



# Microbial formation and degradation of oxygen-containing polycyclic aromatic hydrocarbons (OPAHs) in soil during short-term incubation



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## ABSTRACT

We tested whether OPAHs were formed during 19-wk incubation of a fertile soil at optimum moisture in the dark. The soil had initial mean ( $\pm$ s.e.,  $n = 3$ ) concentrations of  $22 \pm 1.7$  ( $\Sigma 28$ PAHs) and  $4.2 \pm 0.34$   $\mu\text{g g}^{-1}$  ( $\Sigma 14$ OPAHs). After 19 wk, individual PAH and OPAH concentrations had decreased by up to 14 and 37%, respectively. Decreases in % of initial concentrations were positively correlated with their  $K_{OW}$  values for PAHs ( $r = 0.48$ ,  $p = 0.022$ ) and 9 OPAHs ( $r = 0.78$ ,  $p = 0.013$ ) but negatively, albeit not significantly, for 5 OPAHs ( $r = -0.75$ ,  $p = 0.145$ ) suggesting net formation of some OPAHs. The latter was supported by significantly increasing 1-indanone/fluorene ratios while the other OPAH to parent-PAH ratios remained constant or tended to increase. We conclude that OPAHs are formed in soils during microbial turnover of PAHs in a short time.

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## 1. Introduction

Reported close correlations between concentrations of PAHs and their oxygen-containing PAH derivatives (OPAHs) suggest that OPAHs are similarly ubiquitous in the environment as their parent-compounds, the polycyclic aromatic hydrocarbons (PAHs) (Bandowe et al., 2010, 2011; Lundstedt et al., 2007). OPAHs are nevertheless not considered as priority pollutants (e.g., US EPA, 2008) in spite of their partly even higher toxicity (Lampi et al., 2006; Lundstedt et al., 2007), bioavailability (Matscheko et al., 2002) and mobility (Weigand et al., 2002; Lundstedt et al., 2007) in soil compared with the PAHs which are classified as priority pollutants. Because OPAHs are not included in environmental legal regulations, they are frequently not determined and thus not considered when e.g., the success of a remediation effort and natural attenuation of PAH-contaminated sites is assessed.

Soil is the major storage compartment of PAHs in the environment (Wild and Jones, 1995). Therefore, it can be expected that soils host many OPAHs. Previous work mainly focused on former gasworks, coking, wood preservation, and industrial sites (Meyer et al., 1999; Oda et al., 2001; Lundstedt et al., 2007; Biache et al., 2008; Bandowe et al., 2010, 2011). There are also a few reports on urban soils illustrating that many different OPAHs are present at considerable concentrations. Some OPAHs such as 1-indanone, 9-

fluorenone or 9,10-anthraquinone even occurred at higher concentrations in urban soils than their parent-PAHs fluorene and anthracene (Niederer, 1998; Oda et al., 2001; Bandowe et al., 2011). The possible contamination of urban soils with OPAHs is of particular importance for the urban population because of the close contact to soil e.g., via inhalation of soil-derived dusts, direct ingestion by playing little children or urban gardening.

OPAHs in the environment originate directly from combustion sources (primary sources) and photochemical and microbial degradation of parent-PAHs (Vione et al., 2004; Bamforth and Singleton, 2005; Lundstedt et al., 2007). Little is known about the kinetics, mechanism and formation/accumulation of OPAHs from microbial degradation of aged PAHs in soils that have not been bioaugmented or biostimulated (i.e. intrinsic biodegradation of aged PAHs in soil) (Wischmann and Steinhart, 1997; Lundstedt et al., 2003; Lohs and Mossmann, 2005). This knowledge is, however, particularly important with respect to the assessment of toxicity and to judge the success of remediation and natural attenuation of PAH-contaminated soils which might remove parent-PAHs but produce the more toxic and mobile OPAHs. The higher water solubility, higher bioavailability and higher susceptibility to abiotic reactions (photolysis, photochemical and chemical reactivity) suggest that OPAHs may be faster degraded in soil than their related parent-PAHs. Previous studies however indicate that some OPAHs are persistent dead-end metabolites probably stabilized by strong interaction of their polar functional groups with soil solid phase (Lundstedt et al., 2003, 2007; Novoszad et al., 2005).

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**Table 1**  
Physico-chemical and biological properties of the study soil.

Water content [%]	28.09
pH	6.84–6.93
Sand [%]	49.9
Silt [%]	41.4
Clay [%]	8.6
Organic C [mg g <sup>-1</sup> ]	49.1
Inorganic C [mg g <sup>-1</sup> ]	6.6
Total N [mg g <sup>-1</sup> ]	4.8
C/N	10.2
Total S [mg g <sup>-1</sup> ]	0.8
Initial total microbe cells [CFU/g dry soil]	66 × 10 <sup>6</sup>

Therefore, the assessment of the microbial formation of OPAHs in soil requires the consideration of the relationship between water solubility (and thus bioavailability) of the OPAHs as approximated by the octanol–water partitioning coefficients ( $K_{OW}$ ) and their degradation/formation kinetics and the OPAH/parent-PAH ratios (Matscheko et al., 2002; Lundstedt et al., 2003; Maliszewska-Kordybach, 2005; Thiele-Bruhn and Brümmer, 2005). The rate of degradation of a polycyclic aromatic compound (PAC) in soil depends on the inherent degradability, degree of aging and the compound's bioavailability (Semple et al., 2003). A dissipation rate of OPAHs smaller than suggested by their  $K_{OW}$  values and an increasing OPAH to related parent-PAH concentration ratio over a study period can be considered as a first indication of the formation of OPAHs (Lundstedt et al., 2003).

Our objective was to determine to which degree OPAHs are degraded and/or formed from their parent-PAHs during short-term incubation (19 weeks) of an urban soil sample. We assume that OPAHs are mainly formed from microbial conversion of parent-PAHs although we are aware that some OPAHs could be formed from other precursor compounds present in soil organic matter (Uchimiya and Stone, 2009). We hypothesized that (i) the extent of degradation of PAHs and OPAHs is related to the  $K_{OW}$  value, (ii) there will, however, be some compounds which are much less net degraded than expected from their  $K_{OW}$  value or even net formed, and (iii) that incubation of soil leads to increasing OPAH/parent-PAH ratios in spite of the higher degradability of OPAHs because OPAHs are microbially produced.

## 2. Materials and methods

### 2.1. Soil sampling and soil properties

We sampled topsoil (0–10 cm) in a Park area under grassland in the city of Berne, Switzerland (N 46°57.044', E 007°26.176'). The soil was formed from an urban deposit consisting of a mixture of native moraine material of the last glacial age (Wuerm) and urban debris. The sample was sieved in field-fresh state and then separated into one aliquot for incubation, a second aliquot which was frozen as reference and a third aliquot which was air-dried for basic characterization. We determined the equilibrium pH in a soil:solution suspension (v:v, 1:2.5) with a glass electrode (Sentix HWS, WTW GmbH, Weinheim, Germany) and total C concentrations and C:N ratios with an elemental analyzer (vario EL, Elementar Analysensysteme, Hanau, Germany). Organic C concentrations were calculated as difference between the total C concentration before and after combustion of organic matter in a muffle oven at 560 °C. Texture analysis was carried out on dry and sieved (<2 mm) samples. For texture analysis, organic matter was oxidized with 30% H<sub>2</sub>O<sub>2</sub> and micro-aggregates <63 μm were dispersed with Na<sub>2</sub>CO<sub>3</sub>. The sand fractions (63–2000 μm) were separated by wet sieving and the silt (2–63 μm) and clay (<2 μm) fractions were quantified using a SediGraph 5100 (Micromeritics, Norcross, GA, USA, Ryzak and Bieganowski, 2011). Furthermore, we estimated soil microbial activity by determining the number of viable heterotrophic bacteria with the plate count method (Andreoni et al., 2004). Serial dilutions of soil extract were spread on R2A-agar plates, incubated at 21 ± 2 °C and colonies were counted after 6 days. A summary of the physical, chemical and biological properties of the soil is shown in Table 1.

### 2.2. Incubation

Fifty grams of field-fresh and sieved (<2 mm) soil were placed in beakers ( $n = 3$ ), closed with parafilm, and incubated in the dark at 21 ± 2 °C (microcosms). On a weekly basis, each microcosm was aerated, water content checked, and if necessary, gravimetrically adjusted with sterile deionized water. Concentrations of 28 PAHs and 14 OPAHs were determined in three replicate frozen reference samples together with replicate incubated samples ( $n = 3$ ) after 19 ( $n = 3$ ) weeks. Each replicate of the frozen reference samples was analyzed in duplicate and of the incubated samples in triplicate depending on sample size.

### 2.3. OPAH and PAH analysis

We determined the concentrations of 14 carbonyl-OPAHs including 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), 4H-cyclopenta[d,e,f]phenanthrene-4-one (CPHENone), 2-methyl-9,10-anthraquinone (2-MANQ), benzo[a]fluorenone (B(A)FLUone), 7H-benz[d,e]anthracene-7-one (BANTone), benzo[a]anthracene-7,12-dione (7,12-B(A)A), 5,12-naphthacenequinone (5,12-NACQ), 6H-benzo[c,d]pyrene-6-one (B(P)Rone) and 28 PAHs including naphthalene (NAPH), 2-methylnaphthalene (2-MNAPH), 1-methylnaphthalene (1-MNAPH), biphenyl (BP), 1,3-dimethylnaphthalene (1,3-DMNAP), acenaphthylene (ACENY), acenaphthene (ACEN), fluorene (FLUO), phenanthrene (PHEN), anthracene (ANTH), 1-methylphenanthrene (1-MPHEN), 3,6-dimethylphenanthrene (3,6-DMPHEN), fluoranthene (FLUA), pyrene (PYR), 1-methyl-7-isopropylphenanthrene (retene, RET), 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).

Fifteen grams of each sample was extracted by pressurized liquid extraction using an ASE 200 (Dionex, Sunnyvale, CA, USA). Each sample was extracted with CH<sub>2</sub>Cl<sub>2</sub>, followed by a second cycle of extraction using CH<sub>3</sub>COCH<sub>3</sub>/CH<sub>2</sub>Cl<sub>2</sub>/CF<sub>3</sub>COOH (250:125:1 v/v/v). The extracts from each sample were combined, rotary evaporated and cleaned up/fractionated on a silica gel column into OPAH and PAH fractions. Details of ASE extraction conditions were as outlined in Bandowe and Wilcke (2010). The target compounds were separated and quantified with an Agilent 7890A gas chromatograph coupled to an Agilent 5975C mass spectrometer in the electron impact ionization mode. Seven deuterated-PAHs (NAPH-D<sub>8</sub>, ACEN-D<sub>10</sub>, PHEN-D<sub>10</sub>, PYR-D<sub>10</sub>, CHRY-D<sub>12</sub>, PERY-D<sub>12</sub>, B(GHI)-D<sub>12</sub>) and benzophenone-D<sub>5</sub> were added to soils before extraction as internal standards for quantification of PAHs and OPAHs, respectively. Sample extraction, clean-up/fractionation and instrumental analysis followed the earlier reported protocol (Bandowe and Wilcke, 2010; Bandowe et al., 2011).

### 2.4. 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, two blanks made of inert bulk sorbent (Isolute HM-N, Biotage, Upsalla) were processed simultaneously with each batch of samples. The concentrations of all target compounds in the blanks were on average 1.2% of the NAPH concentrations at the beginning of the experiment and <0.5% of the concentrations of all other compounds. The precision as assessed by the relative standard deviations (RSDs) of the duplicate or triplicate samples was 2–28% and 4–53% for the 28 PAHs and 14 OPAHs, respectively. However, DIBE was quantified with an elevated uncertainty because the RSD of the duplicate frozen control samples was only 40%. The accuracy of our measurement of PAH concentrations was checked by analyzing the certified European reference material ERM<sup>®</sup>-CC013a-Polycyclic aromatic hydrocarbons in soil (BAM, Berlin, Germany) analyzed in duplicate. Mean recovery of the compounds, for which certified and indicator values were provided, was 106% ranging from 41% (FLUA) to 180% (B(BJK)), which in our analyses included three compounds (b, j and k) but for only two reference values are certified, i.e. b and k. To confirm the accuracy of the OPAH measurements we compared the concentrations of the OPAHs to the published concentration of some of these OPAHs in the certified reference material-ERM<sup>®</sup>-CC013a. OPAHs in the ERM<sup>®</sup>-CC013a (measured together with our samples) averaged 83% of the previously published concentrations (Bandowe and Wilcke, 2010). The individual recoveries ranged from 71 to 117% except 2-BPCD which was 33% of the published concentration. Furthermore the mean recovery of benzophenone-D<sub>5</sub> spiked to samples before extraction and used as internal standard for the OPAHs was 67% (50–95%).

### 2.5. Calculations and compound properties

The sum of the concentrations of all 28 PAHs was termed  $\sum$ 28PAHs, that of the 16 priority PAHs of the U.S. Environmental Protection Agency (US EPA, 2008)  $\sum$ 16EPA-PAHs and that of 7 OPAHs  $\sum$ 7OPAHs. We calculated concentration ratios of

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