1} in EI and from 3 to 52 pg g^{-1}in NCI. The LOD of BDE-209, measured on the shorter column and with NCI only, was one order of magnitude higher than those of the other PBDE congeners. The LODs in NCI were clearly lower than those in EI for both analytical procedures and both soils. Because of the higher sensitivity in NCI, which was also reported by Covaci et al. (2007), we used NCI for determination of PBDEs in Bratislava soils, although the Br^- ions (m/z = 79 and 81) used as quantifier and qualifier are less selective compared with the ions formed in EI. Our LODs of the optimized method using NCI are comparable with those reported by other authors for soils ranging 1 to 50 pg g^{-1} (Li et al., 2008a), 1 to 40 pg g^{-1} (Yun et al., 2008), and 3 to 49 pg g^{-1} (Luo et al., 2009). LODs decreased in the optimized compared with the basic method, especially for the frequently measured congeners BDE-47, BDE-99 and BDE-153. This decrease in LODs illustrates that the GPC step was not only important to improve the accuracy of the method but also to lower the LODs.

Table E2-3 shows the linear regression equations and coefficients of determination of the linearity test for the Mainz and Manaus soils using the optimized method. For both ionization techniques a good linearity was reached with coefficients of determination ranging 0.995-1.000 in EI and 0.990-1.000 in NCI. This almost perfect linearity illustrates the wide application range of the optimized method covering differently contaminated soils. For the congeners BDE-17, BDE-47, BDE-66, BDE-100, BDE-153, and BDE-183, our findings are in line with those of Sanchez-Brunete et al. (2006).

Table E2-2: Mean recoveries (± standard deviations) of spiked target compounds using the basic and optimized analytical procedures in EI and NCI modes for soil samples from Manaus and Mainz.

	PLE-SPE-GC-MS (basic procedure)				PLE-GPC-SPE-GC-MS (optimized procedure)			
	Recovery [%]				Recovery [%]			
	EI		NCI		EI		NCI	
Analyte	Manaus soil	Mainz soil	Manaus soil	Mainz soil	Manaus soil	Mainz soil	Manaus soil	Mainz soil
BDE-3	n.m.	100 ± 2		113 ± 1	110 ± 1	100 ± 2	120 ± 3	110 ± 12
BDE-17	99 ± 4	86 ± 5	87 ± 9	130 ± 1	84 ± 1	86 ± 5	96 ± 1	100 ± 2
BDE-28	110 ± 3	90 ± 5	140 ± 6	150 ± 1	86 ± 1	90 ± 5	95 ± 3	110 ± 7
BDE-71	95 ± 5	100 ± 1	120 ± 8	150 ± 3	100 ± 1	100 ± 1	110 ± 12	89 ± 1
BDE-47	96 ± 2	97 ± 11	140 ± 10	170 ± 4	100 ± 4	97 ± 11	100 ± 7	110 ± 22
BDE-66	97 ± 3	94 ± 9	140 ± 9	180 ± 3	89 ± 1	94 ± 9	99 ± 9	100 ± 3
BDE-100	55 ± 10	91 ± 18	120 ± 10	170 ± 5	100 ± 1	91 ± 18	110 ± 4	97 ± 5
BDE-99	98 ± 1	100 ± 1	150 ± 18	220 ± 2	100 ± 3	90 ± 1	130 ± 1	110 ± 31
BDE-85	120 ± 10	86 ± 3	140 ± 16	240 ± 1	92 ± 1	86 ± 3	110 ± 5	100 ± 8
BDE-154	51 ± 5	110 ± 1	130 ± 11	230 ± 1	74 ± 2	110 ± 1	110 ± 11	98 ± 9
BDE-153	59 ± 7	110 ± 4	180 ± 29	270 ± 12	85 ± 3	110 ± 4	120 ± 1	100 ± 7
BDE-138	49 ± 5	98 ± 11	180 ± 15	280 ± 11	77 ± 3	98 ± 11	130 ± 16	98 ± 5
BDE-183	120 ± 11	91 ± 18	160 ± 10	260 ± 8	93 ± 7	91 ± 18	120 ± 23	96 ± 7
BDE-190	120 ± 3	72 ± 4	150 ± 8	200 ± 15	95 ± 10	72 ± 4	110 ± 4	94 ± 9
BDE-209	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	120 ± 2	88 ± 21
Average	89 ± 26	95 ± 10	140 ± 25	200 ± 54	92 ± 11 $^{\text{n.s.}}$	94 ± 10$^{\text{s}}$	111 ± 10$^{\text{s}}$	92 ± 8$^{\text{s}}$

n.m. is not measured with this method n.s.: not significant

s:statistically different from the same sample and ionization mode in the basic analytical procedure (P≤0.01 probability level Wilcoxon rank-sum test)

Table E2-3: Limits of detection of the basic (PLE-SPE-GC-EI/NCI-MS) and optimized methods (PLE-GPC-SPE-GC-EI/NCI-MS) and linearity of the optimized method.

	LOD of the basic method		LOD of the optimized method		Linearity of the optimized method			
	EI	NCI	EI	NCI	NCI		NCI	
					Manaus soil		Mainz soil	
	[pg g^{-1}]	[pg g^{-1}]	[pg g^{-1}]	[pg g^{-1}]	R^2	Equation	R^2	Equation
BDE-17	52	40	19	17	0.999	$y = 0.1737 x - 0.1043$	0.993	$y = 0.1783 x + 0.2200$
BDE-28	36	17	11	15	1.000	$y = 0.0191 x - 0.0099$	0.996	$y = 0.2030 x + 0.0603$
BDE71	380	20	59	3	0.999	$y = 0.1720 x - 0.0144$	0.999	$y = 0.1870 x - 0.0925$
BDE47	310	60	49	10	1.000	$y = 0.1464 x + 0.0909$	0.999	$y = 0.1714 x - 0.0043$
BDE66	340	31	47	6	0.998	$y = 0.1451 x - 0.1357$	0.998	$y = 0.1652 x - 0.3986$
BDE100	78	20	73	22	0.999	$y = 0.1538 x - 0.1771$	0.997	$y = 0.1711 x - 0.3371$
BDE99	180	58	110	7	0.998	$y = 0.1411 x - 0.2083$	0.997	$y = 0.1571 x - 0.2542$
BDE-85	170	40	66	23	0.998	$y = 0.1067 x - 0.1159$	0.993	$y = 0.1276 x - 0.4245$
BDE-154	370	25	39	22	1.000	$y = 0.1255 x - 0.0165$	0.994	$y = 0.1799 x - 0.6393$
BDE-153	410	32	47	14	0.997	$y = 0.1252 x - 0.1940$	0.990	$y = 0.1788 x - 0.7897$
BDE138	120	50	60	16	0.995	$y = 0.0924 x - 0.2240$	0.992	$y = 0{,}0990 x - 0.2703$
BDE183	130	60	230	16	0.998	$y = 0.0881 x - 0.0208$	0.991	$y = 0.1055 x - 0.2918$
BDE-190	130	100	100	52	1.000	$y = 0.0590 x - 0.0624$	0.996	$y = 0.0629 x - 0.1277$
BDE-209	n.m.	n.m.	n.m.	370	0.991	$y = 0.0032 x - 0.0749$	0.992	$y = 0.0019 x - 0.0679$

n.m. is not measured - BDE-209 was only measured with NCI

4.2 PBDE concentrations in urban soils of Bratislava.

The concentrations of the tri to hepta brominated congeners (BDE-17, BDE-28, BDE-71, BDE-47, BDE-66, BDE-100, BDE-99, BDE-85, BDE-154, BDE-153, BDE-138 and BDE-190) in the surface horizons of Bratislava soils ranged from 86 to 625 pg g^{-1}, with an average of 311 pg g^{-1} (Tab. E2-4). The highest concentrations were found at sampling sites BA-11 (confluence of the rivers Morava and Danube) and in three garden soils (BA-09 in the vicinity of a chemical plant, BA-12 in the center of the old city, and BA-13 a garden of a villa near Karlova Ves). Sampling site BA-11 is likely polluted by PBDE-contaminated sediments and water from the Danube and Morava rivers while the garden soils may be affected by the use and disposal of household equipment and materials (furniture, building material, plastics and textiles) containing flame retardants. Urban garden soils frequently show elevated POP concentrations because of the regular application of fertilizer, composted organic matter and ashes of wood- or coal-based domestic heating (Krauss and Wilcke, 2003). The surface horizon of the botanic garden soil in Mainz had higher PBDE concentrations than all surface horizons of Bratislava soils. The average concentration of the tri to hepta brominated congeners in the Terra firme region near Manaus was also higher than the tri to hepta brominated PBDE concentrations in Bratislava soils. Elevated PBDE concentrations in the Manaus soil likely originate from Manaus city although the same soils low PAH concentrations resulting in the classification as a remote site by Krauss et al. (2005).

The total PBDE concentrations (i.e. sum of 15 congeners) in all studied surface horizons of Bratislava soils were at the lower end of the range reported in previous studies for remote British grassland (747 pg g^{-1}), British woodland (2500 pg g^{-1}), and Norwegian woodland (970 pg g^{-1}). To eliminate the possible influence of differing C_{org} concentrations in the above comparison of concentration levels from different locations we additionally normalized the PBDE concentrations to the C_{org} concentrations. The organic carbon-normalized PBDE concentrations in surface horizons of Bratislava soils ranged from 250 to 5500 (mean: 1500) pg $(g\ C_{org})^{-1}$ and were lower than the 10 to 37,000 (2500) pg $(g\ C_{org})^{-1}$ in British grassland and 3023 to 110,900 (22,700) pg $(g\ C_{org})^{-1}$ in British woodland soils (Hassanin et al., 2004; Harrad and Hunter, 2006). The PBDE concentrations in surface horizons of Bratislava soils were similar as in the city of Kohtla-Järve in Estonia, where PBDE concentrations from below the detection limit to 1030 pg g^{-1} were detected and in Chinese farmland with, on average, 390 pg g^{-1} (Hassanin et al., 2004; Harrad and Hunter, 2006; Luo et al., 2009). This suggests a more intensive use of PBDE flame retardants in Mainz and other Western Europe locations than in Bratislava, other eastern European cities and China. This finding is in line with the historically more stringent laws concerning fire hazards and the more pronounced use of electronic equipment in western than in Eastern Europe (Jeward et al., 2004).

In most published reports, BDE-209 was the most abundant congener in soils (Mai et al., 2005), which can be attributed to the predominant use of the technical deca mixture in electronic equipment such as e.g., Saytex 102E and Bromkal 82-0DE which contain 92% and 96% BDE-209, respectively (La Guardia et al., 2006). Consequently, BDE-209 is usually also the main compound in soils from e-waste polluted areas (Li et al., 2008a; Zhang et al., 2009). In Bratislava soils, BDE-209 was found at two sampling sites (BA-07 and BA-11) with an average concentration of 735 pg g^{-1}. At these two sites BDE-209 contributed between 61 and 77% to the sum of PBDE concentrations. BA-07 and BA-11 were under meadow-use and in an anti-flooding embankment area, respectively, and to our knowledge not affected by e-waste disposal. It is probable that the anti-flooding embankment received higher BDE-209 burdens from sediments which is in line with the finding that also the tri to hepta brominated PBDEs had elevated concentrations at these sites. In previous studies, BDE-209 accounted for 68 to 93% of the total PBDE concentration in Michigan floodplain soils (Cetin and Odabasi, 2007) and for more than 70% in most urban and industrial soil samples from Taiyuan, China (Li et al., 2008a). The detection of BDE-209 in only two soils at low concentrations further supports our conclusion that Bratislava soils are generally less contaminated with PBDEs than some soils of the western part of Europe.

In Bratislava soils, BDE-47, BDE-100, BDE-99, BDE-153, BDE-154, and BDE-209 dominated the PBDE patterns (Fig. E2-2). The composition of the mixture of tri to hepta brominated congeners varied among the studied locations. BDE-47 was most abundant in surface horizons of Bratislava soils except at sampling sites BA-6, BA-9 and BA-13. The PBDE abundances in the surface horizons of BA-5, BA-8, BA-10 and BA-11 showed the same order, BDE-47 > BDE-99 > BDE-154 > BDE-153 > BDE-100. This suggests that these sites were contaminated by joint sources, which emit the PBDEs in ratios of the technical formulations. The comparison between the concentration ratios of selected congeners in the surface horizons of Bratislava soils and the technical penta mixes DE-71 and Bromkal 70-5DE suggest that the lower brominated congeners BDE-47, BDE-99 and BDE-100 originate from these mixtures (Fig. E2-3). The small deviation of the BDE-99/BDE-100 ratio in the soil compared to the technical mixture may be attributable to congener-specific transport in soil, plant uptake (Müller et al., 2006) and microbial degradation processes (Robrock et al., 2008). The high ratios of BDE-153/BDE-100 and BDE-154/BDE-100 in the Bratislava soils indicate that these congeners originate from an additional source, like the technical octa mixtures

(Bromkal 79 and Bromkal 79-8DE), which, however, could not be included in Fig. E2-3 because they are free of BDE-100. La Guardia et al. (2006) reported that the two mixtures contain 8.66 and 0.15% (w/w) of BDE-153 and 0.8 and 0.38% (w/w) of BDE-154, respectively. Both octa formulations are therefore likely sources of the congeners BDE-153 and BDE-154 detected in Bratislava soils. We conclude that the ratios of the lower brominated congeners in the surface horizons of Bratislava soils illustrate that the technical mixtures penta-mix and octa-mix are sources of PBDEs in the surface horizons. The predominance of the five lower brominated congeners was also seen in European background soils in UK and Norway (Hassanin et al., 2004).

The highest sum of tri to hepta brominated congener concentrations occurred in the surface horizons or layers, except for the sampling sites BA-06 and BA-09 (Tab. E2-4). At most study sites, the sum of tri to hepta brominated congeners concentrations decreased with increasing depth (BA-5, BA-7, BA-8, BA-10, BA-11, and BA-12). This is typical for POPs which are deposited to the soil surface and can be attributed to the strong retention by SOM as a result of the low polarity of PBDEs with K_{OW} values of 10^5-10^8 (Li et al., 2008b) impeding leaching to greater soil depth (Moeckel et la., 2008). Exceptions were the vertical PBDE distribution in the soils at sites BA-06 and BA-09 where subsoil horizons had the highest PBDE concentrations (Tab. E2-4). A likely explanation for these deviating depth distributions is the burial of contaminated surface horizons with less contaminated material which frequently occurs in urban areas as consequence of construction, gardening and landscape shaping activities

To investigate the role of the SOM concentration in explaining the BDE concentration variability in the surface horizons of Bratislava soils, the PBDE concentrations were regressed on C_{org} concentrations (Fig. E2-4). To improve normality of the PBDE and Corg concentration distribution we ran the regression on log-transformed data. The concentrations of the congeners BDE-47 and, BDE-99 were significantly correlated with that of C_{org}, the BDE-66 concentration was marginally significantly correlated while the concentrations of higher brominated congeners such as BDE-154 were not significantly correlated with the C_{org} concentrations. In the literature, there are two possible explanations for correlations between POP and SOM concentrations. Either a correlation is interpreted as indication of near-equilibrium of the air-soil partitioning of gaseous POPs (Wilcke and Amelung, 2000; Meijer et al., 2003; Hassanin et al., 2004) or as the consequence of POP scavenging from the atmosphere by plants and later incorporation into soil (Horstmann and McLachlan, 1998). We consider the first explanation as more likely in our study soils because we only observed a correlation between PBDE and C_{org} concentrations for the relatively volatile congeners BDE-47 and BDE-99 with even a steeper slope of the regression line for the more volatile BDE-47 than BDE-99, while the vegetation scavenges also particle-associated less volatile PBDEs. Furthermore, the vegetation was consistently grassland with likely similar biomass production and differences in C_{org} concentrations had historic reasons.

Table E2-4: Concentration of PBDEs in soil samples from Bratislava (BA-05 to BA-13), Mainz and Manaus in pg g^{-1} dry weight.

	BA05_1	BA05_2	BA05_3	BA05_4	BA06_1	BA06_2	BA06_3	BA07_1	BA07_2	BA08_1	BA08_2	BA08_3	BA08_4	BA08_5	BA09_1	BA09_2	BA09_3
Depth [cm]	0–23	23–60	70–96	>96	0–20	33–50	50–72	0–25	50–120	0–2	2–18	18–66	66–94	>94	0 – 38	38 – 54	70– 96
	[pg g^{-1}]																
BDE-3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BDE-17	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	18	n.d.	18	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BDE-28	20	16	16	n.d.	n.d.	n.d.	17	17	n.d.	20	17	16	17	n.d.	18	n.d.	16
BDE-71	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	27	n.d.	n.d.	32	73	31
BDE-47	41	13	13	14	21	12	31	41	15	65	47	15	13	14	92	21	19
BDE-66	61	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	82	140	66
BDE-100	12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	24	170	34
BDE-99	32	23	n.d.	26	26	23	25	27	23	34	29	24	25	23	38	n.d.	23
BDE-85	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	33	n.d.	n.d.
BDE-154	28	n.d.	n.d.	n.d.	23	n.d.	n.d.	23	n.d.	26	24	n.d.	n.d.	n.d.	26	n.d.	n.d.
BDE-153	20	n.d.	n.d.	n.d.	16	n.d.	n.d.	20	17	16	21	51	21	28	120	230	60
BDE-138	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	27	22	21	19	20	n.d.	n.d.	16
BDE-183	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BDE-190	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BDE-209	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	470	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sum of tri-hepta	214	52	29	40	86	35	91	141	73	188	160	154	95	85	465	634	265
Total sum	214	52	29	40	86	35	91	611	73	188	160	154	95	85	465	634	265

n.d. is not detected

Table E2-4: Continued.

	Ba10_1	Ba10_2	Ba10_3	Ba10_4	BA11_1	BA11_2	BA12_1	BA12_2	BA12_3	BA13_1	BA13_2	BA13_3	BA13_4	Mainz	Manaus
Depth [cm]	0–30	30–75	75–102	>102	0–8	19–50	0–42	42–70	>70	0–48	48–90	90–120	>120	0–10	0 – 10
	[pg g $^{-1}$]														
BDE-3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BDE-17	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BDE-28	n.d.	17	n.d.	n.d.	n.d.	17	n.d.	n.d.	n.d.	18	n.d.	n.d.	n.d.	18	n.d.
BDE-71	n.d.	n.d.	n.d.	n.d.	n.d.	26	n.d.	n.d.	n.d.	31	n.d.	n.d.	n.d.	n.d.	n.d.
BDE-47	30	23)	12	14	280	52	100	35	11	150	17	13	11	490	n.d.
BDE-66	49	n.d.	n.d.	n.d.	82	50	51	n.d.	n.d.	64	n.d.	n.d.	n.d.	150	n.d.
BDE-100	n.d.	n.d.	n.d.	n.d.	100	13	18	n.d.	n.d.	38	n.d.	n.d.	n.d.	92	n.d.
BDE-99	25	23	26	n.d.	65	28	36	27	n.d.	36	23	n.d.	n.d.	91	71
BDE-85	24	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	31	23	n.d.	n.d.	n.d.	65
BDE-154	23	n.d.	n.d.	n.d.	36	36	24	23	n.d.	37	n.d.	n.d.	n.d.	43	33
BDE-153	21	n.d.	16	n.d.	62	21	210	44	n.d.	38	26	n.d.	n.d.	290	330
BDE-138	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BDE-183	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	19	n.d.
BDE-190	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BDE-209	n.d.	n.d.	n.d.	n.d.	1000	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	760	500
Sum of tri-hepta	181	63	54	14	625	243	439	129	11	443	89	13	11	1193	499
Total sum	181	63	54	14	1625	243	439	129	11	443	89	13	11	1953	999

n.d. is not detected

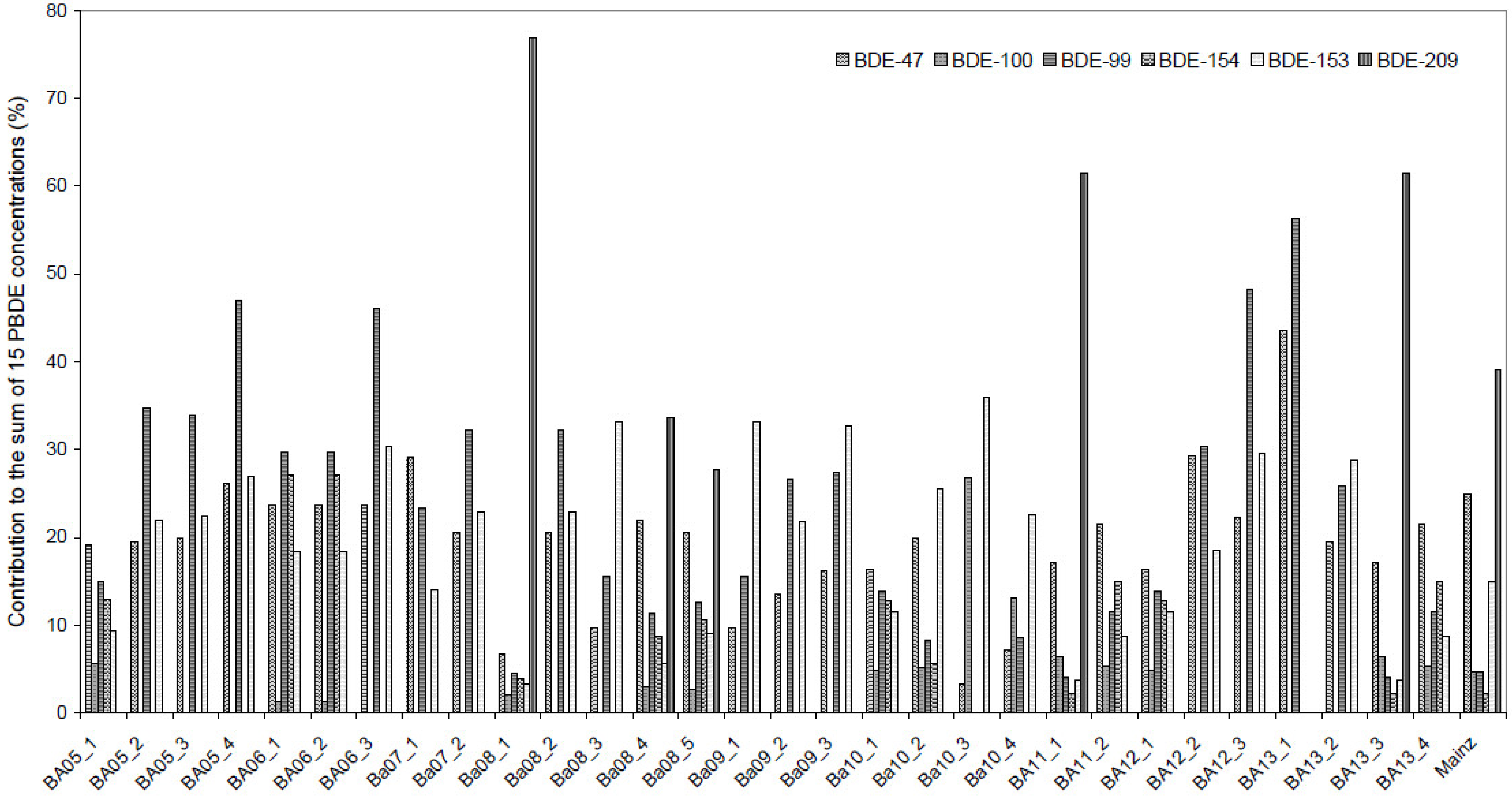


Figure E2-2: Composition of the PBDE mixtures in soils of Bratislava and Mainz. The Manaus soil is not shown, because we did not detect BDE-47and BDE-100 in this sample.

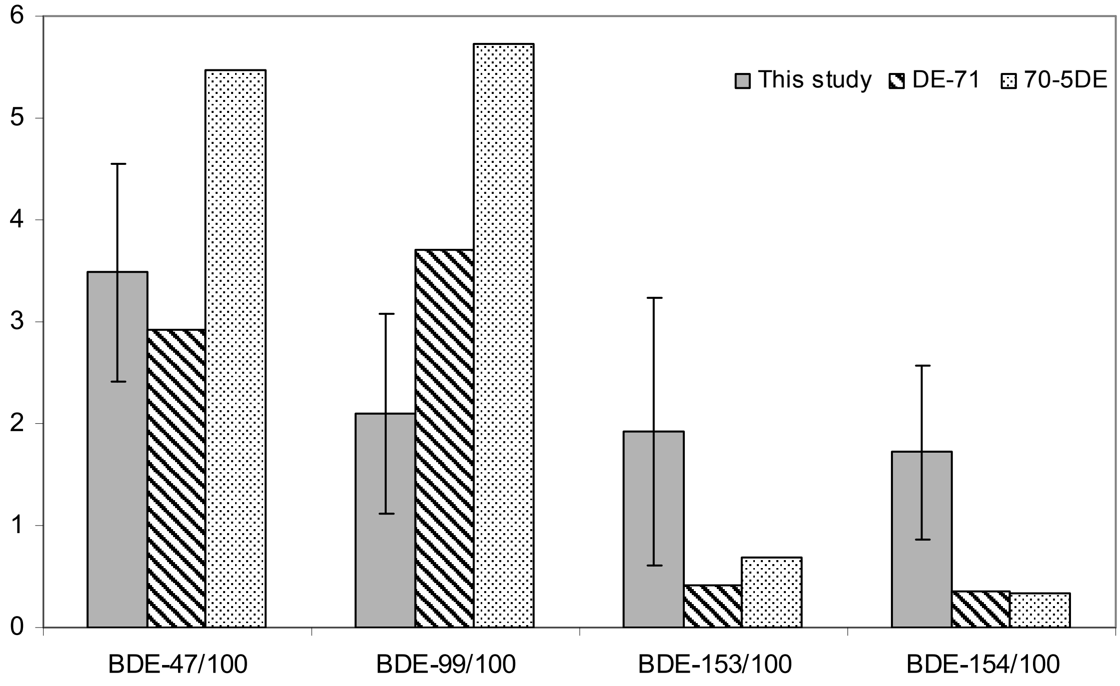


Fig. E2-3: Ratios of selected PBDEs in Bratislava soils and technical PBDE mixtures (penta-mixes: DE-71 and Bromkal 70-5DE (La Guardia et al., 2006).

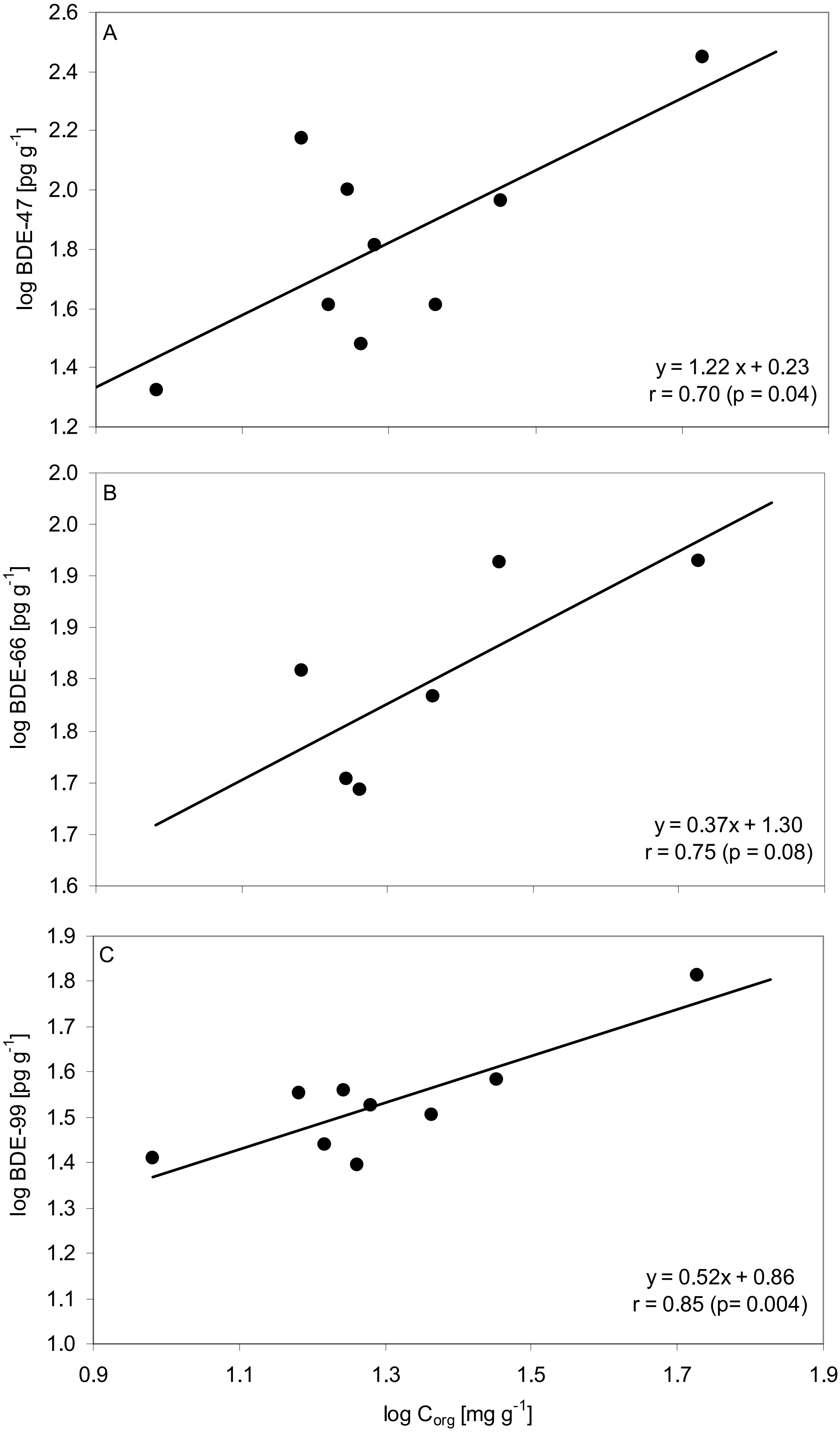


Figure E2-4: Relationship between soil C_{org} concentrations and concentrations of A. BDE-47, B. BDE-66 and C. BDE-99 in surface horizons of urban soils of Bratislava.

5 Conclusion

Our results demonstrate that GPC as additional clean-up step prior to SPE does not affect target compound recoveries but reduces matrix enhancement in the NCI mode and lowers limits of detection. The resulting optimized PLE-GPC-SPE-GC-NCI-MS method for quantification of PBDE concentrations in soil offers low detection limits (<25 pg g^{-1} for most compounds), high accuracy (spike recoveries >88%), high precision (<20% RSD), and a broad application range because of a wide concentration range in which the detector responses are linear.

PBDEs are ubiquitous in soil and can even be detected in the Amazon basin near Manaus. Surface horizons of Bratislava soils had sum of PBDE concentrations in the range of several 100 pg g^{-1} which is a low burden compared to concentrations published for other soils.

The congener pattern in the surface horizons was dominated by BDE-47, BDE-100, BDE-99, BDE-154, BDE-153 and BDE-209, suggesting that the PBDEs in Bratislava soils resulted from the technical penta-, octa- and deca-mixes. PBDEs are strongly sorbed in the surface horizons, likely to SOM, as illustrated by the decreasing sum of tri to hepta brominated congeners with increasing soil depth, associated with the decreasing SOM content. The soil organic matter concentrations explained a large part of the variation in the concentrations of the volatile BDE-47 and BDE-99 concentrations but were not correlated with less volatile PBDEs such as BDE-154.

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E-3 Appendix tables

Table E-3a: Structure and properties of OPAHs under standard condition (25 °C, 101.3 kPa). Data were taken from the PhysiProp data base or estimated using estimation tools from EPI-SuiteTM version 4.0. (USEPA: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm, acessed on 5/8/2009). Bold numbers are experimental data and others are estimated data.

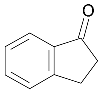
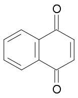
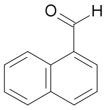
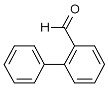
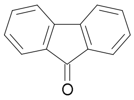
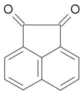
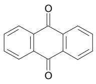
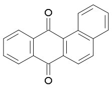
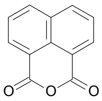
Name	CAS #	Structure	Mw [g mol^{-1}]	Log Kow	Solubility [mg L^{-1}]	Vapor pressure [Pa]
1-Indanone	83-33-0		132.2	2.11	1427	3.56
1,4-naphthoquinone	130-15-4		158.2	**1.71**	2417	0.0225
1-naphthaldehyde	66-77-3		156.2	2.89	244.2	0.334
2-biphenylcarboxaldehyde	1203-68-5		182.2	3.47	0.0247	57.95
9-fluorenone	486-25-9		180.2	**3.58**	3.74	0.00762
1,2-acenaphthenequinone	82-86-0		182.2	**1.95**	90.14	4.5x10^{-5}
9,10-anthraquinone	84-65-1		208.2	**3.39**	**1.35**	**1.6x10^{-5}**
Benzo[a]anthracene-7,12-dione	2498-66-0		258.2	**4.40**	0.2885	5.2x10^{-6}
1,8-naphthalic anhydride	81-84-5		198.2	3.24	5.878	5.5 x 10-5

Table E-3a continued.

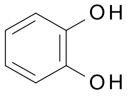
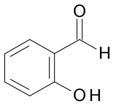
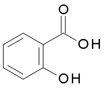
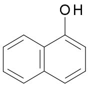
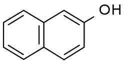
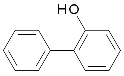
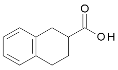
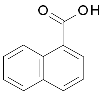
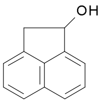
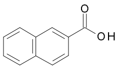
Name	CAS #	Structure	Mw [g mol^{-1}]	Log Kow	Solubility [mg L^{-1}]	Vapor pressure [Pa]
1,2-hydroxybenzene	120-80-9		110.1	**0.88**	**4.61x10^5**	**0.488**
2-hydroxybenzaldehyde	90-02-8		122.1	**1.81**	**1.07x10^4**	**0.791**
2-hydroxybenzoic acid	69-72-7		138.1	**2.26**	**2240**	**0.0109**
1-hydroxynaphthalene	90-15-3		144.2	**2.85**	**866**	**0.0365**
2-hydroxynaphthalene	135-19-3		144.2	**2.70**	**755**	**0.0427**
2-hydroxybiphenyl	90-43-7		170.21	**3.09**	**700**	**0.267**
1,2,3,4-tetrahydro-2-naphthoic acid	53440-12-3		176.2	3.14	298	0.0219
1-naphthoic acid	86-55-5		172.2	**3.09**	**86**	**0.00118**
1-hydroxyacenaphthene	6306-07-6		170.2	2.61	90.45	0.00129
2-naphthoic acid	93-09-4		172.2	**3.28**	**47**	**0.000884**

Table E-3a continued.

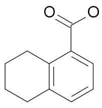
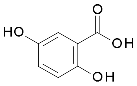
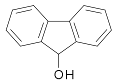
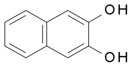
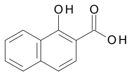
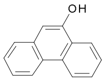
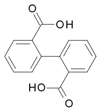
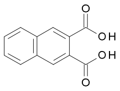
Name	CAS #	Structure	Mw [g mol^{-1}]	Log Kow	Solubility [mg L^{-1}]	Vapor pressure [Pa]
5,6,7,8-tetrahydo-2-naphthoic acid	1131-63-1		176.2	3.84	30.26	0.00417
2,5-dihydrobenzoic acid	490-79-9		154.1	**1.74**	**5000**	4.36x10-5
9-hydroxyfluorene	1689-64-1		182.2	2.71	65.44	8.79x10-5
2,3-dihydroxynaphthalene	92-44-4		160.2	**2.24**	**293**	0.000258
1-hydroxy-2-naphthoic acid	86-48-6		188.2	3.42	229	1.76x10-5
9-hydroxyphenanthrene	484-17-3		194.2	3.86	6.914	0.000237
Biphenyl-2´,2´-dicarboxylic acid	482-05-3		242.2	**2.07**	**100**	3.81x10-7
2,3-naphthalene-dicarboxylic acid	2169-87-1		216.2	2.25	434.7	8.53x10-6

Table E-3b: Physical properties of alkyl/parent-PAHs. LogKow values are from Neff et al. (2005) and Mackay et al. (2006), solubility and vapour pressure values were taken from PhysiProp database or estimated using estimation tools from EPI-SuiteTM version 4.0. (USEPA:http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm, accessed on 5/8/2009). Bold numbers are experimental data and others are estimated data.

Name	Mw [g mol^{-1}]	Log Kow	Solubility [mg L^{-1}]	Vapour pressure [Pa]
1,2,3,4-THNAPH	132.2	3.49	47	32.8
NAPH	128.2	3.37	**31**	**11.3**
C1-NAPH	142.2	3.86	**24.6**	7.33
BP	154.2	3.95	**6.94**	**1.19**
1,3-DMNAPH	156.2	4.42	8.0	3.09
ACENY	152.2	3.94	**16.1**	0.89
ACEN	154.2	3.92	**3.9**	**0.29**
2,3,5-TMNAPH	170.3	4.81	4.78	0.299
FLUO	166.2	4.18	**1.69**	**0.080**
2,6-DIISOPNAPH	212.3	6.08	0.11458	0.0755
PHEN	178.2	4.57	**1.15**	**0.0161**
ANTH	178.2	4.54	**0.0434**	**0.00087**
C1-PHEN	192.2	5.14	**0.26**	**0.00889**
C2-PHEN	206.2	5.51	0.07133	0.00101
FLUA	202.2	5.22	**0.26**	**0.00123**
PYR	202.2	5.18	**0.135**	**0.0006**
RETENE	234.3	6.51	0.00848	0.000352
B(A)A	228.3	5.91	**0.0094**	**2.80×10^{-5}**
CHRY	228.3	5.60	**0.002**	**8.31×10^{-7}**
B(BJK)	252.3	5.80	**0.0015**	**6.67×10^{-5}**

Table E-3b continued.

Name	Mw [g mol^{-1}]	Log Kow	Solubility [mg L^{-1}]	Vapour pressure [Pa]
B(E)P	252.3	6.04	**0.0063**	**7.60x10^{-7}**
B(A)P	252.3	6.04	**0.00162**	**7.32x10^{-7}**
PERY	252.3	6.25	**0.0004**	**7.00x10^{-7}**
IND	276.3	7.0	**0.00019**	1.67x10^{-8}
DIBE	278.3	6.75	**0.00103**	**1.27x10^{-7}**
B(GHI)	276.3	6.50	**0.00026**	**1.33x10^{-8}**
COR	300.3	6.75	**0.00014**	**2.89x10^{-10}**

Table E-3c: Concentrations [ng g^{-1}] of indidual OPAHs and alkyl/parent-PAHs in soils from Angren (Uzbekistan). See Fig. C-1 and Tab. C-1 for description of sampling sites/location. Sampling depth 1 is at 0-0.1 m and 2 is 0.1-0.2 m.

Site/depth	A1	A2	B1	B2	C1	C2	D1	D2	E1	E2	F1	F2	G1	G2	H1	H2	I1	I2	J1	J2	K1	K2
NAPH	306.7	62.1	407.0	44.7	514.4	271.8	580.3	178.8	166.5	49.6	118.5	77.5	105.0	68.9	521.4	57.8	17.8	19.0	55.2	37.6	137.1	67.7
2-MNAPH	106.2	20.6	154.4	16.6	165.6	89.3	222.9	71.6	68.2	25.1	48.0	29.2	39.1	21.0	114.5	13.8	6.9	6.3	15.8	10.3	43.9	27.0
1-MNAPH	60.5	11.1	92.3	9.6	103.5	58.9	157.3	51.8	46.6	17.1	27.8	18.6	22.8	13.6	65.1	8.0	4.3	4.1	9.8	6.3	28.2	19.2
1,3-DMNAPH	38.1	7.0	47.4	5.5	63.7	34.5	94.2	38.1	29.9	13.6	18.6	11.4	13.1	7.8	25.9	3.4	2.7	2.7	5.9	3.4	14.9	13.1
ACENY	11.1	8.0	10.1	2.6	17.9	14.4	9.5	6.2	5.5	2.1	6.2	3.3	4.1	2.5	6.2	0.6	0.6	1.1	2.6	1.6	6.2	3.6
ACEN	9.6	3.1	8.6	1.4	9.8	5.6	18.7	6.0	4.9	2.0	3.7	2.0	2.8	1.3	5.2	0.7	0.6	0.4	1.3	0.7	2.9	1.9
2,3,5-TMNAPH	26.3	5.6	34.1	5.0	33.4	21.6	33.6	26.1	24.6	12.1	16.0	8.2	12.2	5.1	11.5	2.0	2.5	2.0	4.5	3.0	10.6	8.8
FLUO	9.4	4.5	24.3	3.3	14.5	8.5	24.8	6.3	7.6	2.6	8.4	3.5	6.5	2.4	2.8	0.5	1.1	0.6	2.1	1.1	5.0	3.3
PHEN	299.5	157.4	377.2	69.6	444.5	310.0	1083.2	144.6	121.8	52.7	137.1	62.5	118.5	54.7	226.0	27.8	20.5	14.7	48.0	29.7	89.1	51.8
ANTH	8.5	6.4	21.8	2.9	13.7	10.0	25.8	2.6	3.9	1.3	2.9	1.9	2.1	1.7	4.4	1.0	0.7	0.8	1.6	1.0	2.3	1.6
2-MPHEN	112.3	45.5	117.6	20.6	147.6	114.0	352.7	58.9	53.4	33.9	44.0	20.0	32.4	14.4	53.7	6.6	5.7	4.0	11.0	6.4	25.0	12.4
1-MPHEN	29.5	10.9	35.4	5.4	44.8	32.8	123.4	20.0	16.3	10.6	12.1	5.6	8.4	3.8	14.9	1.8	1.8	1.3	2.9	1.8	6.9	3.4
3,6-DMPHEN	15.0	4.6	11.6	2.6	18.0	14.6	36.5	0.0	8.1	4.8	5.7	2.8	3.7	1.9	5.2	1.1	1.3	1.1	1.8	1.2	2.9	1.7
2,6-DMPHEN	13.0	5.2	9.3	2.0	16.1	13.9	28.1	6.3	6.6	3.2	4.7	2.4	3.3	1.4	4.8	1.0	0.9	0.9	1.4	1.0	2.7	1.2
1,7-DMPHEN	26.4	8.5	37.0	5.0	43.3	30.6	56.2	15.0	16.1	9.8	11.1	5.1	7.4	3.5	11.3	1.7	2.0	1.5	2.6	1.6	5.3	2.7
FLUA	251.7	296.6	179.5	94.5	226.9	209.8	193.4	39.7	29.3	15.8	42.8	27.9	43.4	30.4	78.2	10.2	6.0	5.5	31.4	16.2	35.6	16.9
PYR	224.3	317.0	172.6	89.1	183.6	188.9	233.0	36.8	30.9	15.6	33.8	21.0	30.5	18.9	55.1	7.5	5.0	5.4	27.8	11.3	29.3	12.6
RETENE	65.3	11.8	40.9	6.5	54.1	50.5	40.1	17.1	77.4	69.0	23.8	7.5	16.0	6.2	12.9	2.2	4.8	4.1	4.8	4.7	7.7	5.9
B(A)A	86.2	179.5	78.9	42.3	58.9	82.0	99.8	11.2	7.7	4.9	6.5	5.2	6.9	4.9	8.6	1.4	0.9	1.0	9.7	2.8	6.4	2.4
CHRY	201.0	298.3	150.7	74.2	195.5	243.5	548.1	64.2	29.9	16.4	35.2	23.5	36.2	25.7	40.3	5.7	3.6	3.2	22.1	8.9	22.7	9.1
B(BJK)	489.1	949.5	331.9	197.0	366.8	540.5	670.6	109.5	73.3	41.5	58.0	43.6	69.9	58.7	78.5	11.9	10.8	10.3	51.4	21.6	42.3	18.7
B(E)P	159.4	374.8	130.9	76.8	150.5	224.8	447.8	58.1	35.0	16.0	22.2	16.4	26.9	19.3	30.8	4.2	4.7	3.3	20.0	7.3	17.1	6.7
B(A)P	128.3	372.1	137.1	68.8	91.3	153.5	159.5	20.1	18.1	8.4	10.3	8.5	12.8	8.4	12.7	2.7	2.3	2.4	16.9	4.6	7.1	3.1
PERY	65.9	95.5	44.6	19.7	38.1	48.9	38.9	8.0	20.4	7.6	3.5	3.0	4.9	4.3	5.1	1.3	0.0	0.0	4.5	1.6	2.0	1.3
IND	286.7	256.2	128.5	69.2	155.9	222.9	246.6	43.4	48.9	18.6	22.2	15.9	27.4	19.0	26.4	5.4	4.8	4.0	16.9	8.3	15.3	10.1
DIBE	30.0	15.3	10.6	8.2	17.6	19.2	53.0	7.1	3.5	3.1	3.1	3.1	2.8	3.3	5.0	2.1	0.0	0.0	2.8	2.3	2.4	2.4
B(GHI)	211.7	257.0	127.8	56.6	165.4	251.4	288.4	45.0	49.4	16.3	22.5	13.2	24.5	14.0	26.6	3.7	3.0	2.1	16.7	5.5	14.1	5.5
COR	64.3	68.2	36.3	15.3	57.8	88.2	47.0	11.6	18.3	8.0	14.7	8.6	13.1	7.8	15.0	3.1	2.7	2.5	8.0	4.1	8.4	4.0
∑alkyl/parent-PAHs	3336.0	3852.3	2958.4	1015.3	3413.2	3354.6	5913.2	1104.1	1022.2	481.5	763.5	451.5	696.9	424.9	1468.0	189.2	118.0	104.3	399.6	206.2	593.4	317.8

Table E-3c continued.

OPAHs	A1	A2	B1	B2	C1	C2	D1	D2	E1	E2	F1	F2	G1	G2	H1	H2	I1	I2	J1	J2	K1	K2
1-INDA	32.0	5.9	6.6	20.6	12.3	7.4	27.6	22.1	26.9	15.4	35.0	11.6	30.7	10.2	2.1	1.6	13.5	2.3	9.5	9.4	25.2	29.6
1,4-NQ	2.0	0.6	4.0	0.4	5.0	3.6	6.7	2.3	1.6	0.7	0.8	0.7	0.8	0.8	2.8	0.0	0.0	0.0	0.4	0.3	0.7	0.5
1-NLD	14.4	3.1	4.0	2.4	17.9	11.2	20.6	9.0	7.1	3.4	7.3	3.9	5.6	3.1	14.0	1.9	2.1	1.4	2.5	2.2	5.3	4.1
2-BPCD	0.7	0.4	0.0	0.4	8.8	0.4	15.2	0.4	24.6	0.4	0.4	0.4	0.4	0.4	5.4	0.0	0.4	0.0	0.3	0.4	0.4	0.4
9-FLO	315.8	62.3	409.6	33.7	466.3	252.0	810.0	117.4	107.3	41.2	112.7	53.3	104.5	43.3	349.0	34.0	20.2	13.8	36.7	35.9	79.6	42.5
1,2-ACQ	9.1	3.7	14.0	3.5	5.6	5.9	9.6	5.7	4.5	2.6	3.7	3.4	4.2	2.9	6.7	0.0	3.2	0.0	3.2	3.2	3.5	3.5
9,10-ANQ	376.0	106.9	292.6	44.3	368.7	315.2	958.7	82.3	67.0	31.6	118.5	53.6	90.0	52.9	154.9	23.4	14.7	11.8	36.2	24.9	39.6	41.1
1-SALYD	44.3	6.8	19.7	6.7	20.5	13.5	29.0	13.0	20.1	7.4	17.2	7.2	17.1	6.9	7.0	4.6	4.3	4.6	10.7	7.0	15.8	7.0
2-NAPHTHOL	7.5	0.0	3.8	0.0	4.0	4.2	4.1	0.0	0.0	0.0	3.8	0.0	3.6	3.6	7.1	0.0	0.0	0.0	0.0	0.0	3.7	3.6
2-PP	10.7	0.0	7.1	3.4	7.7	5.0	11.4	3.1	5.6	3.1	6.3	3.9	5.1	3.6	7.6	3.4	3.3	3.4	0.0	0.0	4.3	4.0
1-ACENOL	0.0	0.0	0.0	4.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9-FLUORENOL	0.0	0.0	0.0	2.3	0.0	0.0	0.0	0.0	0.0	0.0	2.6	2.3	0.0	2.2	0.0	0.0	0.0	0.0	0.0	0.0	2.4	2.2
∑OPAHs	812.7	189.7	761.2	122.2	916.8	618.4	1892.8	255.2	264.9	105.8	308.1	140.4	262.1	130.0	556.6	68.9	61.7	37.3	99.4	83.2	180.4	138.5

Table E-3d: Concentrations [ng g^{-1}] of alkyl/parent-PAHs and OPAHs in soils from Bratislava. See Fig. D-1, Tab. D-1 for description of sampling sites/location.

Samling site	BA-05/1	BA-05/2	BA-05/3	BA-05/4	BA-06/1	BA-06/2	BA-06/3	BA-07/1	BA-07/2	BA-08/1	BA-08/2	BA-08/3	BA-08/4	BA-08/5	BA 09/1	BA-09/2	BA-09/3
Depth (cm)	0-23	23-60	70-96	> 96	0-20	35-50	50-72	0-25	50-120	0-2	8-18	18-66	66-94	>94	0-38	38-54	70-96
NAPH	258.2	142.1	44.6	15.5	71.5	36.3	6.8	118.5	68.8	496.8	191.5	247.4	36.6	262.5	629.7	228.2	20.2
2-MNAPH	94.3	51.6	19.3	8.3	27.3	18.0	3.3	46.5	25.7	159.9	72.4	91.5	14.5	79.5	218.0	91.0	12.1
1-MNAPH	76.4	40.0	14.6	6.2	23.8	13.4	2.4	40.4	23.2	90.6	48.1	65.9	10.3	67.2	148.1	71.9	11.1
BP	21.5	13.5	4.6	2.5	9.0	5.9	1.0	16.4	10.8	56.5	22.2	29.7	5.4	23.8	50.5	27.2	4.6
1,3-DMNAPH	17.4	16.3	5.4	4.1	15.8	9.1	2.2	26.6	8.9	44.7	25.9	51.0	8.2	35.6	46.2	22.2	9.4
ACENY	1.0	0.8	0.1	0.1	1.3	0.3	0.2	0.7	0.5	14.3	4.2	9.4	1.3	6.2	2.7	3.1	4.9
ACEN	5.7	2.0	0.8	0.4	3.9	1.2	0.4	4.4	2.3	18.6	8.4	23.4	4.7	14.0	11.6	7.7	33.4
2,3,5-TMNAPH	10.5	6.5	3.3	2.4	8.9	4.0	1.3	10.4	7.5	15.6	8.5	19.2	5.1	15.4	21.1	14.1	337.0
FLUO	5.1	3.0	1.3	0.9	4.6	2.1	0.5	5.7	2.8	20.0	10.8	35.7	5.4	15.8	10.7	7.4	114.7
2,6-DIISOPNAPH	22.8	20.1	13.1	9.6	31.0	10.5	7.5	19.2	14.8	17.5	19.9	13.0	11.0	20.9	38.5	24.4	1601.1
PHEN	144.1	122.8	64.1	35.4	133.4	92.0	26.9	126.2	92.3	1158.2	314.4	619.7	145.5	438.8	255.8	200.9	94.3
ANTH	55.0	45.3	19.1	8.0	50.2	31.4	13.4	46.9	31.7	106.6	131.3	48.1	55.4	100.6	50.4	80.4	40.7
2-MPHEN	19.5	14.4	9.0	4.2	22.9	11.7	6.0	19.3	11.9	773.0	99.4	163.5	41.6	95.6	46.0	29.6	13.7
1-MPHEN	4.7	3.3	2.1	1.0	5.9	2.8	1.6	4.8	2.7	302.8	26.6	36.6	12.8	26.7	12.5	8.1	3.4
3,6-DMPHEN	1.5	1.0	0.9	0.5	2.1	0.9	1.0	1.7	0.9	105.0	10.2	11.7	5.0	8.0	3.3	2.0	1.6
3,5-DMPHEN	1.8	0.5	0.0	0.0	2.8	2.4	0.9	0.0	0.0	172.6	7.0	10.0	13.7	4.5	11.9	2.2	1.4
2,6-DMPHEN	1.6	1.0	0.6	0.4	1.9	0.9	0.7	1.6	1.0	173.0	17.9	17.6	6.1	10.4	3.6	2.4	0.9
1,7-DMPHEN	3.4	1.9	1.1	0.6	3.5	1.5	1.1	3.7	1.9	239.8	23.6	27.7	8.4	15.9	9.5	6.0	1.6
FLUA	40.0	18.4	5.7	1.8	64.7	13.1	10.9	43.5	17.2	59130.9	1048.8	1309.5	322.1	503.1	142.0	96.2	322.7
PYR	49.2	18.7	3.1	0.7	69.5	11.7	11.2	46.5	18.4	78660.7	1356.0	1640.3	407.5	558.0	156.4	91.3	107.9
RETENE	50.3	33.2	32.5	8.8	40.2	19.5	19.0	22.5	24.4	325.4	34.5	57.3	23.8	54.9	51.4	41.1	2668.8
B(A)A	27.6	8.4	0.3	0.2	30.0	3.6	3.8	21.1	6.6	19617.5	717.0	780.0	171.3	263.9	76.3	39.5	1.3
CHRY	48.9	14.7	1.0	0.3	50.7	7.5	6.3	34.8	13.5	39706.7	1102.3	1221.7	271.7	428.6	127.2	80.5	2.7
B(BJK)	60.5	21.0	3.5	1.2	540.9	19.9	8.0	25.3	30.9	14281.5	1917.3	2035.0	276.9	462.5	166.2	110.0	259.1
B(E)P	63.7	13.0	1.1	0.3	562.5	7.3	6.5	37.9	42.5	0.0	990.5	1210.3	237.7	428.3	140.0	91.7	120.0
B(A)P	67.9	13.5	0.8	0.3	235.4	5.5	6.3	28.7	12.1	4249.4	871.5	1394.7	235.6	415.8	151.9	68.2	0.6
PERY	13.5	2.7	0.7	0.2	153.4	1.7	1.5	6.4	4.6	8147.2	213.8	325.9	52.2	75.4	20.8	15.8	26.1
IND	36.4	16.1	1.0	0.7	33.4	6.8	4.4	38.8	16.8	2776.6	675.6	814.1	155.8	273.9	93.7	57.8	6.5
DIBE	1.4	1.5	0.7	0.3	4.3	1.3	0.8	4.6	2.2	418.6	96.4	95.8	33.8	29.6	6.8	6.1	1.5
B(GHI)	33.1	10.0	0.7	0.2	30.9	4.3	3.6	23.5	11.4	11390.4	640.7	805.6	137.8	248.7	80.6	44.0	3.2
COR	28.6	6.4	1.4	0.9	16.8	3.4	2.1	15.9	7.4	2199.3	184.9	219.2	47.9	78.7	45.5	44.2	4.4
∑alkyl/parent-PAHs	1265.4	663.9	256.4	116.1	2252.6	350.1	161.5	842.4	515.6	244869.5	10891.5	13430.4	2764.8	5062.8	2828.8	1615.2	5831.0

Table E-3d continued.

Sampling site	BA-10/1	BA-10/2	BA-10/3	BA 10/4	BA-11/1	BA-11/2	BA-12/1	BA-12/2	BA-12/3	BA-13/1	BA-13/2	BA-13/3	BA-13/4	BAk-21	BAk-22
Depth (cm)	0-30	30-75	75-102	>102	0-8	19-50	0-42	42-70	>70	0-48	48-90	90-120	> 120	surface	surface
NAPH	135.9	87.6	59.9	24.8	425.0	46.3	162.4	34.4	1.5	210.6	138.3	33.8	2.8	137.3	258.5
2-MNAPH	54.9	32.6	25.3	13.2	171.3	20.8	61.1	16.6	1.2	71.6	43.0	12.9	2.4	47.5	83.8
1-MNAPH	46.0	26.8	23.9	10.9	135.3	17.1	48.2	12.9	0.9	58.2	32.9	9.3	2.0	43.5	68.3
BP	19.6	15.4	8.2	4.2	55.7	6.4	21.4	5.5	0.4	27.7	16.1	4.9	0.9	18.7	24.0
1,3-DMNAPH	26.1	19.9	17.9	7.8	58.7	9.0	27.4	8.4	0.9	44.9	19.5	7.1	2.0	39.1	31.6
ACENY	1.7	0.2	0.2	0.3	3.2	0.6	3.2	2.1	0.2	5.9	1.7	0.3	0.1	5.8	3.7
ACEN	4.7	2.8	1.5	0.9	12.8	1.8	8.7	2.6	0.2	63.3	3.3	1.0	0.3	19.4	11.4
2,3,5-TMNAPH	9.6	8.2	6.5	3.1	17.6	3.0	11.3	6.1	0.9	21.9	7.4	3.6	1.1	18.3	10.8
FLUO	4.6	4.7	2.4	1.1	12.1	1.6	13.1	2.8	0.2	53.9	4.1	2.4	0.7	19.9	10.6
2,6-DIISOPNAPH	17.9	21.0	22.4	18.0	61.0	23.2	26.5	30.0	13.2	25.4	31.2	14.8	9.1	34.5	30.0
PHEN	134.3	115.8	87.3	59.8	332.0	81.8	356.8	90.0	12.5	882.4	105.7	74.6	25.4	466.3	275.4
ANTH	51.2	42.5	29.3	17.0	139.2	26.9	150.5	30.4	5.7	71.3	37.7	23.7	11.2	194.7	113.7
2-MPHEN	19.8	12.5	9.3	8.8	47.3	12.4	60.5	15.8	2.3	236.2	22.5	10.8	4.3	123.1	63.9
1-MPHEN	5.6	2.9	2.1	1.9	12.0	3.1	15.4	2.9	0.4	47.8	5.6	2.4	1.0	32.1	16.5
3,6-DMPHEN	1.5	1.1	0.9	0.9	3.3	1.4	4.5	1.7	0.5	11.4	1.8	1.0	0.5	12.0	8.3
3,5-DMPHEN	2.7	3.1	0.9	0.4	2.9	1.4	2.1	2.2	0.5	23.3	1.1	0.5	0.4	7.9	5.2
2,6-DMPHEN	1.7	0.9	0.5	0.7	4.1	1.1	5.0	1.5	0.3	23.0	2.0	0.8	0.5	13.0	7.6
1,7-DMPHEN	3.8	1.4	0.9	1.1	7.6	1.9	9.3	2.5	0.3	27.9	4.4	1.4	0.6	25.7	12.6
FLUA	57.9	7.9	4.2	13.1	231.6	27.4	294.3	36.9	2.8	876.3	54.6	18.2	4.7	567.5	515.8
PYR	68.5	5.9	2.8	12.2	245.6	34.1	314.1	50.4	2.2	923.0	59.4	14.5	2.4	566.1	574.5
RETENE	21.0	19.8	17.1	24.6	37.2	24.5	21.1	27.3	21.9	46.7	19.0	16.5	13.4	58.3	60.8
B(A)A	35.6	0.9	0.4	1.6	87.7	13.5	94.3	23.3	0.6	459.2	28.2	2.9	0.3	211.3	213.1
CHRY	59.2	1.9	0.9	3.2	251.7	39.1	235.2	40.5	1.0	738.2	52.0	6.6	0.9	440.6	516.6
B(BJK)	96.9	4.4	7.2	9.2	270.2	28.5	199.3	823.8	5.5	1350.8	42.4	10.1	1.7	447.5	310.8
B(E)P	71.9	2.1	5.1	4.6	250.3	32.6	223.5	341.6	4.7	1035.7	50.8	3.3	0.6	323.5	386.6
B(A)P	85.8	1.7	1.3	1.1	195.9	21.5	188.8	177.5	0.0	1133.6	39.8	1.9	0.7	286.2	238.6
PERY	13.6	0.6	0.5	0.7	31.2	3.7	34.5	16.2	0.0	221.8	8.7	0.6	0.3	59.8	55.6
IND	49.2	2.3	1.7	1.5	121.3	21.9	130.4	40.1	0.0	431.8	44.3	3.4	0.8	257.6	213.5
DIBE	2.5	0.7	0.7	0.7	5.7	1.2	12.9	1.7	0.0	40.7	5.8	1.0	0.6	30.5	17.8
B(GHI)	46.2	1.3	0.8	0.6	126.4	17.2	124.5	37.4	1.0	477.0	30.0	1.9	0.2	260.2	192.2
COR	36.9	1.8	1.7	1.2	57.8	8.3	50.6	23.3	0.0	200.9	29.0	1.5	0.8	61.7	61.4
Σalkyl/parent-PAHs	1186.7	450.5	343.9	249.1	3413.6	533.3	2910.9	1908.3	81.9	9842.1	942.3	287.8	92.6	4829.6	4393.0

Table E-3d continued.

Sampling site	BA-05/1	BA-05/2	BA-05/3	BA-05/4	BA-06/1	BA-06/2	BA-06/3	BA-07/1	BA-07/2	BA-08/1	BA-08/2	BA-08/3	BA-08/4	BA-08/5	BA 09/1	BA-09/2	BA-09/3
Depth (cm)	0-23	23-60	70-96	> 96	0-20	35-50	50-72	0-25	50-120	0-2		18-66	66-94	>94	0-38	38-54	70-96
1-INDA	53.0	30.6	30.4	22.8	20.3	22.9	14.6	43.6	20.3	71.8	31.0	26.5	30.1	43.8	52.1	38.5	45.5
1,4-NQ	3.1	1.2	1.4	1.0	1.2	1.1	1.6	2.6	2.5	15.7	3.0	4.1	1.4	2.8	4.2	5.0	1.5
1-NLD	7.5	4.9	6.7	3.0	2.1	2.1	1.7	4.6	3.4	9.3	3.4	6.2	2.5	5.2	6.4	7.9	3.1
2-BPCD	8.4	2.3	8.8	0.5	8.1	2.9	1.5	1.8	10.2	7.2	2.8	4.7	0.7	4.3	5.3	1.7	4.6
9-FLO	43.9	25.3	31.0	18.6	24.0	23.1	25.3	30.2	21.3	218.5	54.3	84.9	30.9	81.1	51.7	44.2	30.1
1,2-ACQ	3.4	3.2	2.6	1.6	2.4	2.4	2.0	4.4	3.3	25.0	6.4	7.7	2.9	4.1	5.1	5.5	2.1
9,10-ANQ	29.1	8.4	14.1	2.7	20.0	8.9	9.2	31.2	11.0	729.3	100.4	187.7	40.0	85.1	38.5	38.6	11.2
1,8-NAA	3.9	1.4	1.8	1.0	1.6	1.3	1.0	5.5	2.7	88.8	18.2	19.6	6.8	8.2	8.1	10.0	2.3
7,12-B(A)A	21.2	4.6	0.0	0.5	10.5	3.6	5.2	20.4	9.7	1475.7	153.0	92.5	38.2	59.8	29.3	38.5	3.7
2-SALYD	2.4	3.4	1.7	2.1	0.8	2.4	0.4	6.6	3.3	35.8	3.1	4.7	4.8	4.7	7.1	3.5	1.6
2-NAPHTHOL	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2-PP	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	4.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0
1-ACENOL	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.5	0.0	0.6	0.0	0.0	0.0	0.0	0.0
9-FLUORENOL	0.0	0.0	0.0	0.1	0.2	0.0	0.0	0.0	0.0	2.5	0.0	0.2	0.0	0.0	0.0	0.0	0.4
∑OPAHs	176.0	85.2	98.6	54.0	91.7	70.8	62.3	150.9	87.7	2691.7	375.6	439.3	158.3	299.1	208.0	193.4	106.0

Sampling site	BA-10/1	BA-10/2	BA-10/3	BA 10/4	BA-11/1	BA-11/2	BA-12/1	BA-12/2	BA-12/3	BA-13/1	BA-13/2	BA-13/3	BA-13/4	BAk-21	BAk-22
Depth (cm)	0-30	30-75	75-102	>102	0-8	19-50	0-42	42-70	>70	0-48	48-90	90-120	> 120	surface	surface
1-INDA	19.1	68.4	22.2	20.1	84.2	44.9	51.1	24.6	14.3	48.5	35.6	39.2	23.8	41.8	60.8
1,4-NQ	1.1	0.9	1.1	0.9	5.7	1.9	3.7	1.4	0.8	8.3	1.7	1.6	1.6	6.9	4.4
1-NLD	2.0	3.5	2.8	2.5	6.2	2.6	3.6	2.7	0.8	5.8	4.1	2.9	1.1	7.8	3.9
2-BPCD	7.6	5.7	0.8	4.5	10.2	5.3	3.2	1.0	1.3	11.3	4.1	1.1	0.4	8.5	5.4
9-FLO	22.6	29.4	24.9	27.9	65.0	32.2	65.5	26.0	10.3	72.6	23.0	25.3	22.1	79.7	64.2
1,2-ACQ	2.3	3.3	2.7	2.7	38.1	4.3	7.6	2.9	1.5	8.3	2.8	2.2	0.8	23.4	7.9
9,10-ANQ	18.8	6.4	4.2	10.3	84.8	25.1	98.0	22.1	3.5	202.9	21.4	10.2	6.8	89.6	113.6
1,8-NAA	1.5	1.1	2.1	1.3	21.3	5.6	20.6	2.2	0.7	38.6	3.9	1.4	1.0	22.8	35.5
7,12-B(A)A	9.9	1.0	2.2	2.5	106.7	22.5	64.9	15.2	0.6	105.6	17.9	1.4	6.3	60.3	126.7
2-SALYD	2.7	14.5	0.2	1.8	4.0	3.8	4.3	0.9	0.0	6.3	2.5	4.5	1.1	17.3	7.0
2-NAPHTHOL	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	2.7	0.1
2-PP	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.4
1-ACENOL	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.7	0.0	0.0	0.0	0.0	10.9
9-FLUORENOL	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.5	0.0
∑OPAHs	87.6	134.2	63.3	74.5	426.2	148.1	323.3	99.0	33.8	508.9	116.9	89.9	65.1	363.4	441.0

Curriculum vitae

Personal details:

Name: Benjamin A. Musa Bandowe
Date of birth: 02/03/1976
Nationality: Ghanaian
Tel: +49-015207224028
Email: bandowe@yahoo.com
Address: Hechtsheimer Strasse 37a, /WNr. 1222, 55131 Mainz, Germany

Educational background:

2006-2010 **PhD in Chemistry**

University of Mainz, Germany

PhD thesis: Oxygen-containing polycyclic aromatic hydrocarbons (OPAHs) and their parent-PAHs in soil: Analysis, sources and fate.

Date of PhD examination: 29/04/2010

2002-2005 **Master of Science in Chemistry**

Braunschweig University of Technology (TU Braunschweig), Germany

Master thesis: Development and application of an analytical method to determine oxygenated volatile organic compounds (OVOC) in the atmosphere.

2000–2001 **Master of Philosophy in Chemistry (Part 1)**

University of Ghana, Legon-Accra, Ghana

1995–1999 **Bachelor of Science in Chemistry**

University of Ghana, Legon-Accra, Ghana

Working experience:

2006-2009 **Junior Researcher**

Institute of Geography, University of Mainz, Germany

12/2004-5/2005 **Student Research Assistant**

Institute for Chemistry and Dynamics of the Geosphere, Institute II: Troposphere, Research Centre Jülich, Germany

1999-2001 **Teaching and Research Assistant**

Department of Chemistry, University of Ghana

Memberships

Society of Environmental Toxicology and Chemistry (Europe, Africa, UK and German language branches)

German Soil Science Society

Languages:

English: Native speaker

German: Intermediate

Computer skills:

Microsoft office (Word, Excel, PowerPoint etc), Endnote, SPSS, Chemwin,Chemdraw

Hobbies:

Playing tennis, soccer and solving puzzles

Peer-refereed publications

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