Fate of Polycyclic Aromatic Hydrocarbons in the Environment

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Data sheet

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Abstract: The environmental fate of polycyclic aromatic hydrocarbons (PAH's) is reviewed in order to elucidate the actual chemical, physico-chemical and microbiological processes which determine the behaviour of PAH's in the environment. The review focusses on the terrestrial environment, where the fate of PAH's is determined by an interplay between several soil such as pH, redox conditions, organic matter content and microbial biomass.

Keywords: PAH, Polycyclic Aromatic Hydrocarbons, Fate, Solubility, Evaporation, Sorption, Partitioning, Migration, Metabolism, Bioavailability, Biosurfactants, Biodegradation, Analysis

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Resumé

Skæbnen af polycykliske aromatiske hydrocarboner (PAH'er) er gennemgået med henblik på at belyse de kemiske, fysisk-kemiske og mikrobiologiske processer, der er bestemmende for PAH'ernes opførsel i miljøet. Dette review fokuserer på det terrestriske miljø, hvor skæbnen af PAH'er er kontrolleret gennem vekselvirkning mellem en række parametre, der karakteriserer matricen som f.eks. pH, redox forhold, indholdet af organisk materiale og mikrobiel biomasse.

Rapporten summerer kilderne til PAH forurening (kapitel 2), en lang række af reaktioner, der er bestemmende for tilgængeligheden af PAH'er (kapitel 3) samt mikrobiologien bag PAH nedbrydningen (kapitel 4). Således sammenfattes i kapitel 3 de processer, der kontrollerer tilgængeligheden af PAH'er såsom diffusion/dispersion/ konvektion, opløselighed, fordampning og sorption/desorption, hvor effekterne af temperatur, ionstyrke og organiske co-solutter og overfladeaktive stoffer behandles. Specielt fokuseres på betydningen af naturligt forekommende organiske materiale som f.eks. humus stoffer. Det afsluttende afsnit i kapitel 3 er helliget en teoretisk studie, baseret på beregninger med COLUMN2 computer programmet, der illustrerer PAH'ers migrationspotentiale. Mikrobiologien af PAH'er (kapitel 4) er gennemgået med fokus på bionednedbrydning, herunder 'surfactant-enhanced' nedbrydning, og biotilgængelighed.

I det afsluttende kapitel (kapitel 5) er metoden til analyse af PAH'er i jord, baseret på Soxhlet ekstraktion efterfulgt af kvantificering baseret på koblet gas chromatografi - masse spektrometri, givet.

Arbejdet er blevet finansielt støttet gennem det Strategiske Miljøforskningsprogram inden for rammerne af Dansk Center for Økotoksikologisk Forskning og Statens Teknisk-Videnskabelige Forskningsråd.

Summary

The environmental fate of polycyclic aromatic hydrocarbons (PAH's) is reviewed in order to elucidate the actual chemical, physico-chemical and microbiological processes which determine the behaviour of PAH's in the environment. The review focusses on the terrestrial environment, where the fate of PAH's is determined by an interplay between several soil such as pH, redox conditions, organic matter content and microbial biomass.

The report summarizes the sources for PAH contamination (chapter 2), a wide range of reactions determining the availability of PAH's (chapter 3) as well as the microbiology of PAH degradation (chapter 4). Thus, in chapter 3 processes controlling the availability of PAH's, such as diffusion/dispersion/convection, solubility, evaporation and sorption/desorption, are reviewed with special emphasis on parameters as temperature, ionic strength and the possible presence of organic co-solutes or surfactants. Attention is given to the role of naturally occurring organics such as humic material. The final section of chapter 3 is devoted to a theoretical study, based on calculations using the COLUMN2 computer code, to elucidate the migration potential of PAH's. The microbiology of PAH's (chapter 4) is reviewed with respect to biodegradation of PAH's, including surfactant-enhaced degradation, and bioavailability.

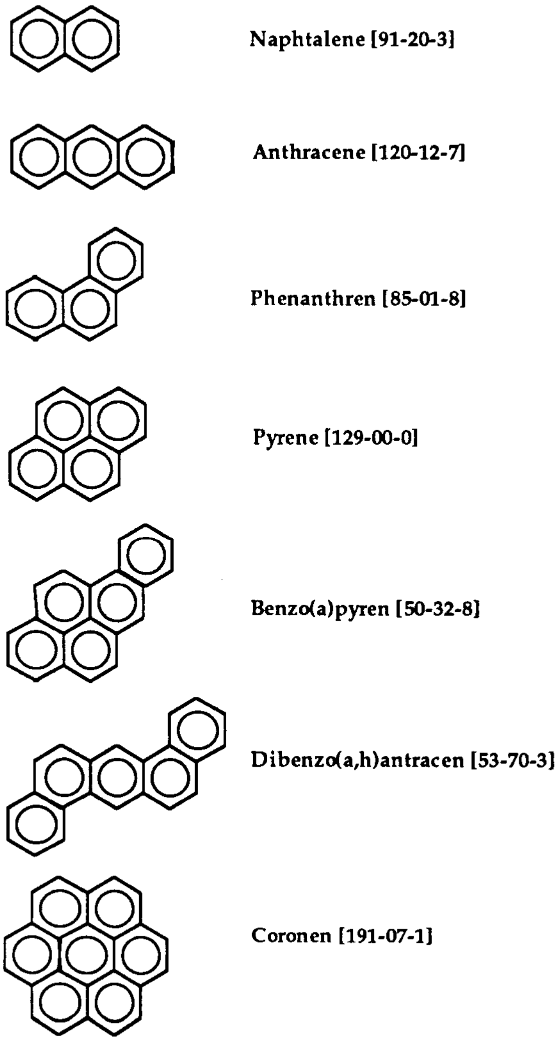
Chapter 5 presents the method for analysis of PAH's in soils based on Soxhlet extraction of the samples followed by quantification using gas chromatography combined with mass spectrometry is given.

The work has been financially supported by the Danish Environmental Research Programme within the frame of the Danish Centre for Ecotoxicological Research, and by the Danish Technical Reseach Council.

1 Introduction

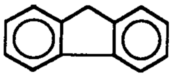
Polycyclic Aromatic Hydrocarbons (PAH's) is the largest single class of chemical carcinogens known today. More than 30 PAH's and several hundred PAH derivatives have been reported to exhibit carcinogenic effects, and potential hazards from the possible occurrence of PAH's in the environment have been noted, resulting in standards for drinking water, food, working atmosphere as well as effluents from industries and mobile sources (*Bjørseth & Ramdahl, 1985*). The present report focusses on the available knowledge concerning the fate of polycyclic aromatic hydrocarbons in the environment, the eventual aim being to provide the scientific background for evaluating/predicting/reducing potential hazards, as well as to disclose the further needs for research activities.

In Scheme 1.1 the structures of a series of PAH's are given with CAS number in brackets.

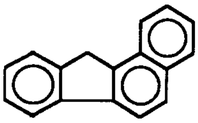


Scheme 1.1

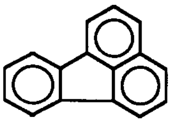
Scheme 1.1 *cont.*



Fluoren [86-73-7]



Benzo(a)fluoren [30777-18-5]



Fluoranthen [206-44-0]

Obviously the potential hazards are closely connected to the fate, e.g. mobility, of PAH's in the environment. In a recent study (*Kiilerich & Arvin, pers. comm.; 1996*) available data for the distribution of naphtalene, methyl napthalene, fluorene, pyrene and benzo(a)pyrene around up to 103 oil and tar polluted sites were collected. In Table 1.1 the reported maximum concentrations are summarized for the 100%, 90%, 75% and 50% fractiles of the sites, respectively.

Table 1.1 Maximum concentration (mg/L) for the 100%, 90%, 75% and 50% fractiles of sites (the numbers in parantheses denote the actual number of sites in the individual fractiles).

		Distance from source (m)		
	fract.	0-10	10-50	>50
Naphtalene	100	19 (103)	14 (63)	23 (44)
	90	8,6 (93)	0,63 (93)	1,0 (40)
	75	1,5 (78)	0,05 (78)	0,004 (33)
	50	0,01 (52)	nd (52)	-
Me-naphtalene	100	1,9 (55)	2,4 (43)	2,0 (21)
	90	0,82 (50)	0,12 (39)	0,0015(19)
	75	0,18 (42)	0,033 (33)	nd (16)
	50	0,029 (28)	nd (22)	-
Fluorene	100	1,2 (19)	0,11 (23)	0,63 (15)
	90	0,13 (18)	0,028 (21)	0,053 (14)
	75	0,060 (15)	0,020 (18)	0,001 (12)
	50	0,02 (10)	nd (12)	-
Pyrene	100	0,17 (24)	0,14 (26)	0,83 (20)
	90	0,094 (22)	0,036 (24)	0,030 (18)
	75	0,050 (18)	0,008 (20)	0,003 (15)
	50	nd (12)	nd (13)	-
Benzo(a)pyrene	100	0,28 (33)	0,027 (34)	0,18 (17)
	90	0,030 (30)	0,0053(31)	0,003 (16)
	75	0,005 (25)	0,0004(26)	nd (13)
	50	nd (17)	nd (17)	-

The data in Table 1.1 unambiguously demonstrate that these species to a certain extent are mobile in the terrestrial environment. However, a general prediction of the mobility based on empirical data like the above is obviously impossible.

A wide range of chemical, physico-chemical and biological reactions can be expected to control the environmental fate of polycyclic aromatic hydrocarbons. The processes comprise diffusion, dispersion and convection, solubility, sorption/desorption, air water exchange, bioavailability as well as (photo)chemical and microbiological transformation and degradation.

Obviously, solubility and sorption/desorption characteristics are crucial features in relation to the availability of PAH's. Thus, several factors have to be considered in order to predict these processes, such as temperature, pH, ionic strength, natural organic matter concentration, particulate matter concentration etc.. In addition the microbial activity has to be evaluated.

For convenience, the roles of all these processes are often summarized in a single coefficient quantifying the equilibrium between the liquid and the solid phase, i.e. the distribution coefficient, K_D. However, to understand, in depth, the environmental pathways and, thus, eventually predict the environmental behaviour of PAH's, a more detailed view seems appropriate. Hence, in Figure 1.1 the general scheme for the possible evaluation of the environmental fate of PAH's is outlined (cf. *Schwarzenbach et al., 1992, chap. 1*).

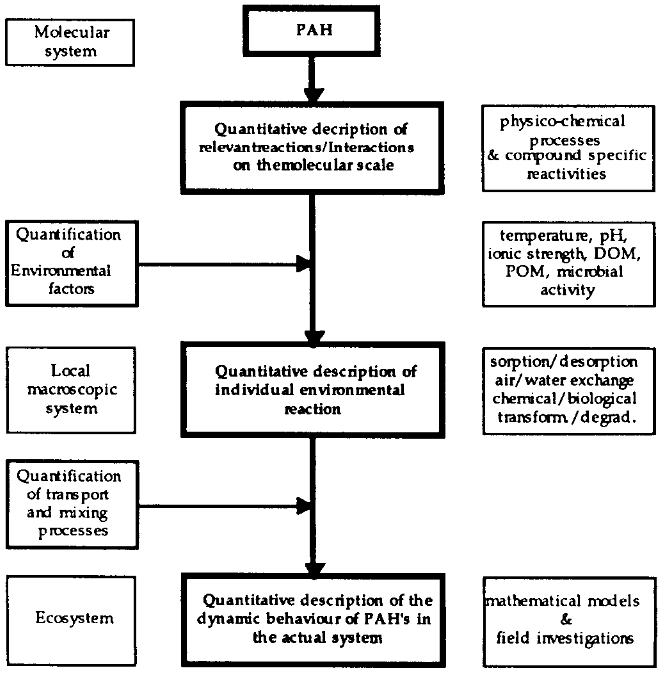


Figure 1.1 Evaluation of environmental fate

2 Sources of PAH's

Thermal decomposition of organic material may lead to the formation of PAH's. Thus, the two major mechanisms for PAH formation are pyrolysis or incomplete combustion and carbonization processes, i.e., PAH's are not only formed at high temperatures (>400°C), but also at rather low temperatures (<200°C) combined with high pressures over geological time scale, e.g. during the generation of coal and mineral oil (*Bjørseth & Ramdahl, 1985*).

In the biosphere, the by far predominant sources of PAH's are anthropogenic, although natural sources, such as vulcanic activity and carbonization mechanisms, have been recognized. Bjørseth and Ramdahl (*1985*) divide the main anthropogenic sources into two categories, i.e., stationary and mobile sources.

The stationary sources are subdivided into residual heating (wood- and coal fired furnaces, fireplaces and stoves, and gas burners), industry (coke, carbon black and asphalt production, petroleum catalytic cracking, aluminium smelting, iron- and steel sintering and ferroalloy industry), power- and heat generation (coal- and oil-fired power plants, wood- and peat-fired power plants, and industrial and commercial boilers), and incineration and open fires (municipal and industrial incinerators, refuse burning, forest fires, structural fires, agricultural burning).

The mobile sources include gasoline-engine automobiles, diesel-engine automobiles, air- and sea-traffic.

Finally, contaminated soils (due to industrial activities, e.g., gas works, oil refining, garage activities) and various types of waste disposal sites may constitute potential sources of PAH pollution.

Emissions of PAH's from the various sources are strongly dependent on the actual PAH producing process. However, a compilation of emission factors is outside the scope of the present work. For emission factors, e.g., Bjørseth & Ramdahl (*1985*) should be consulted.

It should be noted that a variety of fused-ring polycyclic aromatic compounds are naturally produced by bacteria, fungi and higher plants (*Neff, 1985* and references therein). Furthermore, as mentioned above, the carbonization process has resulted in the presence of PAH's in fossil fuels (*Neff, 1985* and references therein). Thus, crude petroleums may typically contain from 0.2 to more than 7% PAH's, whereas shale oils and coal-derived crude oils may contain as much as 15% PAH's. Consequently, crude and refined oils contain significant amounts of PAH's.

3 Availability of PAH's

The availability and, hence, the potential mobility, as well as the bioavailability of PAH's in the environment is closely connected to chemical and physico-chemical parameters, which determine the presence and concentration of PAH's in solution, and subsequently control the distribution in the environment.

3.1 Diffusion, dispersion, convection

All species in solution, e.g., simple dissolved species, complexes, colloids and dissolved as well as particulate organic matter, are subject to diffusion, dispersion and convection.

In cases where a polluted site is in contact with stagnant water, the only possibility for transport of contaminants will be diffusion through the aqueous phase, the mean pathway being given by

$$x = \sqrt{2Dt} \quad m \qquad 3.1$$

where D is the diffusion coefficient (m^2/years) and t the time (years) in which the process has proceeded.

Typically the diffusion coefficient, D, in pure water is found to be in the order of 0.03-0.06 m^2/y. However, in porous media effects as constrictivity and tortuosity may significantly decrease the D values (cf. *Carlsen et al., 1981*).

In near surface layers, where contamination often can be monitored, ground water movement, however, will in general not be neglectible. Hence, dissolved species will be subject to transport of dissolved species in flowing ground water systems, the so-called convection. In addition a series of mixing and dilution processes, one of which is diffusion, takes place. The effect of these processes is summarized as dispersion. The result of dispersion is, as is of diffusion that concentration differences decrease and the dissolved material is diluted. It has been shown (*Marsily et al.,1982*) that the dispersion is varying as a function of time as well as on average travelled distance.

Introducing chemical and physico-chemical processes, e.g. interaction between pollutants and the soil constituents, may significantly change the migration behaviour of the pollutants. The effects of the chemical and physico-chemical processes, which give important contributions to the dispersion, are summarized in the so-called chemical dispersion (*Carlsen et al., 1987*).

Diffusion of a compound in a groundwater flow in a porous media is normally slower than in the unconfined fluid; however, the dispersion coefficient increases with increasing ground water velocity, V. A typical value of dispersion coefficients in water at ambient temperature is around 10^{-9} $m^2/s \approx 0.03$ m^2/y (*vide supra*).

In Fig. 3.1 the effect of varying dispersion coefficients is visualized by calculating the elution concentration profiles of a non-retarded solute from a 100 m porous column, the ground water velocity being 1 m/y (*Nielsen et al., 1985*).

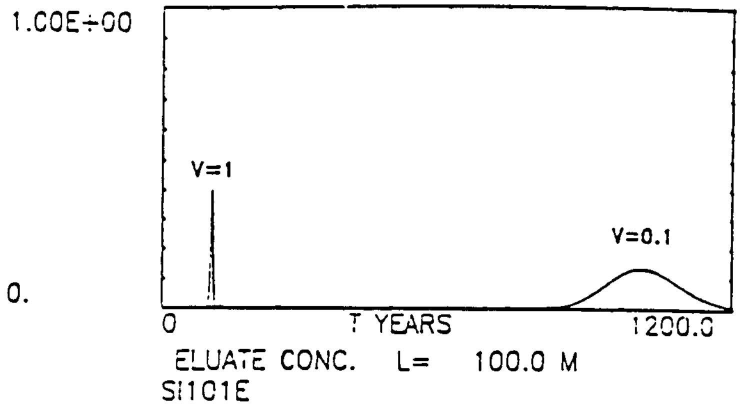


Figure 3.1 Elution curves of compound A. Initial concentrations: $[A_0] = 1$. Retention factor $R_f(A) = 1$. V = 1 and 0.1 m/y, respectively, D = 0.03 m^2/y.

It should be remembered that a decrease in ground water velocity (for constant D) causes pronounced increased dispersion, due to the time effect, i.e. increased residence time in the column. In Fig. 3.2 the calculated elution concentration profiles are depicted for ground water velocities equal to 1 and 0.1 m/y, respectively, D being 0.03 m^2/y.

Finally, retention phenomena influence the dispersion of a given solute during transport in the ground water system. Retention may be due to several physico-chemical processes already mentioned such as sorption/desorption or precipitation/dissolution. The effect of these processes are in general combined in a single retention factor, $R_f(i)$. Assuming equilibrium between the pollutant, i, on the solid phase and in solution, respectively, i.e. reversability of the sorption proceess as well as conditions with no degradation of the pollutant, the corresponding distribution coefficient $K_D(i)$ being related to $R_f(i)$ by:

$$R_f(i) = 1 + K_D(i)(1 - p)d/p \tag{3.2}$$

where p is the porosity and d the bulk density of the solid phase (cf. *Carlsen et al., 1981; Jensen, 1982; Neretnicks, 1980; Anderson et al., 1984; Eriksen & Jacobsen, 1984*).

In Fig. 3.3 the effect of increased retention, i.e. $R_f > 1$ is visualized. It is seen that incresing the R_f values decrease the mobility of the migrating species proportionally to the R_f value and the peaks become broader, as a result of the increased residence time in the column, i.e. due to the so-called chemical dispersion (*Carlsen et al., 1987*).

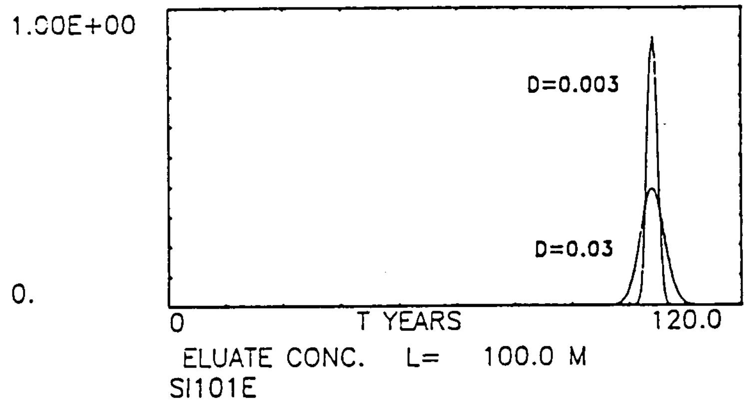


Figure 3.2 Elution curves of compound A. Initial concentrations: $[A_0] = 1$. Retention factor $R_f(A) = 1$. $V = 1$ m/y, $D = 0.03$ and 0.003 m^2/y, respectively.

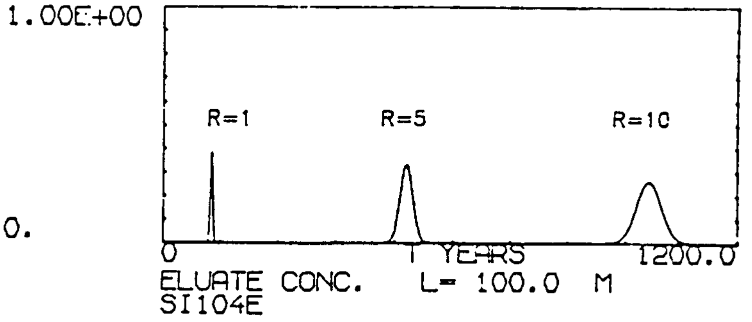


Figure 3.3 Elution curves of compound A. Initial concentrations: $[A_0] = 1$. Retention factor $R_f(A) = 1, 5, 10$, respectively. $V = 1$ m/y, $D = 0.03$ m^2/y.

3.2 Solubility

Often hydrophobic non-polar organic compounds, such as PAH's, are regarded as "insoluble in water". However, all compounds obviously have a finite solubility in water, although it is typically rather low, i.e. the concentration in the saturated solution $S_w(sat) << 1$ at S.T.P., but nevertheless of environmental interest, due to their potential ecotoxicological hazards.

The solubility of PAH's ranges from ca. 10^{-4} to ca. 10^{-10} mol/L (Futoma et al., 1981) in pure aqueous solutions. However, parameters such as temperature, ionic strength (i.e. salt content) and possible dissolved organic solutes and solvents have to be taken into account. The possible presence of dissolved- and/or particulate organic matter (DOM and POM) influences the amount of PAH's in solution as well, however, the speciation being changed by "complex formation". The effects of the presence of DOM and POM will be treated in a seperate section. Thus the present section will deal with the solubility of the pure compounds only.

Not surprisingly, a distinctly decreasing solubility with increasing molecular size is noted. Thus, a linear correlation between geometrically calculated total surface area of the PAH's and the logarithmic water solubility has been observed (*Schwarzenbach et al., 1992, chap. 5*). It should in this connection be remembered that the solubility, $S_w(sat)$, is directly correlated to the activity coefficients, $\text{¥}_w(sat)$, of the PAH's through the formula

$$S_w(sat) = \frac{X_w(sat)}{V_w} = \frac{P}{\text{¥}_w(sat)V_w} \qquad 3.3$$

$X_w(sat)$ being the molecular fraction of the PAH in the saturated water and V_w being the water volume, which for practical purposes roughly can be set equal to 0.018. The constant P is found to be equal to 1 in cases of liquid compounds. For solid compounds, i.e. those with melting points above 25°C, P is found to be equal to the ratio of the fugacity of the solid to the fugacity of the subcooled liquid PAH at the system temperature. If the fugacity of a pure gas arbitrarily is chosen to be 1 atm, P can be determined as $P^\circ(s)/P^\circ(l)$,$P^\circ(s)$ and $P^\circ(l)$ being the vapour pressure of the solid and sub-cooled liquid PAH, respectively (*Futoma et al., 1981; Schwarzenbach et al., 1992, chap. 5*).

Attempts have been made (*Mackay & Shiu, 1997*) to generate a general expression for the P factor. Thus, an expression

$$\ln P = -0.023(T_{tp} - 298.15) \qquad 3.4$$

with T_{tp} being the triple point of the PAH has been found reasonably well to reproduce experimentally derived data (*Mackay & Shiu, 1997; Futoma et al., 1981*). Thus, it may be applied to generate activity coefficient for PAH's not experimentally studied.

Since a simple correlation apparently prevail between the molecular size and solubilities and, hence, activity coefficients, it appears reasonable to derive a simple operational relationship between these features. Thus, the fact that the number of carbon atoms is a very simple measure for molecular size of the PAH's lead Mackay and Shiu (*1977*), based on "a best fit" to suggest the relation

$$\log \yen_{w}(sat) = 3.5055+0.3417(N-6)-0.00264(N-6)^{2} \qquad 3.5$$

between the activity coefficient and the number of carbon atoms, N.

Obviously a combination of the eqn.'s 3.3 - 3.5 will immediately lead to the solubility of a given PAH. However, in cases where the vapour pressures $P^{o}(s)$ and $P^{o}(l)$ are known the solubility can directly be obtained from eqn. 3.3. Vapor pressures and water solubilities of selected PAH's are given in Table 3.1 (data adopted from Schwarzenbach et al., 1992, p. 621, and references therein).

Table 3.1 Vapour pressures and water solubilities of selected PAH's at 25°C.

	log $P^{o}(s)$ (atm)	log $P^{o}(l)$ (atm)	log $S_{w}(sat)$ mol/L
Naphtalene	-3.98	-3.43	-3.61
Anthracene	-8.10	-6.11	-6.46
Phenanthrene	-6.79	-6.05	-5.02
Pyrene	-8.22	-7.40	-6.17
Benzo(a)pyrene	-11.14	-9.63	-8.22
Benzo(a)anthracene	-9.55	-8.20	-7.31
Fluorene	-6.10	-5.22	-4.96
Fluoranthene	-7.91	-7.06	-5.93

Additional solubility data can be found in Maykay and Shiu (*1977*).

3.2.1 Dissolution kinetics

The dissolution kinetics of solid phenanthrene is given by

$$[\text{Phen}]_{\text{sol}} = [\text{Phen}]_{\text{sat}} (1 - \exp(-kt)) \qquad 3.6$$

$[\text{Phen}]_{\text{sol}}$ and $[\text{Phen}]_{\text{sat}}$ being the actual concentration of phenanthrene during the dissolution process and at saturation, respectively (*Kehlen et al., 1986*).

In Fig. 3.4, the dissolution kinetics for finely dispersed phenanthrene in synthetic ground water is shown, the rate constant being 0.104 h^{-1} ($r^2 = 0.983$). The latter increases to ca. 0.11 and 0.16 h^{-1} in the presence of 25 and 50 mg/L humic acids, respectively (*Lassen & Carlsen, 1997*).

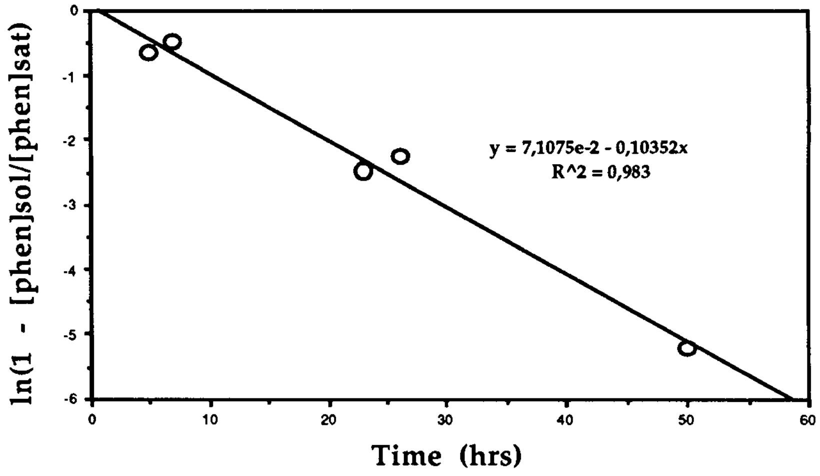


Figure 3.4. Dissolution kinetics of finely dispersed phenanthrene in synthetic ground water.

The rate constant is given by physical factors, i.e.,

$$k = DO/v\partial \qquad 3.7$$

D being the diffusion coefficient of the dissolved phenanthrene in the solution, O the surface area of the solid material, v the volume of the solution and ∂ the thickness of the boundary layer, the latter being assumed as a layer of stangnant fluid around the crystals. Outside the boundary layer a completely mixed solution is assumed. Thus, in the present study the diffusion of dissolved phenanthrene through the boundary layer apparently is the rate determining parameter. Consequently the observed increased rate of dissolution in the presence of humic acids has to attributed to an increased apparent diffusion coefficient.

It is emphasized that the study by Lassen and Carlsen (*1997*) has been carried out with finely dispersed phenanthrene, i.e. crystals exhibiting a relatively large surface area, the results probably representing a conservative estimate of the dissolution rate for solid phenanthrene into groundwater, as crystal size obviously is the rate determining parameter in cases of identical solution composition.

3.2.2 Effect of temperature

Variation in temperature may typically cause changes in the solubilities of dissolved species. Thus, the solubility is given as a function of the enthalpy of solution

$$\log S_w(\mathrm{sat}) = -\frac{\Delta H_s}{2.303RT} + K \qquad 3.8$$

with R and T being the gas constant and the absolute temperature, respectively. K is a constant (*Schwarz, 1977*). In the case of solids the enthalpy of solution is composed by the enthalpy of a phase change (e.g. solid to subcooled liquid) and the excess enthalpy of solution, the enthalpy of the phase conversion typically being the dominant factor.

Since the energy requirement for the solid to liquid phase conversion decreases with increasing temperature, the solubility of solids increases with increasing temperature. Thus, May et al. (*1978*) found linear relationships for 11 PAH's between the logarithmic solubility and the reciprocal temperature within the temperature range of environmental interest, i.e. 5 - 35°C. Thus, the solubility increase being ca. 3 times by changing the temperature from 6 to 26°C.

For a series of PAH's May et al. (*1978*) derived simple polynominal expressions for the water solubility as function of temperature.

$$S_w(\mathrm{sat}) = aT^3 + bT^2 + cT + d \qquad 3.9$$

The temperature is given in °C and the resulting solubility in µg/kg. a, b, c and d are compound specific constants (cf. Table 3.2). In all cases the correlation coefficients were found > 0.99.

Table 3.2 Compound specific constants for deriving temperature dependence of the solubilities (μg/kg) of selected PAH's.

	a	b	c	d
Naphtalene	0	18.9	249.9	13660
Anthracene	0.0013	0.0097	0.8861	8.21
Phenenthrene	0.0025	0.8059	5.413	324
Pyrene	-0.0011	0.2007	-1.051	50.2
Benzo(a)pyrene	0.0003	0.0031	0.1897	1.74
Fluorene	0.0185	0.4543	22.76	543
Fluoranthene	0.0072	-0.1047	4.322	50.4

3.2.3 Effect of ionic strength

Obviously, environmental waters are never pure. Thus, varying content of e.g. dissolved inorganic salts has to be considered in the evaluation of PAH solubilities. Especially in higher saline waters, as sea water and brines, significantly decreased solubilities can be foreseen, as already established by Setschenow as early as 1889 (*Setschenow, 1889*) as the now well-known salting-out effect. Emperically the following relationship between the solubilities of the organic compound in the saline ($S_{w,salt}$(sat)) and in pure (S_w(sat)) waters was established.

$$\log S_{w,salt}(sat) = \log S_w(sat) - K_s[S] \qquad 3.10$$

with K_s being the so-called Setschenow constant and [S] being the total salt concentration. The Setschenow constants are dependent of both the nature of the organic compound and the inorganic salt.

Unambiguously, environmental waters typically contain a variety of salts. Thus, to determine the actual solubilities of PAH's in such systems an effective Setschenow constant has to be applied as suggested by Gordon and Thorne (*1967a, b*).

$$K_s^{eff} = \sum_i K_s(i)X(i) \qquad 3.11$$

where K_s(i) and X(i) the Setschenow constants and molar fractions of the single salts, respectively.

In Table 3.3 Setschenow constants for selected PAH/NaCl combinations are given (*May et al., 1978*)

Table 3.3. Setschenow constants for selected PAH/NaCl combinations.

	K_s
Naphtalene	0.213±0.001
Anthracene	0.238±0.004
Phenanthrene	0.275±0.010
Pyrene	0.286±0.003
Benzo(a)pyrene	0.345±0.002
Fluorene	0.267±0.005
Fluoranthene	0.339±0.010

The variation of the Setschenow constants as function of different salts is visualized in Table 3.4 giving the values corresponding to selected naphtalene/salt combinations (*Gordon & Thorne, 1967a, b*)

Table 3.4. Setschenow constants for selected naphtalene/salt combinations

	K_s
NaCl	0.22
$MgCl_2$	0.30
Na_2SO_4	0.70
$CaCl_2$	0.32
KCl	0.19
$NaHCO_3$	0.32
KBr	0.13
CsBr	0.01

3.2.4 Effect of dissolved organic solutes and solvents

Often PAH polluted waters will contain a variety of other organic compounds as well. Accordingly it is necessary to evaluate the possible effect of dissolved organic solutes and solvents on the solubilities of the PAH's. Unambiguously, PAH's are much more soluble in organic solvent than in water. Thus, the presence of dissolved organic solutes or solvents will generally increase the apparent water solubility of PAH's. It has been demonstrated that phenol concentrations above 50 µg/L increased the solubility of benzo-(a)-pyrene, whereas lower concentrations apparently had no effect (cf. *Futoma et al., 1981*). Likewise did laboratory studies reveal an increased solubility in the presence of organic solvents.

A relatively simple equation (cf. *Lane & Loehr, 1992*) describes the solubilities of PAH's in mixed solvents (S_{mix}(sat)) relative to those in pure water (S_w(sat)).

$$\log S_{mix}(sat) = \log S_w(sat) + \partial f_c \qquad 3.12$$

where ∂ is the so-called cosolvency power and f_c is the volume fraction ($0 \leq f_c \leq 1$) of cosolvent in the solvent mixture.

Obviously, the cosolvency power, ∂, as well as the water solubility, S_w(sat), can be graphically determined by plotting the solubility in the mixed solvent, C_{mix}(sat), as a function of various volume fractions of the cosolvent, f_c, in a log - linear diagram. Based on linear regression ∂ is determined as the slope of the line and the water solubility as the y-intercept.

Cosolvency powers has been determined for a series of PAH's in different soil samples for 2-propanol/water and methanol/water systems (*Lane & Loehr, 1992*). Some inconsistencies for the single PAH's in the different samples are noted, probably due to varying interactions with the solid phase. However, average values can be calculated (Table 3.5)

*Table 3.5.*Average co-solvency powers for selected PAH's in methanol/water (∂_{mw}) and 2-propanol/water (∂_{2pw}) mixtures

	∂_{mw}	∂_{2pw}
Naphtalene	1.83±0.20	2.96±1.01
Anthracene	4.96±0.59	5.95±1.49
Phenanthrene	4.24±0.48	6.18±0.88
Pyrene	4.69±0.80	6.81±1.17
Benzo(a)pyrene	4.05±0.85	5.74±2.54
Benzo(a)anthracene	5.22±2.35	5.68±1.64
Fluorene	3.62±0.40	5.33±0.46
Fluoranthene	4.65±0.74	6.78±1.05

Based on the data obtained a rather good agreement between experimentally and theoretically (cf. eqn. 3.12) determined water solubilities of PAH's was obtained *(Lane & Loehr, 1992)*.

An empirical approach to the cosolvency power was made by Morris et al., (*1988*), who suggested

$$\partial = a\log K_{ow} + b \qquad 3.13$$

with K_{ow} as the octanol-water partition coefficient of the PAH and a and b coefficients unique to the particular solvent.

Eqn 3.13 has successfully been used to calculate the solubility ratio $C_{mix}(sat)/C_w(sat)$ for e.g. pyrene for aqueous solutions containing 10% cosolvent at 20°C (*Morris et al., 1988*): ethylene glycol (1,9), propylene glycol (2,5), 2-propanol (3,3), methanol (2,8), ethanol (3,3), acetonitrile (3,6 and acetone (3,6). Some deviations from the theoretical treatment were noted in agreement with the results of Lane and Loehr (*1992*), whose experimental data were up to a factor ≈2 higher than those derived using the Morris equation (eqn. 3.13).

In general it should be noted that the effect of co-solvents are relatively limited. Thus, rather high concentrations of organics in the aqueous solutions are required in order to increase the solubility significantly. (cf. *Schwarzenbach et. al., 1992*).

3.2.5 Effects of chemical surfactants

Surface-active agents (surfactants, dispersants, detergents and emulsifiers) are a special class of organic solutes, which includes both chemical and biological products. The surfactant molecule is amphiphilic, i.e., consists of a hydrophilic and a hydrophobic part. This characteristic makes it possible for the surface-active compounds to promote the wetting, solubilization and emulsification of various hydrophobic organic compounds (*Amdurer et al. 1986*). In an aqueous medium, emulsifiers affect the formation of an emulsion and its stability, but the presence of emulsifiers does not necessarily, like with the surfactants, cause a reduction in the surface tension of the medium (*Cooper, 1986; Van Dyke, 1991*).

For several years the oil industry has been practicing surfactant-flooded removal of oil from subsurface strata (tertiary oil recovery). Another early application of surfactants has been to break up and disperse spilled oil in order to enhance the biological and photolytic decomposition of oil compounds spilled in marine and costal areas (*Ellis et al., 1985; Amdurer et al., 1986,;Vigon & Rubin, 1989*). Today the application of surfactants is widespread and the surface-active compounds are present in the environment in increasing amounts as a result of indigenous biological activity and of disposal of waste products, and through the discharge of waste waters containing significant levels of these compounds (*West & Harwell, 1992*)

During the last five to ten years the need for research studying the behaviour of surface-active agents in soil and the effect of surfactants on hydrophobic organic compounds has increased. Research has been initiated in order to answer two main questions; 1) how is the mobility of hydrophobic organic compounds affected by the presence of surfactants, when both coexist in the soil as a result of human activities?

and 2) can the use of surfactants enhance the bioavailability and accordingly the biodegradation of hydrophobic organic compounds in soil?

3.2.5.1 Mechanism of mobilization

The amphiphilic structure of the surfactant molecule results in a concentration of the molecule at interphases. At the interphase of an aqueous-solid system (e.g. pore water/soil particles) the hydrophilic part of the surface-active molecule will concentrate at the aqueous phase and the more hydrophobic part of the molecule will orientate toward the nonpolar part of the system. This orientation of the surfactant molecules results in a decreased surface tension of the water. The ability of the water to overcome the capillary forces, which retain the PAH-compounds in the soil pores, and to replace the hydrophobic compounds is increased by decreased surface tension (*Ang & Abdul, 1991*). This characteristic makes surfactants applicable for physical soil washing processes of PAH contaminated soils (*Ellis et al., 1985; Vigon & Rubin, 1989; Abdul et al., 1992*).

Research reported on the use of surfactants for *in situ* aquifer restoration by flushing the contaminated soil, includes a laboratory study conducted by Ellis et al. (*1985*). The extent of solubilization of aliphatic and polycyclic aromatic hydrocarbons, polychlorinated biphenyl (PCB) in chlorobenzene, and di-, tri- and pentachlorophenols from a sandy soil was examined. The removal of the contaminants with the surfactant solution was four orders of magnitude higher than removal obtained by flushing with water alone.

Chiou et al. (*1991*) demonstrated significantly increased solubilities of DDT and PCB's in aqueous solutions containing a commercially available linear alkylbenzenesulfonate (LAS). Furthermore they demonstrated that the small amount of neutral oils in commercially available LAS had a pronounced effect leading to enhanced solubilities as well. Thus, it was concluded that the discharge of waste water containing significant levels of oil and surface-active agents could lead to potential mobilization of organic pollutants in the aquatic environment.

3.2.5.2 Mechanism to increase apparent aqueous solubility

Another characteristic of surfactants is the ability to form micelles. At low concentrations in aqueous solutions (1 ppm, *Swisher, 1987*) the surfactant is present as single molecules. If the surfactant concentration increases to a critical micelle concentration (CMC), the surfactant molecules will aggregate to micelles. (Figure 3.5). In a soil-aqueous system containing hydrophobic contaminants (e.g. PAH's), the micelle formation will results in a colloid suspension of surfactant-PAH micelles in the aqueous phase (an emulsion). The formation of micelles thus results in an increased apparent aqueous solubility of the PAH's.

Edward et al. (*1991*) have studied the solubilization of PAHs in micellar surfactant solutions. They found that aqueous solutions of four commercial, nonionic surfactants increased the apparent aqueous solubility of naphthalene, phenanthrene, and pyrene. Similarly the apparent aqueous solubility of fluoranthene was found to be significantly increased by the presence of the non-ionic surfactant Triton X-100. It was noted that the apparent solubility was only slightly increased at a surfactant concentration less than the CMC (*Willumsen, unpublished*). The solubilization of fluoranthene by varying concentrations of Triton X-100 is presented in Fig. 3.6.

These results are confirmed by several laboratory studies, which have shown that chemical surfactant solutions used in soil-water suspensions were able to increase the aqueous solubility and the mobility of hydrophobic organic compounds, such as oil compounds (*Ang & Abdul, 1991; Abdul et al., 1992*), biphenyl and polychlorinated biphenyls (*Vigon & Rubin, 1989; Abdul et al. 1992*), dodecane (*Pennell et al., 1993; Ambriola et al., 1993*), trichloroethene (Deitsch & Smith 1995), and PAHs (*Vigon & Rubin, 1989; Liu et al., 1991; Edward et al., 1992; Willumsen et al., 1995*). If PAHs pseudo-solubilized in micelles are available to the microorganisms present in soil, the rate and extent of removal of hydrophobic organic contaminants from soil by biological degradation processes can possibly be increased by applying surfactants to the soil-water systems. Surfactant-enhanced biodegradation is discussed in section 4.4.

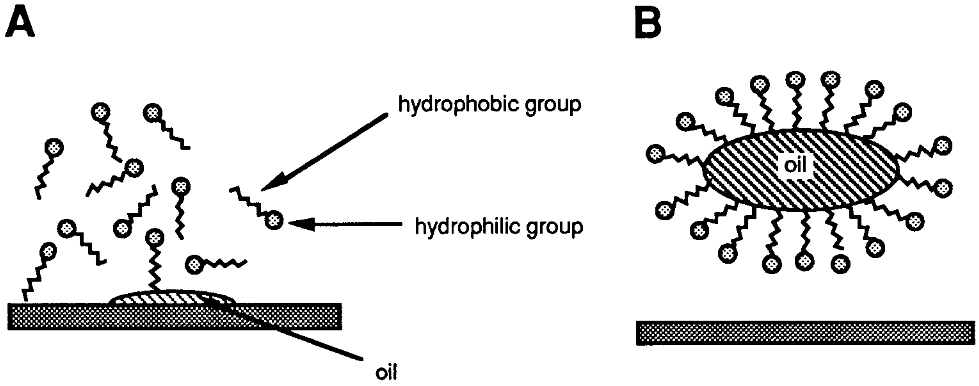


Figure 3.5. Conceptual model of the action of a surfactant on hydrophobic compounds. A: surfactant molecules in low aquoeus concentration (below CMC). B: formation of micelles at surfactant concentrations above CMC.

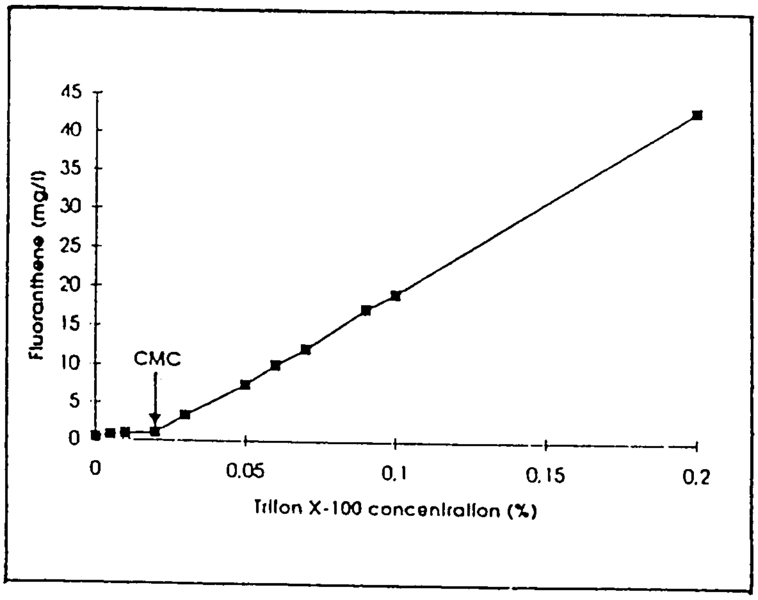


Figure 3.6. Solubilization of fluoranthene by Triton X-100 nonionic surfactant. The CMC is indicated by the intersection of the two linear regions of the solubilization relationship (*Willumsen, unpublished*)

3.3 Evaporation

For non-polar organic compounds evaporation, i.e. transfer of the organic compound from the water solution to the above air, may constitute as an important escape route and, hence, as a process potentially decreasing the water concentration, i.e. the availability of the compound.

The partition between an aqueous solution and the above gas phase is give by the so-called Henry's Law constants, K_H, describing the ratio between the partial pressure of the compound in the gas phase (in atm.) to the molecular concentration in the aqueous phase (in mol/L).

$$K_H = \frac{P_i}{C_w} \text{ atm L/mol} \qquad 3.14$$

which, assuming an ideal gas behaviour of the gaseous fraction, i.e. $P_i = n_iRT/V$, can be rewritten into

$$K_H' = \frac{C_g}{C_w} \qquad 3.15$$

where $K_H' = K_H/RT$ and C_g and C_w the molar concentrations (mol/L) of the compound in the gaseous and aqueous phase, respectively. Thus, K_H' is dimensionless.

Henry's Law constants for PAH's are typically in the range of 1 - 10^{-3} atm L/mol, cf. Table 3.6 giving the calculated values for selected PAH's (cf. *Schwarzenbach et al., 1992, p. 621* and references therein).

Table 3.6. Henry's Law constants, K_H (atm L/mol) for selected PAH's in a water-air system (25°C)

	log K_H
Naphtalene	-0.37
Anthracene	-1.64
Phenanthrene	-1.59
Pyrene	-2.05
Benzo(a)pyrene	-2.92
Benzo(a)anthracene	-2.24
Fluorene	-1.14
Fluoranthene	-1.98

Obviously, both a possible salt concentration in the aqueous phase as well as the temperature of the system will influence the Henry's Law constants. Thus, increasing the salt concentration in the water will (vide supra) lead to a decreased solubility of the PAH's, whereas it will not influence the evaporation. Consequently proportionally increased Henry's Law constants prevail. The temperature effect is somewhat more complicated to assess as both the solubilities and the evaporation rates are affected. However, a thermodynamical analysis (*Schwarzenbach et al., 1992, chap. 6*) lead to the conclusion that Henry's Law constants will increase with increasing temperature.

It should be noted that the simple equations 3.14 and 3.15 refer to an equilibrium situation, which will seldom prevail in environmental systems, as the gaseous molecules typically will be removed due to an air flow caused by the wind (vide infra). However, the removal of the PAH's from the water phase, i.e. the flux from the aqueous to the gaseous phase, can mathematically easily be assessed under certain assumptions (*Maykay et al., 1979*), e.g. 1° a well mixed water phase, 2° an ideal behaviour of the gaseous phase, 3° a constant weater volume, 4° the partial pressure of the PAH's is small compared to the total pressure, and 5° the system is isothermal. In this case the concentration in the water at the time t is given by

$$\ln C = \ln C_o - \frac{K_H F}{VRT} t \qquad 3.16$$

where C_o is the concentration at t = 0, F is the gas flow (m^3/h), V is the water volume (m^3), R is the gas constant and T the system temperature. Thus, eqn. 3.16 can be applied to give a rough estimate of the decrease in aqueous concentration, and consequently availability, of PAH's due to evaporation from open waters as a function of time. However, the technique apprears to more suitable to derive Henry's Law constants by following the concentration C as function of time.

Turning to more realistic environmental systems the flux, $\mathcal{F}$ (mol/cm^2s) of a compound from water to air is given by

$$\mathcal{F} = v_{tot}(C_w - C_a/K_H') \qquad 3.17$$

where v_{tot} (cm/s) is the mass transfer coefficient and C_w and C_a are the concentration in the water and the air, respectively, the unit of the ($C_w - C_a/K_H'$) being mol/cm^3. The mass transfer coefficient can be expressed as a function of the so-called partial transfer velocities v_w and v_a:

$$1/v_{tot} = 1/v_w + 1/v_a K_H' = 1/v_w + 1/v_a' \qquad 3.18$$

The partial transfer velocities of a given compound, $v_w(i)$ and $v_a(i)$, can be assessed through the following two equations using well known values for the the transfer velocities of oxygen in water, $v_w(O_2)$, and water in air, $v_a(H_2O)$, and the corresponding molecular diffusivities, $D_w(O_2)$ and $D_a(H_2O)$[1], as reference values. $D_w(i)$ and $D_a(i)$ are the molecular diffusivities of the actual compounds under investigation.

$$v_w(i) = v_w(O_2)[D_w(i)/D_w(O_2)]^{ß} \qquad 3.19$$

$$v_a(i) = v_a(H_2O)[D_a(i)/D_a(H_2O)]^{\alpha} \qquad 3.20$$

The exponents α and ß have been estimated to be 0.67 and 0.5, respectively (*Mackay & Yeun, 1983*).

Obviously, the transfer velocities, and hence the flux $\mathcal{F}$, will be strongly dependent of the possible removal of the com-

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[1] The values of $D_w(O_2)$ and $D_a(H_2O)$ have been estimated to be 0.26 and 2.1×10^{-5} cm^2/s, respecitively (cf. *Schwarzenbach et al., 1992, chap. 10*)

pound by the wind. However, rather simple empirical relations for the $v_w(O_2)$ and $v_a(H_2O)$ can be derived (cf. *Schwarzenbach et al., 1992, chap. 10*) as given in eqn.'s 3.21 and 3.22.

$$v_w(O_2) = 4x10^{-4} + 4x10^{-5}u_{10}^2 \quad (cm/s) \qquad 3.21$$

$$v_a(H_2O) \approx 0.2u_{10} + 0.3 \quad (cm/s) \qquad 3.22$$

where u_{10} is the wind speed 10 m above the surface (measured in m/s).

Based on the above eqn.'s v_{tot} for naphtalene has been estimated to $2.7x10^{-4}$ and $8.6x10^{-3}$ cm/s for wind speed equal to 1 and 20 m/s, respectively. The analogous values for benzo-(a)pyrene are $9.6x10^{-6}$ and $8.5x10^{-5}$ cm/s, respectively.

3.4 Sorption/Desorption

The sorption of pollutants onto soil and/or dissolved material appears as a predominant factor in controlling the availability of the pollutant. Thus, the sorption/desorption process constitute as a major retardation mechanism influencing the migration behaviour of pollutants with ground water.

Several factors obviously may influence the sorption of pollutants to geological material, such as pH and ionic strength as well as the composition of the solid phase, e.g. clay content and the fraction of organic carbon in the soil.

In cases where the geological media, e.g. soil, exhibit a significant fraction of organic carbon (f_{oc}) the latter plays a major role in determining the sorption of organic pollutants, especially those of non-polar, hydrophobic nature (*Schwarzen-bach, 1982; Schwarzenbach and Westall, 1981, 1984; Frost, 1969; McCall et al., 1981; Means et al., 1980, 1982*).

The sorption has been expressed in terms of partition coefficients, K_D, derived as the ratio between the concentration of the pollutant sorbed on the solid (C_s mol/g) and in solution (C_{sol} mol/L)

$$K_D = C_s/C_{sol} \quad (L/g) \qquad 3.23$$

However, since the fraction of organic carbon in the solid phase apparently plays a crucial role as the dominating solid fraction in the sorption process, the partition coefficients are often normalized to the to the latter

$$K_{oc} = C_s/f_{oc}C_{sol} \qquad 3.24$$

Thus K_{oc} is the organic carbon normalized partition coefficient, corresponding to the partition of a pollutant between a liquid phase and a theoretical solid phase of 100% organic carbon, i.e. $f_{oc} = 1$. In cases of very low, or even zero content of organic carbon, the dominant factors for sorption of organic compounds are the specific surface area and the actual nature of the mineral surface (*Schwarzenbach, 1982*).

It is strongly emphasized that not only the fraction of organic carbon in the solid phase plays an important role in determining the partition between the liquid and the solid phases, but also the dissolved organic matter (DOM) and particulate organic matter (POM) have to be taken into account. Thus, a pronounced binding of non-polar, hydrophobic organic pollutants to dissolved and particulate organic matter has been reported (*Carter & Suffet, 1982, 1983; Caron et al., 1985; Caron & Suffet, 1989; Henry et al., 1989; Hassett & Anderson, 1982; Brownawell & Farrington, 1985; Hunchack-Kariouk & Suffet, 1991*) hereby decreasing the sorption of these compounds to soils and sediments.

Consequently, the migration potential will increase. The concentration of the pollutants in solution can accordingly be regarded as the sum of the concentrations of the free pollutant, C_{aq}, and that of the pollutant "complexed" to the DOM, C_{doc}. Thus K_{oc} can be expressed as

$$K_{oc} = C_s/(C_{aq} + C_{doc})f_{oc} \qquad 3.25$$

Introducing a DOM/water partition coefficient, K_{doc}, the latter being normalized to the carbon fraction of the DOM concentration in the bulk aqueous phase, $[DOC]_w$, i.e.

$$K_{doc} = C_{doc}/C_{aq}[DOC]_w \qquad 3.26$$

gives the following expression for K_{oc}, thus taking into account the "complexation" betwen pollutants and the DOM.

$$K_{oc} = C_s/C_{aq}(1 + K_{doc}[DOC]_w)f_{oc} \qquad 3.27$$

The "complexation" reaction will be discussed in more detail in a proceeding section.

Introducing a suspended solid phase, i.e. particulate matter, a similar set of equations can be derived for the partition between the aqueous phase and the suspended solid phase. Thus,

$$K_{oc}' = C_s'/C_{aq}(1 + K_{doc}[DOC]_w)f_{oc}' \qquad 3.28$$

where K_{oc}', C_s' and f_{oc}' correspond to the partition coefficient for the water/suspended matter system, the concentration of the pollutant sorbed on the suspended matter and the fraction of organic carbon of the suspended matter, respectively.

Combining the two systems, the total concentration of the pollutant sorbed onto the solid phase is given by $C_s + C_s'$. Accordingly, the overall partition coefficient, based on the oganic carbon content of the solid phases can be expressed as

$$K_{oc}^* = \left(\frac{C_s}{f_{oc}} + \frac{C_s'}{f_{oc}'}\right) / \; C_{aq}(1 + K_{doc}[DOC]_w) \qquad 3.29$$

It is emphasized that DOM, e.g. humic matrials, unambiguously is sorbed on mineral surfaces (cf. e.g. *Davis, 1982; Jardine et al., 1989*), whereby the surfaces, and, hence, their properties towards organic pollutants, are significantly changed.

The possible reactions involved in the sorption/desorption process of PAH's can be summarized as follows , **S** denoting the surfaces of the solid/suspended materials, and DOM/PAH the "complex" between dissolved organic matter and PAH's, respectively (cf. Fig. 3.7).

$$\mathbb{S} + \text{DOM} \rightleftharpoons \mathbb{S}\text{-DOM}$$

$$\mathbb{S} + \text{PAH} \rightleftharpoons \mathbb{S}\text{-PAH}$$

$$\text{DOM} + \text{PAH} \rightleftharpoons \text{DOM/PAH}$$

$$\mathbb{S} + \text{DOM/PAH} \rightleftharpoons \mathbb{S}\text{-DOM/PAH}$$

$$\mathbb{S}\text{-DOM} + \text{PAH} \rightleftharpoons \mathbb{S}\text{-DOM/PAH}$$

$$\mathbb{S}\text{-PAH} + \text{DOM} \rightleftharpoons \mathbb{S} + \text{DOM/PAH}$$

$$\mathbb{S}\text{-DOM/PAH} + \text{DOM} \rightleftharpoons \mathbb{S}\text{-DOM} + \text{DOM/PAH}$$

Figure 3.7. Modes of interactions between PAH's and DOM in the presence of solid phases.

Obviously, both the sorption of PAH's onto solid surfaces, possibly normalized to the fraction of organic carbon, as well as the dissollution/precipitation of PAH's shall be regarded as equilibrium reactions. Hence, both $\log K_{oc}$ and $\log S_w$(sat) are linearly related to the Gibbs free energy of sorption and dissolution, respectively (cf. *Alder et al., 1971, chap. 1; Schwarzenbach et al., 1992, chap 11*). Consequently, a linear correlation between $\log K_{oc}$ and $\log S_w$(sat) prevails.

$$\log K_{oc} \;=\; - a \log S_w(\mathrm{sat}) \;+\; b \qquad 3.30$$

Karickhoff (*1981*) reported on calculated values of $\log K_{oc}$, which were found in good agreement with experimentally obtained values (*Karickhoff et al., 1979*).

Comparing these data with the solubility data in Table 3.1 that values of a and b (cf. eqn. 3.30) can be estimated to -0.54 and 1.14 ($r^2 = 0.89$), respectively, which agrees reasonably well with the values -0.686 and 4.273 estimated by Means et al (*1980*) on the basis of 22 PAH's.

Table 3.7. Logarithmic K_{oc} values for selected PAH's.

	$\log K_{oc}$
Naphtalene	2.94
Anthracene	4.20
Phenanthrene	4.08
Pyrene	4.83

Since the sorption, expressed as $\log K_{oc}$, is a function of the solubility, it is obvious that the distribution coefficient will be dependent of the same parameters as the solubility, e.g. temperature (cf. 3.2.2), ionic strength (cf. 3.2.3) and the possible presence of organic co-solvents (cf. 3.2.4). Thus, increasing the temperature will generally cause decreased sorption of PAH's to neutral surfaces, e.g. a 10°C increase in the temperature of the water-solid suspension will results in an approximate 30% decrease in the sorption of pyrene.

In the case of increased ionic strength a simple expression of the distribution coefficient in the presence of an increased bulk salt concentration , $\log K_{oc,salt}$, develops (cf. eqn. 3.10).

$$\log K_{oc,salt} \;=\; \log K_{oc} \;+\; aK_s[S] \qquad 3.31$$

Hence, not surprisingly sorption of PAH's will generally increase with increasing ionic strength.
On the other hand, the possible presence of an organic co-solvents will result in decreased sorption, i.e. lower $\log K_{oc,cos}$

values. Thus, the K_{oc} values for anthracene and pyrene are lowered by a factor of ca. 0.4 and 0.3, respectively, by addition of 10% methanol to the aqueous phase *(Nkedi-Kizza et al., 1985; Schwarzenbach et al., 1992, chap. 11)*.

3.4.1. Octanol - water partition coefficients

The sorption of organic pollutants, as PAH's, i.e. the preference of the latter towards organic carbon, is advantageously addressed through the so-called octanol-water partition coefficient, K_{ow}, which simply express the partitioning of the pollutant between an aqueous phase and an octanol phase. Octanol is generally accepted as the reference organic solvent.

$$K_{ow} = \frac{C_{oct}}{C_w} \qquad 3.32$$

C_{oct} and C_w are the concentrations of the organic compound in octanol and water, respectively.

Obviously $\log K_{ow}$ values reflect the solubility of organic species in the aqueous phase. Based on the same arguments as given above (cf. 3.4) it develops that there is a linear relation between $\log K_{ow}$ and $\log S_w$(sat).

$$\log K_{ow} = - a \log S_w(\mathrm{sat}) + b \qquad 3.33$$

Combining eqn.'s 3.30 and 3.33 it appears that the sorption of e.g. PAH's to solid surfaces, expressed by $\log K_{oc}$ values, can be estimated through the corresponding $\log K_{ow}$ values.

$$\log K_{oc} = x \log K_{ow} + y \qquad 3.34$$

The values of x and y have been estimated based on linear regression analyses taking into account data for more than 20 PAH's. Thus corresponding x,y values have been found to be 1.01,-0.21 (*Karickhoff et al., 1979*), 1.00,-0.317 (*Means et al., 1980*) and 0.989,-0346 (*Karickhoff, 1981*) for $\log K_{ow}$'s ranging from 1.6 to 6.5.

Hence, $\log K_{ow}$ values, which are easily obtainable in the laboratory, e.g. by chromatographic techniques or slow stirring (*OECD, 1989*) apparently give an easy approach to an evaluation of the sorption characteristics of e.g. PAH's in a given environmental compartment.

For PAH's logK_{ow} are typically found in the range of 2 to 6.5, values for selected species being given in Table 3.8 (*Schwarzenbach et al., 1992*)

Table 3.8. Logarithmic octanol-water partition coefficients for selected PAH's

	logK_{ow}
Naphtalene	3.36
Anthracene	4.54
Phenanthrene	4.57
Pyrene	5.13
Benzo(a)pyrene	6.50
Benzo(a)anthracene	5.91
Fluorene	4.18
Fluoranthene	5.22

3.4.2 Influence of dissolved organic matter

The interaction between PAH's and naturally occurring organic matter has been investigated only sparsely. However, the apparent affinity of natural organic matter (NOM) to PAH's as well as to mineral surfaces obviously adds complexity to migration phenomena and may, thus, play an important role in the geochemical behaviour, including transport behaviour, of PAH's in the environment. Hence, the interaction between PAH's and e.g. humic material may lead to significantly increased PAH concentrations in the aqueous phase as well as significantly increased desorption of PAH's bound to mineral surfaces such as Al_2O_3 or SiO_2.

The increasing apparent solubility of phenanthrene in the presence of dissolved humic material, was elucidated by dissolving finely dispersed solid phenanthrene in water with varying amounts of humic acids (*Lassen & Carlsen, 1997*). In Fig. 3.8 the concentration - time profiles for solid phenanthrene in water containing 0, 25 and 50 mg/L of humic acids are shown. It is noted that the presence of dissolved humic material, in agreement with previous *reports (Landrum et al., 1984, Gauthier et al., 1986)*, unambiguously leads to an increase in the apparent aqueous solubility of phenanthrene. However, it should be emphasized that the effect is relatively small. Thus, addition of 25 and 50 mg/L of humic acids to the water phase increased the apparent solubility of phenanthrene by *ca.* 17 and 42%, respectively compared to pure water.

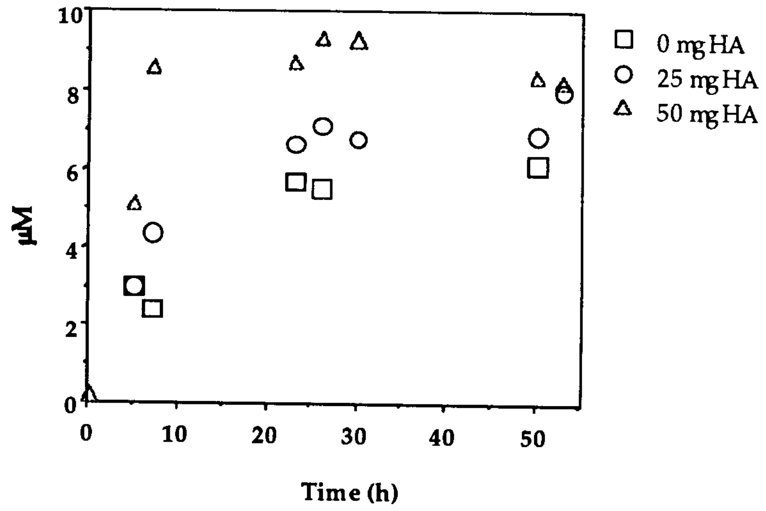

Figure 3.8. Time resolved concentration profiles for the dissolution of finely dispersed solid phenanthrene in water containing varying amounts of dissolved humic acids.

It is noted (Fig. 3.8) that apparently the dissolution of solid phenanthrene is a relatively slow process, which typically requires more than 20 hrs to reach equilibrium (cf. the discussion in section 3.2.1).

The binding of PAH's to humic substances most probably involves electrostatic interactions between the π-electron system of the PAH's and that of the aromatic core of the humic material as suggested for e.g. PCB's (*Leenheer, 1991*).

In agreement with this assumption Gauthier et al. (*1987*) found that K_{oc} values of pyrene correlated strongly with the degree of aromaticity, the correlation being positive, of 14 different humic and fulvic acids. A higher binding capacity of soil humic acid to PAH's relative to aquatic naturally organic matter has analogously been related to the higher aromatic content and lower polarity of the former relative to the latter (*Liu & Amy, 1993*). These results are furthermore consistent with the possible formation of charge-transfer complexes between PAH's and humics (*Kress & Ziechmann, 1977*).

Obviously the interaction (in solution between a PAH and DOM can be expressed as a simple equilibrium reaction

$$\mathrm{DOM + PAH \rightleftharpoons DOM/PAH}$$

the corresponding equilibrium constant being expressed as

$$K_a = \frac{[\mathrm{DOM/PAH}]}{[\mathrm{PAH}][\mathrm{DOM}]} \qquad 3.35$$

which by dividing by the fraction of organic carbon of the dissolved organic matter gives the K_{doc} values (eqn. 3.24), i.e. the interaction constants normalized to the organic carbon content in the studied DOM (cf. section 3.4).

Several techniques have been applied in studying the interaction between DOM and PAH's leading to an apparent solubiliyu enhancement. The techniques comprise luminiscence spectroscopy (*Kress & Ziechmann, 1977*), reverse-phase separation (*Landrum et al., 1984; Morehead et al., 1986*), equilibrium dialysis (*McCarthy & Jimenez, 1985, McCarthy et al., 1989; Carter & Suffet, 1983*), solubility change (*Carter & Suffet, 1983*), changes in sorption behaviour (*Carter & Suffet, 1983*) and fluorescence quenching (*Gauthier et al., 1986, 1987; McCarthy & Jimenez, 1985*).

Both the reverse-phase separation (*Landrum et al., 1984*) and the equilibrium dialysis (*McCarthy & Jimenez, 1985*) techniques rely on a separation of the free and the bound PAH's. Thus, the reverse-phase separation technique is based on the separation of bound PAH's from the free by passing the reaction mixture through a short reverse-phase HPLC column or, in practice a C-18 Sep-Pak cartridge. The humic-bound PAH's passed through the column, while the unbound, i.e. free PAH's were retained.

The equilibrium dialysis technique is based on the fact that the free PAH's can move unhindered through a dialysis membrane, whereas humic bound species are restricted to one side of the membrane. A theoretical treatment of the equilibrium dialysis technique has been given by Carlsen (*1985*). The equilibrium dialysis technique, however, suffers from at least one major disadvantage, since DOM typically contains a low molecular fraction, i.e. the fulvic fraction, which obviously will pass the membrane and hereby possibly masking the results, although the interaction between PAH's and the fulvic acids, which are enriched in oxygen, probably plays a less important role than the interaction with the humic fraction. Furthermore, the fulvic acids may be too small to form the hydrophobic core which would stabilize the PAH's in aqueous solution. A priori, a preliminary dialysis of the DOM solution should remedy this problem. However, it appears that humic acids most probably to a certain extent can be described as an equilibrium system, i.e. by removal of the lower molecular fraction by dialysis shifts the equilibrium in favour of the latter fraction. Consequently a continuous leaching of low molecular weight humics/fulvics can be observed (*Carlsen & Warwick, unpublished*). Furthermore, a preliminary dialysis of the DOM would obviously change the actual nature of the material, which especially in the cases of studies involving site-specific material may be crucial.

Studies on the interaction between DOM and PAH's have been carried out for rather few PAH's only. Landrum et al. (*1984*) studied the interaction between a commercial humic material (Aldrich) and anthracene, phenanthrene, benzo(a)-pyrene and benzo(a)anthracene, respectively. In addition interactions between natural water DOM and anthracene and benzo(a)pyrene, respectively, were investigated. A reasonable correlation between the equilibrium constants and K_{ow} data for the respective PAH's was noted, whereas the binding for a series of hydrophobic organic compounds was found to irreversibly proportional ($\log K_a = -0.648 \log S + 5.72$; $r = -0.97$) to the solubility (*Landrum et al., 1984; Morehead et al., 1986*).

The interaction between anthracene and Boonton and Pakim Pond humic acids, respectively, was studied by Carter & Suffet (*1983*) applying both the equilibrium dialysis-, solubility change- and sorption change behaviour techniques, whereas the equilibrium dialysis technique was applying by McCarthy & Jimenez (*1985*) in their study on the interaction between benzo(a)pyrene and dissolved humic material (Aldrich). The results of the single investigations are summarized in Table 3.9. These data are not normalized to the fraction of organic carbon in the DOM.

Table 3.9 Logarithmic equilibium constant for the interactions between selected PAH and DOM

	HA-1[a]	HA-2[b]	HA-3[c]	DOM[d]
naphtalene	(3.04)[e]			
anthracene	(4.18)[e]			
	4.15[f]	4.81-4.98[g]	4.08-4.61[g]	3.81-4.87[f]
phenanthrene	3.92[f]			
benzo(a)pyrene	5.95[f]			4.26-4.72[f]
	6.31[e]			
benzo(a)anthracene	5.30[f]			
	(5.18)[e]			

[a] Aldrich humic acids, [b] Boonton humic acids, [c] Pakim Pond humic acids, [d] Natural water dissolved organic matter, [e] *Mc-Carthy & Jimenez, 1985;* [f] *Landrum et al., 1984;* [g] *Carter & Suffet, 1983.*

In contrast to the above data, which are not normalized to the fraction of organic carbon, Gauthier et al. (*1986*) reported a series of data for the interaction between anthracene, phenanthrene and pyrene, respectively, with up to 4 different humic acids, the resulting equilibrium constants being normalized. The normalized K_{doc} values for the pyrene HA interaction ranged from 4,74 to 5,23, whereas the values for anthracene and phenanthrene interactions were 4,93 and 4,70, respectively, based on studies on a single type of HA.

A slight tendency towards decreasing equilibrium constants with increasing humic acid concentration was *noted (Landrum et al., 1984; McCarthy & Jimenez, 1985)*, which possibly could be ascribed to conformational differences.

The interaction between phenanthrene and humic acids in solution, which can be expressed as a simple complexation reaction (vide supra) serves as an illustrative example (*Lassen & Carlsen, 1997*)

$$\mathbf{Phen + HA \rightleftarrows Phen{\bullet}HA}$$

the corresponding interaction constant being given by

$$\mathbf{[Phen{\bullet}HA]/[Phen]_f[HA]_f = K} \tag{3.36}$$

Since the total amount of phenanthrene in solution equaled the sum of the free and the complexed phenanthrene, i.e. $[Phen]_{tot} = [Phen]_f + [Phen]_c = [Phen]_f + [Phen{\bullet}HA]$, eqn. 3.36 can be rewritten as

$$\mathbf{[Phen]_{tot}/[Phen]_f = 1 + K\,[HA]_f} \tag{3.37}$$

In the study of Lassen and Carlsen (*1997*) $[Phen]_f$ equaled the concentration of phenanthrene corresponding to saturation in synthetic groundwater without humic acids, as the aqueous solution was kept in equilibrium with solid phenanthrene. The humic acids was assumed to be present in a sufficient excess to allow the assumption $[HA]_{tot} = [HA]_f$.

In Fig 3.9 the $[Phen]_{tot}/[Phen]_f$ ratio as function of the concentration of humic acids in solution is depicted.

The interaction constant, K, between phenanthrene and humic acids in solution, given as the tangential slope of the curve, i.e. $K = d([Phen]_f[HA]_f)/d([HA])$. Thus, the study demonstrated a pronounced decrease in K by increasing humic acids concentration. A rough estimate of K was obtained by assuming linearity between the single measurements. Thus, K was estimated to be 2.4×10^4 mL/g for humic acid concentrations up to 15 mg/L. For comparison Gauthier et al. (*1986*) reported the interaction constant between phenanthrene and fulvic acids to be 3.2×10^4 mL/g, the fulvic acid concentration being kept below 24 mg/L. The interaction constants for the phenanthrene - humic acid interaction for humic acid concentration ranges 15-37 mg/L, 37-79 mg/L and 79-181 mg/L were analogously estimated to be 1.1 x 10^4, 0.69 x 10^4 and 0.12 x 10^4 mL/g, respectively (*Lassen & Carlsen, 1997*).

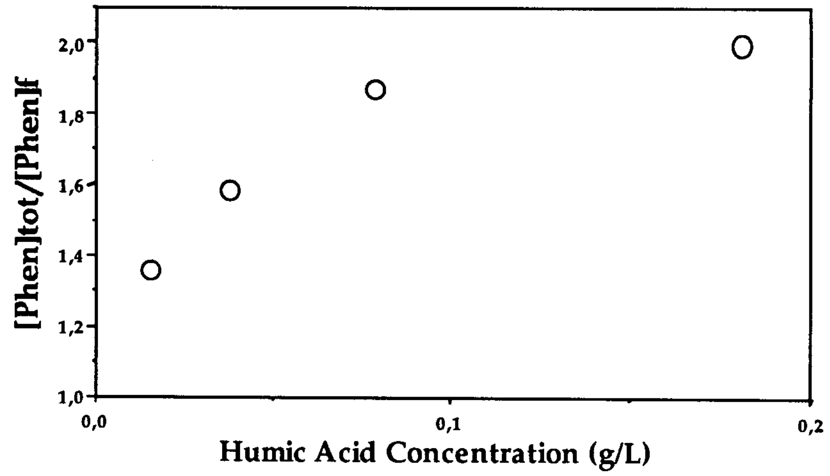

Figure 3.9. $[\mathrm{Phen}]_{tot}$ /$[\mathrm{Phen}]_f$ ratio as function of the concentration of humic acids in solution.

The pronounced decrease in the interaction constant for the reaction between phenanthrene and humic acids in solution was also found to be reflected in an observed decrease in the partitioning coefficient for phenanthrene between water and the dissolved organic matter, K_{OM}, (Fig. 3.10) in agreement with the results of Landrum et al. (*1984*) for low humic acid concentrations.

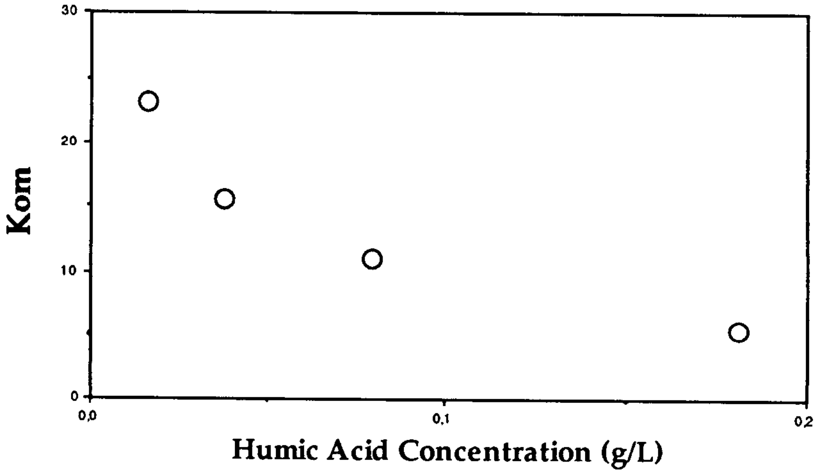

Figure 3.10. Partitioning coefficient, K_{OM}, for phenanthrene between water and the dissolved organic matter as function of humic acid concentration.

In order to explain the decrease in interaction constant, as well as in K_{OM}, possible structural changes in the humic acids was considered (*Lassen & Carlsen, 1997*).

Ghosh and Schnitzer (*1980*) reported on the macromolecular structures of humic materials. It appeared that the macromolecular configurations of humic materials were not unique but varied with changes in the medium. Thus, it was disclosed that the controlling parameters were sample concentration, pH of the system and ionic strength of the medium. The study unambiguously revealed that the humic acid molecules curl-up by increasing humic acid concentrations causing a decrease in the average molecular area as well as in the end-to-end separations. Since the interaction between aromatic hydrocarbons and humic acids can be ascribed to charge-transfer complexation (*Kress & Ziechmann, 1977*), the decrease in interaction constant and K_{OM} is in perfect agreement with the structural changes of the humic acids due to increasing concentration, i.e. the curling-up of the humic molecules apparently leaves a decreased number of sites available for complexation with the phenanthrene molecules.

Increase in pH of the solution of humic acids causes only moderate structural changes (*Ghosh & Schnitzer, 1980*). Thus, an increase in pH from 6.5 to 9.5 caused a decurling of the humic molecules as disclosed by a ca 15% increase in the average molecular area and a ca. 8% increase in the end-to-end separations, respectively. In accordance with this, the present study revealed non-significant variations, apparently within the limits of uncertainty, in the solubility of phenanthrene in synthetic ground water as well as in synthetic ground water containing 100 mg/L of humic acids.

On the other hand the effect of ionic strength apparently is somewhat more pronounced (*Ghosh & Schnitzer, 1980*), as an increase in ionic strength from 0.001 to 0.1 caused a reduction in the average molecular area by 50% and in the end-to-end separations by ca. 28%, respectively. The reduction in solubility of phenanthrene in synthetic ground water from 5.2 µM ($\mu = 0$) to 4.9 µM ($\mu = 0.1$) is in perfect agreement with theory applying a Setschenow constant equal to 0.27 (*Schwarzenbach et al., 1992*). In the presence of 100 mg/L humic acids a significantly more pronouned decrease in phenanthrene solubility was disclosed. Thus, the solubility of phenanthrene was reduced from 9.2 µM ($\mu = 0$) to 9.1 µM ($\mu = 0.001$), 9.1 µM ($\mu = 0.01$) and 8.0 µM ($\mu = 0.1$), respectively, the observed effect obviously related to the curling-up of the humic material by inreasing the ionic strength (*Ghosh & Schnitzer, 1980*).

As discussed above (cf. section 3.4) several other mechanisms have to be taken into account in estimating the migration potential of PAH's in the terrestrial environment. Thus, Murphy et al. (*1992*) investigated how natural organic coatings influence the sorption of hydrophobic organic compounds to solid surfaces as kaolinite and hematite. It was demonstrated that increasing quantities of sorbed humic substances on the mineral surfaces increased the sorption of carbazole, dibenzothiophene and anthracene. In addition, it was shown that the sorption decreased with increasing ionic strength of the bulk solution.

Analogous results were obtained by Liu & Amy (*1993*) who concluded that both aqueous-phase and solid-phase natural organic material (NOM) affect the transport of PAH's in groundwater. Obviously, the degree of effects was linked to the actual nature, i.e. source of origin of the NOM as well as of the inherent transport behaviour of the latter. Thus, it was concluded that 1° interaction between PAH's and NOM is source dependent, 2° interaction between PAH's and minerals is mineralogy dependent, e.g. dependent of NOM sorbed to the surfaces, 3° aqueous-phase NOM facilitates PAH transport and 4° solid-state NOM retards PAH transport.

In order to elucidate the migration potential of PAH's in ground water simple transport calculation has been reported (*Carlsen et al., 1989*) on the influence of complexation on the migration of pollutants in the saturated zone.

3.4.2.1 Migration potential

The general reaction between a PAH and DOM has been discussed above (cf. eqn. 3.33) Typically under environmental conditions the DOM concentration will be significantly higher than the PAH concentration. Thus, the DOM concentration [DOM] can be approximated by the total DOM concentration [$\mathrm{DOM}^{\mathrm{tot}}$], which means that

$$\frac{[\mathrm{DOM/PAH}]}{[\mathrm{PAH}]} = K_a[\mathrm{DOM}^{\mathrm{tot}}] = K_a' \qquad 3.38$$

where K_a' is the apparent equilibium constant of the PAH - DOM equilibium system, which equals the ratio between the apparent first order rate constant for the complex formation, $k_1' = k_1[\mathrm{DOM}^{\mathrm{tot}}]$ and for the dissociation, k_{-1}, respectively.

Migration of PAH's were calculated through a column of 100 m length, the ground water velocity is assumed to be 1 m/y and the dispersion coefficient is fixed to 0.03 m^2/y. The initial concentrations at the entrance of the column of the free, $[\mathrm{PAH}]_o$, and the bound, $[\mathrm{DOM/PAH}]_o$, PAH's were assumed to be 1 mol/m^3 and 0 mol/m^3, respectively. The pollutant, PAH, was introduced to the column as a single pulse.

Equilibrium systems exhibiting apparent equilibrium constants ranging from 0, i.e. no interaction, to 1000 have been reported (*Carlsen et al., 1989*). In all cases, taking a study of Pagenkopf (*1978*) into account, both reactions involved are fast compared to the residence time of the equilibrium system in the column under investigation.

In general it is assumed that the bound PAH's migrate through the column without retention, i.e. R_f(DOM/PAH) = 1, whereas the retention factor of the free PAH's was varied in the range from 1 to 100. However, one set of calculations, assuming a certain retention of the DOM/PAH, applying R_f(DOM/PAH) = 5, has been carried out.

First of all it can stated that independently of the ratio between the rate constants, i.e. the apparent equilibrium constant, as well as of the applied retention factors for the free and the bound PAH, respectively, both parties of the equilibrium system migrate through the column with the same speed, controlled be an *effective retention* factor. Hence, the two species of the system will accordingly be eluted from the exit of the column simultaneously. This fact is demonstrated by the representative set of elution curves depicted in Figure 3.11. The curves in Fig. 3.11 visualizes the elution of an equilibrium system exhibiting an apparent stability constant equal to 100, the retention factors of the free PAH being 2 and 10, respectively. In both cases the retention factor of the complex was fixed equal to 1. It is noted that the PAH/(DOM/PAH) equilibrium system of course is subject to the increase in dispersion by increased retention, i.e. increased residence time in the column.

Unambiguously it can be noted that the elution time increases for an increased PAH retention. The calculated values, referring to the retention factors mentioned in Table 4 are 102, 110, 115, 150, and 200 years, respectively. It can additionally be noted that the actual value of the apparent equilibrium constant is nicely reflected by the ratio between the eluate concentrations of the bound and free PAH's. This feature appears as a common trend for fixed equilibrium constants as summarized in Table 3.10.

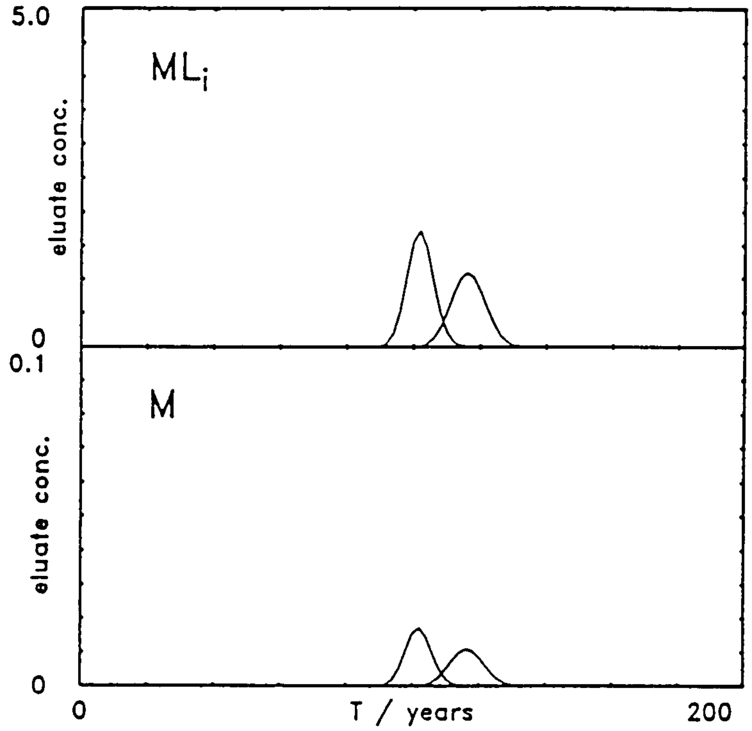

Figure 3.11. Elution curves for the components of a equilibrium system PAH/(DOM/PAH), (apparent stability constant equal to 100) exhibiting R_f(PAH) equal to 2 and 10, respectively, R_f(DOM/PAH) being equal to 1 in both cases.

Apart from the above mentioned effect of increased PAH retention, the figures in the two tables below unambiguously demonstrate, for fixed retention factors for the species involved, a significant decrease in elution time as function of increased apparent stability of the complex. The calculated average elution times given in the above two tables should be compared to the teoretically obtained values for the two species involved, assuming no interaction. These values are given by L R_f(i)/V, L and V being the column length and ground water velocity, respectively. For the system studied the values are given by 100 R_f(PAH) and 100 R_f(DOM/PAH), respectively. The two mentioned effects are visualized in Figure 3.12 and 3.13, showing the calculated average elution time as function of the retention factor (fixed complex stability) for the free pollutant and the apparent equilibrium constant (fixed PAH retention), respectively.

Table 3.10 Average elution times as a function of apparent equilibrium constant and retention factors

k_1'	k_{-1}	K_a'	R_f(PAH)	R_f(DOM/PAH)	$T_{elution}$
1	1	1	2	1	151
			5	1	300
			10	1	561
			50	1	2657
			100	1	5171
5	1	5	2	1	117
			5	1	168
			10	1	257
			50	1	959
			100	1	1780
10	1	10	2	1	110
			5	1	143
			10	1	185
			50	1	500
50	1	50	2	1	104
			5	1	115
			10	1	125
			50	1	200
			100	1	294
100	1	100	2	1	102
			5	1	110
			10	1	115
			50	1	150
			100	1	200
500	1	500	5	1	103
			10	1	103
			100	1	121
1000	1	1000	5	1	102
			10	1	102
			50	1	107
			100	1	112

Table 3.11 gives the calculated average elution times for a series of equilibrium systems, the apparent equilibrium constant varying from 1 to 10000. In all cases the retention factors for the free and the bound PAH were fixed equal to 100 and 5, respectively.

Table 3.11 Average elution times as a function of apparent constant.

k_1'	k_{-1}	$K\text{-}_a'$	$R_f(PAH)$	$R_f(DOM/PAH)$	$T_{elution}$
1	1	1	100	5	5308
5	1	5	100	5	2175
10	1	10	100	5	1507
50	1	50	100	5	685

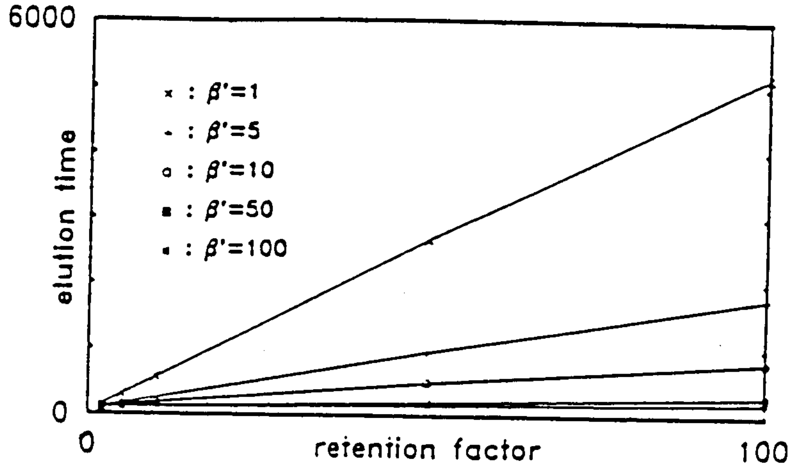

Figure 3.12 Average elution time for the PAH/(DOM/PAH) equilibrium system as function of the retention factor for the free PAH (fixed complex stability).

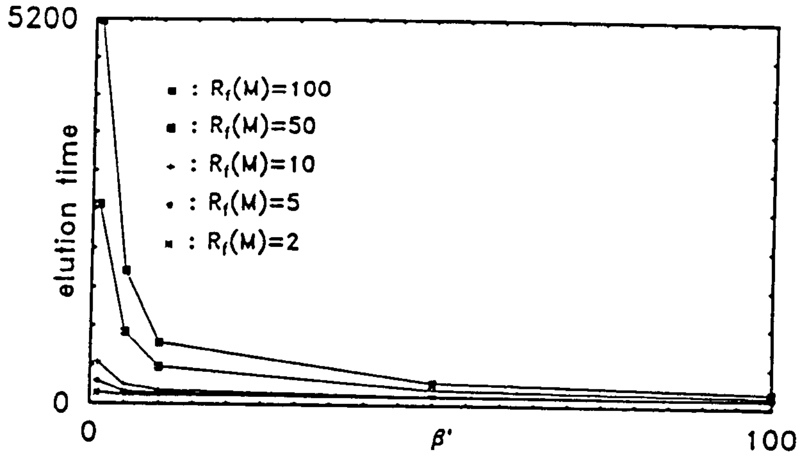

Figure 3.13 Average elution time for the PAH/(DOM/PAH) equilibrium system as function of the apparent stability constant of the system (fixed PAH retention).

Based on the data summarized in Figure 3.13, it is concluded that the following limiting values for the effective retention factors apparently apply to the migration of an equilibrium system through a column:

$$R_f^{eff} \rightarrow R_f(PAH) \text{ for } K_a^{'} \rightarrow 0 \qquad 3.39$$

$$R_f^{eff} \rightarrow R_f(DOM/PAH) \text{ for } K_a^{'} \rightarrow \infty \qquad 3.40$$

It is suggested that the following formula applies to the effective retention factors as function of the individual retention factors for the free pollutant and the complex and the apparent equilibrium constant, respectively. It should be noted that apart from obeying the above mentioned limiting conditions, the formula also agree with that proposed earlier (Carlsen et al., 1987) for the more simple systems, exhibiting only minor differences in retention factors.

$$R_f^{eff} = \frac{R_f(PAH) + R_f(DOM/PAH)}{1 + K_a^{'}} \qquad 3.41$$

Application of eqn. 3.41 gives the following effective retention factors for the equilibrium system summarized in Table 3.10, the values given in parentheses are those obtained by the COLUMN2 calculation: 5250 (5308), 2083 (2175), 1364 (1507), 686 (685), 594 (592), 501 (500). Similar comparisons can be made using the systems summarized in Table 3.9.

In the introductory remarks it was stated that a wide variety of experimental data strongly suggest that humic bound PAH's are subjects to limited sorption only, if any at all. The above described calculations elucidate the possible influence of complexation on the migration of PAH's in the terrestrial environment. Increasing the apparent equilibrium constant, either as a results of high true stability of the complex or by increased DOM concentrations, significantly decrease the effective retention of the equilibrium system. Hence, it is concluded that the naturally occurring organic ligands, i.e. humic acids, which form strong complexes with PAH's (vide supra), apparently must be expected to exhibit a pronounced influence on the migration behaviour of PAH's, leading to an increased migration speed.

4 Microbiology of PAH degradation

4.1 Bacterial metabolism of PAH's

Biodegradation of PAH's has been documented in a variety of laboratory (*Cerniglia, 1992*) and field studies (*Sims & Overcash, 1983*). It was shown that microbial degradation of PAH's is the major process that results in the decontamination of sediments and surface soils (*Sims, et al. 1990*). Recently, it has been recognized that even high molecular weight (HMW) PAH's (those containing more than three fused rings) can undergo biodegradation either through direct mineralization (*Mueller et al., 1990; Weissenfels et al., 1990; Heitkamp et al., 1988; Ho et al., 1996*) or by co-metabolism (a process of fortuitous metabolism of certain PAH's by microbial enzymes used to oxidize other PAH's) (*Cerniglia, 1992; Mueller et al., 1990; Shiaris, 1987*). Bacterial PAH metabolism was exclusively reported for aerobic conditions. Without oxygen, PAH's may persist indefinitely in anaerobic sediments and soils (*Shiaris, 1987; Bauer & Capone, 1985; DeLaune et al., 1981*) For a review of bacterial PAH metabolism see Cerniglia (*1992*). These findings have opened up the possibility of bioremediation as an effective and economical cleanup strategy. Considerable efforts are under way to develop the technology for commercial application (*Tremaine et al., 1994; Ellis et al., 1991; Smith et al., 1989; Mueller et al., 1993, 1995*).

At the same time, the complex process resulting in PAH biodegradation is poorly understood, particularly for HMW PAH's, and has proven difficult to manage consistently in the field. A major problem currently limiting the development of PAH bioremediation technology is the issue of bioavailability (*Alexander, 1994*). PAH's have very low aqueous solubilities and high hydrophobicity. Thus they tend to sorb to particulate hydrophobic surfaces or to be dissolved in an oily phase in soils. These circumstances severely limit their access by bacteria with the requisite metabolic capability. Consequently, PAH's often persist in the environment and resist microbial cleanup efforts (*Beyer et al., 1991; Dechema, 1992; Steilen et al., 1994*). The recalcitrance of PAH's for microbial degradation increases directly with the molecular weight and the octanol-water partition coefficient, K_{OW}, (cf. section 3.4.1) and inversely with aqueous water solubility, because HMW PAH's are more slowly desorbed and, therefore, less available for biological uptake (*Cerniglia, 1992; Aronstein et al., 1991; Gleim, 1994; Shiaris, 1989*).

4.2 Biochemistry and genetics of bacterial PAH degradation

The *nah* genes of *Pseudomonas putida* NCIB 9816-4 that code for the upper catabolic pathway of naphthalene degradation have been cloned and well characterized (*Simon et al., 1993; Denome et al., 1993*). The end metabolite of naphthalene produced by the enzymes encoded by this gene operon is salicylic acid. Transcription of the operon is induced by naphthalene. If, through genetic engineering, the operon is put under the control of alternative promoters, the enzymes encoded by the same genes co-metabolize anthracene to 2-hydroxy-3-naphthoic acid, and phenanthrene to 1-hydroxy-2-naphthoic acid. These water-soluble metabolites are not retained by the bacterial cells containing these gene clones and would not constitute a potential toxicological problem in bioremediation sites, since the indigenous microbiota should readily metabolize them. In fact, these compounds are not considered problem xenobiotics and are not classified as PAH's. The genetics of the lower pathway for naphthalene degradation, i.e., from salicylic acid to CO_2, have not been elucidated yet and gene clones are not available.

The genetics of phenanthrene degradation by bacteria have been partially elucidated. The first two enzymes, phenanthrene dioxygenase and cis-phenanthrene dihydrodiol dehydrogenase, have been cloned and are expressed in heterologous hosts (*Goyal et al., 1996*). The metabolites of these two enzymes are cis-3,4-dihydroxy-3,4-dihydrophenanthrene and 3,4-dihydroxyphenanthrene, respectively. The compound, 3,4-dihydroxyphenanthrene, is a less oxidized metabolite of phenanthrene than 1-hydroxy-2-naphthoic acid, and it is still considered a PAH. Therefore, it would be more practical at present in bioremediation of phenanthrene-polluted soils employing genetically engineered microorganisms, to use *nah* genes than the available phenanthrene degradation genes.

Fluoranthene mineralization has been described for two Gram-negative bacterial strains, EPA 505 and FLA 10-1 (*Yang et al. 1996*), with transient metabolites being acenaphthenone and naphthalene dicarboxylic acid. These strains also mineralize phenanthrene and transform pyrene to several metabolic intermediates. Another Gram-negative fluoranthene degrader, CO6, was isolated and preliminarily characterized (*Yang et al., 1992*). The genes encoded on a 132-kb plasmid in this strain have been cloned into *E. coli*. Work on their characterization and expression has been initiated (*P.H. Pritchard, pers. comm.*).

4.3 Bioavailabilty of sorbed PAH's

According to bioavailability studies, the rate of degradation of a hydrophobic compound is limited by its solubilisation into, and its diffusion through the aqueous phase (*Bosma et al., 1997; Harms, 1996; Harms and Bosma, 1997; Rijnaarts et al., 1990; Volkering et al., 1993; Weissenfels et al., 1992*). However, these investigations generally used PAH-degrading bacteria previously isolated by liquid enrichment cultures. It is possible that such bacterial isolates do not represent the microbiota that has evolved in contaminated soils to degrade PAH's. For 3-chlorodibenzofuran, a mathematical model predicts that bioavailability of the weakly sorbed soil pollutant requires the degrading bacteria to be in close contact with the sorbent (*Harms & Zehnder, 1995; Harms & Bosma, 1997*). Immobilization of the same bacteria on a non-sorbing surface resulted in mass-transfer limitations (*Harms & Zehnder, 1994*). These findings suggest that bacterial preferences for certain surfaces may influence substrate bioavailability. The organism-specific bioavailability of naphthalene in soil was attributed to a similar effect (*Guerin & Boyd, 1992*). One might speculate that bacteria specialized in degrading PAH's need to not only possess the necessary enzymes, but also the capability of finding and interacting efficiently with the sorbed compound in order to facilitate its uptake by the cell. The aqueous concentration of a sorbed compound is expected to be highest at the sorbing surface. A degrader bacterium attached to this surface would benefit from a shorter diffusion distance and a faster mass transfer of substrate (*van Loosdrecht et al., 1990*). Hence, bacteria capable of attaching to hydrophobic surfaces may be better suited to perform degradation of PAH's under realistic soil conditions. Isolation procedures may have to be modified in order to find attaching bacteria. Indeed, the use of PAH sorbing materials was reported to produce different isolates than the use of classical liquid enrichment cultures (*Bastiaens et al., 1996*).

Other bacterial properties might enhance degradation of hydrophobic compounds. In addition to attachment, these include the production of biosurfactants (*Déziel et al., 1996; Willumsen & Karlson, 1997*) and strategies to increase the rate of contaminant desorption (*Guerin & Boyd, 1992; Havel & Reineke, 1995*). Stucki & Alexander (*1987*) proposed that the physiological properties of the organism determine whether the aqueous solubility of the substrate becomes the growth-limiting factor. Most HMW PAH degrading bacteria isolated thus far are closely related to *Sphingomonas* or *Mycobacterium*, two genera of bacteria with special cell wall characteristics. In one study, a pyrene-utilizing *Mycobacterium* sp. was found to grow as a biofilm attached to undissolved pyrene crystals in a liquid culture (*Boldrin et al., 1993*).

Degradation of compounds with low aqueous solubility might also be facilitated by bacterial uptake systems with increased efficiency. Rate-controlling uptake systems or degradative enzymes for hydrophobic compounds were suggested to possess an unusually high affinity for their substrates (*Button, 1992; Harms & Zehnder, 1994*), allowing uptake even at low aqueous concentrations. Such uptake would also reduce substrate concentrations close to the cell surface, which should exert an additional driving force on the diffusive substrate resupply from the non-aqueous phase. However, kinetic data on enzymes involved in the degradation of compounds with low aqueous solubility are scarce and generalisations cannot be made at this point.

An additional adaptation by the cell to compensate for mass-transfer limitation may be an improved oligotrophic capacity of the organism (i.e., a modest substrate requirement for culture maintenance) allowing survival at very low substrate fluxes. Theoretical considerations show that bacterial maintenance requirements determine population densities sustainable in environments of limited substrate supply (*Harms, 1996*). This model also suggests that non-degradable residual concentrations of pollutants in soil result from substrate fluxes falling below the values needed to keep degrading populations viable (*Bosma et al., 1997*). However, this has not been experimentally verified.

Bacterial adaptation to degradation of hydrophobic compounds cannot involve all of the above-mentioned traits in one strain, as some of these properties may be incompatible. For example, excretion of surface-active compounds may interfer with adhesion to hydrophobic surfaces. Such an effect was observed with bacteria attached to heptamethylnonane, which contained the growth substrate hexadecane; adhesion and growth was suppressed by the non-toxic surfactant Triton X-100 (*Efroymson & Alexander, 1991*). Willumsen and Karlson (*1997*) reported that the capability of bacterial isolates from PAH-contaminated soils to produce surface-active compounds was not correlated with the ability of the strains to degrade PAH's.

4.4 Surfactant-enhanced biodegradation

The use of surfactants has been suggested repeatedly as a possible strategy for enhancing bioavailability. Laboratory experiments demonstrating surfactant-enhanced PAH-degradation in aqueous systems have been reported (*Guerin & Jones, 1988; Aronstein et al., 1991; Tiehm, 1994*).

Mineralization of ^{14}C-radiolabelled fluoranthene by *Sphingomonas paucimobilis* strain EPA 505, an organism that uses this compound as a carbon and energy source (*Mueller et al. 1990*), was shown to be enhanced by the presence of the non-ionic surfactant Triton X-100 at up to 34 mM concentration (*Lin et al. 1996*). The bacterium was clearly able to tolerate high surfactant concentrations. However, a mutant of this strain, which has an altered cell surface hydrophobicity, is incapable of tolerating Triton X-100 (*Willumsen & Pritchard, unpubl. information*). This suggests that it is the cell's surface makeup that produces this tolerance toward surfactant-influenced disruption of membrane integrity or functions. Since increasing the surfactant concentration also increases the apparent aqueous PAH concentration, this organism, and others like it, could be excellent candidates for optimizing the bioremediation of PAH-contaminated soils through the use of bioaugmentation procedures.

However, inhibition of PAH-degradation due to surfactant toxicity, reduced bioavailability of micelle-solubilized PAH's, and possibly an insufficient choice of characteristics of the PAH-degrading microorganisms being used together with surfactants, has also been observed (*Laha & Luthy, 1991; Tiehm, 1994; Rouse et al., 1994; Rock & Alexander, 1995; Volkering et al., 1995*). For example, Tiehm (*1994*) has shown that a variety of non-ionic surfactants, at relatively low concentrations (4 - 10 mM), inhibit the mineralization of fluoranthene and pyrene by a pure culture, but not by a mixed microbial community enriched for its ability to mineralize these PAH's. Laha and Luthy (*1992*) showed that a low concentration of the non-ionic surfactant Triton X-100 (0.8 mM) inhibited phenanthrene mineralization by an enriched microbial community.

Successes in the field have been limited. The results reported for surfactant-assisted *in situ* or on-site bioremediation projects support the contradictory findings in the laboratory experiments. Ellis et al. (*1990*) reported enhanced degradation of oil-contaminated soil at a refinery site. The bioremediation strategy was optimized by incorporating microorganisms, nutrients, oxygen and surfactants in various treatment systems. Two field trial programmes were carried out involving the treatment of approximately 2000 m^3 of contaminated soil. The oil contaminants in excavated soil were reduced from 12980 mg/kg to 1273 mg/kg within 34 weeks and from 185 mg/kg to 26 mg/kg in 15 weeks by *in situ* treatment. Similar results have been reported by (*Rittmann & Johnson, 1989; Ducreux et al., 1995*).

In contrast, Deschenes et al. (*1995*) assessed the biodegradation of PAH's in a creosote-contaminated soil using surfactants. Results showed that the surfactant addition did not enhance PAH degradation in the soil. Furthermore, for the four-ring PAH's the presence of surfactants seemed harmful to the biodegradation process, and this effect increased as a function of surfactant concentration. Analogous findings have been reported by Graves and Leavitt (*1991*).

The conclusions of the results reported in the literature are that the effects of surfactants on the biodegradation of hydrophobic organic compounds are difficult to predict. The use of surfactants is obviously very site-specific and various factors need to be considered selecting surfactants (and microorganisms) for every single application. Some factors mentioned in the literature to be of importance are; the biodegradability of the surfactants (*Amdurer et al., 1986; Abdul et al., 1991*), possible toxic effects toward microorganisms and humans (*Amdurer et al., 1986; Vigon & Rubin, 1989; Aronstein et al., 1991; Laha & Luthy, 1991*), the interaction between surfactants and soil/sediment particles (*Jafvert & Heath, 1991; Aronstein et al., 1991*), the possibility of surfactants to act as competitive substrate (*Laha & Luthy, 1991*), and the hydrophobic-lipophilic-balance (HLB) and CMC value of the surfactant (*Vigon & Rubin, 1989; Liu et al., 1991; West & Harwell, 1992*).

4.5 Biologically produced surfactants

Biologically produced surfactants (biosurfactants and bioemulsifiers) are synthesized by bacteria, yeasts and fungi during growth on various carbon sources, in particular during growth on hydrophobic substrates (*Gerson & Zajic, 1979*). Biosurfactants and bioemulsifiers can be divided into two major categories. The low molecular weight surfactants (eg. glycolipids, sophorolipids, trehalose lipids, fatty acids and phospholipids) consist, like chemically produced surfactants, of a hydrophobic and a hydrophilic part. The high molecular weight polymers, the amphiphilic polysaccharide bioemulsifiers, however, do not necessarily consist of hydrophobic/hydrophilic moieties.

Thus, biosurfactant-producing microbiota adapted to PAH-contaminated soils, including those microorganisms that do not degrade PAH's, may have a pronounced effect on the fate of the contaminants by affecting their solubility, hence their transport in the aqueous phase. An alternative to using chemical surfactants in bioremediation may be the addition of biologically produced surface-active agents, or the stimulation of microbial production of biosurfactants and bioemulsifiers in the contaminated soil.

Production of emulsifiers and surfactants by hydrocarbon-degrading bacterial strains has been reported. The environmental sources of these strains were diesel oil and hydrocarbon-contaminated bottom sediment and surface water (*Broderick & Cooney, 1979*); hydrocarbon-contaminated subsurface soil (*Mac Elwee et al., 1990; Francy et al., 1991*); unleaded gasoline-contaminated soil (*Allen et al., 1992*); soil contaminated with petroleum waste (*Déziel et al., 1996*) and coal tar-contaminated soil (*Willumsen & Karlson, 1997*). Only few have investigated the bacterial production of surface-active compounds resulting from PAH metabolism in coal tar-contaminated soil (*Deziel et al., 1996, Willumsen & Karlson, 1997*).

Willumsen and Karlson (*1997*) determined whether fifty-seven bacterial isolates were able to produce surfactants or emulsifiers when grown in a liquid medium containing phenanthrene, anthracene, pyrene and fluoranthene, respectively, as the sole source of organic carbon. In addition, the strains' capacity to mineralize PAH's was determined. Although a significant potential for surfactant production in the microbiota of PAH-contaminated soils was found in this study, degradation of the PAH's did not correlate with the production of surfactants and emulsifiers by the isolates (Table 4.1). These conclusions are comparable with observations reported for aliphatic hydrocarbon-degrading microorganisms, where it has been reported that not all hydrocarbon-degrading strains require production of emulsifiers or surfactants to be able to degrade hydrocarbons (*Gerson & Zajic, 1978; Rapp et al., 1979; Allen et al., 1992*). Results reported by Déziel et al. (*1996*) confirm the above observations. They found that ten out of twenty-three bacterial strains synthesized biosurfactants when grown in mineral salts medium enriched with dextrose, naphthalene or phenanthrene, respectively. Similar to Willumsen and Karlson (*1997*), they found the production of biosurfactants was accompanied by an increase in the aqueous concentration of the PAH's, indicating that the microorganisms were promoting the solubility of their substrate.

Table 4.1 Relationship between surface activity and the ability to degrade selected PAH's, in percent of strains, based on the number of strains (in parantheses) which scored positive in the degradation assay; EI: emulsification index; STR: Reduction in surface tension (nN/m) at 25°C.

A: whole culture samples

	EI ≥ 0.5 STR < 20	EI ≥ 0.5 STR ≥ 20	EI < 0.5 STR ≥ 20	EI < 0.5 STR < 20
Pyrene (12)	56	0	0	44
Fluoranthene (7)	60	20	0	0
Phenanthrene (17)	56	0	0	13

B: culture supernatant samples

	EI ≥ 0.5 STR < 20	EI ≥ 0.5 STR ≥ 20	EI < 0.5 STR ≥ 20	EI < 0.5 STR < 20
Pyrene (12)	33	0	0	33
Fluoranthene (7)	20	0	0	0
Phenanthrene (17)	0	0	7	0

Willumsen and Karlson (*1997*) found a relatively low percentage of emulsifiers among isolates from soil contaminated with PAH's (67%), as opposed to soil contaminated with aliphatic hydrocarbons. This might indicate that growth on PAH's does not require emulsification to the same extent as growth on aliphatic hydrocarbons. Nevertheless, the ability to create a good emulsion appeared to be more pronounced among the PAH-degrading isolates than the ability to lower the surface tension of the culture samples. More research, however, is needed to investigate the usefulness of emulsions in the degradation of PAH.

Biosurfactants and bioemulsifiers are chemically two different kinds of products (*Cooper, 1986*). In the study of Willumsen and Karlson (*1997*) no correlation was found between the reduction in surface tension and the ability to form emulsions. Similarly, no correlation between the reduction in surface tension and the ability to form emulsions has been found for hydrocarbon-grown cells (*Broderick & Cooney, 1979; Bosch et al., 1988; Allen et al., 1992*).

Most published research dealing with these subjects is at laboratory scale. Similar to the results obtained for chemical surfactants, the effects are noted as being either enhancement or inhibition with respect to microbial activity as evidenced by biodegradation of the substrate, oxidation rate or biomass growth (*Rouse et al., 1994*).

Before biologically or chemically produced surfactants can be used routinely to promote bioavailability and bioremediation, research is needed to more fully understand the mechanisms by which surfactants stimulate PAH-degradation. This encompasses estimation of the bioavailability of the micelle-solubilized substrate (*Laha & Luthy, 1991; Tiehm, 1994; Roch & Alexander, 1995; Volkering et al., 1995*) and of the role of specialized microbial cell surface properties for surfactant-enhanced biodegradation of hydrophobic contaminants.

4.6 Bioavailability of PAH's from the Oily Phase of Contaminated Soils

In contaminated field sites, PAH's are likely to be dissolved in the oily (organic) contaminant phase, such as with creosote or coal tar residues. It is not clear how bioavailable these PAH's will be for biodegradation and how surfactants will affect their bioavailability. A recent study by Kohler et al. (*1994*) has shown that a PAH dissolved in an organic phase (heptamethylnonane) was in fact available to the aqueous phase and the bacteria contained therein. Bioavailability depended on the surface area of the organic phase (smaller droplets from vigorous shaking), and the addition of surfactant simply increased the surface area of the organic phase.

In contaminated soils, the organic phase contaminants will be dispersed among soil particles, hence it is not clear how much surface area this represents relative to bioavailability. This may present a much different system, as the organic phase, despite its distribution over extensive surface area, is quite static compared to the physical agitation experienced in shake flask experiments. The importance of surfactants, and of bacteria capable of interacting with the PAH-containing micelles under these conditions could, therefore, be questioned. It will be critical for further development to determine if the bacteria that are optimized relative to their interactions with surfactant micelles, can truly enhance PAH biodegradation in contaminated soils in the presence of surfactants.

4.7 Soil inoculation to stimulate degradation of PAH's

A major challenge in soil bioremediation is contaminant bioavailability (*Alexander, 1994*). For reasons discussed above, diffusive flow of contaminant molecules to inoculated bacterial cells will be limiting and uptake may be negligible if the bacterial inoculum is not placed in direct physical contact with the target contaminant (*Koch, 1990*). Enhancement of uptake by frequent mixing and high inoculation densities is impractical for economic reasons. If plant rhizosphere-competent PAH-degraders could be identified or engineered, growing plant roots could be used to distribute bacterial inocula in the soil and to place them in closer physical contact with the contaminants, thus improving bioavailability. In addition, massflow of the dissolved portion of the contaminants (away from the sorbing surfaces/oily phases to the degrader-inoculated root surface), caused by plant water consumption, should significantly improve contaminant desorption/solubilization kinetics and bioavailability.

5 Analysis of PAH's in soils

Obviously, the analytical chemical procedure is crucial in order to obtain reliable data for PAH concentrations in the environment as input for the evaluation of potential risks from possible PAH contaminations. Several techniques have been suggested including simple extraction procedures based on shaking or stirring, ultrasonic enhanced extraction or Soxhlet extraction. The latter may further be modified as suggested by Hüttenhain and Windrich (*1996*) introducing a medium-pressure liquid extraction of PAH's from soil. The technique further included the modification of a given soil by grinding it with silica in order to destroy the interactions between the humic matter and the PAH contaminants. However, a comparison to conventional Soxhlet extraction gave virtually identical results. Hüttenhain and Windrich suggested cyclohexane/acetone (9:1) as the optimal eluent of PAH's from soils.

Very recently, Dean (*1996*) reviewed the extraction of PAH's from environmental matrices focussing on practical considerations for supercritical fluid extraction (SFE). Dean (*1996*) concludes the appropriate use of the SFE technique for the extraction of PAH's from environmental samples and set up a series of recommendations for quantitative SFE of PAH's from environmental matrices. These include recommendation for the selection of extraction fluid, modifier or modifier mixtures, particle size, extraction time and -temperature.

Within the frame of the present study various techniques for PAH analyses have been reviewed and evaluated. It was concluded that Soxhlet extraction using toluene as eluent followed by analysis by gas chromatography combined with mass spectrometry appeared to be the optimal choice taking the given experimental facilities into account.

In the following a detailed description of a method suitable for the extraction and subsequent analysis of a series of PAH's, as well as oxarenes, thiarenes and azarenes, in soils is given.

The method allows determination of single components such as naphthalene, anthracene, phenanthrene etc.. However, the methylated derivatives of naphthalene, phenanthrene and dibenzothiophene is normally only determined as group-sums as e.g. dimethylphenanthrenes (C2-phenanthrene's), methyldibenzothiophenes, the so-called C1-dibenzothiophene's etc.. In Table 5.1 the compounds typically included in the analyses is summarized together with the characteristic mass applied for the mass spectrometric detection.

Table 5.1. PAH's typically included in the analysis scheme together with the characteristic mass used for the mass spectrometric detection.

PAH	Mass
Naphthalene	128
Methylnaphthalenes	142
Dimethylnaphthalenes	156
Trimethylnaphthalenes	170
Acenaphthylene	152
Acenaphthene	154
Fluorene	166
Anthracene	178
Phenanthrene	178
Methylphenanthrenes	192
Dimethylphenanthrenes	206
Trimethylphenanthrenes	220
Dibenzothiophene	184
Methyldibenzothiophenes	198
Flouranthene	202
Pyrene	202
Benz(a)anthracene	228
Chrysene	228
Triphenylene	228
Benzo(b+j+k)flouranthene	252
Benzo(e)pyrene	252
Benzo(a)pyrene	252
Perylene	252
Indeno(1,2,3-cd)pyrene	276
Benzo(ghi)perylene	276
Dibenzo(a,h)anthracene	278

In addition to the determination of the concentrations of single components and components groups and, thus, calculation of total contents and toxicity equivalents, the method to a certain extent enables the identification of the source from which a given PAH contamination originates.

The sample preparation described in this method is further applicable for the determination of PAH's in solid sediment and sludge samples, the latter samples, however, will typically not have to be sieved.

5.1 Method

Prior to the extraction, all the samples were spiked with a recovery spike mix (cf. Table 5.2) containing appropriate deuteriated and ^{13}C- labelled compounds. Approximately 10 g of a sieved (fraction < 2 mm) soil sample is mixed with the same amounts of glass-beds (to avoid caking of the sample during extraction) and Soxhlet extracted for 24 hours with 550 ml toluene added 2 g HCl-activated copper powder. The Soxhlet is equipped with a modified Dean-Stark to remove possible water successively from the sample. After solvent exchange by rotary evaporator to n-hexane, the sample is eluted through 2 g activated silica with dichloromethane. The dichloromethane elute is evaporated under nitrogen blow, the residue being redissolved in 1 mL toluene. 100 µL of the solution is mixed with 100 µL volume spike (Table 5.3).

Table 5.2. Composition of the recovery spike mixute together with the masses used for the mass spectrometric detection

Labelled PAH	Label	Mass
Naphthalene	$^{13}C_6$	134
2-methylnaphthalene	$^{13}C_6$	148
Acenaphthylene	$^{13}C_6$	158
Acenaphthene	$^{13}C_6$	160
Flourene	$^{13}C_6$	172
Anthracene	$^{13}C_6$	184
Phenanthrene	$^{13}C_6$	184
Dibenzothiophene	D_8	192
Pyrene	$^{13}C_3$	205
Flouranthene	D_{10}	212
Benz(a)anthracene	$^{13}C_6$	234
Chrysene	$^{13}C_6$	234
Benzo(b)flouranthene	$^{13}C_6$	258
Benzo(k)flouranthene	$^{13}C_6$	258
Benzo(e)pyrene	D_{12}	264
Benzo(a)pyrene	$^{13}C_4$	256
Perylene	D_{12}	264
Indeno(1,2,3-cd)pyrene	$^{13}C_6$	282
Benzo(ghi)perylene	$^{13}C_{12}$	288
Dibenzo(a,h)anthracene	$^{13}C_6$	284
Dibenzo(a,e)pyrene	$^{13}C_6$	308

Table 5.3. Composition of the injection (volume) spike mixture together with the masses used for the mass spectrometric detection

Labelled PAH	Label	Mass
Naphthalene	D_8	136
Phenanthrene	D_{10}	188
Pyrene	D_{10}	212
Benzo(b)flouranthene	D_{12}	264
Benzo(k)flouranthene	D_{12}	264
Benzo(a)pyrene	D_{12}	264
Indeno(1,2,3-cd)pyrene	D_{12}	288
Dibenzo(a,i)pyrene	$^{13}C_{12}$	314

Analysis is performed by GC/MS in the EI-SIM mode, and calculations are conducted by using a five level calibration with all the reported PAH's (cf. Table 5.1). The calibration standards are added as spikes in the same concentrations as the samples, and the calculations performed using the area ratios and a linear fit.

5.1.1 Quality assurance

The quality is assured by GLP procedures suplementary to the extensive use of marked spikes, simultaneous analysis of certified reference material, laboratory blanks and duplicate analysis of selected samples.

The use of certified reference material (CRM) is recommended. However, for studies involving long series of analyses of the same kind of samples, non-certified internal reference material may be more convenient. The homogeneity and stability of the latter must be carefully documented, through an extensive analysis against a CRM.

Examples of appropriate CRM are: NIST SRM 1941a, NIST SRM 1491, NIST SRM 2260 and BCR SRM 088.

5.1.2 Detection limit

The detection limit, determined as 3 times the standard deviation of the signal originating from a sample with a concentration of the single PAH's close to the expected detection limit. This is for single components and derivate groups typically in the range of 0,05-2 µg/kg.

5.2 Sampling

Samples of at least 100 g are in pre-cleaned (450°C for two hours or solvent rinsed with 3 times acetone and 3 times n-pentane) boxes of alumina or glass jars with Teflon or metal caps.

It is recommended that the sample container is delivered for sampling by the laboratory.

5.3 Sample storage

The samples are stored and transported in the dark at 4°C. Samples should be transported to the laboratory as quickly as possible and within 24 hours of sampling. Samples must be protected against UV-light.

If the storage and transportation time exceeds 24 hours, the sample must be kept at a temperature at or below - 20°C.

5.4 Sample pretreatment

During all handling samples have to be protected against UV-light by using filtered light and/or amber colored glass wares. Further, any contact with plastic components (with the exception of short contact with utensils made of Teflon) should be avoided.

Non-frozen samples should be extracted within 24 hours.

Approximately 30 g is taken as 6-8 sub-samples distributed over the total amount of the sample, and sieved 2 times through a metal sieve with 2 mm mesh diameter to produce a homogeneous soil sample. A sub-sample of 1-10 g is used for extraction. Two further sub-samples of 4 g are dried at 105°C to constant weight for determination of dry matter; this sample might further, if requested, be used for determining of loss by ignition at 450°C for 4 hours.

5.5 Sample extraction and clean-up

5.5.1 Chemicals

5.5.1.1 Reagents

For the analyses the following solvents were applied:

Toluene, Glass Distilled Grade, n-Hexane, HPLC Grade, Dichloromethane, HPLC Glass Distilled Grade, Acetone, Glass Distilled Grade, n-Pentane, HPLC, "low in benzene grade", Further, Glass beds, 3 mm D., 0.5 mm H., Hydrochloric acid (HCl) p. a., 12 M, Copper, granulated 0.2-0.6 mm, anti-bumping granules, Sodium sulfate, anhydrous, p.a., (dried 24 hours at 250 °C and stored in exicator), Glass fiber and cotton wool, and Chromabond Silica glass columns, 1000 mg (dried 1 hour at 105 °C prior to use) were applied.

The solid reagents (with exception of the Silica columns) are cleaned by 24 hours soxhlet with toluene. The Cu granules are further activated for 1 hour with 12 M HCl immediately before use.

All the liquid reagents are checked routinely for purity by GC-MS analysis.

5.5.1.2 Standard solutions

Two internal deuterium and ^{13}C -labelled standard solutions (spikes) are used. One for recovery calculations ("recovery spike"), predominately applying ^{13}C-labelled compounds, and one for determination of the variation in injection volume, instrumental drift etc. ("injection (volume) spike"), predominantely with deuterated compounds. The composition of the spikes is listed in Table 5.2.

Further, an archive spike solution, which contains both spikes in the same concentrations as in the single spike solutions, is prepared. The latter is used for preparing the finale external standard solutions.

The internal standard solutions are made from certified 100 g/ml solutions from Cambridge Isotope Laboratories, diluted with toluene, and stored at - 20°C.

External (quantification) standard solutions are made from stock solutions prepared by weighing the pure substances and dilution with toluene.

From the stock solutions an external standard mix are prepared with the compounds listed in Table 5.1, the single concentrations being in the range of 30-90 g/mL. From this standard mix dilutions are made covering the appropriate concentration range. Prior to analysis, 100 µL of the external standard is mixed with 100 µL of archive spike.

5.5.2 Equipment and facilities

All equipment to be in contact with the samples are prior rinsed 24 hours in a 4 % RBS 35 bath and subsequently washed with water followed by 3 times washing with acetone and 3 times washing with n-pentane. Disposable glass ware is primarily tested for purity, and used without pretreatment. All laboratory light are filtered yellow, and the cleaned up samples are stored in amber colored vials.

Equipment to be used comprise: 250 mL Soxhlet extraction equipment with Dien-Stark water eliminator, Soxhlet thimbles, 33 x 94 mm, Soxhlet rinsed for 24 h with toluene, 250 ml glass beaker, 1 L round-bottomed glass flask, 1000 µL and 100 µL constriction pipettes, Pasteur pipettes (disposable), 1.8 and 10 mL vials with Teflon lined caps (disposable), 1.8 mL amber colored GC-vials with micro-inserts (disposable), Spatulas, sleeves and large pincettes of stainless steel, a 6 places heating block (temp. > 150°C, Rotary evaporator, Vacuum manifold for Chromabond columns and a Nitrogen blow evaporator.

5.5.3 Extraction

1-10 g of the homogenized sample are weighed (± 0,005 g) directly into a Soxhlet thimble placed in a 250 mL glass beaker. Approximately the same amount (by weight) of glass beads is added, and mixed with the sample. The thimble is moved to a 250 mL Soxhlet extractor, and added 1000 µL of recovery spike (Table 5.2) at the top of the sample. The spiked sample is allowed to equilibrate for at least one hour before extraction.

A 1 L round-bottomed flask with 2 g freshly activated Cu granules and 6-10 anti-bumping granules is connected to the extractor, and *via* the 250 mL glass beaker 550 mL toluene is added through the extractor.

The extractor is connected with the Dien-Stark receiver and condenser, and the samples are refluxed for 24 hours. In parallel a dummy, prepared with a spiked empty thimble, is extracted analogously.

5.5.4 Clean-up

The final extract in the 1 L flask is volume-reduced to approximately 1000 µL by rotary evaporation (30°C; 35-40 mm Hg), and subsequently transferred quantitatively to 1.8 mL vials using n-hexane, evaporated to dryness under a gentle blow of nitrogen and redisolved in 0.5 mL n-hexane.

The dried Chromabond silica column is placed in the vacuum manifold, and conditioned with 5 ml n-hexane before application of the sample. The sample is quantitatively (using 1 mL of n-hexane) applied to the top of the column,

which subsequently is eluted with 5 mL n-hexane, followed by 5 mL dichloromethane. The two elutes are collected separately.
The dichloromethane fraction is evaporated in 1.8 ml vials under nitrogen blow to nearly dryness and redissolved in 1000 µL toluene.

Immediately prior to analyses 100 µL of the sample is transferred to an amber colored GC-vial with micro insert, and mixed with 100 µL injection spike (cf. Table 5.2).

5.6 Analysis

5.6.1 Instrumental

The analysis is performed by capillary GC with MS-detection. Typically a low resolution MS detector (quadrupole instrument) will be satisfying.

In the present study the actual instrumentation consists of a Hewlett Packard gas chromatograph HP 5890 with a HP 7673 autosampler and a Finnegan INCOS 50 mass spectrometer. The column used is a 30 m x 0.2 mm ID fused silica, coated with 0.11 µm cross-linked 5% phenyl methyl-silicone.

The GC is operated in the splitless mode, with He carrier gas and an injection volume of 1-2 µl. The MS is operated in the electron impact-selected ion mode. Detailed instrumental conditions applied are as follows:

Gas chromatograph: HP 5890 with autosampler HP 7673.
Column: DB-5, 30m, 0.22 mm ID, 0.11 µm, 5 % cross linked phenylmethylsilicone.
Carrier gas: Helium, 1 mL/min, 20 psi.
Temperature program: 90°C for 2 min - rate 5°C/min. to 190°C - rate 10°C/min. to 290°C - 290°C for 10 min.
Injection: 1 µL, splitless, 300°C.
Detector: MS on line.

Mass spectrometer: Finnegan INCOS 50.
Inlet: Direct GC-column, transfer line at 290°C.
Ionization: Electron impact, 70 eV.
Acquisition mode: Selected ion mode.

5.6.2 Methodology

5.6.2.1 Analytical setup

For the identification and quantification, and for quality assurance purpose, a parallel set of reference and control samples are analyzed together with the single environmental sample. Thus, the individual environmental sample shall be connected to: a) a simultaneous analysis of a proper dummy, b) a doublet of a comparable sample, c) an appropriate certified reference material and d) a sequence of external standards. Each extraction series (batch) contains at a maximum 6 extractions. If more than three samples of the same type are to be analyzed, the control samples (a, b and c) can be spread over the batches, so that each batch contains at least one of them.

The external standard sequence contains a typically five points quantification standard series and a mix of the two internal spikes in concentrations matching the concentrations added to the samples (archive spike, cf section 5.5.1.2).

The analytical sequence is carried out according to the following scheme:

a): All samples and standards are analyzed by single injection

b): The external standards are analyzed both before and after the samples, and, if requested following c, in between.

c): Max. eight samples between the external standard series

d): All the quality insurance samples are analyzed as ordinary samples

e): At least one ordinary sample is analyzed in duplicate.

High attention should be paid to any carry-over effects. Thus, it may be necessary to reanalyze an extract for verification of low contents in samples analyzed immediately after samples with a high amount of PAH's. If information concerning the PAH concentration levels in the samples is available, the samples should be analyzed in order of an increasing PAH concentration. Indication of the PAH concentrations may be obtained from preliminary GC-FID-results, smell of the sample and/or color of the extract prior to clean up.

If the concentrations in a sample is higher than the linear range of the standard curve, the redisolved extract must be diluted with toluene and subsequently reanalyzed. If the concentration in the sample is lower than half the concentration of the lowest point in the calibration curve, a further dilution of the standard solution shall be included.

5.6.2.2 Identification and integration

The identification of the compounds are performed by a combination of molecular mass determination (as determined by the selected ion mode) and the chromatographic retention time (scan numbers). The internal standards are used for quality control of the retention times, observing that the labelled compounds elute slightly ahead the natural, i.e. unlabelled compounds. Up to 8 scans different for D_{12} or $^{13}C_{12}$ labelled compounds, and 5 scans different for D_6 or $^{13}C_6$ labelled compounds, respectively, are acceptable.

Single components and internal standards are determined by manual area integration of the identified peaks by the valley-to-valley principle. Only peaks higher than three times the noise envelope should be integrated.

Following the standard analytical program as described here, chrysene and triphenylene, and the three benzofluoranthenes, respectively, are not sufficient well resolved, and are thus integrated as sums. The methylated derivatives, although the majority of these compounds appears as well resolved peaks, are routinely integrated as sums.

5.6.2.3 Quantification

The principle of the quantification is external calibration by use of internal standards. The internal standards are used to correct for any loss during sample handling (recovery), and for variations in injection and instrumental sensitivity.

The variation in injection volume and instrumental sensitivity should, for each sample, be lower than 20 % compared to the signals from the archive spike. Correction for variation are made in the integrated areas of then single signals in the sample chromatogram.

The areas of the individual compounds in the dummy should be lower than 10 % of the integrated areas in the samples. Corrections for dummy are made in the integrated areas of the single signals in the sample chromatogram.

The recovery of the single compounds compared with the archive spike should be between 60 and 140 %. The correction for recovery is inherent in the calculation procedure.

A calibration curve is established by five point external standard series. It must be linear and pass through origin.

The amount of the single compounds in the samples is calculated from the ratios between the area-ratios between the analyte and the corresponding internal standard for the external standards and the sample by the means of a linear regression analysis.

6. References

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