Bioavailability of polycyclic aromatic hydrocarbons in sediments: experiments and modelling

Haftka, J.J.H.

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Chapter 1

General introduction
Background

Polycyclic aromatic hydrocarbons (PAHs) are generated continuously by incomplete combustion of organic matter in forest fires, fossil fuels and in waste incineration. Diffuse pollution of combustion derived PAHs has resulted in the ubiquitous distribution of these compounds in remote and urban areas. On a local scale in urban regions, extensive coal gasification in most of the twentieth century has caused massive soil contamination with PAHs [1, 2]. Soil and sediment contamination by PAHs can pose a serious threat to ecosystems due to their toxic, mutagenic and carcinogenic properties. A proper assessment of the environmental risk of PAHs therefore depends on a thorough knowledge of the processes acting between the environmental compartments of soil, sediment, biota, water and air. In the past, risk assessment of PAH-polluted environments was performed by taking the total concentration of PAHs present in soils or sediments as a starting point for evaluating the need of site remediation. Sorption of PAHs to amorphous and condensed organic phases in soil or sediments emphasized the importance of distinguishing between bioavailable and total concentrations of PAHs. The bioavailable concentration of PAHs encountered in soils and sediments refers here to the fraction of total PAH concentration present in a soil or sediment that can be directly taken up by terrestrial and aquatic organisms [3]. The bioavailability of PAHs is known to be organism and species dependent [4] and is generally linked to fractions of these compounds that are readily bioavailable at one point in time. An increasing contact time between PAHs and soils or sediments will result in a decreased chemical and biological availability of PAHs, a process called ageing [5] that over time leads to a decrease in bioaccessibility. Bioaccessibility refers here to the amount that is potentially available to organisms, i.e. the integration of availability over time [6].

The bioavailability and bioaccessibility of PAHs in soils and sediments is determined by linear absorption or dissolution into amorphous organic carbon (AOC) and nonlinear, competitive adsorption to condensed organic matter such as black carbon (BC). The concept of dual domain sorption, i.e. absorption and adsorption, has been employed to explain elevated total organic carbon to water distribution coefficients ($K_{TOC}$), variable biota to sediment accumulation factors and limited bioremediation potential of PAHs in soils and sediments. It has been shown that BC is often responsible for over 90% of total soil and sediment sorption of PAHs at environmentally relevant concentrations (i.e. in the ng/L range) [7]. Rapid and slow desorption from these AOC and BC domains, respectively, results into 1. partitioning to dissolved organic matter (DOM) in the pore water, 2. degradation by microbial communities able to degrade PAHs or 3. uptake by terrestrial and aquatic organisms (Figure 1). A lower bioavailability of PAHs due to stronger sorption of PAHs to soil or sediment organic matter and BC will decrease the biodegradability of PAHs by microorganisms as well as the uptake by aquatic and terrestrial organisms. All processes depicted in Figure 1 centre around the freely dissolved concentration of PAHs. These freely dissolved concentrations form a better basis for
risk assessment than total soil and sediment contents and can be directly compared to water quality criteria [7].

Figure 1. Sketch of the distribution of PAHs between pore water, dissolved organic matter, soft amorphous organic matter and condensed organic matter (BC, black carbon) in soils and sediments along with the processes of biodegradation and uptake by terrestrial and aquatic organisms. The dots represent PAH molecules (taken from [7]).

The research presented in this thesis is focused on the processes that affect the environmental fate of PAHs: vaporization, dissolution, biodegradation, partitioning to DOM and adsorption to BC. This introduction will therefore provide a general background on physicochemical properties of PAHs, biodegradation of PAHs, partitioning of PAHs to DOM, molecular modelling to simulate thermodynamic properties of aromatic hydrocarbons and simulation of sorption of PAHs to environmental sorbents. The research questions and outline of this thesis will be presented at the end of this Introduction.
Physicochemical properties

PAHs are rigid aromatic compounds that behave in a consistent manner with regards to their physicochemical properties, depending on the size or molecular weight of the compound. The molecular structures of a number of representative PAHs are shown in Figure 2.

![Representative members of polycyclic aromatic hydrocarbons](image)

The hydrophobicity of PAHs, expressed in the *n*-octanol to water partition coefficient (*K*<sub>ow</sub>), increases as the molecular size of PAHs increases (Table 1). Concomitantly, the liquid vapour pressures, Henry’s Law constants and aqueous solubilities decrease with molecular size. These physicochemical properties therefore govern the environmental behaviour of PAHs. For example, the supercooled liquid vapour pressure is important in the calculation of the aqueous solubility of PAHs. After conversion of the supercooled liquid vapour pressure from the liquid to the solid phase using the entropy of fusion and the melting point temperature, the aqueous solubility of solid PAHs can be calculated from the molecular weight (*MW*), solid vapour pressure (*P*<sub>S</sub>) and Henry’s law constant (*K*<sub>H</sub>) via the following relationship: *c*<sub>S</sub> = *MW* *P*<sub>S</sub>/*K*<sub>H</sub>. Accurate values of these physicochemical properties are therefore required for the prediction of the environmental behaviour of PAHs. This behaviour is mostly determined by molecular interactions of PAHs in their pure or supercooled liquid state, but these interactions can not be determined directly from experiments because the compounds are solids at ambient temperatures. The supercooled liquid vapour pressures of PAHs can however be easily determined from gas chromatographic retention times [8].
Table 1. Physicochemical properties of polycyclic aromatic hydrocarbons at 298.15 K.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>MW (g/mol)</th>
<th>Tmp (K)</th>
<th>log ( K_{ow} )</th>
<th>log ( P_L )</th>
<th>( K_H ) (Pa m³/mol)</th>
<th>( c_S ) (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>C₁₀H₈</td>
<td>128.17</td>
<td>353.4</td>
<td>3.33</td>
<td>1.57</td>
<td>43</td>
<td>31.5</td>
</tr>
<tr>
<td>Fluorene</td>
<td>C₁₃H₁₀</td>
<td>166.22</td>
<td>387.9</td>
<td>4.18</td>
<td>-0.30</td>
<td>7.87</td>
<td>1.90</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>C₁₄H₁₀</td>
<td>178.23</td>
<td>372.4</td>
<td>4.57</td>
<td>-1.13</td>
<td>3.24</td>
<td>1.10</td>
</tr>
<tr>
<td>Anthracene</td>
<td>C₁₄H₁₀</td>
<td>178.23</td>
<td>488.9</td>
<td>4.68</td>
<td>-1.00</td>
<td>3.96</td>
<td>0.045</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>C₁₆H₁₀</td>
<td>202.25</td>
<td>383.3</td>
<td>5.23</td>
<td>-2.26</td>
<td>0.96</td>
<td>0.260</td>
</tr>
<tr>
<td>Pyrene</td>
<td>C₁₆H₁₀</td>
<td>202.25</td>
<td>423.8</td>
<td>5.18</td>
<td>-2.25</td>
<td>0.92</td>
<td>0.132</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>C₂₀H₁₂</td>
<td>252.31</td>
<td>454.2</td>
<td>6.13</td>
<td>-5.08</td>
<td>0.0465</td>
<td>0.003</td>
</tr>
<tr>
<td>Benzo[e]pyrene</td>
<td>C₂₀H₁₂</td>
<td>252.31</td>
<td>454.4</td>
<td>6.44</td>
<td>-5.13</td>
<td>0.0467</td>
<td>0.005</td>
</tr>
</tbody>
</table>

\( MW \) = molecular weight; \( T_{mp} \) = melting point temperature; \( K_{ow} \) = octanol to water partition coefficient; \( P_L \) = vapour pressure of liquid compounds; \( K_H \) = Henry’s law constant and \( c_S \) = aqueous solubility of solid compounds; \( a \) Ref. [9]; \( b \) Ref. [10]; \( c \) Ref. [11]; \( d \) Ref. [12]; \( e \) Ref. [13]; \( f \) Ref. [14]; \( g \) Ref. [15]; \( h \) Calculated from Clausius-Clapeyron equations from [16]. Original literature references of liquid vapour pressures are cited in Table 2 of Chapter 2 from this thesis; \( i \) Recommended values reported in ref. [17].

Biodegradation

Biodegradation of PAHs is believed to be limited by the amounts dissolved in the aqueous phase. The sorbed and crystalline state as well as non-aqueous phase liquid (NAPL)-dissolved PAHs are generally unavailable for biodegradation [1]. In polluted soils and sediments, mass transfer limitations are mostly inhibiting bioremediation because of strong sorption of PAHs to solid organic phases [18]. The degradation of PAHs can further be complicated by a deficiency in molecular oxygen that is required for the initiation of enzymatic attack on the aromatic rings [19]. For water saturated soils and sediments, there is evidence from the literature for the occurrence of anaerobic degradation of PAHs with nitrate and sulphate as terminal electron acceptors [1].

Tenax extraction of soil and sediment suspensions can function as a quasi-infinite sink for organic compounds in the aqueous phase and can therefore be applied to estimate the rapidly desorbing fraction of PAHs [20] (see also Figure 1). The bioremediation potential of PAH-contaminated sediments can be successfully estimated with Tenax extraction, which provides a good estimate of the biodegradation potential for intrinsically biodegradable compounds such as most PAHs. The amount of PAHs extracted from soils or sediments by Tenax is directly related to the amount that is biodegradable within a reasonable time span of some years [21]. Rapidly desorbing fractions of PAHs from sediments, measured by Tenax assisted desorption, have been linked to the extent of biodegradation in a number of studies [22-24]. The rate of biodegradation of the rapidly desorbing fraction has been suggested to be controlled by intra-aggregate mass transport limitations [23]. Recently, it was shown from modelling calculations and desorption experiments that biodegradation rates were higher than desorption rates for rapidly desorbing
PAHs (determined with Tenax), suggesting that microorganisms were able to promote desorption from the rapidly desorbing fraction [21].

An alternative method to measure bioavailability of PAHs is formed by bioreporters which are genetically modified whole-cell bacterial biosensors. These bioreporters have a relatively rapid response, are easy to cultivate and maintain, and engineered response is direct rather than inferred (such as with Tenax extraction) [25]. A recent application of this method to model sorbents and sediments spiked with naphthalene showed a 1:1 correlation between bioreporter response and Tenax extracted amounts [26].

Additional features of bacteria to degrade PAHs include the excretion of biosurfactants that function as carriers of PAHs, high specific affinities for the substrate to deplete PAHs at the cell surface and formation of biofilms with possible concomitant surface cell modifications to regulate attachment of bacteria to substrates [1]. Furthermore, artificial surfactants (and also DOM) can enhance biodegradation of PAHs in the aqueous phase. Surfactants are able to decrease the interfacial tension between solid and aqueous phases and can form stable aggregates or micelles that can increase the solubility of PAHs. This increased solubility facilitates transport from the solid phase and can enhance the rate of mass transfer to bacteria. The mechanism is poorly understood, however, because both enhancing and inhibiting effects have been shown to occur below as well above the critical micelle concentration [27]. In addition, surfactants can be toxic at high concentrations. Natural DOM could therefore be a better candidate for stimulating bioremediation of polluted sites. A number of studies have addressed the potential of DOM in enhancing the degradation of aqueous phase dissolved PAHs [28-31], but the possible mechanism of the effect of DOM on biodegradation of PAHs remains however unclear.

**Partitioning to dissolved organic matter**

DOM present in pore water of soils and sediments is a complex mixture composed of organic (macro)molecules with aliphatic and aromatic structures with varying attached polar functional groups. DOM is involved in biogeochemical cycles in which it serves as a carbon and energy source for biota and controls levels of dissolved oxygen, nitrogen, phosphorus, sulphur, trace metals and acidity [32]. The classical definitions of DOM are operational, *i.e.* divided in fulvic acids (FA), humic acids (HA) and humin, and are based on solubility properties in acidic or basic aqueous extraction media [33]. In recent reviews of DOM properties, it is proposed that HA (and also natural organic matter) behave as supramolecular associations of low molecular weight organic compounds held together by hydrophobic bonds, hydrogen bonds and/or metal-cation bridges [34, 35].

The partitioning of apolar compounds such as PAHs between DOM and water is determined by the relative proportion of hydrophilic and hydrophobic domains of DOM, the molecular weight distribution of DOM, and the hydrophobicity of PAHs [36]. This process directly affects
the freely dissolved concentration of PAHs resulting in a decreased bioavailability for aquatic organisms [37]. More specifically, characteristics of DOM that affect the partitioning behaviour of PAHs include aromaticity (UV absorptivity at 254 or 280 nm) [38, 37, 39, 40], aliphaticity (H/C atomic ratio) [37, 39], polarity (O/H atomic ratio) [40] and molecular size [38, 37].

Measurements of freely dissolved PAH concentrations for the determination of $K_{DOC}$ are usually accomplished by a physical separation of DOM from pore water in soils or sediments by filtration with an operationally defined cut-off of 0.45 μm [41]. This definition for DOM is flawed, however, when filtration artefacts give rise to variable amounts of DOM [42]. Moreover, the way of DOM separation can result in different DOM characteristics that are dependent on factors like: pH, extractant to solid matrix ratio, sampling of different pore spaces and spatial and temporal variation of DOM [41]. The size distribution of colloids and DOM in the sedimentary environment can therefore have a significant effect on the bioavailability of PAHs [43].

Various methods have been presented in the literature to determine $K_{DOC}$ values of PAHs, such as fluorescence quenching, reversed phase separation, apparent solubility, complexation–flocculation and equilibrium dialysis. The advantages and disadvantages of these methods have been discussed by Krop et al. [44]. Methods based on the use of passive samplers indirectly provide the $K_{DOC}$ value without separation of freely dissolved PAHs from the bulk solution and disturbance of equilibria between DOM and PAHs. In the Solid Phase MicroExtraction (SPME) method developed by Arthur and Pawliszyn [45], the extracting phase is an optical silica fiber with a polymer coating such as polydimethylsiloxane (PDMS) that absorbs the freely dissolved PAHs from the aqueous phase. Depending on the thickness of the coating and extracting conditions (stirring, temperature, water volume, etc.), the fiber extracts a negligible amount that is proportional to the freely dissolved concentration. In the method of negligible-depletion SPME, it is required that 1. the extracted amount has to be minimized to prevent disturbance of equilibria between DOM and PAHs and 2. the absorption kinetics towards the SPME fiber should not be influenced by the presence of DOM [46]. This SPME technique has been used to estimate bioconcentration by mimicking uptake by aquatic [47], soil [48], and sediment [49] organisms (biomimetic extraction). In the case of significant depletion of the analytes from solution, the SPME technique can be applied when equilibrium between fiber and water is reached. In this case, equilibria of analytes between DOM and water are re-established and enhanced diffusion of analytes by the presence of DOM through an unstirred boundary layer (UBL) surrounding the fiber is prevented [50, 51]. The SPME method is considered a fast technique and equilibria are disturbed to a minimum compared to other existing techniques, like e.g. reversed phase separation or complexation-flocculation [44].

The effect of temperature on the partition equilibrium involving PAHs and DOM is directly connected to the Gibbs free energy of transfer ($\Delta G^0$) of PAHs from water to DOM, $\Delta G^0 = -RT \ln K_{DOC}$, where $R$ is the ideal gas constant, $T$ is the absolute temperature and $K_{DOC}$ is expressed on a mole fraction basis. The molar enthalpy and entropy of phase transfer can be obtained from the variation of $K_{DOC}$ with temperature, $\ln K_{DOC} = -\Delta H^0/RT + \Delta S^0/R$ [52]. For partition processes,
the molar enthalpy of PAH sorption is constant, independent of the sorbed PAH concentration [53] and the molar entropy of the phase transfer is relatively small and positive [54]. For most hydrophobic organic contaminants (HOCs), partition coefficients decrease with increasing temperature [52]. The \( K_{DOC} \) values for PAHs are only weakly dependent on temperature and vary less than a factor of 2 with every 10 degrees change in temperature [36]. The temperature dependence of partitioning of PAHs to DOM (and passive samplers) has however not been studied to a large extent in the environmental literature. The experimental determination of sorption coefficients and their temperature dependence is required to validate sorption coefficients derived theoretically by computer simulation, which will be treated further below.

**Simulation of thermodynamic properties of aromatic hydrocarbons**

Environmental macroscopic properties of PAHs such as vaporization, dissolution and hydration are directly connected to thermodynamic variables such as enthalpies of phase transfer. Reliable calculation methods based on molecular properties of sorbate (and sorbent) are therefore required to predict the partitioning behaviour of PAHs. Molecular mechanics employing well parameterized force fields provide a means to obtain structural and energetic data on the molecular interactions involved. The interactions are evaluated with a force field equation that calculates the potential energy of a molecular system as a function of the nuclear positions or Cartesian coordinates in three-dimensional space (ignoring electronic motions). The potential energy is calculated from summed contributions of intra- and intermolecular (non-bonded) interactions within and between molecules. The variation of the potential energy as a function of the \( 3N \) Cartesian coordinates is called the potential energy surface [55]. The application of a computational method to explore this potential energy surface offers a way to connect molecular properties of PAHs to their macroscopic properties. Further details on using force field methods to calculate molecular and macroscopic properties can be found elsewhere in excellent textbooks [55-57].

The calculations performed with the Amber force field on molecular structures of PAHs and environmental sorbents are a combination of Geometrical Optimization (GO), Molecular Dynamics (MD) and Monte Carlo (MC) simulations. The Amber force field used in this thesis has been developed by Cornell *et al.* [58] to simulate proteins, nucleic acids and related organic molecules in condensed phases. In the MD and MC simulations, the \( NVT \) or canonical ensemble is used with a constant number of particles, volume and temperature [56]. The methods applied will be shortly explained here.

In GO calculations, the energy of a molecular structure with \( 3N \) Cartesian coordinates is minimised at zero Kelvin to find a local or global minimum on the potential energy surface. In this way, a geometrically optimised structure is generated for subsequent MD or MC simulations [59].

In MD simulations, a trajectory is generated that describes the evolution of positions and velocities of the particles in a system with time by integrating Newton’s equations of motion
MD was used in this thesis to optimise gas and hydrated systems with simulated annealing (gradual cooling of a molecular system) combined with relatively long MD runs prior to MC simulations of these systems. The temperature in an MD simulation is maintained by coupling the system to a (hypothetical) external heat bath that is fixed at the desired temperature [60]. In MC simulations, the potential energy surface is sampled by generating random configurations in $3N$ Cartesian coordinates from a Boltzmann distribution at a given temperature [59]. New configurations with a lower energy compared to its predecessor are directly accepted and are used as the starting point for the next iteration [55]. After equilibration of potential energies, the calculated average corresponds to a thermodynamic ensemble average [59]. This force field method can be used to predict environmental partitioning properties such as the energies of vaporization, solution and hydration of aromatic hydrocarbons.

**Simulation of sorption to environmental sorbents**

Models for environmental sorbents like DOC and BC should mimic the distribution or macroscopic behaviour of PAHs towards these sorbents and can therefore be used as a molecular probe to study their interactions. Different molecular structures of HA extracted from soil and lake water have been derived from pyrolysis-mass spectrometric analysis of HA in combination with elemental analysis of freeze-dried extracts [61, 62]. Apart from models proposed for DOM (structures not shown here), a possible molecular structure has been reported for BC or soot by Akhter et al. [63] based on a variety of spectroscopic studies and gas chromatography coupled to mass spectrometry. This molecular structure of hexane soot (Figure 3, left) consists of linked polyaromatic sheets with polar functional groups at its edges [63]. The hypothetical character as well as the relatively large molecular size of this type of structures limits the application in computer simulations in terms of representativeness and computer capacity. In Chapter 6 of this thesis, a simplified model structure of BC ($C_{54}H_{18}$; Figure 3, right) was used to simulate interactions of phenanthrene, fluoranthene and benzo[a]pyrene (Figure 2) with BC. This model structure is a truncated polyaromatic sheet of carbon and was chosen to enable rapid force field calculations because of its smaller size.
The calculation of macroscopic thermodynamic properties such as the Gibbs or Helmholtz free energy of sorption using force field methods based on statistical mechanics provides a way to directly calculate sorption coefficients for PAHs and BC. The main advantages of this computational approach are the increased insight into molecular mechanisms relevant for sorption and the time gained by not performing sorption experiments with long equilibration times of several weeks to months in the laboratory for every single sorbate. It will however still be necessary to experimentally determine the sorption coefficients of a small number of compounds in order to validate these computer simulations.


Chapter 1

Research questions and outline of this thesis

The research presented in this thesis is part of an inter-laboratory European Commission supported effort to understand the bioavailability of PAHs in soils and sediments. The aim of this European project called ABACUS (evaluation of Availability to BiotA for organic Compounds Ubiquitous in Soils and sediments) is to provide methods to determine actual bioavailable concentrations of HOCs in soils and sediments to improve risk assessment of polluted sites in Europe. The aim of the research presented in this thesis is to gain knowledge on the environmental behaviour of PAHs and this thesis is therefore focused on the environmental fate processes that PAHs undergo in the environment: vaporization, dissolution, biodegradation, partitioning to DOM and adsorption to BC. The following specific research questions were formulated:

1. What is the effect of DOM on the biodegradation of aqueous phase dissolved PAHs by bacteria?

2. Develop a method to measure DOM to water partition coefficients of PAHs in sedimentary pore water.

3. Develop a force field method to study interactions of PAHs with model structures for dissolved and particulate organic matter.

Accurate physicochemical (and thermodynamic) data are required to validate empirically and theoretically derived estimation models that are used for the prediction of the environmental fate and behaviour of PAHs. Supercooled liquid vapour pressures of PAHs and their temperature dependence were therefore determined experimentally from gas chromatographic retention times in Chapter 2. The effect of DOM on the biodegradation of PAHs by bacteria (Research question 1) was studied in Chapter 3 where the kinetics of the biodegradation process were compared to the kinetics of partitioning of PAHs to SPME fibers in the presence of DOM. The development of the method to determine freely dissolved PAH concentrations in the presence of DOM (Research question 2) is described in Chapters 3 and 4. SPME fibers were used to determine DOM to water partition coefficients for freshwater sediments of different origin. In addition, the effect of temperature was studied in Chapter 4 to gain an understanding of the magnitude of temperature change on partitioning of PAHs to SPME fibers or DOM and the thermodynamics involved in partitioning processes. Thermodynamic properties of aromatic hydrocarbons were calculated in Chapter 5 by computer simulation with a force field method based on MC simulations (Research question 3). The enthalpy difference between the gas and liquid phase of fluoranthene determined from the temperature dependence of the vapour pressure in Chapter 2 was used to validate this force field method. The method depended on atomic charge scaling factors derived from MC simulations of calibration compounds. In Chapter 6, this force field
method was extended to simulate interactions between PAHs and environmental sorbents such as BC. A thermodynamic integration procedure was developed for this purpose in order to derive a sorption coefficient based on MC simulated potential energies of PAHs and a model structure for BC. Finally in Chapter 7, the main findings from this thesis are discussed and conclusions are presented.

References


