STELLINGEN

1. Extracties met organische oplosmiddelen en waterige oplossingen van surfactants zijn ongeschikt voor het voorspellen van de biobeschikbaarheid van hydrofobe organische verontreinigingen in bodems en sedimenten.

   Dit proefschrift
   Chung, N.; Alexander, M. *Environ. Sci. Technol.* 1998, 32, 855-860

2. De verregaande acceptatie van modellen die de bodem-organische-stof beschrijven als een samenraapsel van discrete amorfe en gecondenseerde domeinen (amorphous/condensed, rubbery/glassy, soft/hard) is verbazingwekkend, gezien de minieme hoeveelheid onderzoek die is gedaan naar het daadwerkelijk bestaan, de chemische samenstelling en de fysische conformatie van dergelijke domeinen.

   Dit proefschrift

3. Het gebruik van radiaal-diffusiemodellen voor het beschrijven van de desorptie van hydrofobe organische verontreinigingen in bodem- en sedimentsystemen wekt ten onrechte de indruk dat men deze systemen goed genoeg kent om de keuze voor dergelijke mechanistische modellen te rechtvaardigen.

4. Eén van de saneringsdoelstellingen die is geformuleerd in het kader van het Uitvoeringsprogramma Beleidsvernieuwing Bodemsanering is het bereiken van een stabiele eindsituatie in de ondergrond binnen 30 jaar na start van de sanering. Deze soepele doelstelling zou het gebruik van extensieve saneringstechnieken in de hand moeten werken. Helaas is het momenteel nog niet mogelijk om bij de selectie van een saneringsvariant een goed gefundeerde voorspelling te doen over het al dan niet halen van bovenstaande doelstelling met behulp van extensieve saneringstechnieken.

   Kooper, W. (Quintens advies & management) *Van trechter naar zeef: Afwegingsproces saneringsdoelstelling* Sdu Uitgevers: Den Haag, 1990

5. Het driedimensionale humuszuurmodel dat is beschreven door Schulten is niet correct omdat de samenstelling van het humuszuur deels is gebaseerd op onjuist geïnterpreteerde pyrolysedata. Deze onjuiste interpretatie had kunnen worden voorkomen door het in acht nemen van de "pitfalls in analytical pyrolysis" beschreven door Saiz-Jimenez.

   Schulten, H.-R. *Fresenius J. Anal. Chem.* 1995, 351, 62-73
   Schulten, H.-R.; Schnitzer, M. *Soil Sci.* 1997, 162, 115-130
   Saiz-Jimenez, C. *Environ. Sci. Technol.* 1994, 28, 1773-1779

6. Het is in veel gevallen efficiënter het geheugen van een systeembeheerder uit te breiden dan het geheugen van elke afzonderlijke computer onder zijn of haar beheer.

7. Het veel gehoorde excuus dat men een mobiele telefoon heeft om bereikbaar te zijn, is tegelijk het beste excuus om het ding zo min mogelijk te gebruiken.

Stellingen behorende bij het proefschrift "Bioavailability of Polycyclic Aromatic Hydrocarbons in Soils and Sediments: Prediction of Bioavailability and Characterization of Organic Matter Domains"

Chiel Cuypers
Wageningen, 6 juli 2001

Bioavailability of Polycyclic Aromatic Hydrocarbons in Soils and Sediments:

Prediction of Bioavailability and Characterization of Organic Matter Domains

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Bioavailability of Polycyclic Aromatic Hydrocarbons in Soils and Sediments:

Prediction of Bioavailability and Characterization of Organic Matter Domains

Chiel Cuypers

PROEFSCHRIFT

ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
prof. dr. ir. L. Speelman,
in het openbaar te verdedigen
op dinsdag 4 september 2001
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ABSTRACT

Cuypers, C. 2001. Bioavailability of polycyclic aromatic hydrocarbons in soils and sediments: Prediction of bioavailability and characterization of organic matter domains. PhD-thesis, Wageningen University, The Netherlands, 161 pages.

Polycyclic aromatic hydrocarbons (PAHs) constitute a group of priority pollutants which are of increasing environmental concern because of their adverse effects on humans, animals, and plants. Soils and sediments generally serve as a sink for PAHs, which leads to the accumulation of PAHs at contaminated sites. In the last decade, bioremediation has been frequently used for the clean-up of such contaminated sites. However, despite the common use and cost-effectiveness of bioremediation, it is generally observed that a residual fraction remains undegraded even when optimal biodegradation conditions have been provided. In many cases the recalcitrance of this residual fraction is caused by a limited bioavailability of PAHs. The present thesis focuses on the development of simple and rapid laboratory methods for the prediction of PAH bioavailability. As an integrated part of this study, it was aimed to expand the current knowledge on the structure of amorphous and condensed soil/sediment organic matter (SOM) domains. It is believed that PAHs sorbed in amorphous domains are readily bioavailable, while PAHs sorbed in condensed domains are poorly bioavailable.

Three different methods were investigated for the prediction of PAH bioavailability: persulfate oxidation, cyclodextrin extraction, and surfactant extraction. Persulfate oxidation appeared to be a good and rapid method for the prediction of PAH bioavailability. It was demonstrated that a 3 hour oxidation at 70 °C was sufficient for the removal of all bioavailable PAHs. The oxidation method was successfully validated in a study with 14 historically contaminated soil and sediment samples. Cyclodextrin extraction and surfactant extraction were investigated in a study with two sediment samples, using hydroxypropyl-β-cyclodextrin and Triton X-100 (surfactant) as model compounds. It was demonstrated that hydroxypropyl-β-cyclodextrin extracted primarily readily bioavailable PAHs, while Triton X-100 extracted both readily and poorly bioavailable PAHs. Moreover, hydroxypropyl-β-cyclodextrin did not affect the biodegradation of PAHs, while Triton X-100 enhanced the degradation of low molecular weight PAHs. Altogether, it may be concluded that persulfate oxidation currently provides the most rapid validated method for the prediction of PAH bioavailability in soils and sediments.

To study the composition of amorphous and condensed SOM domains, two different approaches were followed: (*i*) samples were subjected to persulfate oxidation to remove amorphous SOM, before and after which the composition of SOM was studied by thermogravimetric analysis, pyrolysis-GC/MS, and CPMAS ^{13}C-NMR; (*ii*) samples were split in two parts, one part was bioremediated to remove bioavailable PAHs, and both the bioremediated and non-bioremediated part were subjected to 9 different chemical treatments with a known effect on SOM structure. Before and after chemical treatment PAH concentrations and PAH bioavailability were measured. The two approaches led to the following general conclusions on the structure of amorphous and condensed SOM domains: (1) Condensed SOM is less polar than amorphous

SOM. (2) Labile components like carbohydrates, peptides, fatty acids, and free alkanes are likely to be associated with the amorphous SOM domain. (3) There is no clear relationship between aromaticity and the degree of SOM condensation. (4) Condensed SOM is situated in the humin fraction, indicating that it has a relatively high C content, a relatively low O content, and a relatively high degree of polymerization. (5) Humic and fulvic acids are primarily associated with amorphous SOM. (6) Coal and soot are specific condensed facies with a high affinity for PAHs. Apart from these general characteristics it appeared that the composition of the SOM domains was highly sample specific.

CONTENTS

CHAPTER 1

General Introduction

1.1 Scope of this Thesis

Polycyclic aromatic hydrocarbons (PAHs) constitute a group of priority pollutants which are produced in high amounts by natural and anthropogenic sources (*1, 2, 4, 5*). Generally, soils and sediments serve as a sink for PAHs, resulting in the accumulation of PAHs at contaminated sites if the input of PAHs exceeds degradation (*4*). The occurrence of PAHs in soils and sediments is of increasing environmental concern because of their toxic, mutagenic, and carcinogenic properties (*11, 13-15*). These properties may result in possible adverse effects on humans, animals, and plants, which may affect ecosystems as a whole (*11*).

In order to prevent the adverse effects of PAHs regulatory standards have been developed. Generally, these regulatory standards focus on the total amount of PAHs which can be liberated by vigorous extraction with an organic solvent. However, in the last decade it has become more and more clear that these so called 'total PAHs' are not a good measure of the exposure of animals and plants to PAHs (*40*). Several studies have demonstrated that exposure is primarily related to the amount of PAHs that are actually (bio)available (*11*). The bioavailable PAHs are loosely sorbed to the matrix and can be taken up easily.

Bioavailability is also important with respect to the biological clean-up of PAH contaminated soils and sediments. As biological clean-up, or bioremediation, relies on the uptake and/or transformation of PAHs by bacteria, fungi, or plants, bioavailability is a prerequisite for successful PAH removal (*2*). Only readily bioavailable PAHs can be removed by bioremediation, while poorly bioavailable PAHs are recalcitrant.

In the past years, much effort has been put into the development of simple laboratory methods for the estimation of PAH bioavailability. This has resulted in the introduction of methods based on the biodegradation and (solid-phase) extraction of readily bioavailable PAHs (*121, 132, 142-146*). Although operational, these methods have the disadvantage that they are quite laborious and time-consuming. Therefore, a need exists for more simple and rapid alternatives. The main objective of the present dissertation was to study and develop such alternative methods for the prediction of PAH bioavailability.

As an integrated part of this study, it was aimed to expand the current knowledge on the relationship between PAH bioavailability and the structure of soil/sediment organic matter (SOM). In a recent conceptual model SOM has been represented as an entanglement of macromolecules which consists of amorphous and condensed domains (*55, 58*). PAHs sorbed in the amorphous domains were considered to be readily bioavailable, while PAHs in the condensed domains were considered to be poorly bioavailable (at least partly). Some evidence for the existence of the amorphous and condensed domains has been published recently (*111-119*), but little information has been reported on the composition of the domains. In this dissertation it was attempted to elucidate some compositional characteristics of the amorphous and condensed SOM domains.

1.2 Polycyclic Aromatic Hydrocarbons

1.2.1 Structure. PAHs constitute a class of hazardous organic chemicals consisting of two or more fused benzene rings in linear, angular, or cluster arrangements (*1, 2*). PAHs are classified as alternant or nonalternant, the former type including molecules that are derived from benzene by fusion of additional benzenoid rings (e.g. naphthalene, phenanthrene, pyrene), the latter containing rings with fewer or more than six carbon atoms, in addition to the six-membered benzenoid rings (e.g. acenaphthylene, fluorene, fluoranthene) (*3*). By definition PAHs contain only C and H atoms, although N, S, and O atoms may readily substitute in the benzene ring to form heterocyclic aromatic compounds (*4*). Because of their toxicity and their frequent occurrence in the environment, PAHs are considered to be priority pollutants. In the present study we focus on the "16 EPA PAHs" (Figure 1), listed by the U.S. Environmental Protection Agency in the Consent Decree Priority Pollutant list. This list is frequently used by regulatory authorities to identify site contamination and to specify monitoring parameters (*1*). The 16 EPA PAHs have, in general terms, become the standard suite of compounds involved in many environmental studies of PAHs (*4*).

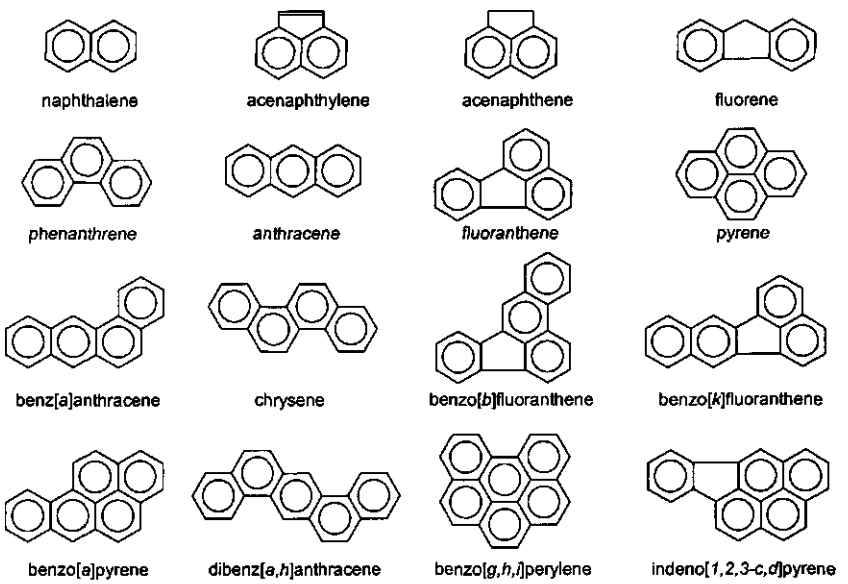

Figure 1. Chemical structures of the 16 EPA PAHs.

1.2.2 Sources. Historically, PAHs have been produced by three different processes: (a) diagenesis of organic material, (b) combustion of organic material, and (c) biogenesis. Generally, these processes are subdivided into natural and anthropogenic sources. Natural sources of PAHs include volcanic eruptions, forest fires, biogenic reactions in plants and bacteria, and thermal geologic reactions associated with fossil-fuel and mineral production.

Anthropogenic sources include the combustion of fossil-fuels as well as non-combustion processes like the production and use of coal tar and its distillation product creosote (*2, 4*). Anthropogenic sources are generally considered the most significant sources of PAH production and introduction into the environment (*5*). Of these anthropogenic sources combustion is thought to account for over 90 % of the environmental concentrations of PAHs (*4*). Perhaps surprisingly, the emission from power generation appears to be relatively minor (generally <1 % of the total emission) alongside the contribution from industrial, residential, and mobile sources. An overview of atmospheric emissions in four countries is given in Table 1.

From the perspective of input of PAHs into the environment, anthropogenic sources are often divided into point sources and low-level input sources. Point source contamination is exceedingly common in many industrially developed countries and is often the focus of significant environmental concern and regulatory complications. Point sources include spills and mismanaged industrial operations (waste treatment lagoons, sludge disposal, leaking tanks, leaking holding ponds), generally resulting in small contaminated areas with relatively high PAH concentrations. In these contaminated areas PAHs are often associated with other types of contaminants like petroleum hydrocarbons, xenobiotic chemicals, and heavy metals (*2*). Examples of industrial activities that may cause point source contamination are: gasification/liquefaction of fossil fuels, coke production, catalytic cracking, carbon black production and use, asphalt production and use, coal-tar production and use, refining/distillation of crude oil and oil-derived products, wood treatment processes, wood-preservative (creosote/anthracene-oil) production, landfill sites, burning/incineration, and fuel/oil storage, transportation, processing, use, and disposal (*1*).

Low-level inputs generally result from atmospheric deposition, although land application of sewage sludge and large-scale spillage and disposal of oily wastes may also contribute. The main source of atmospheric deposits is the incomplete combustion of fossil fuels, typically from industrial activities and automobile exhaust. Besides, some atmospheric input may come from the burning of wood and from incineration operations. Atmospheric PAH depositions are usually very dispersed sources, covering significant amounts of land surface. PAH concentrations from these sources are typically quite low in soil (*2*), although on a global scale atmospheric deposition is the main pathway for PAHs to the terrestrial, aquatic, and marine environment (*4*).

All in all, modern day PAH contamination of soils, sediments, and groundwater originates from four primary sources: creosote, coal tar, petroleum, and industrial effluents and gasses (*2*). Evaluating the contribution of contaminated sites to the total burden of PAHs it may be stated that this contribution can be quite large in some (developed) regions. It was estimated by Wild and Jones (*6*) that, in the United Kingdom, the total amount of PAHs in contaminated sites is approximately equivalent to the total amount of PAHs in 'uncontaminated' areas. Soils and aquatic sediments are generally accepted to represent the largest sink of PAHs in the environment. As a consequence, soils and sediments constitute a potential secondary source of PAHs. Contaminated sites may be very significant secondary sources, affecting particularly the immediate environment (*4*).

Table 1. Estimated atmospheric emissions of total PAHs by source type (ton/year). Compiled by Howsam and Jones (*4*).

Source type	USA (1)	USA (2)[b]	Sweden	Norway	UK (1)	UK (2)[b]
Residential heating						
- coal and wood burning	3939	450	96	48	604	368
- oil and gas burning	17	930	36	15	-	-
Subtotal	3956	1380	132	63	604	368
	(6%)	*(16%)*	*(26%)*	*(21%)*	*(84%)*	*(42%)*
Industrial processes						
- coke manufacturing	632	2490	277	43	0.8	69
- asphalt production	5	4	-	-	-	0.13
- carbon black	3	3	-	-	-	-
- aluminium plants	-	1000	35	160	-	100
- other	2	-	-	-	18.2	33
Subtotal	640	3497	312	203	19	202
	(6%)	*(41%)*	*(62%)*	*(69%)*	*(3%)*	*(23%)*
Incineration						
- municipal	-	-	-	-	*	0.4
- commercial	56	50	2	1	*	0.07
Subtotal	56	50	2	1	0.06	0.47
	(1%)	*(1%)*	*(1%)*	*(0%)*	*(0%)*	*(0%)*
Open burning						
- coal refuse fires	29	100	-	-	-	-
- agricultural fires	1190	400	1	2	6.3	7
- forest fires	1478	600	1	5	-	8
- others	1328	-	-	-	-	23
Subtotal	4025	1100	2	7	6.3	38
	(36%)	*(13%)*	*(1%)*	*(2%)*	*(1%)*	*(5%)*
Power generation						
- power plants	13	1	-	-	5.8	5
- industrial boilers	75	400	7	1	-	-
Subtotal	88	401	7	1	5.8	5
	(1%)	*(5%)*	*(1%)*	*(0%)*	*(1%)*	*(1%)*
Mobile sources						
- gasoline engines	[a]2161	[a]2100	33	13	*	*
- diesel engines	105	70	14	7	*	*
Subtotal	2266	2170	47	20	80	260
	(21%)	*(25%)*	*(9%)*	*(7%)*	*(11%)*	*(29%)*
Total	11031	8598	502	294	715.2	873.6

[a] This figure was not corrected for cars with emission control devices (approximately 50 % when estimated); [b] Values USA 1 and USA 2 as well as values UK 1 and UK 2 were taken from different sources; * Individual values not included.

Table 2. Physical-chemical properties and toxicological properties of PAHs, including molecular weight (MW), LeBas molar volume (V_M), melting point (M_p), boiling point (B_p), vapour pressure (P_v), aqueous solubility (S), Henry's law constant (H), octanol-water partition coefficient (K_{ow}), mutagenicity (Muta Gen), and carcinogenicity to humans (Carc Hum). Values tabulated by Mackay and Callcott (*7*), T=25 °C, unless denoted differently.

PAHs		MW (g/mol)	V_M (cm^3/mol)	Mp (°C)	Bp (°C)	P_v [a,c] (mm Hg)	S (mg/l)	H (Pa·m^3/mol)	K_{ow}	Muta Gen [a,d,e]	Carc Hum [f]
Naphthalene	$C_{10}H_8$	128	148	81	218	$4.92 \cdot 10^{-2}$	31.0	43.01	3.37	-	
Acenaphthylene	$C_{12}H_8$	152		92[a]	265[a]	$2.9 \cdot 10^{-2}$	3.93[b]		4.07[a,b]		
Acenaphthene	$C_{12}H_{10}$	154	173	96	278	$2.0 \cdot 10^{-2}$	3.80	12.17	4.33[a,b]	+	
Fluorene	$C_{13}H_{10}$	166	188	116	295	$1.3 \cdot 10^{-2}$	1.90	7.87	4.18		U
Phenanthrene	$C_{14}H_{10}$	178	199	101	339	$6.80 \cdot 10^{-4}$	1.10	3.24	4.57	-	U
Anthracene	$C_{14}H_{10}$	178	197	216	340	$1.96 \cdot 10^{-4}$	$4.5 \cdot 10^{-2}$	3.96	4.54	-	U
Fluoranthene	$C_{16}H_{10}$	202	217	111	375	$6.0 \cdot 10^{-6}$	0.26	1.04	5.22	+	U*
Pyrene	$C_{16}H_{10}$	202	214	156	360	$6.85 \cdot 10^{-7}$	0.132	0.92	5.18	±	U
Benz[*a*]anthracene	$C_{18}H_{12}$	228	248	160	435	$5.0 \cdot 10^{-9}$	$1.1 \cdot 10^{-2}$	0.58	5.91	+	A
Chrysene	$C_{18}H_{12}$	228	251	255	448	$6.3 \cdot 10^{-7}$	$2 \cdot 10^{-3}$	$1.22 \cdot 10^{-2}$	5.75	+	U*
Benzo[*b*]fluoranthene	$C_{20}H_{12}$	252		167[a]		$5.0 \cdot 10^{-7}$	$1.2 \cdot 10^{-3}$ [b]		6.57[a,b]		B
Benzo[*k*]fluoranthene	$C_{20}H_{12}$	252		217[a]	480[a]	$5.0 \cdot 10^{-7}$	$5.5 \cdot 10^{-4}$ [b]		6.84[a,b]		B
Benzo[*a*]pyrene	$C_{20}H_{12}$	252	263	175	495	$5.0 \cdot 10^{-7}$	$3.8 \cdot 10^{-3}$	$4.6 \cdot 10^{-2}$	6.04	+	A
Dibenz[*a,h*]anthracene	$C_{22}H_{14}$	278	300	267	524	$1.0 \cdot 10^{-10}$	$6 \cdot 10^{-4}$	$1.7 \cdot 10^{-4}$	6.75	+	A
Benzo[*g,h,i*]perylene	$C_{22}H_{12}$	276		222[a]		$1.0 \cdot 10^{-10}$	$2.6 \cdot 10^{-4}$ [b]		7.23[a,b]		U
Indeno[1,2,3-*c,d*]pyrene	$C_{22}H_{12}$	276		163[a]		$1.0 \cdot 10^{-10}$	$6.2 \cdot 10^{-2}$ [c,g]		7.66[a,b]		B

[a] Sims and Overcash (*5*); [b] van Agteren *et al.* (*8*); [c] at 20 °C; [d] Pothuluri and Cerniglia (*9*); [e] Mueller *et al.* (*2*), +: positive Ames assay; - negative Ames assay; [f] IARC (*10*), as tabulated in van Brummelen *et al.* (*11*), A: probably carcinogenic; B: possibly carcinogenic; U: unclassifiable as to carcinogenicity to humans; *: classified as weak carcinogens in Pothuluri and Cerniglia (*9*). [g] Kästner (*12*).

1.2.3 Physico-Chemical Properties. The fate of PAHs in the environment is primarily controlled by their physico-chemical properties and their susceptibility to degradation processes. Key physico-chemical properties are the vapor pressure, aqueous solubility, Henry coefficient, and octanol-water partition coefficient. These properties can be used to deduce a variety of environmental partition coefficients which apply between air, water, soil, sediment, vegetation, and (other) biota (*7*). The physico-chemical parameters exhibit considerable temperature dependency and are usually measured at 20-25 °C. A compilation of some physico-chemical properties of PAHs is presented in Table 2.

At room temperature PAHs are solid crystalline substances (*12*). Boiling points and octanol water partition coefficients of PAHs increase with increasing molecular weight, while their vapor pressures, solubilities, and Henry coefficients decrease with molecular weight. The range of these parameters extends over 4-8 log units (Table 2). In contrast, the melting point of PAHs appears not to be closely correlated with molecular weight.

The vapor pressure of PAHs is rather low, except for PAHs with 2 aromatic rings. As a consequence, most PAHs do not tend to volatilize. Moreover, PAHs are highly hydrophobic, which is reflected in a low aqueous solubility and a high octanol-water partition coefficient. This hydrophobicity indicates a high affinity of PAHs for biota and natural organic matter in soils and sediments (*7*).

1.2.4 Toxicity. PAHs in soils and sediments have been observed to be toxic to the environment (*11, 13-15*). The mechanism of this toxicity is highly complex, complexity being enhanced by the fact that toxicity does not depend on simple physico-chemical characteristics. All in all, PAHs should not be regarded as a homogeneous group of chemicals, despite their apparent similarity in structure. An excellent overview of PAH toxicity was given by van Brummelen *et al.* (*11*). This overview is briefly summarized in the present paragraph.

According to van Brummelen *et al.* (*11*), PAHs do not have one type of toxic action. Different toxicity mechanisms may play a role, which depend on the compound, the type of exposure (acute or chronic), the organism, and the environmental conditions. In general, four toxicity mechanisms are distinguished: nonpolar narcosis, phototoxicity, biochemical activation (and subsequent adduct formation), and disturbance of hormone regulation.

Nonpolar narcosis is an aspecific mode of toxicity caused by non-reactive, non-electrolyte chemicals. It is thought to result from the physical disturbance of the structure of biological membranes, which may lead to the deregulation of essential membrane bound processes such as osmoregulation and neurotransmission. Narcosis can develop quickly during short-term exposure. Therefore, it is typically observed in acute toxicity experiments. Due to its aspecific nature, the toxicity of nonpolar narcotics can be correlated with the octanol water partition coefficient. This partition coefficient determines the accumulation of PAHs in the biological membrane. Nonpolar narcosis is also called baseline toxicity. All PAHs exert baseline toxicity, while PAHs with a more specific mode of action will exert additional toxic effects. Naphthalene, fluorene, and phenanthrene are known to exert nonpolar narcosis only.

Phototoxicity is a specific toxicity mechanism which may occur in the presence of UV light. UV radiation induces the formation of free radicals, which damage a variety of macromolecules, leading to a toxic effect. Anthracene, fluoranthene, pyrene, chrysene, benz[*a*]anthracene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, and benzo[*g,h,i*]perylene have been reported to be phototoxic. Phototoxicity can develop relatively rapidly and can be observed both in acute and chronic toxicity experiments.

Biochemical activation and subsequent adduct formation is the best-known toxicity mechanism of PAHs. During enzymatic transformation certain PAHs are transformed into highly reactive compounds which may form covalent bonds with macromolecules such as proteins and DNA, the adducts. DNA adducts may give rise to mutations which may result in carcinogenesis and teratogenesis. Biochemical activation is a highly specific (chronic) toxicity mechanism, limited to a specific group of PAHs (see Table 2). Not all organisms are equally equipped with the enzyme system needed to carry out biochemical activation.

Only circumstantial evidence exists for the disturbance of hormone regulation by PAHs. Disturbance is thought to occur either by direct interaction of PAH metabolites with hormone receptors or, indirectly, by interference with hormone metabolism. This type of toxicity requires chronic exposure.

It should be noted that toxic effects can only be exerted if organisms can take up the PAHs. This implies that, besides toxicity, the PAH concentration, bioavailability, and uptake mechanism are crucial parameters. Together, these parameters determine the actual risk of PAHs. Obviously, also biotransformation is of crucial importance. Organisms that can metabolize PAHs extensively have relatively low residue concentrations and will be less susceptible to narcosis and phototoxicity. On the other hand, organisms that have a high rate of PAH metabolism are more likely to be the victim of adduct formation or disturbance of the hormone regulation. Therefore, it may be concluded that relationships between PAH toxicity and residue levels in organisms are ambiguous.

1.3 Remediation of PAH Contaminated Soils and Sediments

PAHs in contaminated soils and sediments may pose a risk to humans and ecosystems if their concentrations and mobility are high. To prevent spreading and uptake of PAHs, action should be taken either to decrease PAH mobility or to lower PAH concentrations. The mobility of PAHs can be reduced by isolation and/or immobilization of the contaminant. Removal of PAHs can be accomplished by a variety of remediation techniques, which may be applied in situ or after excavation (ex situ, on-site). Remediation may involve physical-chemical and biological techniques.

1.3.1 Physical-Chemical Remediation. Physical-chemical remediation comprises a large group of techniques that rely on several different principles. For PAH contaminated soils and sediments these principles include the physical separation of contaminated and clean material, the oxidation of PAHs, the extraction of PAHs, and the immobilization of PAHs.

Physical separation techniques aim to separate highly contaminated particles from relatively clean particles, yielding a small fraction of highly contaminated matter and a relatively clean bulk fraction. This bulk fraction generally consists of sandy material that, ideally, meets the regulatory standards for re-use. The contaminated fraction usually is more clayey and organic. It is disposed off or treated further by other remediation techniques. Typically, physical separation relies on differences in grain size, density, and/or surface properties. The most common physical separation is a classification using hydrocyclones or sedimentation basins. Hydrocyclones may be combined with coal spirals, upstream columns, froth flotation, and magnetic separation to increase remediation efficiency (*16*). Obviously, physical separation techniques can only be applied after excavation or dredging.

Oxidation of PAHs may be achieved both thermally and chemically. Thermal oxidation can be carried out under dry conditions (450-650 °C) but also under wet conditions (200-300 °C). Processes involved are thermal desorption, combustion/incineration, and pyrolysis (*16-18*). Chemical oxidation is achieved by reaction of PAHs with peroxide or ozone (*19-21*). Chemical oxidation may be applied in situ or ex situ, whereas thermal oxidation can only be applied ex situ.

PAHs can be extracted from soils and sediments with organic solvents (*22*) and with aqueous solutions of surfactants (*23-27*). In principle, extraction can be applied both in situ and ex situ. However, ex situ extraction is generally more effective and much easier to control.

Immobilization results in the permanent inclusion of a contaminant in the solid matrix. Generally, cold immobilization and thermal immobilization are distinguished (*16*). Cold immobilization is the hardening of a waste material using an organic/inorganic additive. It is not appropriate for the fixation of PAHs. Thermal immobilization (1000 to over 1250 °C) leads to the combustion of organic contaminants and the fixation of heavy metals. For PAHs, thermal immobilization should be seen as a thermal oxidation process rather than an immobilization process. Thermal immobilization can be effective for the treatment of pollution cocktails containing heavy metals and organic pollutants (PAHs).

Not all physical-chemical techniques discussed above are equally fit to treat soils and sediments. Generally, dredged sediments contain a considerable amount of water, which precludes the application of thermal techniques, due to high energy consumption. Furthermore, chemical oxidation of dredged sediments is not considered an operational technique. This means that physical separation processes are the only physical-chemical techniques available for the clean-up of PAH contaminated dredged sediments. Besides physical separation, biological treatment, isolation, and disposal can be applied. Currently, disposal is the cheapest and most popular option for the remediation of contaminated sediments in The Netherlands. However, an increasing amount of sediment is subjected to physical separation before disposal. Separation is performed to reduce dumping costs and to obtain a clean sand fraction that can be used beneficially.

For PAH contaminated soils all physical-chemical remediation techniques can be applied in principle. Nevertheless, only thermal treatment and physical separation are currently operational.

In The Netherlands $700 \cdot 10^3$ tons of soil were remediated thermally in 1998. Another $820 \cdot 10^3$ tons were treated by classification. These amounts are considerable taking in mind that $1250 \cdot 10^3$ tons were disposed at controlled dump sites (*28*). The figures above represent all contaminated soils, including a considerable amount contaminated with PAHs.

1.3.2 Bioremediation. In comparison with the amounts of soil that were remediated physical-chemically in 1998, the $380 \cdot 10^3$ tons of soil that were remediated biologically were relatively minor. These $380 \cdot 10^3$ tons present the soil bioremediated after excavation and do not include the soil that was bioremediated in situ. Therefore, the actual amount of soil that was bioremediated may have been larger than the figure presented above.

Roughly, two bioremediation techniques can be distinguished: phytoremediation and (stimulated) microbial degradation. Phytoremediation has been defined as "the use of green plants and their associated microbiota, soil amendments, and agronomic techniques to remove, contain, or render harmless environmental contaminants" (*29*). Phytoremediation relies on the uptake of contaminants in the plant itself and on the stimulation of degradation and immobilization of contaminants outside of the plant. Plants take up contaminants most readily from the liquid phase, but in some cases also vapor phase uptake occurs. Following uptake, translocation from the roots to other plant tissues may take place, sometimes followed by volatilization. In the plant contaminants may undergo complete degradation or transformation to less toxic compounds which may be bound in plant tissues in nonavailable forms (*30*). Phytoremediation *ex planta* involves the stimulation of microbial activity in the rhizosphere by secretion of organic compounds (root exudates) and the release of enzymes which themselves are capable of degrading organic contaminants (*30*). It is clear that clean-up by phytoremediation is highly complex, involving four-way interactions between contaminants, microbes, plants, and soils/sediments. As stimulation of microbial activity contributes to the remediative action, phytoremediation is closely associated with the second bioremediation technique: stimulated microbial degradation.

Stimulated microbial degradation involves the use of microorganisms to degrade hazardous organic compounds to less harmful substances, like carbon dioxide, water, and non-toxic organics. The degradation process may be enhanced by changing the pH, oxygen content, moisture content, and nutrient content of the material (*1*). There are three general strategies for implementing microbial degradation to treat PAH contaminated soils and sediments: bioreactors, solid-phase operations, and in situ operations. Each strategy has its own unique characteristics.

Bioreactor treatment of PAH contaminated soils and sediments is mainly limited to the use of slurry reactors (*2*). The contaminated material is excavated, slurried with water, and treated in a reactor where conditions for bioremediation are optimized. There is considerable control over the operating conditions (pH, oxygen content, nutrients, mixing), which often results in quick and effective treatment (*1*). Besides, bioreactors may be inoculated successfully with a specially selected, acclimatized microbial enrichment culture. So far, bioreactors have not been extensively used to treat PAH contaminated soils and sediments (*1*). One of the reasons is that

slurry treatment is four to ten times as expensive as solid-phase remediation (*17*). However, remediation time may be more than proportionally lower.

Solid-phase bioremediation is defined as the treatment of contaminated soils or sediments in a contained, aboveground system using conventional soil management practices (tilling, aeration, irrigation, fertilization) to enhance microbial degradation of organic contaminants (*2*). Several solid-phase bioremediation strategies are distinguished, varying from extensive techniques like landfarming to more intensive techniques like engineered soil cells or biopiles. Landfarming involves the application of material to soil, followed by management of the area by fertilization, irrigation, lime addition, and tilling (*1*). Landfarming practice is usually limited to the treatment of the surficial 15-30 cm of soils, although greater depths can be controlled by recent machinery (*2*). A major disadvantage with landfarming is the possibility of contaminant movement from the treatment area. This disadvantage is overcome in the intensive systems which are generally built as prepared beds, lined with low permeability material. The prepared beds are aerated by forced aeration or pile turning (*1*), which allows the construction of piles up to 1-2 m without concern for oxygen transfer limitations (*2*). The solid-phase bioremediation techniques described above are considered to be operational (*17*); hybrids of the extensive and intensive systems are sometimes applied in practice (*2*).

In situ bioremediation can be defined as the stimulation of the native microflora to degrade target organics through the passive management of environmental parameters, without excavation of soil (*2*). The principle of in situ remediation is to deliver essential co-reagents to the indigenous micoflora to facilitate biodegradation. The most important co-reagents are oxygen and inorganic nutrients (principally nitrogen, introduced in aqueous solution). The oxygen may be introduced as air, liquid oxygen, or (in)organic peroxides. Multiple economic, practical, and technical factors favor the use of air as an oxygen carrier. This air may be supplied in the saturated zone (biosparging) or in the unsaturated zone (bioventing). Potential benefits of in situ bioremediation are its noninvasive character and its supposed cost-effectiveness. Recognized limitations are physico-chemical constraints (bioavailability, desorption kinetics), geological constraints (permeability, vertical and horizontal conductivity, water depth), and treatment times. Moreover, the systems may be very heterogeneous and difficult to monitor. As a consequence, effective and complete treatment is difficult to ascertain (*2*).

Altogether, PAH biodegradation will be achieved most rapidly with bioslurry reactors, followed by solid-phase applications and in situ strategies. Site-specific characteristics and performance criteria determine the applicability of each strategy. Of course there is a direct relationship between the rate of PAH degradation, the extent of PAH degradation, and the treatment costs (*2*).

1.3.3 Policy Changes and Perspectives for Bioremediation. In the beginning of the previous paragraph it was shown that the role of bioremediation for the clean-up of contaminated sites in the Netherlands is relatively minor compared to the role of physical-chemical techniques and disposal. Yet, it is expected that bioremediation will become increasingly important in the near

future, due to significant changes in the Dutch soil remediation policy in 1997 (*153*). These changes will result in a drastic change of remediation strategies.

Before 1997, soil remediation in The Netherlands was aimed at the reduction of contaminant concentrations to levels allowing the multifunctional use of soils. Roughly, this implied that the target values for soils in industrial and residential areas were similar, without considering the specific requirements for soils in such areas. Obviously, target values for industrial soils were prohibitively low, which lead to a stagnation of soil sanitation operations due to high remediation costs. In the new policy, the remediation is aimed at a cost-effective reduction of contaminant concentrations to levels related to the specific function of the area. For surface soil, the reduction of risk and the prevention of contact with contaminants are central issues. For deeper soil layers, the objective of remediation is the reduction of undesired contaminant transport to the groundwater, removing contaminants to a residual level that allows for minimal after-care. It was proposed that remediation should be carried out following a standard approach as much as possible, tailor-made solutions being allowed in complicated situations. The standard approach for the surface soils involves the creation of a clean layer with a depth and quality dependent on the function of the area. For the deeper soil layers contaminant concentrations should be reduced to a so called "stable final situation" within 30 years. This implies that sources are removed and plumes are contained to prevent further spreading.

Given the above, it is clear that bioremediation will become increasingly important for the remediation of soils contaminated with organic pollutants. In situ bioremediation and natural biodegradation processes (natural attenuation) are expected to play a key role in future remediation strategies.

For sediments a similar trend is observed, shifting from rigid target levels towards risk reduction, creating more possibilities for treatment by bioremediation. One of the objectives of the new Dutch policy is to reduce the disposal of dredged sediments in controlled dump sites. This policy favors the treatment of dredged sediments and the beneficial reuse of the remediated material afterwards.

1.3.4 Factors Affecting PAH Biodegradation. In this paragraph we focus on the factors which may affect the outcome of bioremediation by stimulated microbial degradation. Phytoremediation is not addressed as it has been seldomly applied on a large scale, so far. Moreover, phytoremediation involves many complex interactions between contaminants, microbes, plants, and the solid matrix. Focusing on stimulated microbial degradation, the following five factors affect the likelihood of successful bioremediation: (a) toxicity, (b) environmental conditions, (c) biodegradability, (d) the presence of suitable microorganisms, and (e) bioavailability.

(a) *Toxicity*: High aqueous-phase concentrations of toxic contaminants such as heavy metals and cyanides may slow down metabolic processes and prevent the growth of biomass. The degree and mechanism of toxicity vary with the specific toxicants, their concentrations, and the microorganisms exposed (*31*).

(b) *Environmental conditions*: Important environmental factors which may influence PAH degradation in soils and sediments are the oxygen content, pH, temperature, moisture content, and nutrient content (*1*). Provision of sufficient oxygen is important to enable aerobic degradation of PAHs. Although anaerobic degradation of certain PAHs (naphthalene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene) has been reported under nitrate and/or sulfate reducing conditions, it is slow in comparison with aerobic degradation (*33-37*). Optimal pH values for PAH degrading bacteria range from 7.0-7.8 (*1*). Many bacteria are fairly insensitive to small pH variations in the range 6-9 (*32*). Fungi generally exhibit an optimal pH of 4-5. Optimal temperatures and moisture contents for PAH degradation vary from 20 to 30 °C and 25 to 90 %, respectively (*1*). Finally, it is important that critical nutrients like nitrogen and phosphorous are available in a usable form, appropriate concentrations, and proper ratios (*1*). In theory C, N, and P should be available in a (molar) ratio of approximately 120:10:1. However, unknown yield factors and the fact that a part of the PAHs may be slowly degraded complicates proper estimation. For PAHs an optimal ratio of 800:13:1 has been reported (*1*).

(c) *Biodegradability*: Microbial degradation of PAHs has been reviewed by several researchers (*8, 9, 12, 38, 39*) who generally distinguish between degradation by bacteria and degradation by fungi. Although degradation may take place under aerobic and anaerobic conditions, we focus primarily on aerobic degradation. A wide variety of bacteria can degrade PAHs aerobically. Roughly, it can be stated that more bacteria are known to degrade low molecular weight PAHs than high molecular weight PAHs. Many bacteria have been isolated that are capable of using 2 and/or 3 ring PAHs as their sole source of carbon and energy. In comparison, only few 4 ring PAHs (pyrene, chrysene) and none of the 5 and 6 ring PAHs can be used as the sole source of carbon and energy. These 4, 5, and 6 ring PAHs are usually converted cometabolically, except for benzo[*g,h,i*]perylene and indeno[1,2,3-*c,d*]pyrene, for which no evidence of bacterial degradation has been found so far. In general, it may be concluded that low molecular weight PAHs may be degraded rapidly, whereas high molecular weight PAHs are more resistant to bacterial degradation (*1, 2, 5*).

Fungal degradation has been reported for all 16 EPA PAHs except benzo[*g,h,i*]perylene and indeno[1,2,3-*c,d*]pyrene. Generally, fungi oxidize PAHs via cytochrome-P450 after uptake. However, white rot fungi are capable of degrading PAHs extracellularly by non-specific radical oxidation. This oxidation is induced by lignolitic enzymes. As a consequence, white rot fungi have a relatively high potential to degrade 4, 5, and 6 ring PAHs, including benzo[*g,h,i*]perylene and indeno[1,2,3-*c,d*]pyrene, provided that these PAHs are readily available (*12, 39*).

(d) *Presence of suitable microorganisms*: The indigenous microbial community in soils and sediments usually has a high capacity for PAH degradation. Prior exposure and acclimatization will enhance the degradation rates (*1*). Still, there are situations in which inoculation of PAH-contaminated sites could potentially enhance bioremediation. These situations include sites at which the removal rate is very low and sites contaminated with high molecular weight PAHs which have to be degraded cometabolically (*2*). Unfortunately, inoculation is a complicated process with relatively little success to encourage its use (*2*).

(v) *Bioavailability*: Bioavailability represents the accessibility of a chemical for assimilation and possible toxicity (*40*). It is often considered to be the most important factor affecting the outcome of a bioremediation operation (*2*). Bioavailability will be addressed in more detail in the next paragraph, in which definitions, mechanisms, and backgrounds are discussed.

1.4 Bioavailability

1.4.1 Defining Bioavailability. Bioremediation of PAH contaminated soils and sediments generally shows a biphasic loss of PAHs: a short period of rapid PAH degradation is followed by a longer period of slow degradation (*41-47*). This biphasic behavior often results in an incomplete removal of PAHs, residual PAHs remaining in the matrix after remediation. In many cases the height of the residual concentrations is related to PAH bioavailability. Thus, bioavailability represents one of the principal factors influencing the extent of PAH degradation in contaminated soils and sediments. Besides the importance for bioremediation, bioavailability is of major importance from the perspective of ecotoxicological risk assessment. In general, only readily bioavailable PAHs will form a risk for the surrounding environment. Given the use of the term bioavailability in such different fields as bioremediation and ecotoxicology it will come as no surprise that several different definitions of bioavailability can be found in literature:

(1) Bioavailability is the complex interactive effect of myriad physico-chemical factors on the rate and extent of biodegradation (*2*).
(2) The bioavailable fraction of a contaminant is the fraction that can desorb or be degraded within a specific period (*48*).
(3) Bioavailability describes the phenomenon that bioremediation of soils contaminated with hydrophobic solutes depends on the rate and extent of desorption from a solid surface or dissolution from a separate phase (*49*).
(4) The bioavailability of a chemical is determined by the rate of mass transfer relative to the intrinsic activity of the microbial cells. A reduced bioavailability of pollutants in soil is caused by the slow mass transfer to the degrading microorganisms (*50*).
(5) Limited bioavailability refers to the situation where the potential biodegradation capacity of the microorganisms exceeds the mass-transfer rate to the aqueous phase where biodegradation can occur (*51*).
(6) Bioavailability (to environmental scientists) represents the accessibility of a chemical for assimilation and possible toxicity (*40*).
(7) A pollutant has a limited bioavailability when its uptake rate by organisms is limited by a physico-chemical barrier between the pollutant and the microorganisms (*52*).
(8) The bioavailable fraction of a chemical is the fraction in soil/sediment and (interstitial) water which can potentially be taken up during the organisms life-time into the organisms tissue (*11*).
(9) The term bioavailability (to mammalian toxicologists) represents the availability for crossing a cell membrane and entering a cell (*40*).

Clearly, the first five definitions have been formulated from a bioremediation perspective, while definitions 6 and 7 are more general and definitions 8 and 9 reflect an ecotoxicological point of view. It must be emphasized that the definitions can not be applied outside the range of their original context; a definition that is useful in one specific study may not be relevant in another. In the present thesis bioavailability will be approached from a bioremediation perspective, predominantly. A definition of bioavailability is formulated below.

In general, the rate at which microbial cells can convert chemicals during bioremediation depends on two factors: (a) the rate of uptake and metabolism by microorganisms (intrinsic activity of the cell); (b) the rate of transfer to the microbial cells (mass transfer) (*50*). Bioremediation is limited by bioavailability if mass transfer to the cells is lower than the capacity of the cells for biodegradation. This can be translated into a bioavailability number (*Bn*), representing the ratio of the mass transfer rate constant to the microbial specific affinity (*50*). In the present thesis the mass transfer aspect of bioavailability is emphasized. Biological experiments were performed in such a way that degradation was primarily limited by the rate of mass transfer, except in a short initial stage, in which mass transfer may have exceeded the microbial degradation capacity. In this situation, bioavailability may be defined as the ability of a contaminant to desorb to the aqueous phase, within the time-frame of an experiment, under infinite dilution conditions. Although sorbed PAHs may be bioavailable to some extent (*53, 54*), it is generally accepted that PAHs in soils and sediments can only be taken up by microorganisms if they are present in the aqueous phase (*52*). Transfer to the aqueous phase is controlled by a number of physical-chemical sorption/desorption processes. In the next two paragraphs equilibrium sorption and desorption kinetics are discussed.

1.4.2 Equilibrium Sorption. Sorption of hydrophobic organic contaminants by soils and sediments has historically been described using linear partitioning models with a distribution coefficient, K_d (L/kg), representing the relationship between aqueous-phase and solid-phase concentrations (*55*). Such models are predicated on the hypothesis that soil/sediment organic matter dominates sorption, which is usually valid if a significant amount of organic matter is present (organic carbon > 0.02-0.1 %) (*56, 57*). Another important condition for the application of partitioning models is that SOM forms a relatively homogeneous and amorphous (gel-like) phase for which sorption isotherms are linear over a wide range of aqueous-phase concentrations (*55*). Furthermore, the assumption of a partitioning model carries with it an expectation that the sorption process is completely reversible and not subject to competition among different solutes. If so, sorption coefficients can be normalized to the organic carbon content (f_{oc}, dimensionless), yielding an organic carbon-water distribution coefficient K_{oc} (L/kg; $K_{oc}=K_d/f_{oc}$). An overview of K_{oc} values for the sorption of PAHs is presented in Table 3. This table includes K_{oc} values for sorption to SOM, dissolved organic matter (DOM), humic acids (HA), and fulvic acids (FA).

The overview in Table 3 shows that K_{oc} values generally increase with increasing molecular weight of PAHs. This has led to the formulation of relationships between K_{oc} values and other physico-chemical properties, like octanol-water partitioning coefficients (K_{ow}) and aqueous

solubilities (S). Several correlations have been presented in literature, usually in the following general form (*70, 84, 85*):

$$\log K_{oc} = a \cdot \log K_{ow} + b \quad (1)$$

$$\log K_{oc} = c \cdot \log S + d \quad (2)$$

where a, b, c, and d are constants obtained by linear regression. These constants exhibit considerable variation from sorbent to sorbent, which is illustrated by the K_{oc} values of individual PAHs in Table 3, varying by 1-3 orders of magnitude. This demonstrates that the regression parameters obtained for one sorbent can not be automatically applied to other sorbents.

The wide variation in K_{oc} values of individual PAHs can be explained by the fact that a simple equilibrium partitioning model may not be appropriate for the description of the sorption process. Over the past decade, an increasingly convincing body of evidence has evolved to show that the partitioning model is conceptually and phenomenologically inconsistent with the observed sorption behavior (*55*). It includes the observation that sorption is (*i*) non-linear, (*ii*) competitive, and (*iii*) hysteretic. Moreover, nonlinearity of sorption has been observed to be dependent on the (*iv*) equilibration time and (*v*) SOM composition.

(*i*) *Sorption nonlinearity*: While partitioning is a linear process, the vast majority of the sorption isotherms of hydrophobic organic contaminants are non-linear if measured over a broad concentration range (*63, 86-104*). Non-linear isotherms have been measured for sorption to soils, sediments, humic materials, and model sorbents, non-linearity being most expressed at relatively low aqueous solute concentrations. Sorption has been described with Freundlich isotherms (*63, 88-95, 98, 99, 103*), Langmuir isotherms (*88, 104*), and combined adsorption-partitioning models, including combinations of linear and Freundlich isotherms (*88, 105*), linear and Langmuir isotherms (= Dual Reactive Domain Model) (*63, 92, 95, 99, 100*), and linear and Polanyi-based isotherms (*63, 102, 103*).

Due to isotherm non-linearity, K_{oc} values may vary significantly with aqueous-phase solute concentrations. K_{oc} values are higher at low solute concentrations, causing all commonly used correlations of K_{oc} with octanol-water partition coefficients and solubility (equations 1 and 2) to underestimate K_{oc} values for low solute concentrations (i.e. < ±10 % of the aqueous solubility) (*92*). The Freundlich isotherm, which is most frequently used in the literature, is given by the following equation:

$$Q = K_f \cdot C^n \quad (3)$$

where Q is the amount of pollutant bound to the solid matrix, K_f is the Freundlich binding coefficient ((mg/kg)·(L/mg)^n), n is the Freundlich exponent which is a measure of the chemical heterogeneity, and C is the aqueous pollutant concentration (mg/L).

Table 3. Organic carbon-water distribution coefficients (K_{oc}) of PAHs. Values for soil/sediment organic matter (SOM), dissolved organic matter (DOM), fulvic acids (FA), and humic acids (HA).

PAHs	log K_{oc}			
	SOM	DOM	FA	HA
Naphthalene	3.1-3.3[a]; 3.3[f]; 3.1-3.5[o]; 2.9[q]; 3.1[r]			3.7[y]
Acenaphthylene	4.6-5.0[j]			
Acenaphthene				
Fluorene	6.5[c]; 4.1-4.4[d]; 4.4[f]; 4.2-5.3[l]			4.7[y]
Phenanthrene	4.4-4.6[b]; 6.5-6.7[c]; 4.1-6.4[e]; 4.9[f]; 4.0-6.8[g]; 5.6-6.7[h]; 4.2-5.9[l]; 6.1-7.0[m]; 5.6-7.7[p]; 4.1[q]; 4.4[r]			4.7[y]; 4.2-4.8[z]
Anthracene	4.6-4.9[b]; 4.9-5.2[d]; 5.6-6.9[h]; 4.7-5.9[l]; 4.2[q]; 4.4[r]		<4.0[v]	<4.0-5.0[v]; 4.7[y]; 4.4-4.7[z]
Fluoranthene	5.1-5.3[b]; 6.2-6.4[c]; 5.6-5.7[d]; 5.9-6.9[h]; 5.3-7.3[l]; 4.5-5.1[j]; 4.7-4.8[k]; 6.1-6.7[m]	5.1-5.2[aa]	3.8-4.25[a]; <4.0-4.8[v]	4.05-4.4[a]; 4.9-5.2[v]; 5.0-5.1[z]
Pyrene	5.2-5.3[b]; 6.6-7.0[c]; 5.8-5.9[d]; 5.6[f]; 6.0-6.8[h]; 5.0-6.5[l]; 5.5-6.6[m]; 4.6-4.9[n]; 5.6-6.3[p]; 4.8[q]; 4.1-5.1[r]	3.6[s]	4.0-4.2[s]; 4.7-5.0[u]; 5.0[w]; <4.0-5.0[v]; 3.8-3.9[z]	4.3-5.2[s]; 4.5-5.5[u]; 4.8-5.4[v]; 5.2[w]; 5.0-5.7[z]
Benz[*a*]anthracene	5.9[b]; 6.7-8.0[h]			5.6[y]
Chrysene	5.8-5.9[b]; 6.9-7.9[h]; 6.0[l]			
Benzo[*b*]fluoranthene	6.1-6.6[b]; 7.6-8.4[h]; 6.3-6.8[l]	6.6[aa]		
Benzo[*k*]fluoranthene	6.1-6.6[b]; 7.8-8.5[h]; 6.0-6.9[l]	6.7-6.9[aa]		
Benzo[*a*]pyrene	6.2-6.6[b]; 7.8-9.0[h]; 6.0-6.3[m]	(4.5-6.3[x]); 6.5-6.6[aa]	4.5-4.9[t]	5.2-5.9[t]; 6.3[y]
Dibenz[*a,h*]anthracene	7.6-8.5[h]			
Benzo[*g,h,i*]perylene	8.7-9.3[h]; 6.6-6.8[l]	6.9-7.1[aa]		
Indeno[1,2,3-*c,d*]pyrene	8.6-9.2[h]; 6.9[l]	6.8-6.9[aa]		

[a]Kopinke *et al.* (*58*); [b]Püschel and Calmano (*59*); [c]Ten Hulscher *et al.* (*60*); [d]Cornelissen *et al.* (*61*); [e]Karapanagioti *et al.* (*62*); [f]Xia and Ball (*63*); [g]Kleineidam *et al.* (*64*); [h]Jonker and Smedes (*65*); [i]Maruya *et al.* (*66*); [j]Brannon *et al.* (*67*); [k]He *et al.* (*68*); [m]McGroddy and Farrington (*69*); [o]Rutherford *et al.* (*71*); [p]McGroddy *et al.* (*72*); [q]Karickhoff (*73*); [r]Karickhoff *et al.* (*74*); [s]Chin *et al.* (*75*); [t] De Paolis and Kukkonen (*76*); [u]Gauthier *et al.* (*77*); [v]Perminova *et al.* (*78*); [w]Herbert *et al.* (*79*); [x]McCarthy *et al.* (*80*), value represents K_{dom}; [y]Nielsen *et al.* (*81*); [z]Laor and Rebhun (*82*); [aa]Lüers and Ten Hulscher (*83*).

(*ii*) *Sorption competition*: Competitive sorption does not take place in partitioning processes, but it has been observed for sorption of hydrophobic organic contaminants (HOCs) to soils, sediments, humic materials, and model sorbents (*87, 89, 97-102*). In general, sorption of HOCs to SOM can be suppressed both by polar and nonpolar cosolutes. This suppression can occur at relatively low aqueous cosolute concentrations, structurally similar molecules competing more strongly (*97*). Overall, competition will result in suppressed sorption and increased isotherm linearity (Freundlich n approaching 1) (*98*). The competitive effects suggest a certain degree of sorption selectivity (*97*).

(*iii*) *Adsorption-desorption hysteresis*: Partitioning is a reversible process, while sorption of HOCs to soils, sediments, and humic materials is often irreversible, exhibiting considerable adsorption-desorption hysteresis (*93, 95,* 96, 103, *106, 107*).

(*iv*) *Time-dependent sorption*: HOC sorption to soils, sediments, and humic materials is a process which takes considerable equilibration time (*61, 67, 91, 94, 97, 98*). In general, the extent of sorption increases with time, isotherms changing from approximately linear to increasingly nonlinear as reaction time increases. Accordingly, Freundlich K_f increases with time, while Freundlich n decreases with time (*91, 94, 97, 98*). The time needed for attainment of apparent equilibrium is highly dependent on the solute concentration. At high solute concentrations apparent equilibrium may be reached within a few hours, whereas at low solute concentrations equilibration may take several days to over a year, depending on the type of sorbent (*94*).

(*v*) *SOM composition dependent sorption*: In the partitioning model SOM is represented as a homogeneous amorphous (gel-like) phase which exhibits linear sorption (*55*). However, several recent studies have shown that SOM is not a homogeneous amorphous phase, but a collection of organic facies with varying sorption isotherms, ranging from practically linear to strongly nonlinear (*62, 64, 86, 87, 90, 93, 94, 97, 102, 108*). These organic facies differ in maturity, which may lead to K_{oc} values varying by 2-3 orders of magnitude (*64, 108*). Young and Weber (*90*) pictured SOM as a macromolecule with a structure ranging from completely amorphous in relatively young SOM (fulvic acids) to one that is increasingly condensed as diagenetic alteration occurs, ultimately becoming highly crystalline in extremely old organic carbon structures like anthracite coals. It has been shown that samples with more physically condensed and chemically reduced SOM exhibit greater solute affinity, more nonlinear sorption isotherms, more pronounced hysteresis, and lower sorption rates (*93, 94*). In addition, it has been demonstrated that strong nonlinear sorption of HOCs may result from adsorption on small amounts of high surface area carbonaceous material such as charcoal-like substances or soot (*62, 64-66, 69, 86, 87, 109, 110*). These materials have been found to exhibit extremely high K_{oc} values.

Three conceptual models have been developed recently to enable interpretation and (quantitative) description of the non-ideal sorption phenomena: (a) Weber *et al.* (*55, 88*) hypothesized that SOM is comprised by two principal types of domains: a highly *amorphous* (soft) and a relatively *condensed* (hard) domain. Sorption of HOCs into the amorphous domain

is linear, fast, completely reversible, and can be described using the linear partitioning model. Sorption in the condensed domain is nonlinear, slow, and hysteretic. (b) Xing and coworkers (*99, 100*) conceptualized SOM to consist of flexible *rubbery* and inflexible *glassy* phases, analogous to the same states that may be assumed by a polymer. A characteristic of the glassy state is the presence of unrelaxed free-volume in the form of internal nanometer size voids or pores. Sorption in rubbery domains occurs by solid-phase dissolution (partitioning), which is linear and non-competitive, whereas sorption in glassy domains occurs by a dual mode mechanism that includes both partition and adsorption-like (hole-filling) interactions. Sorption in glassy domains is nonlinear and shows competitive interactions. (c) Chiou and coworkers (*86, 87*) postulated that high-surface-area carbonaceous material is responsible for strong nonlinear sorption at low HOC concentrations. Sorption to SOM was believed to follow a linear partitioning model.

Within the framework of the present thesis it was chosen to integrate the three models above, combining their valuable aspects. We will use the terms amorphous and condensed to describe the SOM domains, assuming that condensed SOM may contain a certain amount of nanometer size pores (voids) and that high surface area carbonaceous material (coal, soot) constitutes a specific facies in the condensed domain (Figure 2). From the perspective of bioavailability it may be reasoned that HOCs which are sorbed in the amorphous domains are readily bioavailable, whereas PAHs which are sorbed in the condensed domains are poorly bioavailable (at least partly). Evidence supporting the existence of discrete SOM domains has been provided recently by spectroscopic analysis (*111-115*) and differential scanning calometry (DSC) (*116-119*). So far, little information has been reported on the chemical composition of the SOM domains.

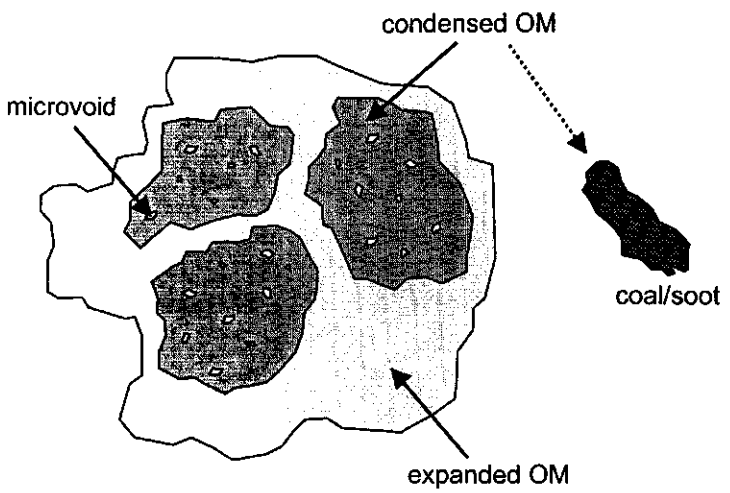

Figure 2. Schematic representation of organic material in soil/sediment.

1.4.3 Desorption Kinetics. The bioavailability of PAHs is related to the ability of PAHs to desorb from the solid matrix into the aqueous phase. Generally, this desorption is biphasic (Figure 3): a short period of rapid desorption is followed by a longer period of slow desorption (*25, 61, 120-123*). In accordance, PAHs are often divided in a rapidly desorbing and a slow desorbing fraction. The division between them is rather arbitrary, but in many cases it occurs at a few hours to a few days (*124*). Obviously, the rapidly desorbing fraction is readily bioavailable, whereas the slow desorbing fraction is poorly bioavailable.

The biphasic desorption of PAHs is seriously affected by the time of contact between PAHs and the solid matrix. Historically contaminated (aged) samples, where contact times may have been months, years, or even decades, usually contain a relatively large slow fraction. As a consequence, contaminants in aged samples are less bioavailable than contaminants in freshly contaminated samples (*40, 124*). This is reflected in a reduced susceptibility to biodegradation, reduced uptake by animals and plants, reduced toxicity, and reduced extractability with mild extractants (*125-133*). The extent of bioavailability reduction during aging is dependent on the contaminant concentration, the aging conditions, and the sample characteristics: sequestration is more extensive at low contaminant concentrations (*131*); wetting and drying cycles increase the extent of sequestration (*127*); and both the rate and the extent of sequestration may vary markedly among different soil and sediment samples (*128*).

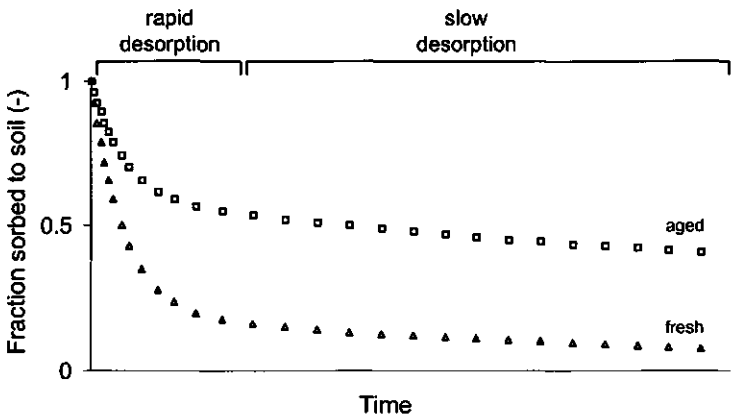

Figure 3. Desorption of PAHs from contaminated soils/sediments: a fresh contamination and an aged contamination.

Two main mechanisms have been proposed to be responsible for the slow desorption of aged HOCs: sorption retarded pore diffusion and organic matter diffusion. Sorption retarded pore diffusion postulates the rate-limiting process to be molecular diffusion in pore water, retarded, chromatographic like, by local sorption on pore walls (*124*). These walls may or may not be composed of or coated with organic matter.

The organic matter diffusion model postulates diffusion through natural organic matter as the rate limiting step for HOC desorption (*124*). In accordance with Figure 2, diffusion through SOM should be divided in diffusion through amorphous and condensed domains. Diffusion through condensed domains may be assumed to be much slower than diffusion through amorphous domains. This is in correspondence with diffusion through glassy and rubbery phases of polymers. Diffusion through the glassy phase of synthetic polymers is much slower than diffusion through the rubbery phase. This is due to the higher viscosity of the glassy phase, which resists molecular motion, and due to the extra time spent sorbed in nanovoids (*97*). Sorption at internal voids may be sterically hindered, which could result in an activated hole-filling (or hole-emptying) process.

According to Pignatello and Xing (*124*), it is quite likely that both organic matter diffusion and sorption retarded pore diffusion operate together in the same aggregates. Organic matter diffusion may predominate in soils and sediments that are high in organic matter and low in aggregation, while surface retarded pore diffusion may predominate in soils and sediments where the opposite conditions exist. Cornelissen *et al.* (*134*), who measured the temperature dependence of slow desorption of HOCs in sediments, observed that the magnitude of the activation enthalpy of slow desorption (60-70 kJ/mole) was in the polymer diffusion range (>60 kJ/mole) rather than in the pore diffusion range (20-40 kJ/mole). They concluded that intra-organic matter diffusion could explain slow desorption from their samples better than pore diffusion.

Biphasic desorption of HOCs has been often described with radial diffusion (e.g. *135-138*) and two-compartment models (e.g. *61, 121, 122, 134, 138, 139*). In this thesis we focus on the latter, as the radial diffusion model is considerably more complicated to apply. Moreover, the radial diffusion model unjustly gives the idea that experimental results can be described mechanistically, while in practice the diffusion model is far from mechanistic due to the poor representation of sample characteristics. This is illustrated by the following points: (*i*) the size distribution of particles is generally unknown; (*ii*) the radial diffusion model does not consider the presence of different SOM domains; (*iii*) the radial diffusion model considers HOCs to be homogeneously distributed over particles, while recent studies with PAH contaminated samples showed lateral variations at the subparticle scale, indicating a heterogeneous distribution that is difficult to characterize (*154, 155*).

The two-compartment model can be represented by the following equation, assuming that no readsorption of desorbed compounds takes place (*61*):

$$Q_t/Q_o = F_{rapid} \cdot e^{-k_{rapid} \cdot t} + F_{slow} \cdot e^{-k_{slow} \cdot t} \qquad (4)$$

In this equation t is time, Q_t and Q_0 are the sorbed amounts at time t and time zero, F_{rapid} and F_{slow} are the fractions of the contaminant in the rapid and slow compartment at time zero, and k_{rapid} and k_{slow} are the rate constants of rapid and slow desorption. Table 4 gives an overview of k_{rapid} and k_{slow} values of some PAHs.

Table 4. Rapid and slow desorption constants of PAHs.

PAHs	k_{rapid} (h^{-1})	$k_{slow} \cdot 10^3$ (h^{-1})
Naphthalene	0.1[c]	0.52-1.2[f]; 1-10[c]
Acenaphthylene		0.66-3.8[f]
Acenaphthene		0.046-3.8[f]
Fluorene	0.404[b]	3.83[d]; 4.08[e]; 1.7[e']; 184[e'']; 0.0076-3.5[f]
Phenanthrene	0.29-0.72[a]	0.43-2.8[a]; 0.21-1.3[f]
Anthracene	0.20[a]; 0.320[b]	0.44[a]; 2.96[d]; 2.62[e]; 1.3[e']; 170[e'']; 0.26-1.4[f]
Fluoranthene	0.116[a]; 0.202[b]	0.41[a]; 3.12[d]; 2.98[e]; 1.2[e']; 128[e'']; 0.53-7.1[f]
Pyrene	0.098[a]; 0.163[b]	0.42[a]; 3.24[d]; 2.74[e]; 1.2[e']; 153[e'']; 0.54-13[f]
Benz[*a*]anthracene	0.043[a]	0.38[a]; 0.15-3.0[f]
Chrysene	0.043[a]	0.32-0.98[a]; 0.77-10[f]
Benzo[*b*]fluoranthene		
Benzo[*k*]fluoranthene		
Benzo[*a*]pyrene		1.2[a]
Dibenz[*a,h*]anthracene		
Benzo[*g,h,i*]perylene		
Indeno[1,2,3-*c,d*]pyrene		

[a]Bonten, 30°C (*48*); [b]Cornelissen (*121*), 20 °C; [c]De Jonge *et al.* (*140*), 25 °C; [d]Cornelissen *et al.* (*61*), 20°C; [e]Cornelissen *et al.* (*134*), 20°C; [e']Cornelissen *et al.* (*134*), 5°C; [e'']Cornelissen *et al.* (*134*), 60°C; [f]Williamson *et al.* (*122*), 20-23°C.

The existence of rapid and slow compartments may be related to three different phenomena (*141*): (a) sites with different accessibility (physical mass transfer resistance); (b) sites with different sorption mechanisms (two or more types of sorption reactions of which at least one is rate limiting); (c) different (chemical) molecular-scale reaction sites (sites differing in affinity for the solute and/or having a different rate of solute-site reaction). Assuming that organic matter diffusion is responsible for the biphasic desorption behavior, it may be reasoned that a combination of the three phenomena takes place. Obviously, amorphous and condensed regions are likely to have a different accessibility due to differences in density, cross-linking, and chain rigidity. Besides, different sorption mechanisms have been observed to take place (*134*). Rapid desorption is associated with a partitioning mechanism that is driven by entropy gain, while slow desorption is associated with a process that is both enthalpy and entropy driven and involves the removal of the highly structured water mantle around the dissolved HOC. Finally, specific sorption of HOCs may occur, sorption sites being provided by the internal voids. As sorption in these voids is likely to involve structural rearrangements in the vicinity of the voids it is likely to be specific, with varying affinities for different types of voids.

Recently, the existence of a third, very slow compartment has been observed (*60, 134, 141*). It is possible that this compartment is associated with a non-linear hole-filling mechanism, while the slow compartment involves mass transfer limited partitioning-like sorption in condensed organic matter.

1.4.4 Measuring Bioavailability. Evidence is compelling that a vigorous extraction with an organic solvent fails to predict the bioavailability of HOCs in soils and sediments (*40*). Generally, a vigorous extraction is applied to recover the total amount of a contaminant. Therefore, it seriously overestimates the bioavailability of HOCs in aged samples. In the last decade the development of new laboratory methods for the prediction of HOC bioavailability has become an important issue. Such methods are needed to predict the performance of bioremediation and the possible adverse effects (toxicity) of contaminants. Existing tests for the prediction of bioremediation potential have traditionally been based on the microbial conversion of contaminants. Although reliable, these tests are long and laborious. Therefore, a considerable amount of research has been devoted to the development of simple non-biological laboratory methods for the prediction of bioavailability. For PAHs this research has yielded several characterization methods. These methods are based on the removal of bioavailable PAHs by (*i*) solid-phase extraction, (*ii*) cyclodextrin extraction, (*iii*) (super)heated water extraction, (*iv*) organic solvent extraction, and (*v*) supercritical CO_2 extraction.

(*i*) *Solid-phase extraction* (SPE) has been studied by several groups (*121, 132, 142-146*) and relies on the extraction of readily bioavailable PAHs with a solid sorbent in an aqueous slurry. This solid sorbent has a high sorption capacity for PAHs and usually consists of hydrophobic beads (e.g. Tenax TA), membrane disks, or coated fibers. In addition, semipermeable membrane devices have been applied (*142, 147*). The solid sorbent is introduced in the samples and keeps the aqueous phase free of PAHs. Thus, a so-called infinite dilution is established, which results in a concentration gradient between the aqueous phase and the soil or sediment particles. This leads to (further) desorption of readily bioavailable compounds. By regular refreshment of the solid sorbent infinite dilution is maintained and readily bioavailable PAHs are removed from the system. The PAHs that remain in the samples after SPE are a measure of the amount of contaminant that is poorly bioavailable. Typically, the removal of readily bioavailable PAHs takes several hours (low MW PAHs) to several days (high MW PAHs). SPE may be considered an operational technique for the measurement of PAH bioavailability and is more rapid than biological characterization. Nevertheless, an accurate prediction of PAH bioavailability by means of SPE is expected to take several days.

(*ii*) *Cyclodextrin extraction* has been investigated by Reid *et al.* (*148*). Cyclodextrins are cyclic polysaccharides with a hydrophilic shell and an apolar cavity, which can form water-soluble inclusion complexes with PAHs. In an aqueous solution, cyclodextrins lower the free PAH concentration, resulting in the formation of a concentration gradient between the aqueous phase and the solid matrix. This concentration gradient causes PAHs to dissolve. Regular refreshment of the cyclodextrin solution is necessary to maintain the concentration gradient and to accomplish complete dissolution of the bioavailable PAHs. The PAHs remaining after extraction are a measure of the amount of PAHs that are poorly bioavailable. Obviously, cyclodextrin extraction is very similar to SPE, with the exception that dissolved molecules are used to extract the PAHs. Up to now, cyclodextrin extraction has only been applied to predict the

bioavailability of spiked PAHs. Successful application to historically contaminated (aged) samples remains to be demonstrated.

(*iii*) *(Super)heated water extraction* has been studied by Johnson and Weber (*146*). The extraction technique relies on the measurement of desorption at high temperatures ranging from 75 °C (heated) to 150 °C (superheated or subcritical). At these high temperatures desorption of PAHs is accomplished rapidly and desorption rate constants (k_{rapid} and k_{slow} in equation 4) can be obtained within a short time. As desorption is performed at several temperatures, the temperature dependency of k_{rapid} and k_{slow} can be calculated using the Arrhenius equation. The rate constants at low temperature can be extrapolated and desorption under field conditions can be predicted, as well as bioavailability. So far, (super)heated water extraction has shown promising results. However, it should be noted that the high temperatures may affect the sorption behavior of PAHs by a structural rearrangement of the organic matter. This may lead to an overestimation of bioavailability.

(*iv*) *Organic solvent extraction* (minutes to hours) has been extensively investigated by the group of Alexander (*126, 128, 130, 133*). Their experiments demonstrated that extractability diminished with increased aging of PAHs, the extent of extraction largely following bioavailability to bacteria. Nevertheless, the correlation between bioavailability and extractability was reported not to be particularly strong in a study with 16 different soils. A possible reason for this is the interaction of organic solvents with SOM. This interaction may influence bioavailability by competition for sorption sites and by swelling. The latter will seriously affect the sorption properties of SOM.

(*v*) *Supercritical CO_2 extraction* relies on the rapid extraction of bioavailable PAHs with CO_2 at elevated temperatures and pressures (*149-152*). By applying sequentially stronger extraction conditions selective extraction of PAHs associated with rapid, moderate, slow, and very slow "sites" can be accomplished. Thus, an estimation of PAH bioavailability can be obtained within a few (1-4) hours (*151, 152*). An artifact that may appear during supercritical CO_2 extraction is swelling of organic matter. This may affect the sorption properties of the material investigated, leading to changes in PAH bioavailability.

Evaluating the five methods described above, only SPE can currently be considered operational. The other four methods still need extensive validation before they can be applied in practice. The extractions with superheated water, organic solvents, and supercritical CO_2 need to be studied critically. It is likely that the conditions applied during these extractions may seriously affect the sorption properties of the material investigated. This may hamper their use for the prediction of PAH bioavailability.

It is concluded that the challenge of developing a rapid characterization test for the prediction of PAH bioavailability remains. Although SPE is considerably more rapid than the biological characterization tests, an accurate prediction of bioavailability may still take several days.

1.5 Outline of this Thesis

This thesis covers two topics: (a) the measurement of (PAH) bioavailability in soils and sediments and (b) the composition of the amorphous and condensed soil/sediment organic matter domains in which PAHs and other organic contaminants are sorbed. Measurement of bioavailability is addressed in Chapters 2-5, while the composition of the organic matter domains is addressed in Chapters 6 and 7.

Chapter 2 introduces a new method for the prediction of PAH bioavailability in soils and sediments. This method is based on the oxidation of bioavailable PAHs with persulfate. The persulfate oxidation method is validated in Chapter 3, comparing PAH oxidation and biodegradation in 14 soil and sediment samples from historically contaminated sites. Chapter 4 evaluates the application of surfactant extraction and cyclodextrin extraction for the prediction of PAH bioavailability. Triton X-100 (surfactant) and hydroxypropyl-β-cyclodextrin were used to extract bioavailable PAHs. In Chapter 5 two methods which had proven to be successful for the prediction of PAH bioavailability were tested for the prediction of petroleum hydrocarbon bioavailability. Solid-phase extraction and persulfate oxidation were conducted on three petroleum contaminated samples.

Chapters 6 and 7 address the composition of amorphous and condensed soil/sediment organic matter domains. Chapter 6 evaluates the effect of persulfate oxidation on the composition of SOM. Chapter 7 deals with the effect of (SOM) extraction, hydrolysis, and oxidation on the concentration and bioavailability of PAHs. The results in Chapters 2-7 are discussed in Chapter 8.

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CHAPTER 2

Development of a Persulfate Oxidation Method for the Prediction of PAH Bioavailability in Soils and Sediments *

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Abstract

To predict PAH bioavailability in soils and sediments a rapid characterization test was developed, relying on the chemical oxidation of readily bioavailable PAHs by persulfate. We studied the effect of oxidation temperature (50-121 °C), oxidation time (1-24 h), and persulfate to organic matter ratio (2.9-28.9 g/g) on the extent of PAH oxidation. Residual PAH concentrations after oxidation were compared with residual concentrations after 21 d of biodegradation in an optimized slurry reactor. It was shown that persulfate oxidation at 50 and 70 °C provided a good estimation of PAH bioavailability if the oxidation time was sufficiently long (≥ 24 and 3 h, respectively) and the persulfate to organic matter ratio ≥5.8 g/g was sufficiently high. Oxidation at 90 and 121 °C overestimated PAH bioavailability. Two methods were selected for further investigation: a 3 h oxidation at 70 °C and a 2 h oxidation at 95 °C (S_2O_8/OM ratio 12 g/g). These methods were tested on 3 field samples containing an aged PAH contamination. It appeared that the residual PAH concentrations after 3 h of oxidation at 70 °C were similar to the residual concentrations after 21 d of biodegradation. A 2 h oxidation at 95 °C overestimated biodegradation. This overestimation could not be attributed to a thermal enhancement of PAH bioavailability. It was likely to result from a more extensive oxidation of organic matter at 95 °C.

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2.1 Introduction

Since 1997, several laboratory methods have been published for the prediction of hydrophobic organic contaminant (HOC) bioavailability in soils and sediments. These methods rely on the removal of bioavailable HOCs by means of solid-phase extraction (*1-4*), cyclodextrin extraction (*5-7*), membrane extraction (*2*), solvent extraction (*8-11*), surfactant extraction (*12*), and supercritical CO_2 extraction (*13-15*) (see Chapter 1). In the present study we investigated the potential of a rapid chemical oxidation method for the prediction of PAH bioavailability. This method is based on the selective chemical oxidation of a part of the soil/sediment organic matter (SOM) by persulfate.

Persulfate decomposes when it is heated and forms sulfate radicals ($SO_4^{\bullet -}$) which can react in a complex radical-chain mechanism (*16, 17*). The kinetics of persulfate decomposition have been widely investigated (*16, 18, 19*), as well as the oxidation of many organic and inorganic substances (e.g. *16, 20-29*). Since the discovery of persulfuric acid in 1878 (*30*), numerous papers have appeared concerning the properties and reactions of persulfate (*19*). Research has yielded several technical applications of persulfate, most important being the initiation of polymerization reactions, the synthesis of grafted copolymers, etching of pressed connections, and catalysis of various organic reactions (*17, 20, 21, 31*). Besides, persulfate has been used in non-detonating blasting-charges, as an oxygen carrier in rocket fuel, and as an oxidizing element in photographic color fixing-baths (*31*).

More recently, persulfate has been applied for the oxidation of complex natural organic macromolecules (*32-43*). Persulfate oxidation was used for the determination of dissolved organic carbon in freshwater (*32, 33*), total organic carbon in aquifer material (*34*), dissolved organic nitrogen in soil extracts (*42*), and carbon and nitrogen in soil microbial biomass (*43*). In a soil chemistry context, persulfate oxidation was studied as a first step in the sequential degradation of fulvic acids, humic acids, and humin (*35-38*). Sequential degradation was used as a method to study the structure of these organic materials. Finally, persulfate oxidation has been used as a method to remove labile, non-recalcitrant, organic matter from soil and sediment samples (*39-41*).

Research demonstrated that persulfate oxidation does not accomplish a complete degradation of complex natural organic macromolecules. Experiments learned that soil organic matter can be typically degraded for 20-40 % (*34, 35, 37*). The type and the relative quantities of the (residual) oxidation products vary strongly with the source of the organic matter (*37, 38*).

We hypothesize that persulfate oxidation predominantly removes amorphous SOM, while condensed SOM is not seriously affected (definitions amorphous and condensed SOM in Chapter 1). In accordance, persulfate oxidation is hypothesized to remove the readily bioavailable PAHs that are sorbed in amorphous SOM, while the poorly bioavailable PAHs that are sorbed in condensed SOM are recalcitrant. In other words, the amount of PAHs that remain in soil/sediment after persulfate oxidation may be a measure of the amount of PAHs that are nonavailable to microorganisms. This hypothesis is supported by the work of Weber *et al.* (*40*) and Young and Weber (*41*), who showed that persulfate oxidation selectively removed soft

(amorphous) SOM. The residual organic matter exhibited a considerably higher organic carbon partitioning coefficient (K_{oc}) for phenanthrene than the original material. Further support is found in literature on polymer functionalization. Functionalization of polymers can be achieved by oxidation with persulfate, which introduces hydroxyl or hydroperoxide groups at the polymer surface (*44-46*). Experiments with crystalline polypropylene demonstrated that persulfate oxidation was restricted to the outer layers of the crystalline polymer particles (*46*). The interior of the particles was not affected. Given the correspondence between crystalline polymers and condensed SOM (Chapter 1), it may be reasoned that condensed regions in SOM are highly resistant to persulfate oxidation.

In the present chapter we tested the hypothesis that persulfate oxidation is capable of selectively removing readily bioavailable PAHs from contaminated soil and sediment samples. Thus, we assessed the potential of persulfate oxidation for the prediction of PAH bioavailability. First, a parameter study was performed to investigate the effect of oxidation temperature (50-121 °C), oxidation time (1-24 h), and persulfate to organic matter ratio (2.9-28.9 g/g) on the extent of PAH oxidation. Residual PAH concentrations after oxidation were compared with residual PAH concentrations after biodegradation. On the basis of the results two methods were selected for further investigation. These methods were tested on one soil and two sediment samples, all polluted with PAHs. Oxidation was compared with biodegradation.

In addition to the oxidation studies, we investigated the effect of short-term heating on the bioavailability of the PAHs. It has been shown by Bonten *et al.* (*47, 48*) that heating of soil to a temperature higher than 65 °C can significantly enhance PAH bioavailability, even if the high temperature is maintained only for a short period of 1-2 h. This bioavailability enhancement was attributed to a rearrangement of the organic matter structure at high temperature. Such a rearrangement of organic matter is confirmed by the discovery of glass transition phenomena in humic acids, fulvic acids, and soil organic matter (*49-51*). Glass transitions in these materials are reported to occur in the temperature range 43-97 °C. Altogether, it may be expected that during persulfate oxidation a similar rearrangement of the organic matter structure may take place. Such a rearrangement would lead to an increase of PAH bioavailability, possibly resulting in an overestimation of PAH bioavailability by oxidation. To investigate the effect of high temperature on PAH bioavailability, we bioremediated one soil sample and two sediment samples, both after receiving a thermal pretreatment and without receiving thermal pretreatment. The residual PAH concentrations after biodegradation were compared with residual concentrations after persulfate oxidation.

2.2 Background

In general, the radical chain process of an aqueous persulfate oxidation, in the presence of organic substrates and variable valence metal ions, may be represented as a kinetic scheme including the following initiation, propagation, and termination reactions (*17, 20*):

Initiation

$S_2O_8^{2-} \rightarrow 2\ SO_4^{\bullet-}$ (1)

$S_2O_8^{2-} + HQH \rightarrow SO_4^{\bullet-} + HQ^{\bullet} + HSO_4^-$ (2)

$S_2O_8^{2-} + Me^{n+} \rightarrow SO_4^{\bullet-} + Me^{(n+1)+} + SO_4^{2-}$ (3)

Propagation

$Me^{(n+1)+} + HQH \rightarrow HQ^{\bullet} + Me^{n+} + H^+$ (4)

$SO_4^{\bullet-} + HQH \rightarrow HQ^{\bullet} + HSO_4^-$ (5)

$SO_4^{\bullet-} + H_2O \rightarrow {}^{\bullet}OH + HSO_4^-$ (6)

${}^{\bullet}OH + HQH \rightarrow HQ^{\bullet} + H_2O$ (7)

$HQ^{\bullet} + S_2O_8^{2-} \rightarrow SO_4^{\bullet-} + HSO_4^- + Q$ (8)

$SO_4^{\bullet-} + Me^{n+} \rightarrow Me^{(n+1)+} + SO_4^{2-}$ (9)

${}^{\bullet}OH + Me^{n+} \rightarrow Me^{(n+1)+} + OH^-$ (10)

Termination

$HQ^{\bullet} + Me^{(n+1)+} \rightarrow Me^{n+} + Q + H^+$ (11)

$2\ HQ^{\bullet} \rightarrow$ *products* (12)

$SO_4^{\bullet-} + HQ^{\bullet} \rightarrow$ *products* (13)

${}^{\bullet}OH + HQ^{\bullet} \rightarrow$ *products* (14)

${}^{\bullet}OH + SO_4^{\bullet-} \rightarrow$ *products* (15)

$2\ {}^{\bullet}OH \rightarrow$ *products* (16)

$2\ SO_4^{\bullet-} \rightarrow$ *products* (17)

Here, HQH, $HQ^{\bullet}$, and Q are an organic substrate (e.g. PAH), its radical, and the oxidation product, respectively. $Me^{(n+1)+}$ and Me^{n+} are metal ions. If the $Me^{(n+1)+}/Me^{n+}$ redox potential is high, the system contains mainly Me^{n+} metals. In that case $Me^{(n+1)+}$ is rapidly reduced by the organic substrate or its radicals. If the $Me^{(n+1)+}/Me^{n+}$ redox potential is low, the system contains mainly $Me^{(n+1)+}$ ions. In this second case Me^{n+} ions are rapidly oxidized by persulfate ions. If the $Me^{(n+1)+}/Me^{n+}$ redox potential is low, initiation occurs by reactions 1, 2, and 4; propagation by reactions 3, 5-8, and 11; and termination by reactions 9, 10, and 12-17.

2.3 Materials and Methods

2.3.1 Soil and Sediment Characteristics. One soil sample and three sediment samples were studied. The soil sample (Griftpark) was collected at a former gas plant site in Utrecht, The Netherlands. The sediment samples were dredged from harbors and waterways at several locations in The Netherlands. All samples were homogenized, passed through a 2-mm sieve, and stored at 4 °C in the dark until use. Properties of the samples are presented in Table 1. It should be noted that the PAH concentration in PH sediment was very high in comparison to the PAH concentrations in the other samples. Besides, PH sediment contained a substantial amount of mineral oil.

Table 1. Characteristics of the soil and sediment samples.

	Clay:Silt:Sand (%)	OM (%)	OC (g/g OM)	PH-KCl (-)	Oil[b] (mg/kg)	PAHs[c,d] (mg/kg)
Berkenwoude (BW)	-[a]	32.7	-	7.0	-	130 (19%)
Griftpark (GR)	-	4.9	-	7.5	-	100 (36%)
Overschie (OR)	5:3:92	17.5	0.45	7.2	880	167 (0%)
Petroleum Harbor (PH)	21:15:64	9.7	0.63	7.8	13,600	1,770 (80%)

[a] -: not measured; [b] total petroleum hydrocarbons, C_{10}-C_{40}; [c] 16 EPA PAHs; [d] parentheses indicate PAH bioavailability (%) as reported in Cuypers *et al.* (*52*).

2.3.2 PAH Analysis. Samples were mixed with acetone and water up to an acetone/water ratio of 4:1 (v/v) and a solid/liquid ratio of 1:10 g/mL. The resulting slurry was sonicated for 15 min (Retsch UR 2) and shaken at room temperature for 1 h (150 rpm, Gerhardt Laboshaker). A sample of the extract (1.5 ml) was centrifuged (5 min, 13,000g) and analyzed for PAHs by HPLC.

PAHs in the acetone/water mixture (20μL of extract) were separated on a reverse-phase C18 column (Vydac 201TP54, 5μ) with external guard column (Vydac 102GD54T, 5μ) using a mixture of acetonitrile and water as an eluens. The separation was performed at a constant flow of 1 mL/min, varying the acetonitrile/water ratio between 1:1 and 99:1 (v/v) (0-5 min, 1:1; 5-20 min, linear increase from 1:1 to 99:1; 20-40 min, 99:1; 40-45 min, linear decrease from 99:1 to 1:1; 45-50 min, 1:1). PAHs (16 EPA) were detected by UV absorbance at 254, 264, 287, 300, 305, and 335 nm (Gynkotek UVD 340S). We measured phenanthrene [PHE], anthracene [ANT], fluoranthene [FLT] (3 ring PAHs), pyrene [PYR], benz[*a*]anthracene [BaA], chrysene [CHR], benzo[*b*]fluoranthene [BbF], benzo[*k*]fluoranthene [BkF] (4 ring PAHs), benzo[*a*]pyrene [BaP], dibenz[*a,h*]anthracene [DBA], benzo[*g,h,i*]perylene [BPE], and indeno[1,2,3-*c,d*]pyrene [IPY] (5-6 ring PAHs). Naphthalene [NAP] and fluorene [FLU] (2 ring PAHs) were only measured in the extracts of PH sediment. Acenaphthylene and acenaphthene were not analyzed because their concentrations showed large variations within the bulk samples.

Following the procedure above more than 95 % of the PAHs can be extracted in one extraction step (*53*).

2.3.3 Persulfate Oxidation. *Optimization of persulfate oxidation:* PH sediment was oxidized under various conditions to study the effect of oxidation temperature (50, 70, 90, 121 °C), oxidation time (1, 3, 8, 24 h), and persulfate to organic matter ratio (2.9, 5.8, 11.6, 28.9 g/g) on the extent of PAH removal. The set-up of the parameter study is presented in Table 2. Sediment samples (~2.5 g dm) were thoroughly mixed with $K_2S_2O_8$ (Aldrich, analytical grade) and demineralized water to obtain a slurry with an aqueous persulfate ($S_2O_8^{2-}$) concentration of 0.0357 g/mL. This slurry was heated in a water bath shaker (50, 70, and 90 °C; 120 rpm) or a pressure cooker (121 °C). After heating, the samples were cooled under running water and the slurry was filtered (S&S 589[1] ashless). The solids were extracted with acetone for PAH analysis. All oxidations were carried out in triplicate.

Testing of 2 oxidation procedures: Soil/sediment samples (BW, GR, OR; ~2.5 g dm) were oxidized at 70 °C (3 h) and 95 °C (2 h), applying an aqueous persulfate concentration of 0.0357 g/mL. The persulfate to organic matter ratio was 12.0 g/g, which is based on the (optimal) 11.6 g/g in the parameter study. Oxidations (quadruplicate) were carried out as described above.

Table 2. Set-up of the persulfate oxidation parameter study.

Temperature (°C)	Time (h)	S_2O_8/OM (g/g)
50	1	11.6
	3	11.6
	8	11.6
	24	11.6
70	1	11.6
	3	2.9/5.8/11.6/28.9
	6	2×11.6[a]
	8	11.6
	24	2.9/5.8/11.6/28.9
90	1	11.6
	3	11.6
	8	11.6
	24	11.6
121	3	11.6

[a]Persulfate was added at t=0 h and t=3 h to study the effect of additional persulfate addition on the extent of PAH oxidation.

2.3.4 Biodegradation. Biodegradation experiments (triplicate) were carried out following a method which has been described by Bonten *et al.* (*47*). Briefly, wet soil or sediment (5 g dm) was mixed with an aqueous mineral medium (0.3g/L NH_4NO_3, 0.1 g/L $MgSO_4 \cdot 7H_2O$, 0.1 g/L $CaCl_2 \cdot 2H_2O$, 40 mg/L KH_2PO_4, 160 mg/L K_2HPO_4, 5 mg/L $FeCl_3$) up to a water content of 1.5 mL/g. The samples were inoculated with 2.5 mL of an active microbial enrichment culture, which was pre-cultured on PH sediment. The enrichment culture contained a mixture of microorganisms which showed a clear degradation potential for all 16 EPA PAHs except

benzo[*g,h,i*]perylene and indeno[1,2,3-*c,d*]pyrene. This degradation potential was demonstrated during growth of the enrichment and during experiments in which the inoculum was added to sterilized contaminated soil.

The inoculated samples were incubated at 30 °C and mixed on a rotary tumbler at 22 rpm. After 21 d, the samples were sacrificed for PAH analysis. During biodegradation, headspace oxygen and carbon dioxide concentrations were measured regularly. An oxygen concentration >10 % (vol.) was maintained in the headspace by regular flushing with air. Continuous mixing in the bottles assured that the O_2 concentration in the slurry was sufficiently high for biodegradation.

To investigate the effect of short-term heating on PAH bioavailability biodegradation experiments were also carried out on samples that had been subjected to a thermal pretreatment. Thermal pretreatment was carried out as follows: Samples (BW, GR, OR; 5 g dm) were mixed with an aqueous mineral medium to form a slurry with a water content of 1.5 mL/g. This slurry was heated in a water bath at 70 °C for 3 h, or at 95 °C for 2 h. After heating, samples were cooled under running water and stored until inoculation for biological treatment.

2.4 Results and Discussion

2.4.1 Optimization of Persulfate Oxidation. The effect of oxidation temperature and oxidation time on the extent of PAH oxidation was investigated in an experiment in which the S_2O_8/OM ratio was kept constant (11.6 g/g). The results of this experiment are presented in Figure 1.

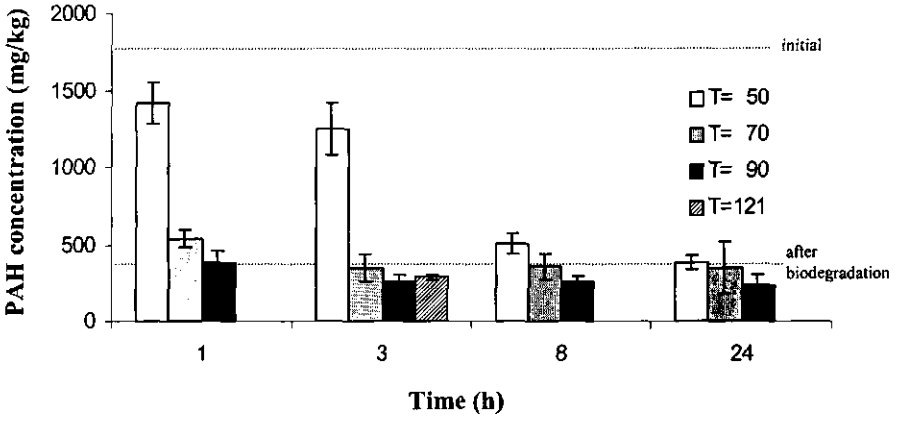

Figure 1. Residual PAH concentrations in PH sediment after persulfate oxidation at different temperatures. Initial PAH concentration and concentration after biodegradation are included (dotted lines).

Figure 1 demonstrates that rapid PAH removal occurred in the first hours of the experiment. After this rapid PAH removal, PAH concentrations stabilized at a more or less constant value. In the temperature range studied, the rate of PAH oxidation increased with temperature, as is most clearly observed between 50 and 90 °C. Stabilization of PAH concentrations occurred within 3 h at 70 and 90 °C, while at 50 °C PAH concentrations stabilized between 8 and 24 h. The increase of the oxidation rate with temperature is in correspondence with information in the literature. It has been demonstrated that a temperature increase has a positive effect on the rate of persulfate decomposition and on the rate of organic substance oxidation (*16, 19, 31*).

It is observed in Figure 1 that the residual PAH concentrations after stabilization were very similar in the experiments at 50 °C and 70 °C (i.e. after 24 and 3 h, respectively). These concentrations were not significantly different from the residual concentrations after biodegradation (*t*-test, 95%). In comparison, the (stable) residual concentrations in the experiments at 90 and 121 °C (i.e. after 3 h) were slightly lower than the residual concentrations in the experiments at 50 and 70 °C. Besides, they were significantly lower than the residual concentrations after biodegradation. The results demonstrate that oxidation at 50 and 70 °C may give a good indication of the extent of PAH degradation during bioremediation, provided that the oxidation time is sufficiently long. Oxidation at 90 and 121 °C leads to an overestimation of PAH degradation. This overestimation might be attributed to the rearrangement of the organic matter structure at high temperatures, leading to an enhancement of PAH bioavailability, as has been described by Bonten *et al.* (*47*). Altogether, it seems preferable to perform persulfate oxidation at the lowest temperature possible, within the shortest time possible. Therefore, it may be concluded that a 3 h oxidation at 70 °C is optimal.

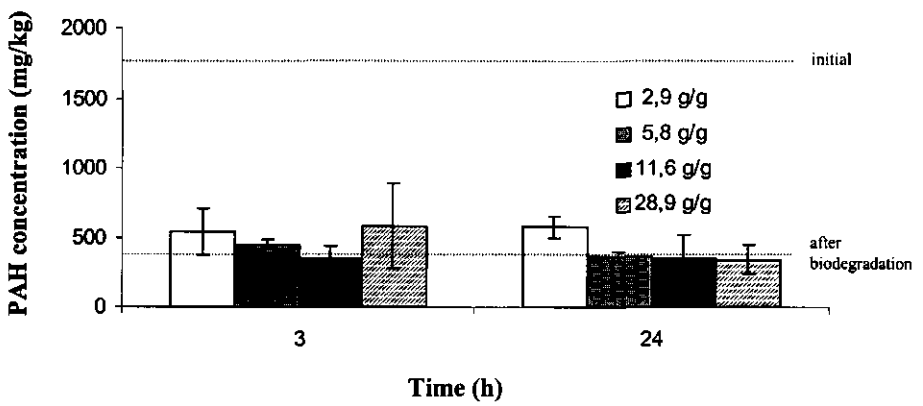

Figure 2. Residual PAH concentrations in PH sediment after persulfate oxidation at different S_2O_8/OM ratios. Initial PAH concentration and concentration after biodegradation are included (dotted lines).

The effect of the S_2O_8/OM ratio on the extent of PAH oxidation was investigated at 70 °C (3 and 24 h). The results in Figure 2 demonstrate that the S_2O_8/OM ratio hardly influences the extent of PAH oxidation within the range studied. Residual PAH concentrations after 3 h of oxidation were similar for all S_2O_8/OM ratios and did not differ significantly from the residual concentration after biodegradation. A slight difference between the 2.9 g/g experiment and the 5.8, 11.6, and 28.9 g/g experiments was observed after 24 h. Residual PAH concentrations in the experiments with the lowest persulfate addition were slightly higher. Repeated persulfate addition after 3 h (11.6 g/g at t=0 and t=3 h) did not result in additional PAH removal (data not shown). It is concluded that a S_2O_8/OM ratio ≥5.8 g/g provided a satisfactory excess for the oxidation of all bioavailable PAHs. A S_2O_8/OM ratio of 12 g/g was selected for the optimized oxidation procedure.

2.4.2 Biodegradation versus Oxidation. Two oxidation procedures were selected for further investigation: a 3 h oxidation at 70 °C and a 2 h oxidation at 95 °C (S_2O_8/OM ratio: 12 g/g). The former procedure was selected as the most optimal procedure in the parameter study. The latter procedure could possibly provide a more rapid alternative. In Figure 3 residual PAH concentrations after oxidation are compared with residual concentrations after biodegradation. Biodegradation was performed with and without thermal pretreatment at 70 and 95 °C. Thermal pretreatment was performed to study the effect of short-term heating on PAH bioavailability.

Biodegradation: Figure 3 demonstrates that substantial biodegradation of PAHs took place in GR soil, whereas hardly any biodegradation took place in BW and OR sediment. Biodegradation was highest for 3 ring PAHs and decreased progressively with increasing molecular weight. High molecular weight PAHs (5-6 ring) were hardly degraded. Most likely this was due to a combination of strong sorption to the matrix and intrinsic recalcitrance of individual PAHs like benzo[*g,h,i*]perylene [BPE] and indeno[1,2,3-*c,d*]pyrene.

Thermal pretreatment slightly enhanced the degradation of 3 and 4 ring PAHs, although enhancement was not significant (*t*-test, 95%). The effect of thermal pretreatment increased with increasing temperature, as has been observed before (*47*). Biodegradation of 5-6 ring PAHs was not enhanced by thermal pretreatment. Altogether, it may be concluded that a 3 h thermal pretreatment at 70 °C and a 2 h thermal pretreatment at 95 °C did not lead to a significant enhancement of PAH bioavailability. This conclusion seems to be in conflict with the work of Bonten *et al.* (*47*) who observed a significant enhancement of PAH bioavailability after a short thermal pretreatment (1 h, 95 °C). However, it should be noted that this enhancement was found for only one of the two samples studied. The other sample was not affected. In a later study, Bonten *et al.* (*54*) demonstrated that bioavailability in this second sample could be enhanced by applying longer treatment times or higher treatment temperatures. Obviously, the effect of thermal treatment differs between samples with a different origin. This difference is likely to result from a difference in organic matter structure. Such a difference affects the thermal behavior of organic matter, as is illustrated by Leboeuf and Weber (*49*), who found a marked difference in glass transition temperature for humic acids from a different origin.

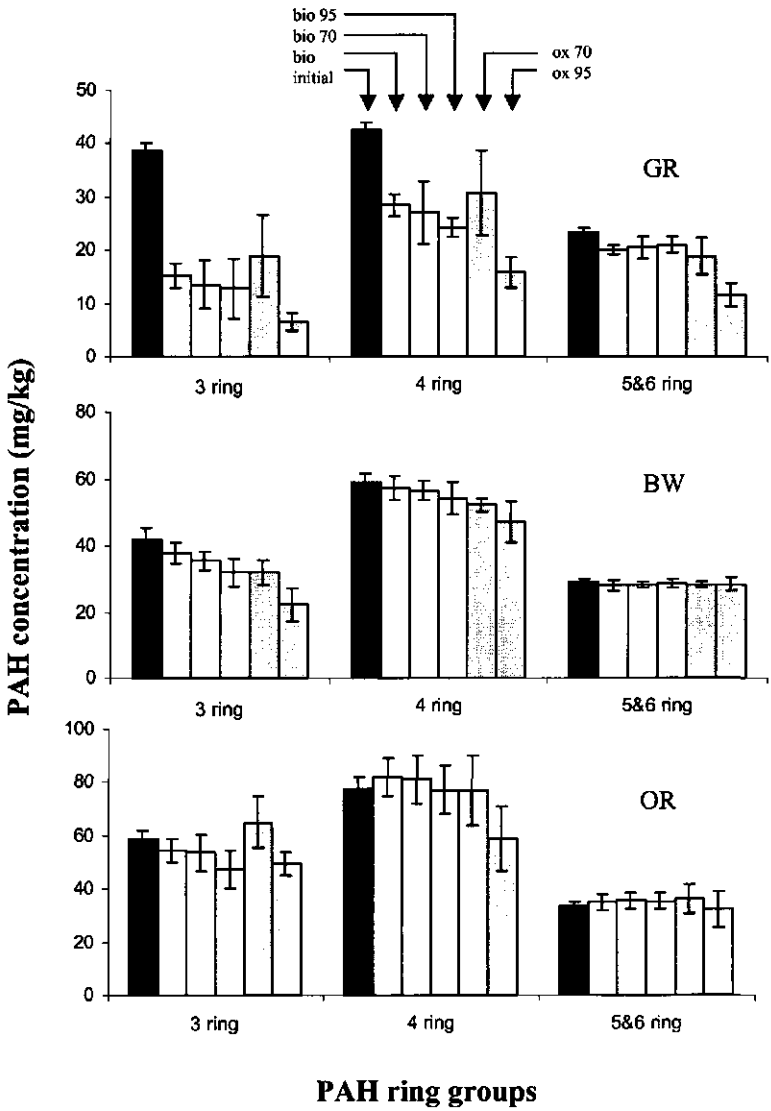

Figure 3. PAH concentrations in GR, BW, and OR samples. Bars represent initial concentrations (*initial*), concentrations after biodegradation (*bio*), concentrations after biodegradation with thermal pretreatment at 70 and 95 °C (*bio 70; bio 95*), and concentrations after persulfate oxidation at 70 and 95 °C (*ox 70; ox 95*).

Persulfate Oxidation: Figure 3 shows that there is a considerable difference between persulfate oxidation at 70 °C and persulfate oxidation at 95 °C, which is in correspondence with the results in Figure 1. PAH concentrations after oxidation at 70 °C were similar to the concentrations after

biodegradation. The extent of PAH removal decreased with increasing molecular weight of the PAHs. In general, the residual PAH concentrations remaining after oxidation at 70 °C provided a good estimate of the residual concentrations after biodegradation.

In comparison, oxidation at 95 °C often resulted in residual PAH concentrations that were lower than the concentrations after biodegradation. The differences between residual PAH concentrations after oxidation and biodegradation were significant in many cases (*t*-test 95%). As a consequence, persulfate oxidation at 95 °C can not be applied for the prediction of the extent of PAH biodegradation.

The differences between oxidation at 70 °C and 95 °C can not be explained by a thermal enhancement of PAH bioavailability. The biological experiments with thermal pretreatment showed that the thermal effect was much too small to account for the substantial difference in PAH removal. We assume that the difference between PAH removal at 70 and 95 °C is caused by a more extensive oxidation of organic matter at 95 °C. This assumption is supported by data in Chapter 6. In this chapter it was demonstrated that oxidation at 70 °C (3h) hardly affected lignin in soil organic matter. In contrast, it was shown by Saiz-Jimenez and de Leeuw (*55*) that oxidation at 140 °C (2 h) drastically reduced the lignin content of soil organic matter. This discrepancy illustrates that temperature enhancement may promote the oxidation of structures that are originally recalcitrant. Moreover, it appears that different organic matter structures are not equally susceptible to oxidation at a certain temperature. Such a difference in susceptibility is reflected in the activation energy for oxidation of organic compounds (*20*). Kislenko *et al.* (*20*) tabulated the activation energies of several simple organic compounds for persulfate oxidation (e.g. ethanol, gluconic acid, benzaldehyde) and observed marked differences between them. Altogether, we conclude that the higher temperature (95 °C) may have enabled the oxidation of organic matter that was unaffected at 70 °C. As a result, part of the organic matter that contained poorly bioavailable PAHs may have been oxidized at 95 °C.

2.5 Conclusions

Considering the application of persulfate oxidation for the prediction of PAH bioavailability, the optimal process conditions are an oxidation temperature of 70 °C, an oxidation time of 3 h, and a persulfate to organic matter ratio of approximately 12 g/g. Oxidation under these conditions provided a good estimate of the extent of PAH biodegradation in an optimized slurry reactor. A 2 h oxidation at 95 °C overestimated PAH biodegradation, most likely due to a more extensive oxidation of organic matter. Validation of the 70 °C oxidation procedure is described in Chapter 3.

Acknowledgments

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CHAPTER 3

Rapid Persulfate Oxidation Predicts PAH Bioavailability in Soils and Sediments *

Chiel Cuypers, Tim Grotenhuis, Jan Joziasse, Wim Rulkens

Abstract

Persulfate oxidation was validated as a method to predict polycyclic aromatic hydrocarbon (PAH) bioavailability in soils and sediments. It was demonstrated for 14 historically contaminated soils and sediments that residual PAH concentrations after a short (3 h) persulfate oxidation correspond well to residual PAH concentrations after 21 days of biodegradation. Persulfate oxidation of samples that had first been subjected to biodegradation yielded only limited additional PAH oxidation. This implies that oxidation and biodegradation removed approximately the same PAH fraction. Persulfate oxidation thus provides a good and rapid method for the prediction of PAH bioavailability. Thermogravimetric analysis of oxidized and untreated samples showed that persulfate oxidation was likely to have affected amorphous organic matter primarily. The results indicate that this amorphous organic matter contained only readily bioavailable PAHs.

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3.1 Introduction

Bioremediation of soils and sediments contaminated with PAHs often results in high residual concentrations (*1*) which do not satisfy the standards for clean soil. In many cases these high residual concentrations are caused by limited bioavailability of PAHs, resulting from strong sorption to the soil matrix (*2*).

To predict whether PAH bioavailability is sufficient to apply bioremediation, a characterization test is needed. Existing characterization tests are generally based on the microbiological conversion of PAHs. Although reliable, these tests are long and laborious. Therefore, alternative tests have been and are being developed, most of them based on the desorption of readily available PAHs to the water phase. In these tests desorption of PAHs is followed by immobilization of dissolved PAHs on a solid sorbent (*3-5*), enclosure of dissolved PAHs in cyclodextrins (*6*), or transport of dissolved PAHs over a semi-permeable membrane, followed by entrapment in a hydrophobic solvent phase (*4*). Although these desorption tests are faster than biological characterization tests, an accurate prediction of PAH bioavailability is expected to take at least days to weeks, depending on the PAHs of interest and the length and type of the bioremediation process considered.

A faster prediction of PAH bioavailability may be achieved by solvent extraction (*7-10*) or supercritical CO_2 extraction (*11, 12*). Solvent extraction has been extensively investigated by the group of Alexander. Their experiments show that extractability of PAHs with mild extractants diminishes with increasing time of PAH aging, the extent of extraction largely following bioavailability to bacteria. However, correlations between bioavailability and extractability were reported not to be particularly strong in a study with 16 different soils (*8*). What is more, experiments have always been carried out on lab contaminated (spiked) soil samples which had aged for 200 days, maximally. For historically contaminated soil samples the applicability of solvent extraction as a tool for the prediction of PAH bioavailability still remains to be demonstrated.

As for supercritical CO_2 extraction, research is still in an early stage and has not yielded a validated method yet. So far, experiments have shown that aging of an artificial PAH contamination can significantly reduce extractability, the general trend following biodegradability and toxicity.

An artifact that may appear during solvent extraction and supercritical CO_2 extraction is swelling of organic matter (*13, 14*). Swelling of organic matter changes the structure of organic matter and thereby the sorption properties of the material investigated (*15*). Consequently, swelling may influence PAH bioavailability and has to be taken into account when applying solvent extraction or supercritical CO_2 extraction for the prediction of PAH bioavailability.

Recently, we developed a fast chemical oxidation method for the prediction of PAH bioavailability in soils and sediments (*16*, Chapter 2). This method is based on the partial selective chemical oxidation of soil organic matter by persulfate ($S_2O_8^{2-}$). In the present study we validated this oxidation method, comparing residual PAH concentrations after persulfate oxidation with residual PAH concentrations after biodegradation. To this purpose, oxidation and

biodegradation experiments were performed on 14 soils and sediments with a wide variation in PAH concentrations. For six of these soils and sediments persulfate oxidation was also carried out on material that had first been subjected to biodegradation. Additionally, a solid-phase extraction was performed on two soils in order to directly compare the extent of oxidation and biodegradation with the extent of PAH desorption.

3.2 Background

3.2.1 PAH Sorption and Bioavailability. Sorption of PAHs to the soil matrix is dominated by sorption to soil organic matter if the organic carbon content exceeds approximately 0.1 wt % (*17*). In this context, soil organic matter has been described as a polymeric material consisting of amorphous and condensed regions, which exhibit different sorption behavior for PAHs (*15, 18, 19*). Typically, sorption of PAHs to amorphous organic matter is linear and displays a relatively low affinity, whereas sorption to condensed organic matter is non-linear and shows high affinity, especially at low concentrations. Desorption of PAHs from soil exhibits biphasic behavior: an initial phase of rapid desorption is followed by a phase of slow desorption. This biphasic behavior also occurs during biodegradation. In the initial phase of biodegradation desorption of PAHs is fast and the rate of PAH removal is primarily limited by microbial degradation kinetics. In the second phase desorption is slow and the rate of PAH removal is limited by desorption (*20*). Combining the above it may be reasoned that poorly bioavailable PAHs desorb slowly and are primarily sorbed to condensed organic matter, whereas readily available PAHs desorb rapidly and are primarily sorbed to amorphous organic matter.

3.2.2 Persulfate Oxidation. Persulfate oxidation has been proposed as a method to predict PAH bioavailability in soils and sediments (*16*). Persulfate decomposes when it is heated and forms sulfate radicals ($SO_4^{\cdot-}$) which can react with organic substances in a complex radical-chain mechanism (*21, 22*). It has been demonstrated that persulfate oxidation of soil organic matter and dissolved organic matter is incomplete (*23-27*). Soil organic matter can typically be degraded for 20-40%, leaving a recalcitrant residue (*25-27*). The type and relative quantities of the oxidation products vary strongly with the source of the organic matter (*26*). Experiments by Weber *et al.* (*28*) and Young and Weber (*29*) showed that persulfate oxidation selectively removes soft, presumably amorphous organic matter, the residual organic matter exhibiting a considerably higher organic carbon partitioning coefficient (K_{oc}) for phenanthrene than the original material.

In relation to the prediction of PAH bioavailability we hypothesize that persulfate oxidation predominantly removes amorphous organic matter and the readily available PAHs that are sorbed in it. Condensed organic matter is assumed not to be seriously affected. Hence, the amount of PAHs that remain in soil after persulfate oxidation may be a measure of the amount of PAHs that are non-available for microorganisms. If the above hypothesis is valid persulfate oxidation can be applied as a tool for the prediction of PAH bioavailability in soils and sediments.

The optimal process conditions for persulfate oxidation have been assessed in Chapter 2 (*16*). The optimal conditions were concluded to be an oxidation time of 3 h, an oxidation temperature of 70 °C, and a persulfate to organic matter ratio of 12 g/g. The high temperature only slightly enhanced PAH bioavailability under these conditions (*30*, Chapter 2).

3.3 Experimental Section

3.3.1 Chemicals. Potassium persulfate with a purity of more than 99% was obtained from Aldrich. All other chemicals were analytical grade and purchased from Merck. The porous polymer sorbent Tenax TA, 20-35 mesh, was obtained from Chrompack. Before use, the Tenax was rinsed with acetone and hexane and dried at 45°C.

3.3.2 Soils and Sediments. The properties of the soil and sediment samples are presented in Table 1. The samples were provided by AB-DLO (sample 1), Bion Biological Soil Remediation (sample 2), TNO (samples 3 and 8-14), Arcadis Heidemij (sample 4), and TOP Soil Management (sample 5). Samples 6 and 7 were collected on-site.
The different soil samples originated from a railroad site (sample 1), a wood preservation site (sample 6), and four former gas plant sites (samples 2-5 and 7). The sediments (samples 8-14) were dredged from harbors and waterways at several locations in The Netherlands.
Before use, the soil samples 4-7 were air dried and all soil and sediment samples were passed through a 2-mm sieve. Next, the sediment samples were centrifuged to increase the dry matter content and Kralingen hotspot soil (sample 5) was mixed with an uncontaminated sandy soil (1/4, w/w) to increase soil homogeneity and to prevent the occurrence of extremely high PAH, heavy metal, and cyanide concentrations. All samples were stored at 4°C in the dark until use.

3.3.3 PAH Extraction and Analysis. Soil and sediment samples were mixed with acetone and water up to an acetone/water ratio of 4:1 (v/v) and a solid/liquid ratio of 1:10 g/mL. The resulting slurry was sonicated for 15 minutes and shaken at room temperature for 1 h (150 rpm, Gerhardt Laboshaker). The extracts were centrifuged (5 min, 13,000g) and analyzed for PAHs by HPLC. It has been demonstrated that over 95 % of the PAHs can be extracted in one step using this procedure (*33*).
PAHs (20µl of extract) were separated on a reverse-phase C18 column (Vydac 201TP54, 5µ) with external guard column (Vydac 102GD54T, 5µ) using a mixture of acetonitrile and water as an eluens. The separation was performed at a constant flow rate of 1 mL/min, varying the acetonitrile/water ratio between 1:1 and 99:1 (v/v) (0-5 min, 1:1; 5-20 min, linear increase from 1:1 to 99:1; 20-40 min, 99:1; 40-45 min, linear decrease from 99:1 to 1:1; 45-50 min, 1:1). PAHs were detected by UV absorbance at 254, 264, 287, 300, 305, or 335 nm (Gynkotek UVD 3405).

Table 1. Dry matter content (DM), organic matter content (OM), PAH concentrations, and previously reported biodegradation percentages in the soil and sediment samples.

Soils	DM (% m/m)	OM [a] (% m/m)	PAHs [b] (mg/kg)	Biodegradation PAHs [c] (%)
1 Railroad Maarn	92	4.7	36	
2 Stadskanaal	84	6.0	64	
3 Griftpark Utrecht	71	4.9	100	
4 Kralingen Rotterdam	99	13.1	121	10-20
5 Kralingen hotspot	94	5.3	161	
6 Schijndel	99	1.7	123	50-70
7 Sint Oedenrode	99	3.0	68	
Sediments				
8 Assendelft	41	13.3	122	61
9 Berkenwoude	27	32.7	130	27
10 IJsselstein	44	33.8	28	
11 Lemster Rien	46	13.5	117	0
12 Overschie Rotterdam	55	17.5	167	9
13 Petroleum Harbor Amsterdam	44	9.7	2036	70-90
14 Slibkoek	65	5.9	7	

[a] Organic matter content measured as weight loss after combustion at 550°C for 5 h. [b] Sum of 16 EPA PAHs, except naphthalene, acenaphthylene, and acenaphthene. Concentrations of these PAHs regularly showed large variations within soil samples. Naphthalene is included for Petroleum Harbor sediment as measurements could be well reproduced. [c] Biodegradation as measured by Bonten *et al.* (*31*) (soils 4 and 6) and STOWA (*32*) (sediments 8, 9, 11, 12, 13).

3.3.4 Biodegradation. Biodegradation experiments were carried out following a method which has been described by Bonten *et al.* (*31*). Briefly, soil and sediment samples (5 g of dry material) were mixed with an aqueous mineral medium up to a water content of 1.5 mL/g. The samples were inoculated with 2.5 mL of an active microbial enrichment culture, which was pre-cultured on sediment from the Amsterdam Petroleum Harbor. The enrichment culture contained a mixture of microorganisms which showed degradation potential for all 16 EPA PAHs except benzo[*g,h,i*]perylene and indeno[123-*c,d*]pyrene. This potential was demonstrated in experiments with sterilized PAH contaminated soil and during growth of the enrichment. The degradation potential was limited for benzo[*a*]pyrene and dibenz[*a,h*]anthracene, which are degraded cometabolically and need a suitable cosubstrate (*34, 35*).

The inoculated samples were incubated at 30 °C and mixed on a rotary tumbler (22 rpm). After 21 days samples were extracted with acetone and the solvent was analyzed for PAHs by HPLC. It was assumed that after 21 days all rapidly desorbing (readily bioavailable) PAHs had been degraded, so that PAH biodegradation was primarily controlled by slow desorption. This is in line with results of other slurry-phase biodegradation experiments (*31, 36-38, Ch.5*).

During the course of biodegradation, the headspace oxygen content was monitored regularly. It was assured that the O_2 content was sufficiently high (>10 % vol.) by regular flushing with air. Biodegradation experiments were carried out in quadruplicate.

3.3.5 Persulfate Oxidation. Persulfate oxidation was performed as previously described by Cuypers *et al.* (*16*, Chapter 2). Soil and sediment material (5 g wet wt) were thoroughly mixed with $K_2S_2O_8$ and demineralized water to obtain a persulfate ($S_2O_8^{2-}$) to organic matter ratio of 12 g/g and an aqueous persulfate concentration of 0.0357 g/mL. The resulting slurry was placed in a water bath shaker at 70°C and shaken end-to-end for 3 hours (120 rpm). After 3 hours the slurry was filtered (S&S 589[1], ashless) and PAHs were extracted from the solids with acetone. The extract was analyzed for PAHs by HPLC. Oxidations were carried out in quadruplicate.
For soils 2, 4, and 6 and sediments 11-13, oxidation was also performed on material that had first been subjected to biodegradation. After 21 days of biodegradation the samples were filtered and solids were oxidized as described above.

3.3.6 Solid-phase Extraction. Solid-phase extraction was performed on two sediments: Assendelft and Petroleum Harbor Amsterdam. The extraction method was adapted from Bonten *et al.* (*39*) and Cornelissen *et al.* (*40*). Extractions were carried out in 50 mL separatory funnels, which were filled with sediment (Assendelft 2.5 and Petroleum Harbor Amsterdam 1.5 g dry matter), 40 mL of 0.01 M $CaCl_2$ solution, 20 mg of NaN_3, and 0.5 g of Tenax-TA adsorbent. The separatory funnels were shaken end-to-end at room temperature at such a speed that the slurry and Tenax beads were well mixed. After 1, 3, 6, 12, 24, 48, 72, 120, and 192 h, the sediment suspension was separated from the Tenax and 0.5 g of fresh Tenax was added to the suspension. The contaminated Tenax was extracted with 20 mL of acetone and the acetone was analyzed for PAHs by HPLC. Finally, after 264 h, the soil suspension was separated from the Tenax, centrifuged, and the solids were extracted with 20 mL of acetone, which was analyzed for PAHs by HPLC. The desorption experiments were carried out in duplicate.

3.3.7 Thermogravimetric Analysis. The composition of soil organic matter before and after persulfate oxidation was studied using thermogravimetric analysis (Du Pont 951 Thermogravimetric Analyzer, 10 °C/min, 20-950 °C, air flow 50 mL/min) and differential thermal analysis (Du Pont High Temperature DTA Cell, 10 °C/min, 20-950 °C, air flow 50 mL/min). Samples 5, 8, and 11-13 were analyzed.

3.4 Results and Discussion

3.4.1 Persulfate Oxidation versus Biodegradation. The results of the oxidation and biodegradation experiments are presented in Figure 1 and Figure 2, both for the sum of the 16 EPA PAHs and for three different ring groups (2&3, 4, and 5&6 ring PAHs). In Figure 1 microbiological PAH removal is plotted against oxidative PAH removal. In Figure 2 residual PAH concentrations after biodegradation are plotted against residual concentrations after persulfate oxidation. Petroleum Harbor sediment has been omitted from Figure 2 as the PAH concentrations were high and fell outside the range of the axes. Regression parameters for the data in Figure 1 and Figure 2 are given in Table 2.

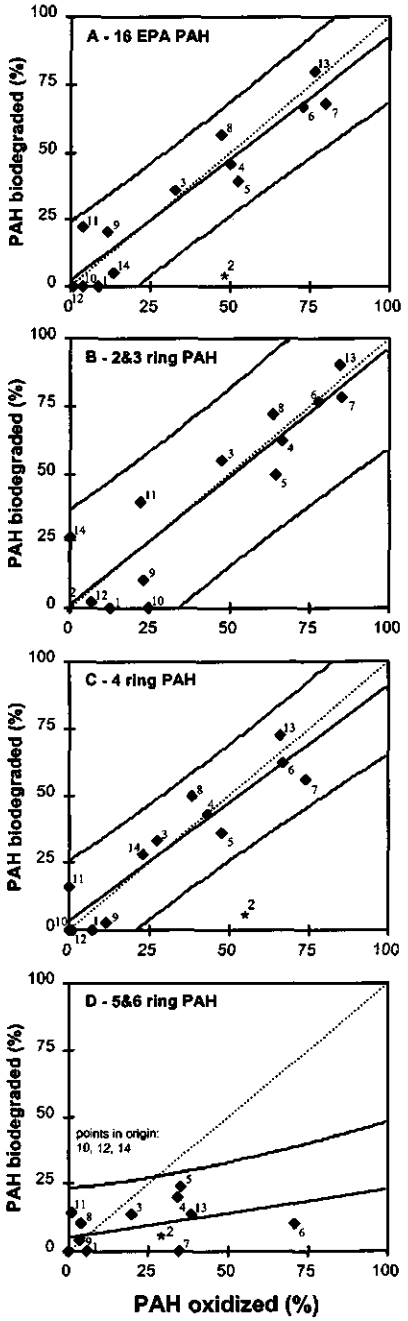

Figure 1. PAH removal during bioremediation versus PAH removal by persulfate oxidation. Numbers refer to soils and sediments in Table 1. Solid lines represent linear regressions and their 95% confidence intervals. Dotted lines represent hypothesized 1:1 correlations.

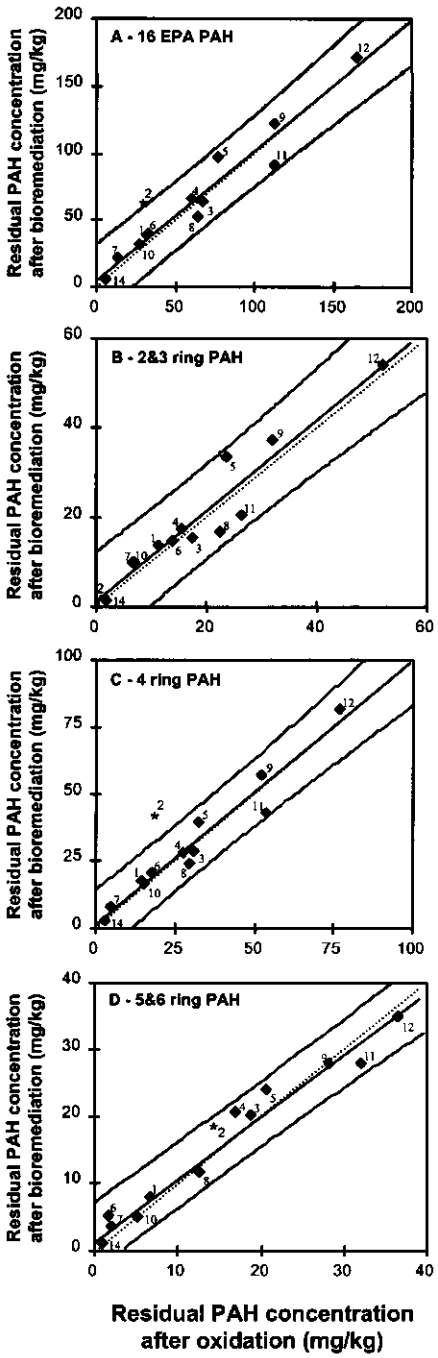

Figure 2. Residual PAH concentrations after bioremediation versus residual PAH concentrations after persulfate oxidation. Numbers refer to soils and sediments in Table 1. Solid lines represent linear regressions and their 95% confidence intervals. Dotted lines represent hypothesized 1:1 correlations.

Table 2. Regression parameters for data in Figures 1 and 2.

	Removal percentages			Residual concentrations		
PAHs	slope	intercept	R^2	slope	intercept	R^2
16 EPA [a]	0.91	2.22	0.90	0.97	4.35	0.95
2&3 ring [b]	0.95	0.78	0.82	1.02	0.76	0.91
4 ring [c]	0.88	3.08	0.87	0.99	1.18	0.95
5&6 ring [d]	0.18	5.12	0.22	0.91	2.11	0.97

[a] Sum of 16 EPA PAHs, except naphthalene, acenaphthylene, and acenaphthene. Concentrations of these PAHs regularly showed large variations within the samples. Naphthalene is included for Petroleum Harbor sediment as measurements could be well reproduced. [b] Fluorene, phenanthrene, anthracene, and fluoranthene; naphthalene is included for Petroleum Harbor sediment. [c] Pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, and benzo[*k*]fluoranthene. [d] Benzo[*a*]pyrene, dibenz[*a,h*]anthracene, benzo[*g,h,i*]perylene, and indeno[1,2,3-*c,d*]pyrene.

It appears from Figure 1 that the extent of PAH removal by persulfate oxidation was in agreement with the extent of PAH removal by biodegradation for all PAHs except the 5&6 rings. The only clear exception was Stadskanaal, which showed considerable PAH oxidation but negligible biodegradation. However, the poor biodegradation in Stadskanaal soil was likely to be caused by an inhibitory low pH rather than by limited PAH bioavailability. Stadskanaal soil had an initial pH of approximately 1, which could not be sufficiently compensated by the phosphate buffer in the mineral medium. The optimum pH values for hydrocarbon degradation by bacteria and fungi are 6-8 and 4-5, respectively. Therefore, the low pH must have inhibited the biodegradation of PAHs. This was confirmed by negligible O_2 consumption and CO_2 production. Hence, Stadskanaal soil has been omitted from the regression in Figure 1 and Figure 2.

In Figure 1 the 95% confidence intervals of regression enclose the 1:1 correlation lines for 2&3 ring, 4 ring, and 16 EPA PAHs. This demonstrates that the relation between oxidation and biodegradation does not differ significantly from the hypothesized 1:1 correlation. In contrast, it is clear from Figure 1 that 5&6 ring PAHs were better oxidized than biodegraded. A similar difference between biodegradation of 5&6 ring PAHs and the predicted bioavailability has been found by others using solid-phase extraction (*3, 4*). It has been suggested that 5&6 ring PAH biodegradation was limited by (unspecified) microbial factors rather than bioavailability (*3*). According to Bonten *et al.* (*20*) these microbial factors may include: (i) an unfavorable Gibbs free energy, (ii) a high activation energy, (iii) slow transport of 5&6 ring PAHs over the microbial cell membrane, and (iv) the inability of microorganisms to grow on low aqueous 5&6 ring concentrations. However, so far, no conclusive explanation for the poor degradation of 5&6 ring PAHs has been given. For now, it is clear that microorganisms can not use these PAHs as the sole carbon and energy source and can only convert them cometabolically (*34*).

Figure 2 shows a good correlation of the residual concentrations after biodegradation with the residual concentrations after oxidation for all PAH ring groups, including 5&6 rings. Statistical analysis shows that the regression lines do not differ significantly from the 1:1 correlation lines (2-sided t-test, 95%). It appears from Table 2 that the regression lines for the residual concentrations are closer to the hypothesized 1:1 correlation than they are for the removal percentages. Besides that, R^2 values are higher and, consequently, 95% confidence limits of

regression are more narrow. The above suggests that residual PAH concentrations can be more accurately predicted than removal percentages. The reason for this is that an inaccuracy in the low concentration range will only have a minor effect on the fit of the residual concentrations, whereas the inaccuracy in terms of removal percentages may be high. However, this can only partly explain the distinct differences between the 5&6 ring data in Figure 1 and 2. For 5&6 ring PAHs it should be taken into account also that both PAH removal by oxidation and PAH removal by biodegradation were very low for the majority of the soil and sediment samples. Obviously, this low PAH removal results in a good correlation of the residual PAH concentrations. Yet, it has the effect that the few samples with a relatively high oxidative PAH removal (e.g. soils 6 and 7) dominate linear regression.

In summary, persulfate oxidation provides a fast method for the prediction of PAH bioavailability. It should be noted, however, that in practice the outcome of bioremediation also depends on other factors than bioavailability. Other important factors are toxicity, environmental conditions, and the presence of suitable microorganisms. In principle, the latter two factors can be controlled to a certain extent; the importance of toxicity must be stressed. Although persulfate oxidation provides specific information on PAH bioavailability, it gives no information about toxicity. This implies that persulfate oxidation and biological experiments are best applied in combination for the prediction of the actual bioremediation potential. First, a persulfate oxidation should be carried out, which gives a rapid indication of PAH bioavailability. Second, a biological characterization has to be performed, but only if PAH bioavailability is significant and bioremediation is expected to be cost-effective. This biological characterization gives an additional indication of possible toxicity effects. The advantage of the combination is that no time-consuming biological tests have to be carried out if persulfate oxidation has shown that PAH bioavailability is insufficient to allow satisfactory biodegradation.

3.4.2 Persulfate Oxidation after Biodegradation. A persulfate oxidation was performed on material that had first been subjected to biodegradation in order to assess whether the fraction of PAH that is oxidized by persulfate is the same fraction that is degraded by microorganisms. The results (Table 3) indicate that generally only a very limited amount of PAHs could be oxidized after biodegradation. Significant oxidation of all ring groups was only observed in Stadskanaal and Schijndel soil. In Kralingen soil only 5&6 ring oxidation was significant.

The additional oxidative PAH removal was expected for Stadskanaal soil and for the 5&6 ring PAHs in other samples. In Stadskanaal soil microbiological PAH removal had been severely inhibited by the low initial pH and, consequently, not all bioavailable PAHs had been biodegraded. The biodegradation of 5&6 ring PAHs may have been seriously hampered by microbiological factors, resulting in incomplete removal of bioavailable PAHs.

In contrast, the additional oxidative removal of PAHs from Schijndel soil was not expected. Although no good explanation for this result can be given, the cause should either be an incomplete biodegradation of rapidly desorbing PAHs, or the oxidation of some slowly desorbing PAHs in addition to the rapidly desorbing PAHs.

Based on the results of all samples except Schijndel soil we conclude that the fraction of PAH that was removed by persulfate oxidation was largely similar to the fraction that was removed by biodegradation. This means that the organic matter that was affected by persulfate oxidation mainly contained PAHs that could desorb rapidly. Furthermore, it indicates that PAHs that were non-available to microorganisms were principally sorbed in organic matter that was not affected by persulfate oxidation.

Table 3. Oxidative PAH removal after bioremediation (% of the initial PAH concentration before bioremediation)[a].

Soils	16 EPA PAHs	2&3 ring PAHs	4 ring PAHs	5&6 ring PAHs
Stadskanaal	42 ± 2 (5)[b]	49 ± 1 (0)[b]	44 ± 2 (6)[b]	33 ± 4 (7)[b]
Kralingen	6 ± 8 (46)	3 ± 8 (62)	4 ± 8 (43)	15 ± 8 (20)[b]
Schijndel	19 ± 4 (67)[b]	13 ± 4 (77)[b]	20 ± 5 (62)[b]	-
Sediments				
Lemster Rien	15 ± 15 (22)	28 ± 24 (39)	7 ± 19 (16)	17 ± 16 (14)
Overschie Rotterdam[c]	0 ± 12 (0)	0 ± 13 (2)	0 ± 13 (0)	0 ± 14 (0)
Petroleum Harbor Amsterdam[c]	0 ± 6 (80)	0 ± 5 (79)	0 ± 8 (72)	0 ± 17 (14)

[a] Average values and standard deviations are presented; parentheses indicate percentage of PAHs removed during bioremediation. [b] Residual concentrations after persulfate oxidation are significantly lower than residual concentrations after bioremediation (t-test, 95%). [c] Average residual concentrations after persulfate oxidation are slightly higher than average residual concentrations after bioremediation.

3.4.3 Solid-phase Extraction. Besides persulfate oxidation, several alternative non-biological characterization tests have been developed for the prediction of PAH bioavailability. Of these tests, aqueous solid-phase extraction has been most extensively studied in relation to the prediction of bioavailability during bioremediation (*3*). Here, we compared aqueous solid-phase extraction to persulfate oxidation and biodegradation (504 h). It was assumed that after 504 h of biodegradation all rapidly desorbing PAH had been degraded, PAH degradation being primarily controlled by slow desorption.

In Figure 3 residual PAH fractions after oxidation, biodegradation, and solid-phase extraction (24, 72, and 264 h) are presented. Residual fractions were also measured at intermediate desorption times but these measurements are not included in Figure 3 as they followed a similar trend.

It is shown in Figure 3 that after 24 h and 72 h of solid-phase extraction the residual PAH fractions seriously overestimated the residual fractions after three weeks of biodegradation. In comparison, the residual fractions after 264 hours of extraction approached the residual fractions after biodegradation and thus gave a good prediction of PAH bioavailability. This prediction was of similar quality as the prediction with persulfate oxidation.

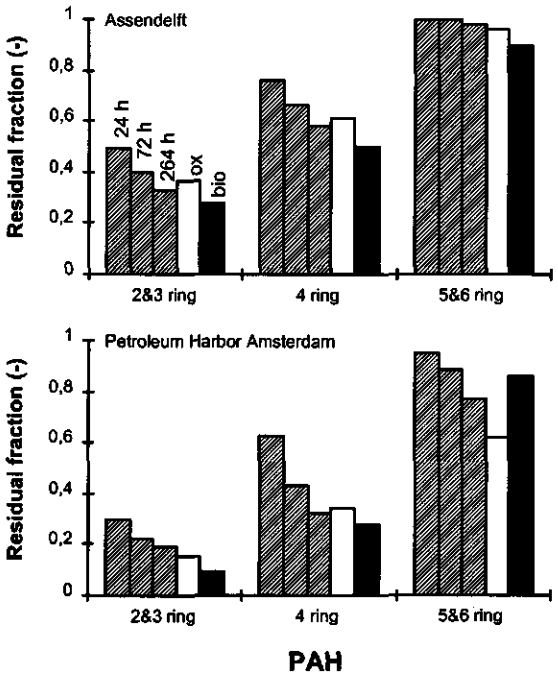

Figure 3. Residual PAH fractions after 24, 72, and 264 h of solid-phase extraction (pattern), after persulfate oxidation (white), and after bioremediation (black).

Altogether, it appears that a short solid-phase extraction accomplished insufficient PAH desorption to predict PAH bioavailability in a long-term biodegradation process. A similar observation has been reported by Cornelissen *et al.* (*3*), who observed that calculated rapidly desorbing fractions seriously underestimated PAH bioavailability. These calculated rapidly desorbing fractions are comparable to fractions desorbed after 24 h in this study.

The discrepancy between short-term solid-phase extraction and long-term biodegradation originates from a difference in the progress of PAH desorption. In an optimized slurry bioreactor PAH degradation is governed by PAH desorption shortly after startup of the reactor. Therefore, it can be assumed that for an accurate prediction of PAH bioavailability the length of a solid-phase extraction must be close to the length of biodegradation in a slurry reactor. In principle, this applies to all characterization processes in which bioavailability is estimated by desorption of PAHs into the water phase. Short desorption experiments can only give a rough (under)estimate of long-term PAH biodegradation. However, the desorption time becomes considerably less important after all rapidly desorbing PAHs have been removed and desorption proceeds only slowly.

It is concluded that besides persulfate oxidation also solid-phase extraction provides a good characterization method for the prediction of PAH bioavailability in historically contaminated

soils and sediments. However, desorption times needed were considerably longer than the oxidation time. Consequently, persulfate oxidation currently is the most rapid method for the prediction of PAH bioavailability.

3.4.4 PAH Bioavailability Related to Organic Matter Structure. The reluctant PAH oxidation after biodegradation and the good correlation between PAH removal by oxidation and biodegradation indicate that there may be a direct relationship between PAH bioavailability and the organic matter domain in which PAHs are sorbed. In order to study this relationship the organic matter structure before and after persulfate oxidation was investigated by thermogravimetric analysis (TGA). A typical result of TGA is presented in Figure 4.

Briefly, TGA experiments show that 10-35% of the original organic material was oxidized by persulfate, which is in agreement with values that have been reported by others (*25-27*). For untreated samples weight loss was observed up to 800 °C. Below 600 °C this weight loss was caused by the removal of organic matter. At higher temperatures the weight loss was caused by the endothermic (DTA results) decomposition of carbonate (*41, 42*). In oxidized samples no weight loss was observed above 600°C. The reason for this is that carbonate was removed by acidification during oxidation.

It appears from Figure 4 that the organic matter that was oxidized by persulfate was typically removed between 250 and 350 °C during TGA. This organic matter is generally considered to be less resistant to microbiological decomposition and chemically less condensed than the organic matter that is removed between 350 and 600 °C (*41*). In other words, the results confirm the hypothesis that persulfate oxidation preferentially removes amorphous organic matter.

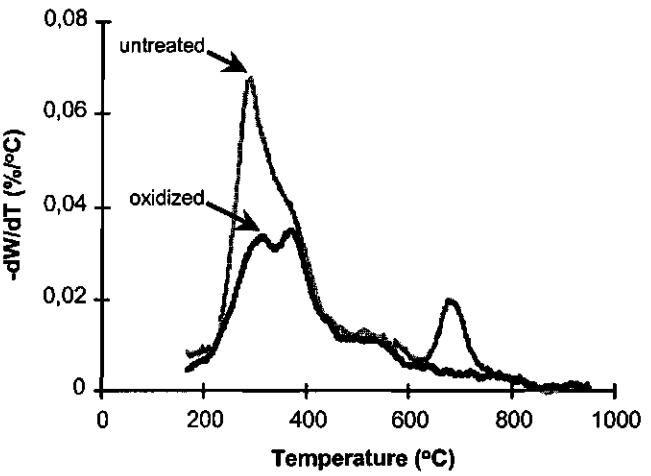

Figure 4. Rate of weight loss during thermogravimetric analysis of Lemster Rien sediment. Untreated sediment (gray), oxidized sediment (black).

If we consider the limited amount of PAH that was oxidized after bioremediation it can now be reasoned that the amorphous organic matter that was oxidized contained primarily readily bioavailable PAHs. However, the results do not prove that *all* readily bioavailable PAHs were sorbed in amorphous organic matter. If part of the readily bioavailable PAHs had been in condensed organic matter this part would likely also have been oxidized, as the high oxidation temperature enables (partial) desorption of readily bioavailable PAHs during oxidation. In contrast, the results do indicate that the poorly bioavailable PAHs were predominantly sorbed in condensed organic matter.

So far, the relationship between bioavailability and sorption to a specific organic matter domain could only be indirectly deduced from sorption isotherms. These isotherms were measured for spiked contaminants in untreated soils and in soils which had been oxidized with persulfate or peroxide (*28, 29, 43-46*). To our knowledge this is the first study in which bioavailability is related more directly to the organic matter structure, using a combination of biodegradation and oxidation experiments and a variety of field aged contaminated soils and sediments.

Acknowledgments

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CHAPTER 4

Extraction with Hydroxypropyl-β-cyclodextrin and Triton X-100 for the Prediction of PAH Bioavailability in Contaminated Sediments *

Chiel Cuypers, Tessa Pancras, Tim Grotenhuis, Wim Rulkens

Abstract

A study was conducted to investigate whether cyclodextrins and surfactants can be used to predict polycyclic aromatic hydrocarbon (PAH) bioavailability in contaminated sediments. Two sediment samples were extracted with aqueous solutions of hydroxypropyl-β–cyclodextrin (HPCD) and Triton X-100. PAH removal during extraction was compared with PAH removal during solid-phase extraction and biodegradation. It was demonstrated that HPCD extracted primarily readily bioavailable PAHs, while Triton X-100 extracted both readily and poorly bioavailable PAHs. Moreover, HPCD did not affect the degradation of PAHs in biodegradation experiments, while Triton X-100 enhanced the biodegradation of low molecular weight PAHs. It was concluded that HPCD extraction may provide a good method for the prediction of PAH bioavailability. Triton X-100 extraction is unfit for the prediction of PAH bioavailability.

* Accepted for publication in *Chemosphere* in a slightly modified form

4.1 Introduction

PAHs are pollutants of great environmental concern because of their toxic, mutagenic, and carcinogenic properties (Pothuluri and Cerniglia, 1998; Van Agteren *et al.* 1998). The biodegradation of PAHs, particularly those of low molecular weight, has been widely investigated. It has been shown that PAHs can be totally degraded or partially transformed either by a community of microorganisms or by a single microorganism (Cerniglia, 1992; Mueller *et al.*, 1996; Pothuluri and Cerniglia, 1998; Van Agteren *et al.*, 1998).

Microbial degradation of PAHs is thought to be the major process involved in effective bioremediation of contaminated soils and sediments (Cerniglia, 1992). Unfortunately, PAH removal during bioremediation is often incomplete and residual concentrations after bioremediation are often too high to satisfy the standards for clean soil (Beck *et al.*, 1995). In many cases, these high residual PAH concentrations are caused by the limited bioavailability of PAHs. In general, the rate at which microorganisms can convert chemicals during bioremediation depends on two factors: (*i*) the rate of uptake and metabolism; (*ii*) the rate of transfer to the cell. The so-called bioavailability of a chemical is determined by the rate of mass transfer relative to the intrinsic activity of the microbial cells. It is controlled by a number of physical-chemical processes such as sorption/desorption, diffusion, and dissolution. Reduced bioavailability of pollutants is caused by the slow mass transfer to the degrading microorganisms (Bosma *et al.*, 1997).

In soils and sediments contaminant mass transfer is often described in terms of desorption kinetics. Generally, desorption of PAHs is biphasic, whereby a short period of rapid desorption is followed by a longer period of slow desorption (Beck *et al.*, 1995; Yeom *et al.*, 1996; Cornelissen *et al.*, 1997; Cornelissen *et al.*, 1998; Williamson *et al.*, 1998). This biphasic behavior also occurs during bioremediation. In the initial phase of bioremediation the rate of PAH removal is high and PAH removal is primarily limited by microbial degradation kinetics. In the second phase the rate of PAH removal is low and PAH removal is limited by slow PAH desorption (Bonten, 2001). It is evident that the poorly bioavailable PAH fraction is constituted by the slowly desorbing fraction.

In the last decade a considerable amount of research has been devoted to the development of methods for the measurement of PAH bioavailability. This research has yielded lab tests based on the microbiological conversion of bioavailable PAHs, solid phase extraction (SPE) of bioavailable PAHs (Cornelissen *et al.*, 1998; MacRae and Hall, 1998; Tang *et al.*, 1999), and persulfate oxidation of bioavailable PAHs (Cuypers *et al.*, 2000). In addition, alternative tests have been suggested based on the removal of readily bioavailable PAHs by solvent extraction (Kelsey *et al.*, 1997; Chung and Alexander, 1998; Nam *et al.*, 1998; Chung and Alexander, 1999) and supercritical CO_2 extraction (Loibner *et al.*, 1997; Loibner *et al.*, 1998). Recently, also cyclodextrin extraction (Reid *et al.*, 1998; Reid *et al.*, 1999; Reid *et al.*, 2000) and surfactant extraction (Volkering *et al.*, 1998a) have been proposed for the prediction of bioavailability of hydrophobic organic contaminants (HOCs).

The application of cyclodextrin extraction for the prediction of PAH bioavailability was first studied by Reid *et al.* (1998; 1999; 2000), who demonstrated that PAH extractability with hydroxypropyl-β-cyclodextrin (HPCD) was closely related to PAH mineralization by microorganisms. It was demonstrated that extractability decreased with increased aging of spiked PAHs. So far, only experiments with spiked PAHs have been reported. Consequently, the applicability of cyclodextrin extraction for the prediction of PAH bioavailability in aged samples from contaminated sites remains to be demonstrated.

The application of surfactant extraction for the prediction of bioavailability was studied by Volkering *et al.* (1998a), who showed that mineral oil bioavailability could be successfully predicted by an extraction with Triton X-100. Wahle and Kördel (1997) applied surfactants for the measurement of the ecotoxicological relevance of a HOC contamination. So far, surfactant extraction has not been used for the estimation of PAH bioavailability.

It should be noted that both cyclodextrins and surfactants have not only been proposed for the prediction of PAH bioavailability, they have also been reported to increase PAH bioavailability (Volkering *et al.*, 1998b; Wang *et al.*, 1998; Jordan and Cunningham, 1999). Wang *et al.* (1998) claimed that HPCD could significantly enhance the bioavailability of a spiked PAH contamination, thus seriously enhancing its biodegradation. Volkering *et al.* (1998b) and Jordan and Cunningham (1999) studied the application of surfactants for the enhancement of bioremediation. They described several mechanisms via which surfactants can enhance HOC bioavailability. Altogether, the application of surfactants for the increase of bioavailability seems to be in conflict with the application for the prediction of PAH bioavailability. In the present study it was aimed to clarify this apparent contradiction.

To this purpose, we investigated the application of cyclodextrins and surfactants for the prediction of PAH bioavailability. Sediment samples from 2 different contaminated sites were extracted with HPCD and Triton X-100 solutions. Besides, the samples were subjected to biodegradation and solid-phase extraction. Biodegradation and solid-phase extraction were used as reference methods to establish which part of the PAHs could be biodegraded and to what extent biodegradation was governed by bioavailability. Biodegradation experiments were also carried out with samples to which HPCD or Triton X-100 was added.

4.2 Background

In this study 3 methods were used for the prediction of PAH bioavailability. These methods rely on the aqueous extraction of bioavailable PAHs. The main driving force for extraction is the (PAH) concentration gradient between the sediment particles and the aqueous phase. This driving force is maintained by enhancement of the apparent solubility of PAHs by addition of a solid sorbent (Tenax-TA), HPCD, or Triton X-100.

Tenax-TA is a solid porous polymer sorbent based on 2,6-diphenyl-p-phenylene oxide. It has been applied successfully for the extraction of rapidly desorbing PAHs from contaminated sediments. As such, it could be used for the prediction of the extent of bioremediation

(Cornelissen *et al.*, 1998). The affinity of PAHs for Tenax is approximately similar to the affinity for organic carbon (Cornelissen *et al.*, 1997).

HPCD is a cyclic oligosaccharide with a hydrophilic shell and a toroidal-shaped apolar cavity. It can form water-soluble inclusion complexes by incorporating PAHs in its cavities. An obvious prerequisite for the formation of the inclusion complexes is that the size and shape of the target molecule (PAH) and the HPCD cavity are complementary. Although this is the case for low molecular weight PAHs, it can be calculated that high molecular weight PAHs are too large to fit in the HPCD cavity. Wang and Brusseau (1995) calculated the size of naphthalene (width-length: 0.5-0.71 nm), phenanthrene (0.58-0.78 nm), anthracene (0.5-0.92 nm), fluoranthene (0.71-0.92 nm), and pyrene (0.71-0.89 nm). The sizes indicate that naphthalene and phenanthrene fit into the HPCD cavity completely (diameter-depth: 0.75-0.78 nm), whereas anthracene, fluoranthene, and pyrene can be only partially included. However, it has been reported by Wang and Brusseau (1995) that molecules that are too large to form 1:1 inclusion complexes with β-cyclodextrin can form 2:1 (cyclodextrin:PAH) inclusion complexes.

The following HPCD/water partition coefficients (K_{HPCD}) can be calculated from data reported in the literature: K_{HPCD} = 508-1470 L/kg for naphthalene (Wang and Brusseau, 1993; Brusseau *et al.*, 1994; McCray and Brusseau, 1998; Ko *et al.*, 1999), K_{HPCD} = 1230-3628 L/kg for phenanthrene (Brusseau *et al.*, 1997; Wang *et al.*, 1998; Ko *et al.*, 1999), K_{HPCD} = 2936-2967 L/kg for anthracene (Wang and Brusseau, 1993; Brusseau *et al.* 1994), and K_{HPCD} = 11400 L/kg for pyrene (Brusseau *et al.*, 1994). Comparison of K_{HPCD} values with organic matter/water partition coefficients (K_{om}) (Van der Meijden and Driessen, 1986) shows K_{HPCD}/K_{om} ratios to vary between 2 (naphthalene) and ~0.3 (phenanthrene, anthracene, pyrene). K_{HPCD}/K_{OM} ratios calculated from data reported by Reid *et al.* (2000; Fig. 2A) were 0.17 (phenanthrene), 0.024 (anthracene), and 0.0072 (benzo[a]pyrene). The data show that incompatibility of the high molecular weight PAHs with the cyclodextrin cavity is reflected in the K_{HPCD} values. K_{HPCD} values for high molecular weight PAHs do not increase with molecular weight as much as K_{om} and K_{oc} values do. A similar conclusion can be drawn from the formation constants of β-cyclodextrin inclusion complexes reported by Blyshak *et al.* (1989).

Triton X-100 is a nonionic surfactant which forms micelles above its critical micelle concentration (CMC). PAH molecules can be included in these micelles, which leads to a dramatic increase of the apparent solubility. Comparison of K_{om} values (Van der Meijden and Driessen, 1986) with (micellar) surfactant/water partition coefficients for Triton X-100 (K_{surf}, calculated from Edwards *et al.*, 1991) learned that K_{surf}/K_{om} ratios are slightly higher than 1 (phenanthrene, pyrene).

4.3 Materials and Methods

4.3.1 Chemicals. Triton X-100 ($C_{34}H_{62}O_{11}$, 646.9 g/mol, purity 98-102%), HPCD (purity 97+%), and acetonitrile (HPLC grade) were purchased from Merck, Acros Organics, and Lab-Scan, respectively. All other chemicals were analytical grade and obtained from Merck. The

porous polymer sorbent Tenax-TA, 20-35 mesh, was purchased from Chrompack. Before use, the Tenax was rinsed with acetone and hexane and dried at 45°C.

4.3.2 Sediments. The sediments used in this study were dredged from the 1st Petroleum Harbor in Amsterdam, The Netherlands, and from a waterway near Assendelft, The Netherlands. Dredged samples were, homogenized, passed through a 2-mm sieve, and stored at 4 °C in the dark until use.

Assendelft sediment (Ad) had dry matter (dm) content of 41 % (w/w), an organic matter content of 13.3 % (w/w), and a pH of 7.1. The dry matter had the following composition: sand 79 %, silt 11 %, clay 10 %. The sediment contained 122 mg/kg PAHs (16 EPA).

Petroleum Harbor sediment (PH) had a dry matter content of 44 % (w/w), an organic matter content of 9.7 % (w/w), and a pH of 7.8. The dry matter consisted of 64 % sand, 15 % silt, and 21 % clay. The sediment was contaminated with PAHs (16 EPA, 2036 mg/kg) and mineral oil (13600 mg/kg). The mineral oil was (partly) present in sticky tar-like particles, which formed a NAPL phase in which PAHs could partition.

4.3.3 PAH Analysis. Sediment samples were mixed with acetone and water up to an acetone/water ratio of 4:1 (v/v) and a solid/liquid ratio of 1:10 g/mL. The resulting slurry was sonicated for 15 min (Retsch UR 2) and shaken at room temperature for 1 h (150 rpm, Gerhardt Laboshaker). Extracts were centrifuged (5 min, 13,000g) and analyzed for PAHs by HPLC. It has been demonstrated that over 95% of the PAHs in soils and sediments can be extracted in one step using this extraction method (Noordkamp *et al.*, 1999).

PAHs (20µL of extract) were separated on a reverse-phase C18 column (Vydac 201TP54, 5µ) with external guard column (Vydac 102GD54T, 5µ) using a mixture of acetonitrile and water as an eluens. The separation was performed at a constant flow of 1 mL/min, varying the acetonitrile/water ratio between 1:1 and 99:1 (v/v) (0-5 min, 1:1; 5-20 min, linear increase from 1:1 to 99:1; 20-40 min, 99:1; 40-45 min, linear decrease from 99:1 to 1:1; 45-50 min, 1:1). PAHs were detected by UV absorbance at 254, 264, 287, 300, 305, and 335 nm (Gynkotek UVD 340S). We measured phenanthrene [PHE], anthracene [ANT], fluoranthene [FLT] (3 ring PAHs), pyrene [PYR], benz[*a*]anthracene [BaA], chrysene [CHR], benzo[*b*]fluoranthene [BbF], benzo[*k*]fluoranthene [BkF] (4 ring PAHs), benzo[*a*]pyrene [BaP], dibenz[*a,h*]anthracene [DBA], benzo[*g,h,i*]perylene [BPE], and indeno[1,2,3-*c,d*]pyrene [IPY] (5-6 ring PAHs). Naphthalene, acenaphthylene, and acenaphthene were not measured because their concentrations showed large variations within the bulk samples. Fluorene was not measured because Triton X-100 disturbed its analysis.

4.3.4 Biodegradation. Biodegradation experiments (triplicate) were carried out following a method which has been described by Bonten *et al.* (1999b). Briefly, wet sediment (5 g dm) was mixed with an aqueous mineral medium up to a water content of 1.5 mL/g. The samples were inoculated with 2.5 mL of an active microbial enrichment culture, which was pre-cultured on PH

sediment. The enrichment culture contained a mixture of microorganisms which showed clear degradation potential for all 16 EPA PAHs except benzo[*g,h,i*]perylene and indeno[1,2,3-*c,d*]pyrene. This degradation potential was demonstrated during growth of the enrichment and during experiments in which the inoculum was added to sterilized PAH contaminated soil. The degradation potential was slightly limited for benzo[*a*]pyrene and dibenz[*a,h*]anthracene, which have to be degraded cometabolically and need a suitable cosubstrate (Keck *et al.*, 1989; Van Agteren *et al.*, 1998). The inoculated samples were incubated at 30 °C and mixed on a rotary tumbler at 22 rpm. After 7, 14 (PH only), and 21 days, three samples were sacrificed for analysis. Acetone was added to the bottles, samples were extracted, and acetone was analyzed for PAHs by HPLC.

Biodegradation experiments (triplicate) were also carried out with samples to which HPCD (0.18 and 0.89 g) or Triton X-100 (Ad: 0.113, 0.138, and 0.247 mmol; PH: 0.138, 0.162, and 0.272 mmol) was added. The Triton concentrations corresponded with aqueous surfactant concentrations of 1, 10, and 50×CMC.

During the course of biodegradation the headspace oxygen content was measured regularly. An oxygen content >10 % (vol.) was maintained by regular flushing with air. Continuous mixing in the bottles assured that the O_2 content in the slurry was sufficiently high for biodegradation.

The set-up of the biodegradation experiments was optimized in a preliminary study (data not shown). In this study it was assured that 21 days of biodegradation was sufficient for the removal of the rapidly desorbing (readily bioavailable) PAHs. It was observed that only 4 % additional PAH removal occurred after 21 days of biodegradation (84 day experiment). This limited PAH removal after 21 days is in line with results reported by others (Lewis, 1993; Pinelli *et al.*, 1997; Rutherford *et al.*, 1998; Bonten *et al.*, 1999b).

A temperature of 30 °C was maintained to create optimal conditions for the degradation of the PAHs. This temperature is higher than the temperature at which desorption experiments were performed (20 °C and room temperature), but nevertheless the results of the biodegradation and desorption experiments may be compared. It has been demonstrated by Bonten *et al.* (1999a,b) that only temperatures higher than 65-70 °C can significantly increase the fraction of PAHs that desorb rapidly. In other words, biodegradation at 30 °C instead of 20 °C may increase the rate at which readily bioavailable PAHs are degraded but it does not increase the amount of PAHs that are readily bioavailable.

4.3.5 Solid-Phase Extraction. The solid-phase extraction (SPE) method was adapted from Bonten *et al.* (1999a) and Cornelissen *et al.* (1997). Extractions were carried out in 50 mL separatory funnels, which were filled with wet sediment (amount equivalent with 2.5 g dm and 1.5 g dm for Ad and PH, respectively), 40 mL of 0.01 M $CaCl_2$ solution, 20 mg of NaN_3, and 0.5 g of Tenax-TA adsorbent. The separatory funnels were shaken end-to-end at room temperature at such a speed that the slurry and Tenax beads were well mixed. After 1, 3, 6, 12, 24, 48, 72, 120, and 192 h, the sediment suspension was separated from the Tenax and 0.5 g of fresh Tenax was added to the suspension. The contaminated Tenax was extracted with 20 mL of acetone and

the acetone was analyzed for PAHs by HPLC. After 264 h, the soil suspension was separated from the Tenax and centrifuged. The solids were extracted with 20 mL of acetone, which was analyzed for PAHs by HPLC. The experiment was carried out in duplicate.

4.3.6 HPCD Extraction. Extractions were carried out in 60 mL serum bottles which were filled with wet sediment (amount equivalent with 2.5 g dm), a 0.5 g/L NaN_3 solution (50 mL), and HPCD (Ad 7.26 g, PH 5.28 g). The bottles were sealed with Teflon-lined silicone septa and shaken end-to-end (150 rpm) at room temperature. After 2, 6, 12, 24, 48, 72, and 120 h, bottles were centrifuged, supernatant was decanted, and fresh HPCD and NaN_3 solutions were added. After 0, 24, 72, and 172 h, bottles were sacrificed for PAH analysis (duplicate). Solids were extracted with acetone after centrifugation.

An important factor in the HPCD extraction is the HPCD concentration. For bioavailability prediction the HPCD concentration should be high enough to enable desorption of all readily bioavailable PAHs. Here, on a weight basis 20 times more HPCD than organic matter was added per extraction step. This is in accordance with the amount used by Reid *et al.* (2000). Equilibrium calculations (using K_{HPCD}/K_{om} ratios calculated from Reid *et al.*, 2000; see Background Section) indicate that the following amounts of PAHs could be extracted from the samples in 8 extraction steps, assuming that all PAHs were bioavailable: phenanthrene > 99.9 %, pyrene ± 96 %, benzo[*a*]pyrene ± 66 %. This calculation demonstrates that HPCD provides sufficient sorption capacity for the extraction of bioavailable PAHs with 2-4 aromatic rings. The extraction of PAHs with 5-6 aromatic rings could have been limited by the sorption capacity of HPCD. Nonetheless, capacity limitations were not expected as only 5 % (Ad) and 22 % (PH) of the 5-6 ring PAHs were observed to be bioavailable in a previous study (Cuypers *et al.*, 2000).

4.3.7 Triton X-100 Extraction. To optimize the Triton X-100 extraction in terms of the Triton X-100 concentration, the following two parameters were studied: (*i*) Triton X-100 sorption to the sediment, (*ii*) the effect of the Triton X-100 concentration on the PAH extraction efficiency.

(*i*) Triton X-100 sorption was calculated from the amount of surfactant needed to reach the critical micelle concentration (CMC) in the presence and absence of sediment material. Sorption experiments (duplicate) were carried out in Schott bottles (300 mL), which were thoroughly cleaned with detergent, rinsed with demineralized water, and filled with a 200 mg/L $HgCl_2$ solution, wet sediment (amount equivalent with 2.76 g dm and 3.79 g dm for Ad and PH, respectively), and Triton X-100 (0-3.0 mmol/L), allowing for no headspace. The bottles were equilibrated for 48 h on a rotary tumbler (16 rpm) at 20 °C. After equilibration, samples were centrifuged and the surface tension of the supernatant was measured using the Wilhelmy plate method (MacRitchie, 1990). The surfactant dose needed to reach the CMC was determined as the amount of surfactant above which an increase of the surfactant concentration did not lead to a further decrease of the surface tension.

(*ii*) The effect of the Triton X-100 concentration on the PAH extraction efficiency was studied in a triplicate equilibrium desorption study. Schott bottles (300 mL) were filled with a 200 mg/L

$HgCl_2$ solution, wet sediment (amount equivalent with 2.76 g dm and 3.79 g dm for Ad and PH, respectively), and Triton X-100 (0-240×CMC and 0-190×CMC in the presence of Ad and PH, respectively), allowing for no headspace. Bottles were equilibrated for 120 h on a rotary tumbler (16 rpm) at 20 °C. After 120 h the suspensions were filtered, the solids were extracted with acetone, and the extracts were analyzed for PAHs.

Based on the outcome of the study described under (*i*) and (*ii*), the following optimized extraction procedure was chosen: Schott bottles (130 mL) were filled with a 200 mg/L $HgCl_2$ solution, wet sediment (amount equivalent with 2.76 g dm and 3.08 g dm for Ad and PH, respectively), and Triton X-100 (10.3 mmol), allowing for no headspace. The bottles were mixed on a rotary tumbler (16 rpm) at 20 °C. After 2, 6, 24, 48, 72, 120, and 172 h, bottles were sacrificed for PAH analysis (duplicate). The sediment suspensions were centrifuged and solids were extracted with acetone.

4.4 Results and Discussion

4.4.1 Optimization Triton X-100 Extraction. ***(i) Triton X-100 sorption:*** The CMC of Triton X-100 was measured to be 0.22 mmol/L, which is in correspondence with values reported by others (Laha and Luthy, 1992; Fu and Alexander, 1995). The amount of surfactant added to reach the CMC in the presence of Assendelft and Petroleum Harbor sediment was 0.42 mmol/L and 0.56 mmol/L, respectively. Triton X-100 sorption was calculated to be 22.1 mmol/kg for Assendelft and 26.9 mmol/kg for Petroleum Harbor sediment.

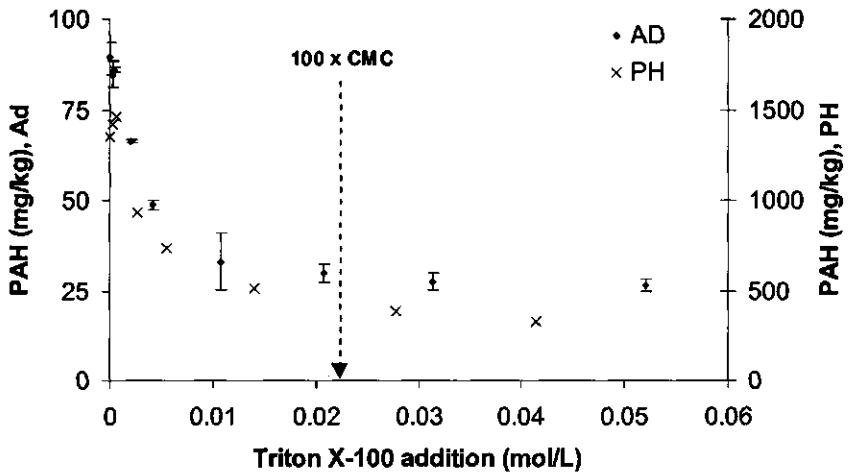

Figure 1. Residual PAH concentrations in Assendelft (Ad) and Petroleum Harbor (PH) sediment after Triton X-100 extraction (120 h) versus the amount of Triton X-100 used for extraction. Error bars represent standard deviations. Symbols may cover the error bars (PH sediment).

(ii) Triton X-100 concentration: The relationship between the Triton X-100 concentration and the extraction efficiency is graphically represented in Figure 1. Figure 1 demonstrates that residual PAH concentrations decreased with an increasing surfactant dose, PAH concentrations leveling off at high surfactant doses. Based on the results, a surfactant dose corresponding with 0.034 mol/L (Ad; 155×CMC) and 0.042 mol/L (PH; 190×CMC) was chosen for the Triton X-100 extraction. On an organic matter basis this was 18.1 (Ad) and 22.3 (PH) g/g. These amounts provided sufficient sorption capacity for the desorption of (almost all) bioavailable PAHs.

4.4.2 HPCD Extraction and Triton X-100 Extraction Compared with Biodegradation and SPE. The course of the PAH concentrations during biodegradation is presented in Table 1. Table 1 shows that PAH degradation was considerable both in Assendelft and in Petroleum Harbor sediment. In both sediments biodegradation was highest for 3 ring PAHs and it decreased progressively with increasing molecular weight of the PAHs. Residual 3, 4, and 5-6 ring concentrations represented 35, 58, and 98 % of the concentrations initially present in Assendelft sediment and 20, 40, and 100 % of the concentrations initially present in Petroleum Harbor sediment.

Table 1. Residual PAHs after 7, 14, and 21 days of biodegradation.

		Residual fraction (-) [a]		
Sediment	**PAHs**	**7 d**	**14 d**	**21 d**
Assendelft	3 ring	0.64 ± 0.11	-	0.35 ± 0.04
	4 ring	0.85 ± 0.13	-	0.58 ± 0.10
	5 and 6 ring	1.00 ± 0.15	-	0.98 ± 0.12
Petroleum Harbor	3 ring	0.32 ± 0.09	0.23 ± 0.05	0.20 ± 0.04
	4 ring	0.77 ± 0.11	0.41 ± 0.03	0.40 ± 0.03
	5 and 6 ring	0.99 ± 0.10	1.00 ± 0.07	1.00 ± 0.03

[a] Average values and standard deviations are presented.

The course of the PAH concentrations during SPE, HPCD extraction, and Triton X-100 extraction is presented in Figures 2 and 3. In these figures also the residual PAH concentrations after 21 days of biodegradation are included.

Figures 2 and 3 show that SPE exhibited biphasic behavior: PAH concentrations dropped rapidly in the first 20-50 h; PAH concentrations decreased only slowly thereafter. Residual 3, 4, and 5-6 ring concentrations after SPE (264 h) represented 33, 58, and 97 % of the concentrations initially present in Assendelft sediment and 20, 34, and 78 % of the concentrations initially present in Petroleum Harbor sediment.

Comparison of the residual PAH concentrations after SPE with the residual concentrations after biodegradation shows that the extent of 3 and 4 ring biodegradation could be rather well predicted by SPE. This indicates that (after 21 days) biodegradation of 3 and 4 ring PAHs was primary limited by slow desorption. Residual 5-6 ring concentrations after SPE were not in

complete agreement with the concentrations after biodegradation. Although correspondence was good for Assendelft sediment, SPE overestimated 5-6 ring biodegradation in Petroleum Harbor sediment. Most likely 5-6 ring biodegradation in Petroleum Harbor sediment was limited by microbial factors rather than slow desorption. Such a limitation of 5-6 ring biodegradation by microbial factors has been reported by others (Cornelissen *et al.*, 1998; Van Agteren *et al.*, 1998). Microbial factors may include an unfavorable Gibbs free energy, a high activation energy, slow transport over the cell membrane, and the inability of microorganisms to grow on low aqueous 5-6 ring concentrations (Bonten, 2001).

Figures 2 and 3 show that HPCD extraction largely followed SPE. After a rapid initial decrease, PAH concentrations slowly approached the residual concentrations after biodegradation. Residual PAH concentrations after 172 h of HPCD extraction were almost similar to the concentrations after biodegradation.

Extraction of PAHs with Triton X-100 was biphasic: residual PAH concentrations leveled off after a rapid initial decrease. In comparison with SPE (and HPCD extraction), Triton X-100 extraction was rapid and residual PAH concentrations were low. A similar difference between surfactant extraction and SPE has been reported by Yeom *et al.* (1996). Residual PAH concentrations after 172 h of Triton X-100 extraction were considerably lower than the residual PAH concentrations after biodegradation. This was most evident for PAHs with a high molecular weight. Figures 2 and 3 show that the percentage of PAHs removed by Triton X-100 was almost equal for all ring groups (approx. 70-80 %). In comparison, the percentage of PAHs removed by SPE, HPCD extraction, and biodegradation decreased with increasing molecular weight of the PAHs.

The difference between SPE and HPCD extraction on the one hand and Triton X-100 extraction on the other indicates that there is a principle difference in the mechanism which underlies these extraction methods. In all three methods PAHs were removed from the aqueous phase by sorption to or inclusion in a specific sink (Tenax-TA, HPCD, Triton X-100 micelles). By regular refreshment of the sink (or by addition of an overdose), aqueous PAH concentrations were kept low and a maximum concentration gradient was maintained between the solids and the aqueous phase. This concentration ensured that desorption took place at the highest possible rate. The principle difference between the methods lies in the way Tenax-TA, HPCD, and Triton X-100 interact with the sediment matrix.

Visual observation of the SPE experiments learned that the solid sorbent Tenax-TA did not interact with the sediment matrix. Literature information learned that HPCD does not interact with the sediment matrix. It has been shown by Brusseau *et al.* (1994) and McCray and Brusseau (1998) that HPCD does not sorb to soils and sediments. Furthermore, HPCD does not partition appreciably to NAPL phases (McCray and Brusseau, 1998). Therefore, it may be reasoned that HPCD changes the system only by an increase of the apparent solubility, a (slight) reduction of the surface tension (Wang and Brusseau, 1993; McCray and Brusseau, 1998), and a reduction of the NAPL/water interfacial tension (Boving *et al.*, 1999). Contrary to Tenax-TA and HPCD, Triton X-100 showed considerable interaction with the solid matrix. This interaction

was demonstrated in the sorption experiments and is likely to have changed the desorption behavior of PAHs, resulting in enhanced PAH bioavailability.

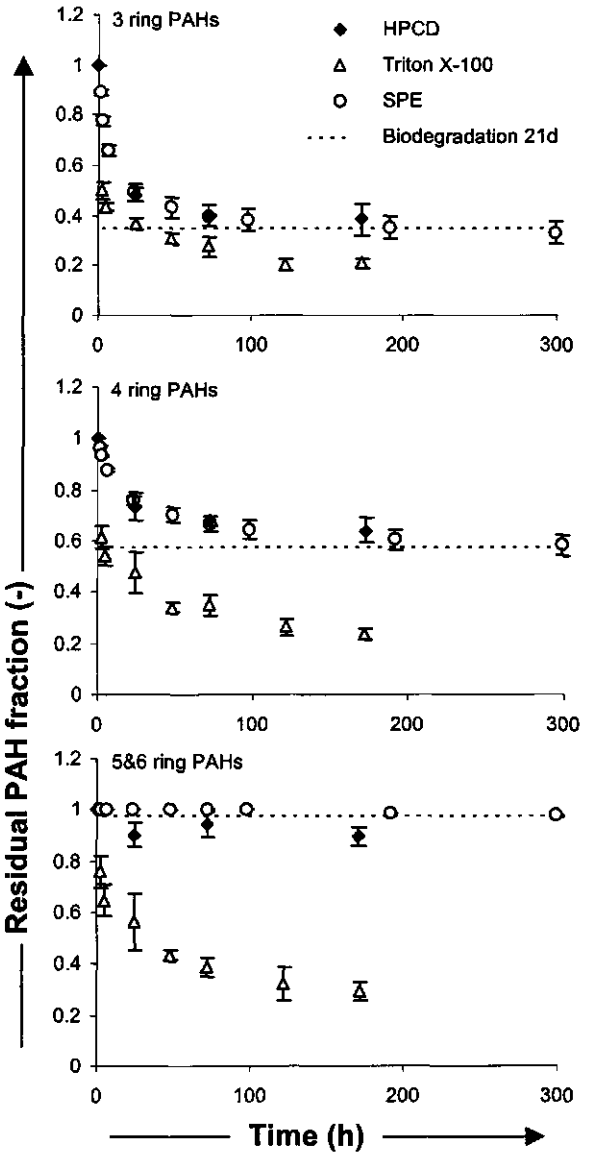

Figure 2. Desorption of PAHs from Assendelft sediment during HPCD extraction, Triton X-100 extraction, and solid-phase extraction. The dotted line indicates the residual PAH concentration after biodegradation. Error bars represent duplicate samples. Symbols may cover the error bars.

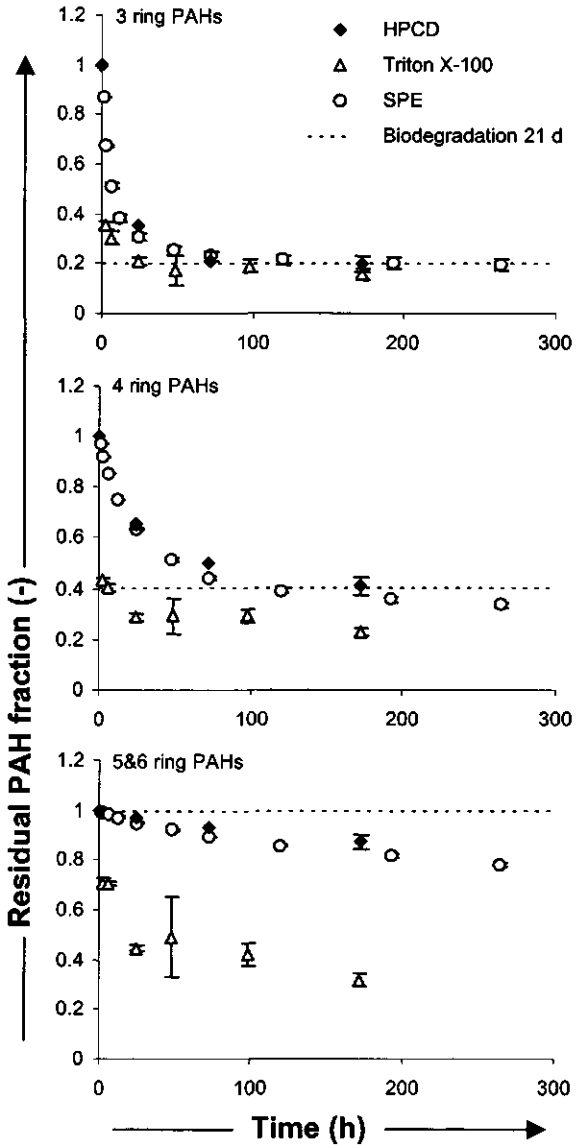

Figure 3. Desorption of PAHs from Petroleum Harbor sediment during HPCD extraction, Triton X-100 extraction, and solid-phase extraction. The dotted line indicates the residual PAH concentration after biodegradation. Error bars represent duplicate samples. Symbols may cover the error bars.

In the literature several mechanisms have been described via which surfactants can enhance the bioavailability of HOCs. These mechanisms have been reviewed by Volkering *et al.* (1998b) and Jordan and Cunningham (1999). They can be divided in three main categories: (i) enhancement of the apparent solubility, (ii) facilitated transport, and (iii) emulsification of NAPLs. The relative contribution of the mechanisms is dependent on the system characteristics and on the physical state of the contaminants.

Evaluating the results of our experiments it may be concluded that enhancement of the apparent PAH solubility is not the mechanism which enhanced PAH bioavailability in the Triton X-100 extraction. The results of SPE and HPCD extraction demonstrated that a sole enhancement of the apparent solubility did not result in the extraction of more PAHs than were removed by biodegradation. A similar conclusion was drawn by Yeom *et al.* (1996).

Facilitated transport of PAHs covers several different processes. These processes include the interaction of surfactant monomers with PAHs, the interaction of surfactant with sorbed PAHs, the interaction of surfactant with the surface, mobilization of PAHs trapped in ganglia by lowering the surface tension of pore water, mobilization of NAPLs in pores by reduction of the interfacial tension, and mobilization of the PAHs by swelling of the organic matrix (Volkering *et al.*, 1998b; Jordan and Cunningham, 1999). Altogether, these processes either change the sorption properties of the matrix itself or increase transport through the (un)changed matrix. In our experiments facilitated transport is the only mechanism which can explain bioavailability enhancement in Assendelft sediment. In Petroleum Harbor sediment, which contains a NAPL phase (tar-like particles), enhanced PAH availability may also be caused by emulsification of the NAPL. At this point, it is not possible to distinguish between the contribution of emulsification and facilitated transport and between the different processes which cause facilitated transport.

Altogether, the results in Figures 2 and 3 demonstrated that HPCD extracted primarily readily bioavailable PAHs, whereas Triton X-100 extracted both readily and poorly bioavailable PAHs. It may be concluded that extraction with HPCD was a suitable method for the prediction of PAH bioavailability in contaminated sediments. Extraction with Triton X-100 enhanced PAH bioavailability.

4.4.3 Effect of HPCD and Triton X-100 on Biodegradation of PAHs. The residual PAH concentrations after biodegradation are presented in Figures 4 and 5. It should be noted that these PAH concentrations are the sum of the concentrations in the solid phase and the concentrations in the liquid phase of the slurry. Residual concentrations are expressed in mg/kg solid material.

Figures 4 and 5 show that HPCD addition hardly enhanced PAH degradation in Assendelft and Petroleum Harbor sediment. In Assendelft sediment biodegradation enhancement was significant only for chrysene at the highest HPCD concentration (*t*-test, 95%). In Petroleum Harbor sediment biodegradation enhancement was significant for phenanthrene, fluoranthene, benz[*a*]anthracene, chrysene, and indeno[1,2,3-*c,d*]pyrene at the lowest HPCD concentration and for benzo[*g,h,i*]perylene and indeno[1,2,3-*c,d*]pyrene at the highest HPCD concentration.

However, despite the significance of the differences for some PAHs, it is clear that the general effect of HPCD addition was small. Thus, it is concluded that HPCD addition neither stimulated nor inhibited PAH biodegradation. This implies that, besides its negligible effect on PAH bioavailability, HPCD did not stimulate the cometabolic degradation of poorly degradable 5-6 ring PAHs. Cometabolic degradation could have been stimulated by conversion of HPCD, which was observed as an increased O_2 consumption and CO_2 production during the whole experiment. Furthermore, HPCD addition did not inhibit PAH degradation. PAH degradation could have been inhibited as a result of a decreased free aqueous PAH concentration or as a result of HPCD biodegradation, which could have caused nutrient deficiency or the development of a microbial community unfit for the degradation of PAHs.

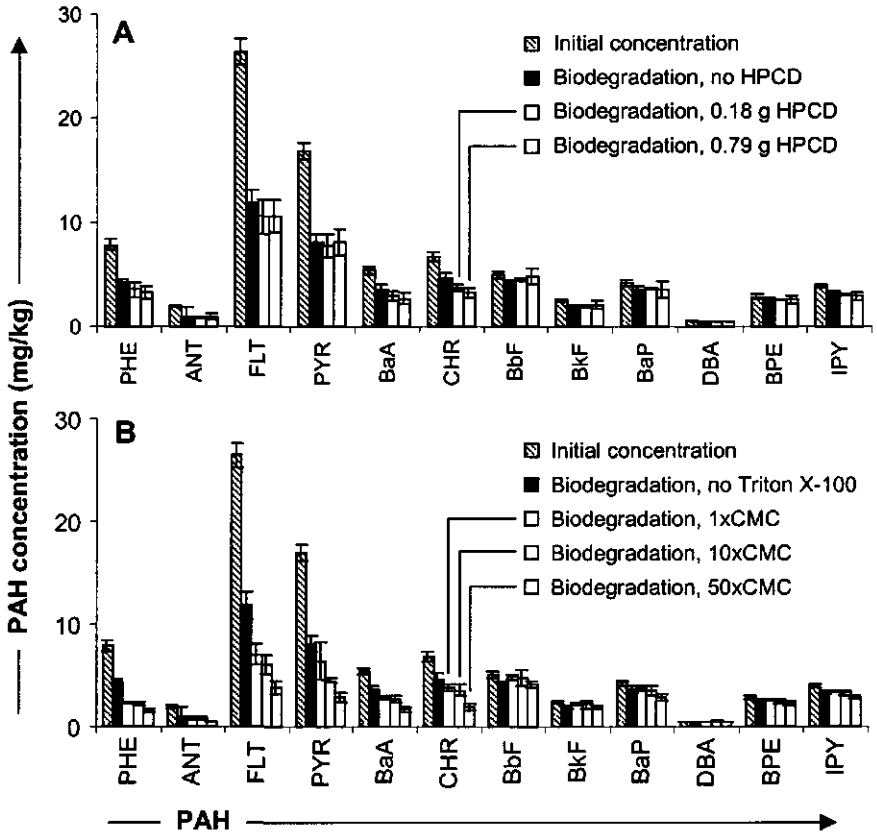

Figure 4. PAH concentrations in Assendelft sediment before and after biodegradation. Biodegradation experiments were carried out with and without addition of HPCD (A) and Triton X-100 (B). Error bars represent standard deviations.

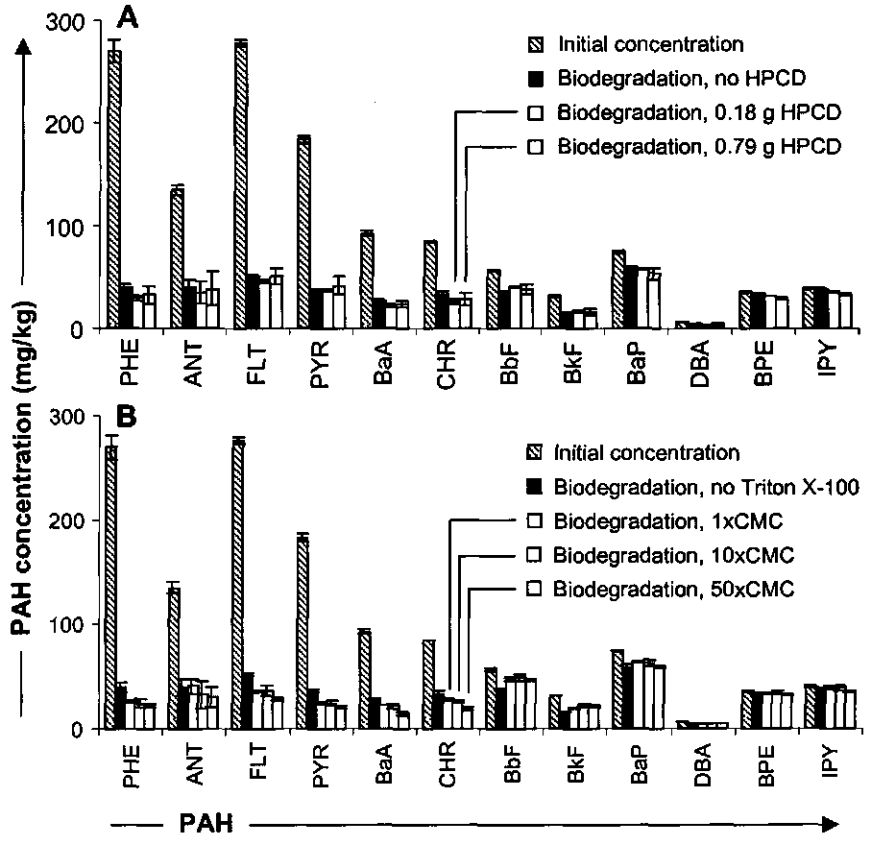

Figure 5. PAH concentrations in Petroleum Harbor sediment before and after biodegradation. Biodegradation experiments were carried out with and without addition of HPCD (A) and Triton X-100 (B). Error bars represent standard deviations.

Conversely to HPCD addition, Triton X-100 addition did enhance PAH biodegradation in Assendelft and Petroleum Harbor sediment. Biodegradation enhancement was evident for the low molecular weight PAHs and increased with increasing Triton X-100 concentration. Significant biodegradation enhancement primarily occurred for PAHs with a molecular weight lower than benzo[*b*]fluoranthene. For PAHs with a molecular weight similar to or higher than benzo[*b*]fluoranthene biodegradation was hardly enhanced and in some cases even inhibited.

The limited enhancement of biodegradation of high molecular weight PAHs by Triton X-100 is in contrast with the results of the extraction experiments which demonstrated that especially the bioavailability of high molecular weight PAHs was enhanced. This discrepancy between the extraction experiments and biodegradation experiments can be explained by a limited capacity of the microbial community for 5-6 ring degradation. Besides this limited degradation capacity, also limitation by a Triton X-100 induced inhibition effect may have occurred. Research has

shown that Triton X-100 and other nonionic surfactants can both positively and negatively affect biodegradation of PAHs and other hydrophobic organic contaminants (Rouse *et al.*, 1994; Volkering *et al.*, 1998b; Jordan and Cunningham, 1999). Positive effects have been attributed mainly to increased bioavailability; negative effects have been attributed to several inhibitory factors, including surfactant toxicity, poor bioavailability of micellar PAHs, inhibition of bacterial attachment, increased PAH sorption as a result of surfactant sorption, nutrient deficiency resulting from surfactant biodegradation, and the development of a microbial community unfit for PAH biodegradation. It has often been stated that Triton X-100 is inhibitory at concentrations above the CMC, inhibition being reversible if concentrations are reduced to below CMC concentrations (Laha and Luthy, 1991; Laha and Luthy, 1992; Volkering *et al.*, 1998b; Jordan and Cunningham, 1999). Our experiments demonstrated that supra-CMC concentrations were inhibitory for the biodegradation of some PAHs, whereas they stimulated the biodegradation of other PAHs.

4.5 Conclusions

The present study demonstrated that HPCD solutions extracted primarily readily bioavailable PAHs from sediment samples containing an aged PAH contamination. This result confirms the work of Reid *et al.* (1998; 1999; 2000) who suggested that HPCD extraction may be applied for the prediction of PAH bioavailability. It seems to be in conflict with the work of Wang *et al.* (1998) who reported that HPCD enhanced the bioavailability of spiked PAHs. It is apparent that experimental results obtained with spiked PAHs could not be automatically extrapolated to systems containing strongly sorbed (aged) PAHs. Altogether, HPCD may accelerate the desorption of readily bioavailable PAHs, but it does not increase the amount of PAHs that are readily bioavailable. This conclusion was supported by biodegradation experiments which showed that HPCD addition had a negligible effect on the extent of PAH degradation.

Conversely to HPCD, Triton X-100 enhanced PAH bioavailability. This bioavailability enhancement was likely to be caused by the interaction of Triton X-100 with the sediment matrix. Obviously, Triton X-100 extraction can not be applied for the prediction of PAH bioavailability, which is in contrast with the results of Volkering *et al.* (1998a), who successfully predicted mineral oil bioavailability using Triton X-100. Enhancement of PAH bioavailability by Triton X-100 was in line with the work of others (Jordan and Cunningham, 1999). Biodegradation experiments showed that bioavailability enhancement by Triton X-100 may stimulate the biodegradation of poorly bioavailable PAHs, provided that these PAHs are biodegradable (3 and 4 rings).

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CHAPTER 5

Prediction of Petroleum Hydrocarbon Bioavailability in Contaminated Soils and Sediments *

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Abstract

Recently, several laboratory methods have been developed for the prediction of contaminant bioavailability. So far, none of these methods has been extensively tested for petroleum hydrocarbons. In the present study we investigated solid-phase extraction and persulfate oxidation for the prediction of total petroleum hydrocarbon (TPH) bioavailability. One sediment and two soil samples were subjected to solid-phase extraction, persulfate oxidation, and biodegradation, after which hydrocarbon removal was compared. It was demonstrated that a short solid-phase extraction (168 h) provided a good method for the prediction of the extent of TPH degradation in an optimized slurry reactor (84 d). Solid-phase extraction slightly underestimated the degradation of readily biodegradable hydrocarbons, whereas it slightly overestimated the degradation of poorly biodegradable hydrocarbons. Persulfate oxidation appeared to be unfit for the prediction of TPH bioavailability as persulfate was unable to oxidize hydrocarbons with a high ionization potential. Hydrocarbons that were affected were likely to be transformed rather than completely oxidized. Nevertheless, persulfate oxidation provided a good method for the prediction of polycyclic aromatic hydrocarbon (PAH) bioavailability.

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5.1 Introduction

In the last decade, bioremediation has been frequently used to treat soils and sediments contaminated with petroleum hydrocarbons. However, despite the common use and cost-effectiveness of bioremediation, it has been observed that a residual fraction remains undegraded even when optimal biodegradation conditions have been provided (Huesemann, 1997). The extent of hydrocarbon degradation is critically dependent upon four factors: (*i*) the presence of hydrocarbon degrading bacteria, (*ii*) environmental conditions (temperature, electron acceptors, nutrients, pH), (*iii*) the recalcitrance of individual compounds in the hydrocarbon mixture, and (*iv*) bioavailability (Huesemann, 1995; De Jonge *et al.*, 1997). In principle, the first two factors can be controlled to a certain extent. The latter two factors reflect intrinsic properties of the individual samples and hydrocarbon contaminants.

The rate at which hydrocarbon degrading microorganisms can convert chemicals depends on the rate of transfer to the cell and the rate of uptake and metabolism by the microorganisms. The so-called bioavailability is determined by the rate of mass transfer relative to the intrinsic activity of the microbial cells. It is controlled by a number of physical-chemical processes such as sorption/desorption, diffusion, and dissolution (Bosma *et al.*, 1997).

Contaminant mass transfer in soils and sediments is often described in terms of desorption kinetics. Generally, desorption of hydrocarbons is biphasic, whereby a short period of rapid desorption is followed by a longer period of slow desorption (Beck *et al.*, 1995; Yeom *et al.*, 1996; Cornelissen *et al.*, 1998; Williamson *et al.*, 1998). This biphasic behavior also occurs during the bioremediation of petroleum hydrocarbons (Huesemann and Moore, 1993; Chaîneau *et al.*, 1995; De Jonge *et al.*, 1997; Huesemann, 1997; Salanitro *et al.*, 1997). In the initial phase of bioremediation the rate of hydrocarbon removal is high and removal is primarily limited by microbial degradation kinetics. In the second phase the rate of hydrocarbon removal is low and removal is generally limited by slow desorption. Altogether, the poorly bioavailable fraction of a hydrocarbon contamination is formed by the hydrocarbons that desorb slowly in the second phase of bioremediation.

In recent years, a considerable amount of research has been devoted to the development of laboratory methods for the prediction of hydrocarbon bioavailability. This research has resulted in several characterization methods, based on the removal of readily bioavailable hydrocarbons by solid phase extraction (SPE) (Cornelissen *et al.*, 1998; MacRae and Hall, 1998; Tang *et al.*, 1999; Morrison *et al.*, 2000), persulfate oxidation (Cuypers *et al.*, 2000), solvent extraction (Kelsey *et al.*, 1997; Chung and Alexander, 1998; Nam *et al.*, 1998; Chung and Alexander, 1999), supercritical CO_2 extraction (Loibner *et al.*, 1997; Hawthorne and Grabanski, 2000), and cyclodextrin extraction (Reid *et al.*, 2000). Of these methods, only SPE and persulfate oxidation have been extensively validated with historically contaminated (aged) samples. In such samples the contact time between the contaminants and the solid matrix may have been months, years, or even decades. So far, none of the methods has been tested for a complex mixture of organic compounds like total petroleum hydrocarbons (TPH). They have only been applied for the prediction of PAH, PCB (polychlorinated biphenyl), and pesticide bioavailability.

In the present study SPE and persulfate oxidation were investigated for the prediction of TPH bioavailability in contaminated soils and sediments. SPE is based on the extraction of readily bioavailable hydrocarbons with water. The main driving force for extraction is the concentration gradient between the soil and sediment particles and the aqueous phase. This driving force is maintained by enhancing the apparent solubility with the addition of the solid sorbent Tenax-TA. Tenax-TA is a porous polymer based on 2,6-diphenyl-p-phenylene oxide. It has been applied successfully for the extraction of rapidly desorbing PAHs from contaminated sediments (Cornelissen *et al.*, 1998; MacRae and Hall, 1998) and for the extraction of rapidly desorbing DDT (1,1,1-trichloro-2,2-bis[*p*-chlorophenyl]ethane), DDE (1,1-dichloro-2,2-bis[*p*-chlorophenyl]ethane), DDD (1,1-dichloro-2,2-bis[*p*-chlorophenyl]ethylene), and dieldrin from contaminated soils (Morrison *et al.*, 2000). As such, it could be used for the prediction of PAH biodegradation and for the estimation of DDT, DDE, DDD, and dieldrin availability to earthworms. The affinity of organic contaminants for Tenax is approximately similar to the affinity for organic carbon (Cornelissen *et al.*, 1997).

The persulfate oxidation method is based on the removal of readily bioavailable hydrocarbons by means of oxidation. Persulfate decomposes when it is heated and forms sulfate radicals which can react with organic substances in a complex radical chain mechanism (House, 1962; Kislenko *et al.*, 1996). In soil and sediment samples, typically 20-40 % of the organic material can be degraded by persulfate oxidation (Martin *et al.*, 1981; Martin and Gonzalez-Vila, 1984; Powell *et al.*, 1989). It has been shown that persulfate selectively oxidizes bioavailable PAHs in contaminated field samples. PAHs remaining after oxidation are a measure of the amount of PAHs that are poorly bioavailable (Cuypers *et al.*, 2000).

We studied TPH bioavailability in one sediment sample and two soil samples collected at TPH contaminated field sites. These samples were subjected to SPE, persulfate oxidation, and biodegradation. The removal of TPH by SPE and persulfate oxidation was compared to the removal of TPH by biodegradation. Compositional changes resulting from SPE and biodegradation were also investigated. Furthermore, SPE was applied to samples which had first been subjected to biodegradation.

5.2 Materials and Methods

5.2.1 Chemicals. Acetonitrile (HPLC grade) and petroleum ether (analytical grade) were purchased from Lab-Scan Analytical Sciences (Dublin, Ireland); hexane (HPLC grade) was purchased from Rathburn (Walkerburn, Scotland); and potassium persulfate (analytical grade) was purchased from Sigma-Aldrich (Steinheim, Germany). All other chemicals were analytical grade and obtained from Merck (Darmstadt, Germany). The porous polymer sorbent Tenax-TA (20-35 mesh) was purchased from Chrompack (Bergen op Zoom, The Netherlands). Before use, it was rinsed with water, acetone, and hexane (3×10 mL/g each) and dried over-night at 45°C.

5.2.2 Soil and Sediment Characteristics. One sediment and two soil samples were studied. All samples were homogenized, passed through a 2-mm sieve, and stored in the dark under nitrogen at 4 °C until use.

The sediment sample was dredged from the 1st Petroleum Harbor (PH) in Amsterdam. PH sediment had a dry matter (*dm*) content of 44 % (w/w), an organic matter content of 9 % (w/w), and a pH of 7.1. The dry matter consisted of 64 % sand, 15 % silt, and 21 % clay. The sediment sample as well as the two soil samples were analyzed as described in the *Extraction and Analysis* section. The sediment contained 13,640 mg/kg petroleum hydrocarbons and 2,036 mg/kg PAHs (sum of 16 PAHs listed by the U.S. Environmental Protection Agency in Consent Decree Priority Pollutant list). The TPH extract was dominated by a wide variety of PAHs and alkyl PAHs. In addition, small amounts of hopanes and recalcitrant branched alkanes (e.g. pristane and phytane) were identified. N-alkanes were not detected.

Arcadis Petroleum (AP) soil had a dry matter content of 89.1 % (w/w), an organic matter content of 3.5 % (w/w), and a pH of 7.2. AP soil was contaminated with 8,420 mg/kg petroleum hydrocarbons. The TPH extract was dominated by branched alkanes/alkenes and alkyl PAHs. Besides, it contained alkylbenzenes, light alcohols, and traces of n-alkanes.

Van Velde Buren (VVB) soil had a dry matter content of 75.9 % (w/w), an organic matter content of 9.5 % (w/w), and a pH of 7.3. VVB soil contained 34,100 mg/kg petroleum hydrocarbons and 40 mg/kg PAHs. The TPH extract of VVB soil was dominated by linear and branched alkanes. Hopanes and (alkyl-)PAHs were detected.

5.2.3 Extraction and Analysis. Sediment/soil samples were mixed with acetone and water up to an acetone/water ratio of 4:1 (v/v) and a solid/liquid ratio of 1:10 g/mL. The resulting slurry was sonicated for 15 min (Retsch UR 2) and shaken at room temperature for 1 h (150 rpm, Gerhardt Laboshaker). Aliquots of 1.5 mL were then taken, centrifuged (5 min, 13,000*g*), and analyzed for PAHs by high pressure/performance liquid chromatography (HPLC) as described below. Petroleum ether (6 mL/g *dm*) was added to the rest of the slurry, which then was mixed on a rotary tumbler for 30 min (16 rpm). The slurry was allowed to settle for 5 min and the liquid phase was decanted over a vacuum filter (glass filter, Schleicher & Schuell GF 50). Petroleum ether (4mL/g) was added to the (non-decanted) residue, the slurry was mixed for 30 min (16 rpm) and decanted over the glass filter. The combined filtrates were transferred to a separatory funnel and shaken twice with tap water (water:solvent 20:3 mL/mL). The petroleum ether phase was transferred into a 250 mL Schott bottle to which dried Na_2SO_4 (16 h, 550 °C, 1:10 g/mL) and florisil (16 h, 110 °C, 0.85:1 g/g *dm*) was added. The bottle was mixed for 30 min (16 rpm). Finally, petroleum ether was filtered (Schleicher & Schuell, GF 50), weighed, and analyzed for TPH by gas chromatography (GC).

PAHs in the acetone/water mixture (20μL of extract) were separated on a reverse-phase C18 column (Vydac 201TP54, 5μ) with external guard column (Vydac 102GD54T, 5μ) using a mixture of acetonitrile and water. The separation was performed at a constant flow of 1 mL/min, varying the acetonitrile/water ratio between 1:1 and 99:1 (v/v) (0-5 min, 1:1; 5-20 min, linear

increase from 1:1 to 99:1; 20-40 min, 99:1; 40-45 min, linear decrease from 99:1 to 1:1; 45-50 min, 1:1). PAHs were detected by UV absorbance at 254, 264, 287, 300, 305, and 335 nm (Gynkotek UVD 340S). The following PAHs were measured: (a) 3 rings: phenanthrene [PHE], anthracene [ANT], fluoranthene [FLT]; (b) 4 rings: pyrene [PYR], benz[*a*]anthracene [BaA], chrysene [CHR], benzo[*b*]fluoranthene [BbF], benzo[*k*]fluoranthene [BkF]; and (c) 5-6 rings: benzo[*a*]pyrene [BaP], dibenz[*a,h*]anthracene [DBA], benzo[*g,h,i*]perylene [BPE], and indeno[1,2,3-*c,d*]pyrene [IPY]. Naphthalene, acenaphthylene, acenaphthene, and fluorene were not analyzed because their concentrations showed large variations within the bulk samples.

TPH analysis was performed on a Hewlett-Packard gas chromatograph (5890 Series II). The hydrocarbons (3µL of extract) were separated on a WCOT fused silica column (Chrompack-Varian, 10 m, 0.32 mm i.d.) coated with CP SimDist (film thickness 0.1 mm). Helium was used as a carrier gas (3.1 mL/min). The initial oven temperature of 40 °C was maintained for 5 min, after which the oven was heated at 10 °C/min, up to 300 °C. This temperature was maintained for 10 min. The GC was equipped with an FID detector.

Detailed analysis of petroleum hydrocarbons (1µL of extract) was performed on a GC-MS system (gas chromatograph + mass spectrometer). Hydrocarbons were separated on a Hewlett-Packard 6890 GC equipped with a J&W DB 5ms column (30 m × 250 µm × 0.25 µm). Helium was used as a carrier gas (1.2 mL/min). The injector temperature was 250 °C and the initial oven temperature was 50 °C. After 5 min the oven was heated at 4 °C/min up to 300 °C. This temperature was maintained for 10 min. The GC was coupled to a Hewlett-Packard 5973 MSD (*m/z* 50-800, 2 scans/s, ion source temperature 250 °C). The extract was analyzed in the selected ion mode for alkanes (*m/z* = 57, 71), hopanes (*m/z* = 191), and steranes (*m/z* = 217).

Chromatograms of the TPH extracts of PH, AP, and VVB samples are presented in Figure 1. This figure demonstrates that PH sediment and AP soil contained a weathered petroleum contamination (storage tanks in the Amsterdam Petroleum Harbor were destroyed during World War II), whereas VVB soil contained a fresh contamination.

5.2.4 Biodegradation. Biodegradation experiments were carried out following a method which has been described by Bonten *et al.* (1999a). Briefly, wet soil/sediment (5 g *dm*) was weighted into 80 mL glass serum bottles and mixed with an aqueous mineral medium (0.3g/L NH_4NO_3, 0.1 g/L $MgSO_4 \cdot 7H_2O$, 0.1 g/L $CaCl_2 \cdot 2H_2O$, 40 mg/L KH_2PO_4, 160 mg/L K_2HPO_4, 5 mg/L $FeCl_3$) up to a water content of 2 mL/g. The samples were inoculated with 2.5 mL of an active microbial enrichment culture, which was pre-cultured on PH sediment. The enrichment culture contained a mixture of microorganisms which showed clear degradation potential for petroleum hydrocarbons and all 16 EPA PAHs except benzo[*g,h,i*]perylene and indeno[1,2,3-*c,d*]pyrene. This degradation potential was demonstrated during growth of the enrichment and during experiments in which the inoculum was added to sterilized contaminated soil.

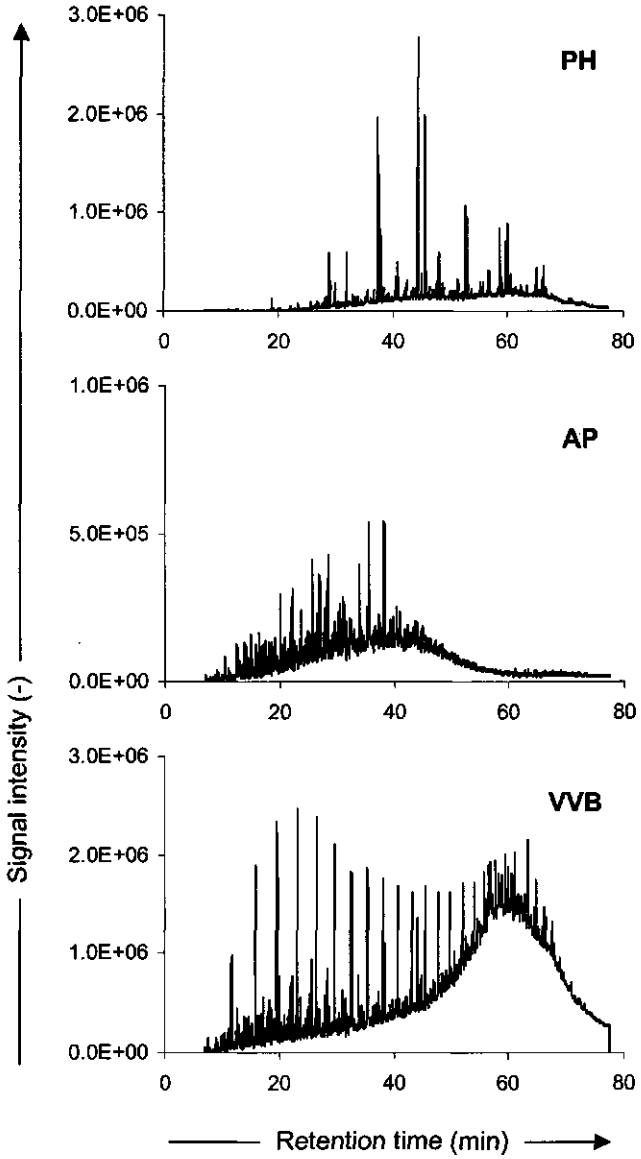

Figure 1. Chromatograms of the TPH extracts of PH sediment, AP soil, and VVB soil (GC-MS, total ion count).

After inoculation bottles were capped and incubated at 30 °C. Samples were mixed on a rotary tumbler at 22 rpm. After 7, 14, 21, 35, 49, 63, and 84 days, samples were analyzed for TPHs. GC-MS analysis was performed on samples obtained on days 0 and 84. The PAH concentration was monitored in the extracts of PH sediment.

Biodegradation experiments were also conducted with samples to which Triton X-100 (0.1 mL) or a concentrated nutrient solution (doubling original nutrient concentrations) was added at day 49 in order to investigate whether biodegradation (at that point) was limited by slow desorption or nutrient deficiency, respectively.

During biodegradation, oxygen and carbon dioxide concentrations were measured regularly. An oxygen concentration >10 % (vol.) was maintained in the headspace by regular flushing with air. Continuous mixing in the bottles assured that the O_2 concentration in the slurry was sufficiently high for biodegradation.

A temperature of 30 °C was maintained to create optimal conditions for biodegradation. This temperature is higher than the temperature at which solid phase extraction experiments were performed (room temperature), but, nevertheless, the results of the biodegradation and desorption experiments may be compared. It has been demonstrated for PAHs (Bonten *et al.*, 1999a,b) that only temperatures higher than 65-70 °C can significantly increase the fraction of the contaminants that desorb rapidly. In other words, biodegradation at 30 °C instead of room temperature may increase the rate at which readily bioavailable petroleum hydrocarbons are degraded but it does not increase the amount of petroleum hydrocarbons that are readily bioavailable.

5.2.5 Solid-Phase Extraction. The SPE method was adapted from Bonten *et al.* (1999b) and Cornelissen *et al.* (1997). Extractions were carried out in 50 mL separatory funnels, which were filled with wet soil/sediment (amount equivalent to 3 g *dm* for PH and VVB, 2 g *dm* for AP), 40 mL of 0.01 M $CaCl_2$ solution, 1 g of $HgCl_2$, and 2 g of Tenax-TA adsorbent. The separatory funnels were shaken end-to-end at room temperature at such a speed that the slurry and Tenax beads were well mixed. After 8, 24, 48, 72, 96, 168, 192, 216, and 240 h, the sediment suspension was separated from the Tenax and 2 g of fresh Tenax was added to the suspension. After 4, 8, 24, 48, 96, 168, and 264 h, samples were analyzed. The soil suspension was separated from the Tenax, the suspension was centrifuged, and the solids were extracted with acetone and petroleum ether. Extracts were analyzed for TPHs (all extracts), PAHs (all extracts of PH sediment), and petroleum hydrocarbon composition (168 h extracts). Hydrocarbon composition was analyzed in the 168 h extracts as TPH removal was observed to be limited after this point. Accordingly, compositional changes were expected to be minor after 168 h.

SPE (168 h) was also performed on material that had first been subjected to 49 days of biodegradation.

5.2.6 Persulfate Oxidation. The persulfate oxidation method was adapted from Cuypers *et al.* (2000). Soil/sediment (3.5 g *dm*) was thoroughly mixed with $K_2S_2O_8$ and demineralized water to obtain a persulfate ($S_2O_8^{2-}$) to organic matter ratio of 12 g/g and an aqueous persulfate concentration of 0.0357 g/mL. The resulting slurry was placed in a water bath shaker at 70°C and shaken end-to-end for 3 hours (120 rpm). After 3 hours the slurry was filtered (Schleicher & Schuell 589[1], ashless) and solids were extracted.

The effect of the oxidation temperature, oxidation time, persulfate to organic matter ratio, and aqueous persulfate concentration on the extent of TPH oxidation was investigated. An overview of the parameters studied is presented with the results in Table 4.

5.3 Results and Discussion

5.3.1 Biodegradation. The course of TPH degradation is presented in Figure 2. This figure shows that considerable TPH degradation occurred in all samples, with biodegradation exhibiting biphasic behavior. TPH concentrations dropped rapidly in the first period of biodegradation, after which concentrations leveled off. Rapid TPH degradation lasted 2-3 weeks in PH sediment and AP soil, and 7-9 weeks in VVB soil, which contained a considerably higher amount of TPHs. After 84 days, TPH concentrations in PH, AP, and VVB had decreased to 49, 16, and 18 % of their initial values, respectively. CO_2 production during the experiments (Figure 3) followed the degradation patterns in Figure 2. CO_2 production was high in the first weeks but leveled off as TPH removal plateaued.

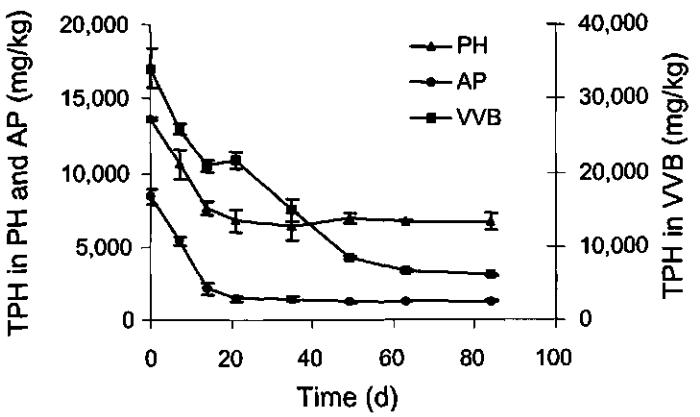

Figure 2. TPH concentration during biodegradation of petroleum hydrocarbons in PH sediment, AP soil, and VVB soil. Data points represent mean values of three samples; error bars represent standard deviations (day 49: two samples, average and range).

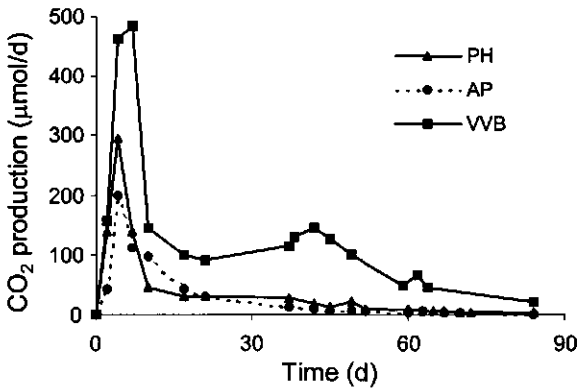

Figure 3. CO_2 production during biodegradation of petroleum hydrocarbons in PH sediment, AP soil, and VVB soil.

In Figure 4 the degradation of specific hydrocarbon fractions is presented. These fractions represent the hydrocarbons with a residence time between the n-alkanes indicated by the carbon numbers. Figure 4 demonstrates that low molecular weight hydrocarbons were degraded faster and to a larger extent than high molecular weight hydrocarbons. Figure 4 shows that C_{10}-C_{12} hydrocarbons were almost completely degraded within 35 days, whereas a large portion of the C_{24}-C_{40} hydrocarbons was recalcitrant throughout the experiment. The relative contribution of low molecular weight hydrocarbons to the TPH concentration decreased during the experiment, while the relative contribution of high molecular weight hydrocarbons increased. This relative enrichment in high molecular weight hydrocarbons was most evident for the samples which contained a weathered TPH contamination (PH, AP). A similar enrichment in high molecular weight hydrocarbons during biodegradation has been reported in the literature for TPHs (Salanitro *et al.*, 1997), normal and branched alkanes (Kennicutt, 1988; Huesemann, 1995), and aromatic compounds (Huesemann, 1995; Cuypers *et al.*, 2000).

GC-MS analysis revealed several compositional changes upon biodegradation. It was demonstrated that alkanes were virtually absent after degradation. In PH sediment no alkanes could be detected after 84 days and in AP soil only traces of recalcitrant compounds like phytane and pristane were preserved. In VVB soil traces of alkanes/alkenes were recovered after degradation, but even higher n-alkanes, like nonacosane, and relatively recalcitrant compounds, like pristane and phytane, were degraded by more than 95 %. Besides the alkanes, alkyl benzenes and low molecular weight PAHs were also degraded to a large extent. In contrast, hopanes, steranes, and high molecular weight PAHs were well-preserved in all samples. The compositional changes upon TPH degradation corresponded well with changes reported in the literature. Several researchers observed extensive degradation of n-alkanes and isoprenoids (Fedorak and Westlake, 1981; Ballerini and Vandecasteele, 1982; Kennicutt, 1988; Butler *et al.*,

1991; Chaîneau *et al.*, 1995), whereas steranes (Kennicutt, 1988), hopanes (Kennicutt, 1988; Butler *et al.*, 1991; Prince *et al.*, 1994), and high molecular weight PAHs (Cuypers *et al.*, 2000) were reported to be highly recalcitrant.

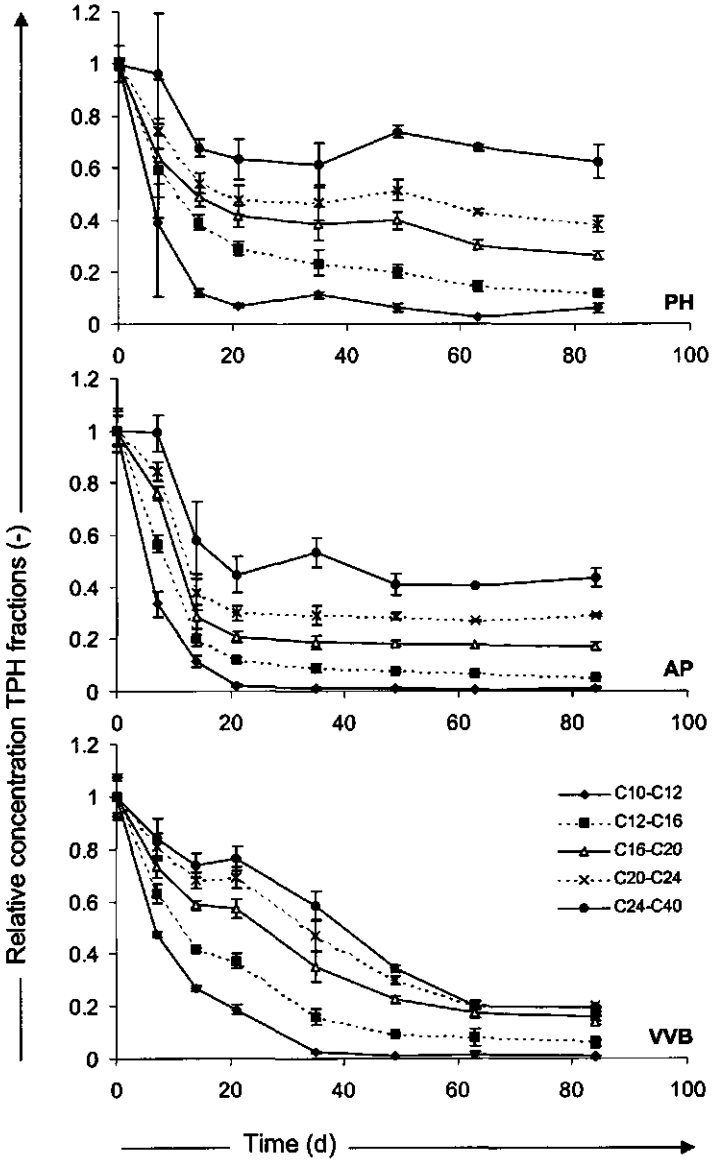

Figure 4. Relative concentration (C_t/C_0) of hydrocarbon fractions during biodegradation of petroleum hydrocarbons in PH sediment, AP soil, and VVB soil. Data points represent mean values of three samples; error bars represent standard deviations (day 49: two samples, average and range).

Table 1. PAH concentrations (mg/kg) during biodegradation and SPE of Petroleum Harbor sediment

	Biodegradation [a, c]									SPE [b, c]	
	0 d	7 d	14 d	21 d	35 d	49 d	63 d	84 d		168 h	
PHE	303.7 ±33.8	45.3 ±11.4	35.8 ±1.4	28.7 ±2.4	26.4 ±1.6	25.5 ±3.6	24.4 ±1.2	21.3 ±0.8	*(7)*	48.5 ±5.5	*(16)*
ANT	130.3 ±13.7	45.7 ±16.2	44.1 ±11.3	31.1±6.8	28.0 ±6.6	29.6 ±9.2	20.6 ±1.8	21.3 ±2.0	*(16)*	43.3 ±9.3	*(33)*
FLT	277.4 ±9.1	64.5 ±8.0	48.6 ±0.8	42.4 ±2.6	45.0 ±3.0	39.5 ±2.2	41.0 ±0.7	37.9 ±2.2	*(14)*	56.5 ±3.0	*(20)*
PYR	186.9 ±7.5	95.7 ±15.4	37.3 ±0.6	31.8 ±2.0	32.8 ±1.6	29.8 ±1.2	30.1 ±0.1	28.3 ±1.6	*(15)*	35.6 ±2.4	*(19)*
BaA	90.1 ±4.6	34.3 ±2.3	26.6 ±0.3	24.2 ±1.8	25.2 ±2.1	23.1 ±1.1	25.3 ±1.9	22.8 ±2.1	*(25)*	29.6 ±2.0	*(33)*
CHR	73.8 ±3.9	33.5 ±1.8	31.7 ±1.0	28.1 ±2.6	27.3 ±3.8	26.5 ±1.5	27.8 ±3.2	25.7 ±3.4	*(35)*	32.7 ±3.8	*(44)*
BbF	54.6 ±2.1	50.8 ±2.6	29.3 ±1.0	24.8 ±1.5	24.5 ±1.1	23.4 ±0.8	24.7 ±1.9	22.6 ±1.5	*(41)*	27.3 ±1.6	*(50)*
BkF	29.4 ±1.2	22.3 ±3.3	13.1 ±0.3	12.2 ±0.7	13.2 ±0.7	12.6 ±0.5	13.5 ±1.0	12.4 ±0.9	*(42)*	15.9 ±1.0	*(54)*
BaP	73.5 ±3.0	72.9 ±2.2	54.3 ±0.5	45.2 ±2.0	35.4 ±1.3	35.0 ±0.9	30.9 ±1.5	27.9 ±1.8	*(37)*	34.7 ±2.0	*(47)*
DBA	5.5 ±0.4	5.1 ±0.1	3.7 ±0.1	3.4 ±0.2	3.1 ±0.1	3.3 ±0.4	3.5 ±0.2	3.2 ±0.2	*(58)*	4.5 ±04	*(82)*
BPE	31.8 ±1.6	32.5 ±1.0	28.5 ±0.4	28.9 ±0.7	29.9 ±0.6	30.1 ±0.1	31.3 ±1.9	30.5 ±0.7	*(96)*	25.3 ±2.3	*(80)*
IPY	37.0 ±1.2	37.9 ±0.7	33.4 ±0.4	33.4 ±0.8	34.7 ±0.7	35.6 ±0.5	36.9 ±2.2	35.2 ±1.1	*(95)*	29.2 ±2.5	*(79)*

[a]Mean ± standard deviation of three samples. [b]Mean ± range of two samples. [c]Values in brackets represent percentage PAHs left after 84 d of biodegradation and 168 h of SPE.

PAH degradation was monitored in PH sediment. The results in Table 1 show that considerable PAH degradation occurred, degradation being significant for all PAHs except benzo[*g,h,i*]perylene and indeno[1,2,3-cd]pyrene. The bulk of the PAH degradation took place in the first 3 weeks of the experiment. After that, degradation proceeded only slowly. The extent of PAH removal after 84 d was highest for 3 ring PAHs and it decreased progressively with increasing molecular weight. A similar decrease in PAH degradation with increasing molecular weight has been reported before (Ballerini and Vandecasteele, 1982; Kennicutt, 1988; Huesemann, 1995; Cuypers *et al.*, 2000).

In order to investigate whether biodegradation after 49 days was limited by nutrient deficiency or slow desorption, batches were amended with a concentrated nutrient solution or a Triton X-100 solution. Nutrient addition slightly enhanced CO_2 production in all samples, but it did not result in a significant (*t*-test, 95%) decrease of residual TPH concentrations after 84 days (data not shown). In comparison, Triton X-100 addition dramatically enhanced both CO_2 production and TPH degradation. This resulted in significantly lower TPH concentrations after 84 days (Table 2). In the batches to which Triton X-100 was added, additional TPH degradation was approximately one thousand mg/kg in all samples. TPH concentrations decreased to 43, 3, and 16 % of their initial values (PH, AP, and VVB, respectively). The above indicates that after 49 days biodegradation was limited by slow desorption rather than nutrient deficiency.

Table 2. Biodegradation of TPHs in original samples and in samples to which Triton X-100 was added after 49 days.

		TPH concentration (mg/kg) [a]		
Time (d)	**Triton X-100 added**	**PH**	**AP**	**VVB**
0		13,637 ±134	8,416 ±503	34,096 ±2,682
49		6,922 ±304	1,218 ±72	8,556 ±274
84	*no*	6,719 ±561	1,307 ±63	6,182 ±88
84	*yes*	5,813* ±85	287* ±29	5,337* ±177

[a]Mean ± standard deviation of three samples (t= 0 and t= 84 d), mean ± range of two samples (t= 49 d). *TPH concentrations after 84 d significantly lower than concentrations in samples without Triton X-100 addition (*t*-test, 95%).

5.3.2 Solid-Phase Extraction. The course of TPH concentrations during SPE is presented in Figure 5. This figure shows that SPE exhibited biphasic behavior: TPH concentrations dropped rapidly in the first 24-100 h, after which they decreased only slowly. Residual TPH concentrations after 168 h represented 46, 15, and 12 % of the concentrations initially present in PH, AP, and VVB samples, respectively. Comparison of the residual concentrations after SPE with the residual concentrations after biodegradation showed that the extent of TPH degradation could be rather well predicted by a short (~168 h) solid-phase extraction. This indicates that biodegradation was primarily limited by slow desorption at the end of the experiment. The above was confirmed by SPE of bioremediated samples. After bioremediation (49 days), no TPH removal by SPE occurred in PH and VVB samples. AP samples exhibited only 8 % additional TPH removal.

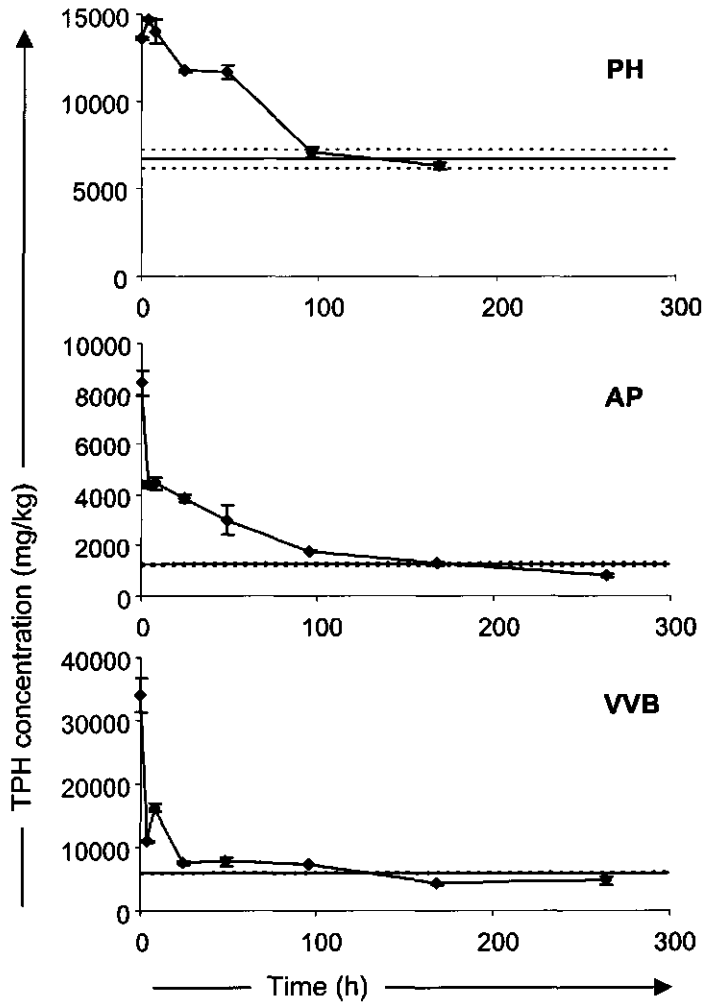

Figure 5. TPH concentration during solid-phase extraction of PH sediment, AP soil, and VVB soil. Data points represent mean values of two samples; error bars represent the two measurements. Horizontal lines represent TPH concentrations after 84 d of biodegradation (solid lines), including standard deviations (dotted lines).

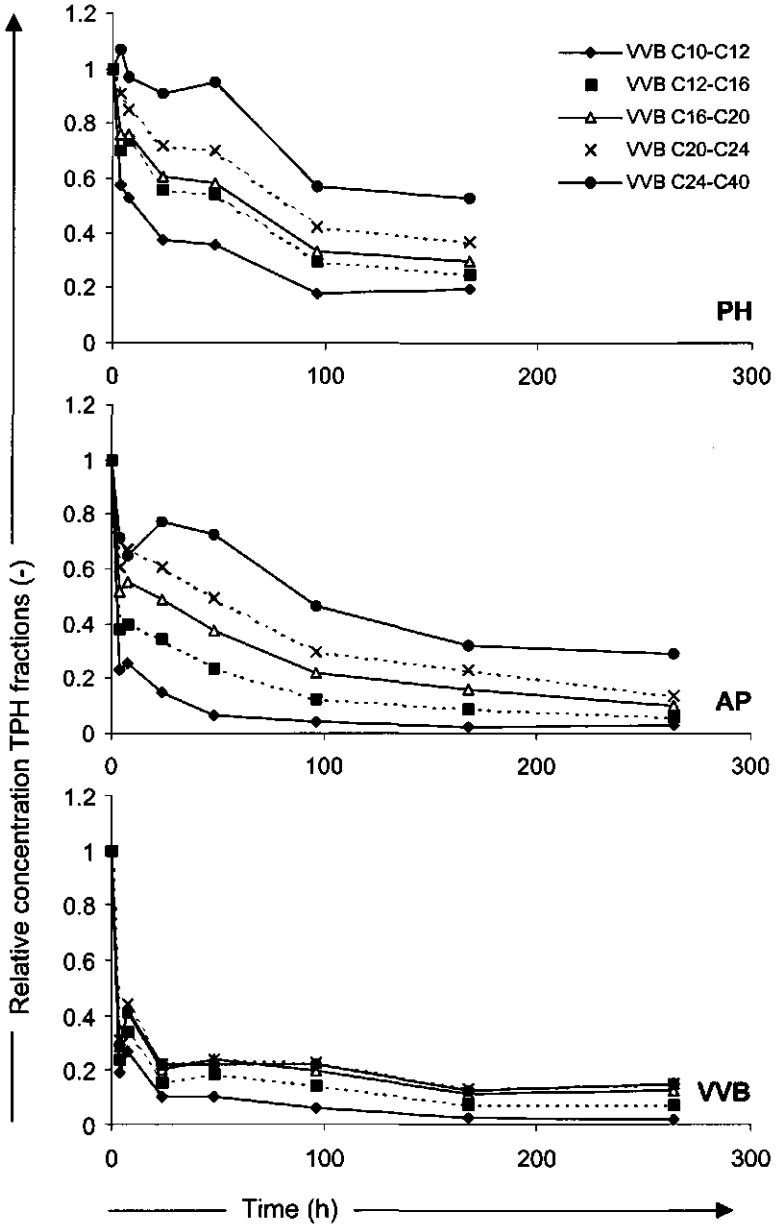

Figure 6. Relative concentration (C_t/C_0) of hydrocarbon fractions during solid-phase extraction of PH sediment, AP soil, and VVB soil.

The decrease of TPH fractions during SPE is presented in Figure 6. This figure shows that low molecular weight hydrocarbons were extracted more rapidly than high molecular weight hydrocarbons. Also, a larger fraction of the low molecular weight TPHs was extracted after 168/264 h. This difference between high and low molecular weight hydrocarbons is most expressed in samples containing a weathered TPH contamination. The weathered contaminants in PH sediment and AP soil desorbed more slowly than the fresh contaminants in VVB soil. It can be seen in Figure 6 that especially high molecular weight components were relatively poorly bioavailable in the samples with a weathered TPH contamination. This decreased bioavailability of weathered contaminants is likely to be caused by progressive sequestration during aging. Such a decreased bioavailability of hydrophobic organic contaminants due to aging has been extensively described in the literature (Pignatello and Xing, 1996; Kelsey *et al.*, 1997; Luthy *et al.*, 1997; Chung and Alexander, 1998; Tang *et al.*, 1998; Alexander, 2000; Morrison *et al.*, 2000)

Comparison of desorption in Figure 6 with biodegradation in Figure 4 learns that the hydrocarbon patterns after SPE (168/264 h) were largely similar to the patterns after biodegradation (84 d). Nevertheless, two differences were observed (see Table 3). First, SPE removed slightly less low molecular weight hydrocarbons than biodegradation. This difference is likely to result from the difference in extraction/biodegradation time. Second, high molecular weight compounds were slightly better desorbed than they were degraded. This difference is likely to be caused by the recalcitrance of certain (bioavailable) high molecular weight hydrocarbons against microbial degradation. This recalcitrance resulted in the persistence of bioavailable hydrocarbons during biodegradation, a phenomenon which has been discussed in the literature before (Huesemann, 1997; Cornelissen *et al.*, 1998; Cuypers *et al.*, 2000).

In the present study recalcitrance of certain hydrocarbons against biodegradation was confirmed by GC-MS analysis. Comparison of the TPH extracts after SPE (168h) with the TPH extracts after biodegradation (84 d) showed that the hydrocarbons which were recalcitrant against biodegradation (steranes, hopanes, high molecular weight PAHs) were removed (partly) by SPE. In contrast, some traces of readily biodegradable components (n-alkanes) had not been removed completely after 168 h of SPE. These components had disappeared completely after 84 d of biodegradation.

Table 3. Relative concentration (C_t/C_0) of hydrocarbon fractions after SPE (168/264 h)[a] and biodegradation (84 d).

	PH		AP		VVB	
	SPE	Bio	SPE	Bio	SPE	Bio
C_{10}-C_{12}	0.19	0.06	0.03	0.01	0.02	0.01
C_{12}-C_{16}	0.25	0.12	0.06	0.05	0.07	0.07
C_{16}-C_{20}	0.30	0.26	0.10	0.17	0.12	0.16
C_{20}-C_{24}	0.37	0.38	0.14	0.29	0.15	0.20
C_{24}-C_{40}	0.53	0.62	0.29	0.43	0.15	0.20

[a]PH, 168h; AP and VVB, 264 h.

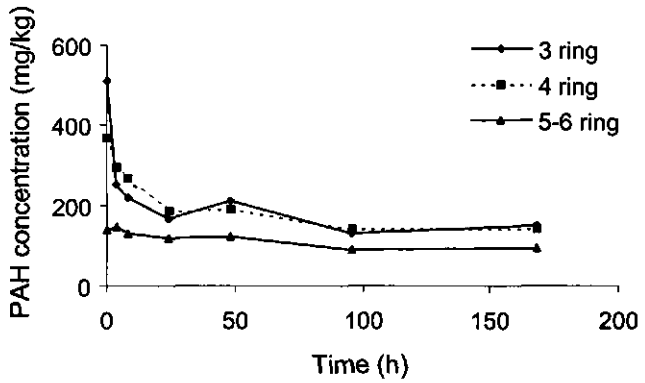

Figure 7. PAH concentrations during solid-phase extraction of PH sediment. Values presented for 3 ring, 4 ring, and 5-6 ring PAHs.

A similar observation was made for PAHs in PH sediment (Figure 7, Table 1). For readily biodegradable PAHs, SPE slightly underestimated biodegradation, because the extraction time was relatively short compared to the biodegradation time. For poorly biodegradable PAHs, degradation was negligible, although these PAHs were partly desorbed in the SPE experiment.
Altogether, it can be concluded that SPE provides a rapid method for the prediction of residual TPH concentrations after biodegradation. This method slightly underestimates the degradation of readily bioavailable hydrocarbons, whereas it slightly overestimates the degradation of poorly biodegradable hydrocarbons. This agrees with data for PAH contaminated soils and sediments (Cornelissen *et al.*, 1998; Cuypers *et al.*, 2000).
The results further indicate that the extent of TPH biodegradation was primarily governed by TPH bioavailability. The good correspondence between the residual concentrations after SPE and the residual concentrations after biodegradation indicates that only a minor fraction of the bioavailable hydrocarbons was recalcitrant against biodegradation in the samples studied.

5.3.3 Persulfate Oxidation. The residual TPH concentrations after persulfate oxidation are presented in Table 4. This table shows that oxidation under standard conditions removed far less petroleum hydrocarbons than were removed by biodegradation. TPH removal was significant in PH sediment and AP soil (*t*-test, 95 %), but oxidation was insufficient to affect all bioavailable hydrocarbons.
Table 4 further demonstrates that a change of one of the individual oxidation parameters (temperature, time, persulfate/OM ratio, aqueous persulfate concentration) did not lead to an enhancement of TPH oxidation in PH sediment. On the other hand, the adjustment of all four oxidation parameters at the same time seriously enhanced TPH removal. This enhancement was

significant in all samples, although oxidation was still insufficient to remove all bioavailable hydrocarbons.

In contrast to the results for petroleum hydrocarbons, oxidation of PAHs (in the same samples) led to the removal of all PAHs that were bioavailable. Results presented in Table 4 show that residual PAH concentrations after persulfate oxidation were similar to residual concentrations after biodegradation. Residual PAH concentrations after persulfate oxidation were not significantly affected by changes of the oxidation parameters (*t*-test, 95%). Nevertheless, a clear decrease was observed in the experiments in which a high aqueous persulfate concentration was applied.

The difference in behavior between PAHs and TPHs can be explained by a combination of two factors: (*i*) a difference in ionization potential between individual hydrocarbons, (*ii*) incomplete oxidation of target compounds. The ionization potential of PAHs is lower than the ionization potential of many other hydrocarbons (James and Lord, 1992; Lide, 1995). The reason for this is that the pi electrons of aromatic molecules are held more loosely than the electrons of carbon-carbon or carbon-hydrogen sigma bonds (Solomons, 1980). As a consequence, molecules without pi electrons (like alkanes) are much less susceptible to oxidation than aromatic molecules. This is reflected in the recalcitrance of aliphatic compounds in the oxidation of soil organic matter with mild oxidants like persulfate (Saiz-Jimenez and De Leeuw, 1987; Saiz-Jimenez, 1992).

In addition, hydrocarbons were likely to be transformed rather than completely oxidized (to CO_2 and H_2O). As a consequence, oxidation products still contributed to the TPH concentration. In comparison, the oxidation products of PAHs did not contribute to the residual PAH concentration, as they were not identified as one of the target PAHs during HPLC analysis.

Altogether it can be concluded that the persulfate oxidation method which has been successfully applied for the prediction of PAH bioavailability (Cuypers *et al.*, 2000) was unfit for the prediction TPH bioavailability. Persulfate oxidation was confirmed to be a good method for the prediction of PAH bioavailability, also in the presence of high TPH concentrations.

Table 4. TPH and PAH concentrations after persulfate oxidation under various conditions.

Oxidation parameters [a]				TPH (mg/kg) [b]			PAH (mg/kg) [a]
T (°C)	t (h)	$S_2O_8^{2-}$/OM (g/g)	water/$S_2O_8^{2-}$ (ml/g)	PH	AP	VVB	PH
70	3	12	28	12,380 ±961	5,940 ±346	34,232 ±2633	285 ±145
95	3	12	28	12,671 ±1417	-	-	217 ±110
70	24	12	28	12,489 ±567	-	-	310 ±158
70	3	36	28	13,667 ±881	-	-	304 ±153
70	3	12	14	12,808 ±1077	-	-	169 ±86
95	24	36	14	10,060 ±298	2,456 ±267	23,645 ±2193	130 ±65
Initial concentration				*13,637 ±134*	*8,416 ±503*	*34,096 ±2682*	*1,294 ±82*
After biodegradation (84 d)				*6,719 ±561*	*1,307 ±63*	*6,182 ±88*	*289 ±17*

[a] Standard conditions: 70 °C, 3 h, 12 g/g $S_2O_8^{2-}$/OM, 28 mL/g water/$S_2O_8^{2-}$. [b] Mean ± standard deviation of three samples.

5.4 Conclusions

The present study demonstrated that an estimation of TPH bioavailability by SPE provides a good measure of the extent of TPH degradation in an optimized slurry experiment. Although it was observed that TPH degradation was both affected by bioavailability and biodegradability, it was concluded that bioavailability was the principal factor governing the extent of biodegradation in the aged soil and sediment samples. A short (~168 h) SPE experiment slightly underestimated the degradation of readily biodegradable TPHs, whereas it overestimated the degradation of poorly biodegradable TPHs. We expect that SPE can be successfully applied to predict TPH bioavailability in a wide range of soils and sediments with various compositional properties.

Persulfate oxidation was unfit for the prediction of TPH bioavailability. Persulfate was unable to oxidize hydrocarbons with a high ionization potential (alkanes, alkyl structures), which, therefore, were recalcitrant against oxidation. Moreover, most hydrocarbons were likely to have been transformed rather than completely oxidized. This may also have contributed to the incomplete removal of bioavailable petroleum hydrocarbons. Still, persulfate oxidation provided a good method for the prediction of PAH bioavailability. This implies that persulfate oxidation may be applied for the prediction of bioavailability of all sorts of components with a low ionization potential.

Acknowledgments

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CHAPTER 6

Amorphous and Condensed Soil/Sediment Organic Matter Domains: 1. The Effect of Persulfate Oxidation on the Composition of Soil/Sediment Organic Matter *

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Abstract

The composition of amorphous and condensed soil/sediment organic matter (SOM) domains was investigated for 1 soil sample and 4 sediment samples. These samples were oxidized with persulfate to remove amorphous SOM, before and after which the composition of SOM was studied by thermogravimetric analysis, pyrolysis-GC/MS, and CPMAS ^{13}C-NMR. Comparison of the SOM composition before and after oxidation showed that condensed SOM was more thermostable and less polar than amorphous SOM. Condensed SOM was relatively low in O-alkyl C and carboxyl C and it was likely to contain only small amounts of labile organic components (carbohydrates, peptides, fatty acids). Apart from these general characteristics, the composition of the condensed and amorphous domains appeared to be highly dependent on the origin and nature of the SOM investigated. Condensed domains in relatively undecomposed SOM were enriched in aliphatic C, whereas condensed domains in relatively weathered SOM were enriched in aromatic C. Altogether, the compositional changes upon persulfate oxidation were similar to the compositional changes upon humification, which supports the idea that weathered SOM is more condensed than the original material.

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6.1 Introduction

SOM has been described as the whole of the organic material in soils and sediments, including litter, microbial biomass, water-soluble organics, stabilized organic matter (humus), and plant residues in varying stages of decomposition (*1*). Except in the litter zone, the bulk of SOM is thought to consist of humic substances that bear little physical and chemical resemblance to their precursor biopolymers (*1, 2*).

The prevailing physical concept of SOM is one of a polymer-mesh phase, which can be visualized as a three-dimensional entanglement of macromolecules that is perfused with water (*2*). Recently, a conceptual model has been proposed in which SOM is described as being comprised by highly amorphous (soft, rubbery) and relatively condensed (hard, glassy) domains (*3-7*). Evidence for the existence of such domains has been provided by spectroscopic analysis (*8-12*) and by the discovery of a glass transition in humic acid and soil samples (*13-16*). Up to now, the knowledge on the (chemical) composition of the SOM domains is rather limited.

In the present study we investigated the composition of amorphous and condensed SOM domains. To this purpose, we studied the SOM composition in 5 soil and sediment samples, both before and after removal of (part of the) amorphous SOM by persulfate oxidation. Persulfate oxidation has been shown to be a relatively mild oxidation method which decomposes typically 20-40% of the SOM (*17-19*). After oxidation, a recalcitrant residue remains which has a high affinity for hydrophobic organic contaminants (HOCs) (*3, 20*). It has been demonstrated that persulfate oxidation removes readily bioavailable PAHs from PAH contaminated soils and sediments, while poorly bioavailable PAHs are unaffected (*20*). This indicates that amorphous SOM is oxidized by persulfate, while condensed SOM remains unchanged. Up to now, it has not been verified whether all amorphous SOM is removed by persulfate oxidation. Nonetheless, it may be stated that the organic matter remaining after oxidation is "more condensed" than the original material.

In this study SOM composition was investigated by thermogravimetric analysis (TGA), pyrolysis-GC/MS, and CPMAS ^{13}C-NMR. Compositional characteristics of the amorphous and condensed domains were derived from changes in SOM composition upon persulfate oxidation.

6.2 Background

6.2.1 Amorphous and Condensed SOM Domains. In soils and sediments contaminated with HOCs SOM is the principal HOC sorbent if it is present above trace levels (i.e. organic carbon content > 0.02-0.1 %) (*21, 22*). For the interpretation of sorption data specific conceptual models of SOM have been introduced (*4-7*). In literature two models have received much attention: Weber *et al.* (*4, 5*) hypothesized SOM as being comprised by highly amorphous and relatively condensed domains, whereas Xing *et al.* (*6*) conceptualized SOM as an amalgam of rubbery and glassy phases. In practice, these two conceptual models correspond to a large extent. It has been hypothesized (and verified) that sorption of HOCs in amorphous/rubbery domains is linear, fast, and completely reversible, whereas sorption in condensed/glassy domains is nonlinear, slow,

and hysteretic (*2, 4, 6, 7,*). In accordance, it can be reasoned that HOCs sorbed in amorphous/rubbery SOM are readily bioavailable, whereas HOCs sorbed in condensed/glassy SOM are poorly bioavailable (at least partly).

6.2.2 Composition of Amorphous and Condensed Domains. Some compositional characteristics of the amorphous and condensed SOM domains have been reported in the literature. These compositional characteristics have been derived from correlations between the affinity of SOM for HOCs (expressed as K_{oc}, the organic carbon normalized sorption coefficient) and descriptors of the SOM composition. It was assumed that the degree of SOM condensation is higher if K_{oc} values are higher.

Following this line of reasoning, the following composition related characteristics of amorphous and condensed SOM can be derived from the data reported in literature:

(i) Condensed SOM is less polar than amorphous SOM; polarity expressed as O/C, (O+N)/C, or O/H atomic ratios (*23-31*).

(ii) Condensed SOM is more aromatic than amorphous SOM (*23, 26, 32-35*). This is typically observed for humic acids, fulvic acids, and dissolved organic matter. SOM as a whole exhibited a poor correlation between K_{oc} and aromaticity (*31*).

(iii) SOM that has been subjected to more extensive diagenetic alterations is more condensed than the original material (*3, 27, 29, 30*). The structure of the macromolecules ranges from completely amorphous in relatively young SOM to one that is increasingly condensed as diagenetic alteration occurs (*3*). Variations in origin and maturity of SOM may yield spectacular differences in K_{oc} values for different organic facies (*36, 37*).

(iv) SOM which has been subjected to NaOH extraction (*27*), peroxide oxidation (*28, 38*), or persulfate oxidation (*3*) is more condensed than the original material.

(v) Humic acids are more condensed than fulvic acids and less condensed than humin (*25, 26, 28, 32-34, 39, 40*).

(vi) Coal and soot are specific condensed facies which exhibit an extremely high affinity for (planar) HOCs (*36, 37, 40-43*).

6.3 Experimental Section

6.3.1 Soil and Sediment Samples. Experiments were carried out on 1 soil sample and 4 sediment samples. The soil sample (Kralingen) originated from a former gas plant site in Rotterdam, The Netherlands. The sediment samples were dredged from harbors and waterways at several locations in The Netherlands. Properties of the samples are presented in Table 1. Besides these properties, Assendelft and Lemster Rien sediment showed traces of poorly decomposed plant material, Overschie sediment contained woody and coal-like particles, and Petroleum Harbor sediment contained sticky tar-like aggregates, probably resulting from a weathered oil contamination. The oil and PAHs in Petroleum Harbor sediment contributed 14 % and 2 % to the total organic material, respectively. In the other samples the contribution of oil and PAHs was much lower (oil $\leq$ 4 %, PAHs $<$ 0.1 %). It is expected that oxidation of

contaminants will hardly contribute to the removal of organic matter by persulfate oxidation. PAHs form a relatively small part of the organic material, while oil is poorly oxidized by persulfate (Chapter 5).

Before analysis, samples were homogenized, passed through a 2 mm sieve, and split in two parts. One part was oxidized by persulfate, the other part was stored for direct analysis of the SOM composition. Persulfate oxidation was carried out as described previously (*20*). In brief, soil and sediment samples were mixed with $K_2S_2O_8$ and demineralized water to obtain a slurry with a persulfate ($S_2O_8^{2-}$) to organic matter ratio of 12 g/g and an aqueous persulfate concentration of 0.0357 g/mL. The slurry was shaken for 3 h at 70 °C (Julabo SW-20C water bath shaker, 120 mpm) and filtered afterwards. Solids were rinsed with ample demineralized water and dried at 104 °C. Dried samples (original and oxidized) were ground with a mortar and pestle before further analysis.

Table 1. Characteristics of soil and sediment samples.

	clay:silt:sand (%)	OM (%)	OC (g/g OM)	PH-KCl (-)	Oil (mg/kg)	PAHs[a] (mg/kg)
Assendelft	10:11:79	13.3	0.53	7.1	350	122 (57%)
Kralingen	20:34:46	13.1	0.68	7.5	5800	121 (46%)
Lemster Rien	14:21:65	13.5	0.60	7.3	1900	117 (22%)
Overschie	5:3:92	17.5	0.45	7.2	880	167 (0%)
Petroleum Harbor	21:15:64	9.7	0.63	7.8	13600	2036 (80%)

[a]Parentheses indicate PAH bioavailability (%) as reported in Cuypers *et al.* (*20*).

6.3.2 Thermogravimetric Analysis (TGA). TGA was carried out on a Du Pont 951 Thermogravimetric Analyzer. Weight loss of the samples (25-35 mg) was recorded while samples were heated from 20 to 950 °C at 10 °C/min under an air flow of 50 mL/min.

In order to determine whether weight loss during TGA was due to exothermic or endothermic transitions, differential thermal analysis (DTA) was performed. During DTA differences in temperature between samples and an inert reference material were recorded while samples were heated at a constant rate. The decrease or increase of the sample temperature in comparison to the temperature of the reference material was attributed to endothermic or exothermic transitions occurring in the sample. DTA was carried out on a Du Pont High-Temperature DTA Cell (20-950 °C, 10 °C/min, 50 mL/min).

6.3.3 Pyrolysis-GC/MS. Pyrolysis was carried out on a Horizon Instruments Curie-Point pyrolyser. Samples were pressed onto a flattened ferromagnetic wire and heated inductively for 5 s (610 °C). The pyrolysis unit was connected to a Carlo Erba gas chromatograph in which pyrolysis products were separated on a fused silica column (Chrompack, 25 m, 0.25 mm i.d.) coated with CP-Sil 5 (film thickness 0.40 μm). Helium was used as a carrier gas. The initial oven temperature of the GC was 40 °C and after pyrolysis the oven was heated at 7 °C/min, up

to 320 °C. This temperature was maintained for 20 min. The end of the GC column was coupled to a Fisons MD 800 mass spectrometer (*m/z* 45-650, ionization energy 70 eV, cycle time 1 s).
For Assendelft and Kralingen pyrolysis was also carried out after thermally assisted hydrolysis and methylation (THM). THM was performed by adding a droplet of an aqueous tetramethylammonium hydroxide solution (25 %) to samples that were pressed onto a ferromagnetic wire. After addition of the solution the samples were slightly dried, inserted into the pyrolysis probe, and analyzed. The conditions for pyrolysis-GC/MS analysis were similar to the conditions described above.

6.3.4 CPMAS ^{13}C-NMR. Prior to NMR measurements, soil and sediment samples were extracted with hydrofluoric acid in order to remove paramagnetic metals. HF extraction was carried out as described by Schmidt *et al.* (*44*). Samples (5g) were weighed into 50 mL polyethylene bottles to which 40 mL of a 10 % (v/v) HF solution was added. The bottles were closed and vigorously shaken for 30 s. After shaking, the suspension was allowed to settle for 12 h, after which the supernatant was removed. The complete extraction procedure was repeated twice and after the third extraction the solid residue was washed with distilled water and filtered. The solid residue was freeze–dried and ground for NMR analysis. HF extraction as described above has been reported to enhance the signal-to-noise ratio of NMR spectra, causing minimal change of the SOM composition, except for a possible loss of carbohydrates (*44*).
The solid state ^{13}C-NMR spectra were acquired on a Bruker AMX 300 spectrometer, operating at 300 MHz proton frequency (^{13}C, 75 MHz), applying the cross polarization magic angle spinning technique. The rotor spin rate was set at 5000 Hz and a recycle time of 1 s, an acquisition time of 13 ms, and a contact time of 1 ms were applied. For each spectrum 10,000-30,000 scans were recorded, the exact number of scans depending on the sensitivity of the sample.
The relative abundance of functional groups was calculated for the following chemical shift regions: 0-50 (alkyl), 50-110 (O-alkyl), 110-160 (aromatic), and 160-220 (carboxyl). For an accurate assessment of the relative abundance of the functional groups the integrated signal of each chemical shift region was corrected for the signal decay during the time of contact. Correction was carried out as described by Kinchesh *et al.* (*45*):

$$A_0 = \frac{A_t}{\left(\frac{T_{1\rho H}}{T_{1\rho H} - T_{CH}}\right)\left(e^{-t/T_{1\rho H}} - e^{-t/T_{CH}}\right)}$$

Here, A_0 is a measure of the signal when carbon relaxation is absent, A_t is the signal intensity at 1 ms (resultant of signal decrease due to relaxation and signal increase due to cross-polarization), t is the contact time (1 ms), $T_{1\rho H}$ is the proton relaxation time in the rotating frame, and T_{CH} is the cross-polarization time.

Correction as described above is important for solids that are heterogeneous at a molecular level. These solids consist of domains of different structures, each type of domain having a different $T_{1\rho H}$ value (*46*). For SOM, $T_{1\rho H}$ values have been reported to vary widely for different carbon types (*47*). Accordingly, optimal contact times for the measurement of different carbon types may not be similar (*48*). It has been reported that even samples from the same origin can yield different $T_{1\rho H}$ values for a certain carbon type (*49*). Therefore, correction factors have to be determined for every single sample.

In this study the $T_{1\rho H}$ and T_{CH} values for the chemical shift regions were obtained from an experiment in which the contact time was varied from 0.10 to 10 ms (3600 scans per contact time). The magnitude of $T_{1\rho H}$ and T_{CH} was determined by fitting the equation to the experimental data (minimizing the sum of squared errors).

6.4 Results and Discussion

6.4.1 Thermogravimetric Analysis. In Figure 1 the rate of weight loss during TGA is presented, which is the derivative of the original weight data collected. Data for temperatures below 180 °C were discarded because weight loss below 180 °C was primarily due to the (endothermic) loss of water. Such a loss of water up to 200 °C without significant loss of carbon has been reported by others (*50, 51*) and can be attributed to dehydration of organic and inorganic compounds (*52*).

The thermograms in Figure 1 are dominated by 4 peaks, which had a maximum value in the temperatures ranges 290-310 °C (peak 1), 370-390 °C (peak 2), 530-540 °C (peak 3), and 680-730 °C (peak 4). These temperature ranges largely correspond with the ranges reported by Turner and Schnitzer (*53*) and Schnitzer and Hoffman (*54*). The exact position and height of the peaks differed for each individual sample. In some cases peak 2 was present as a shoulder on peak 1.

Comparison of the thermograms of the original (non-oxidized) samples demonstrates that Assendelft and Lemster Rien exhibited a highly similar TGA pattern. Thermograms of these samples were dominated by peak 1, while peak 3 was almost absent. The thermograms of Kralingen and Overschie were also very similar. Figure 1 shows that peak 3 was well-expressed in these samples. Moreover, Kralingen and Overschie were the only samples that showed exothermic decomposition at temperatures higher than 600 °C (DTA, data not shown). In Assendelft, Lemster Rien, and Petroleum Harbor decomposition at temperatures higher than 600 °C was endothermic. Petroleum Harbor differed from all other samples. Its TGA pattern was an average of the two extreme cases described above.

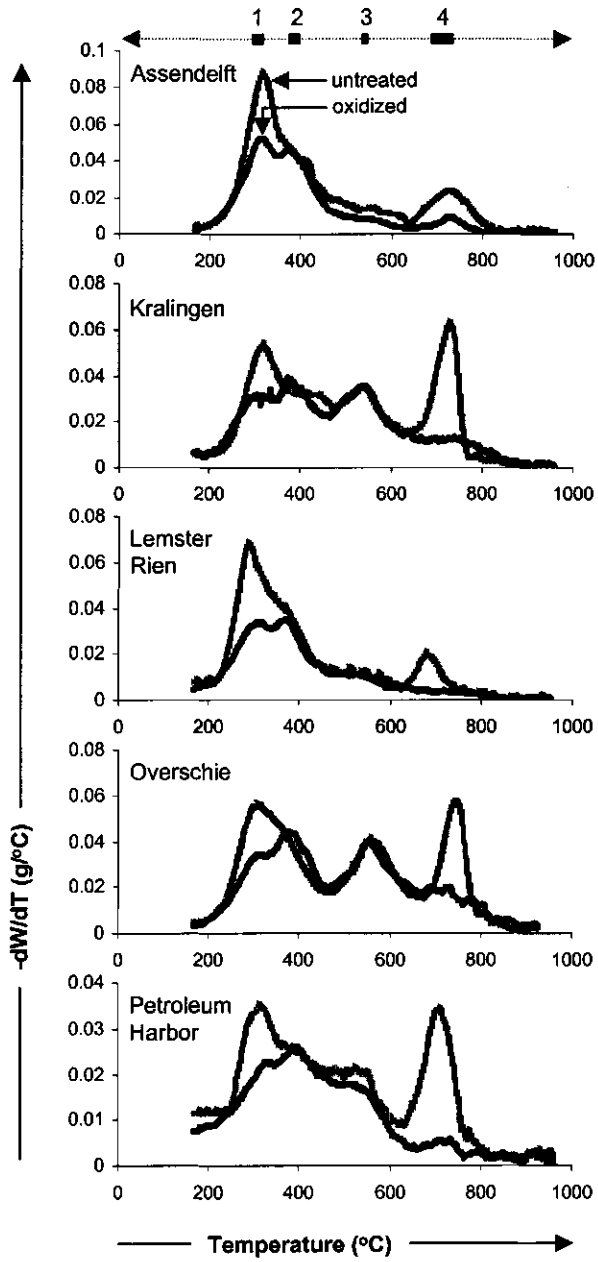

Figure 1. Rate of weight loss during thermogravimetric analysis of untreated (gray) and oxidized (black) samples.

Persulfate oxidation clearly changed the TGA pattern of all samples. Typically, peaks 1 and 4 were reduced by oxidation, whereas peaks 2 and 3 were unaffected. Based on the weight loss up to 650 °C, it was calculated that approximately 10-35 % of the SOM was oxidized by persulfate, which is in agreement with values reported by others (*17-19*). Oxidative removal was relatively high in Assendelft and Lemster Rien and relatively low in Kralingen and Overschie (30, 33, 12, and 13 %, respectively). Petroleum Harbor displayed 23 % oxidative SOM removal.

To be able to draw conclusions on the compositional characteristics of amorphous and condensed SOM domains the TGA patterns in Figure 1 were interpreted in terms of SOM composition. The following composition related characteristics of peaks 1-4 have been reported:

Peak 1 is generally assigned to labile structures and relatively simple organic matter components (*54-57*). These components include fatty acids, peptides, and carbohydrates such as monosaccharides, cellulose, and hemicellulose (*55-57*). They are readily biodegradable and can be easily extracted by (mild) solvents. Schnitzer and Hoffman (*54*) reported that the height of peak 1 is inversely proportional to the degree of humification. The peak becomes broader and smaller as organic matter becomes more humified. Experiments with cellulose have shown that peak 1 shifts to a higher temperature if the crystallinity of cellulose increases (*57*).

Peaks 2 and 3 are generally assigned to more humified organic substances. Both humic acids and fulvic acids yielded peaks in a temperature range corresponding with peaks 2 and 3, humic acids being more thermostable than fulvic acids (*58*). Organic matter which was resistant to NaOH dissolution was found to produce a relatively high signal in the range of peak 3 (*59*). This indicates that the humin fraction of SOM is highly thermostable. Besides humified materials, also non-humified materials may contribute to peaks 2 and 3. It has been shown that lignin and wood can produce peaks up to 520 °C (*55, 57*). For lignin and wood the relative abundance of peaks 1-3 depends on their source and age. Altogether, peaks 2 and 3 are reported to origin from SOM with a relatively high C/H ratio (*50, 55*). Peak 3 has been addressed to the decomposition of aromatic nuclei (*53, 58*) and calcium-OM complexes (*54*). However, also aliphatic compounds may seriously contribute to peak 3, as follows from the high thermostability of humin fractions.

Peak 4 has almost exclusively been attributed to the endothermic decomposition of carbonate (*54, 55, 58*). Nonetheless, peak 4 can also (partially) result from the exothermic decomposition of very condensed graphite-like substances like coal and soot (*55*).

Altogether, literature information indicates that substances that are humified or diagenetically altered are more thermostable than the original plant compounds (*50, 55, 59*). It has been shown that low temperature peaks decrease and high temperature peaks increase upon humification (*51, 53, 54*). This increased thermostability corresponds with recalcitrance against microbial degradation and extraction (*50, 51, 57*).

Evaluating Figure 1 in view of the literature information above, it may be concluded that Kralingen and Overschie contained a certain amount of coal- or soot-like material, which decomposed exothermally at very high temperatures. SOM in Assendelft and Lemster Rien was likely to be less humified than SOM in Petroleum Harbor, Kralingen, and Overschie. This is

supported by visual inspection of the samples, revealing the presence of poorly decomposed organic material in Assendelft and Lemster Rien samples. Changes in the TGA patterns after oxidation lead to the following indications on the compositional characteristics of the amorphous and condensed SOM domains: (*i*) Fatty acids, peptides, and carbohydrates (monosaccharides, cellulose, hemicellulose) are likely to be associated with amorphous SOM domains. This follows from the decrease of peak 1 upon oxidation. (*ii*) Humified plant compounds, lignin, and wood may be part of the condensed domain. This follows from the recalcitrance of peaks 2 and 3. (*iii*) Coal and soot are likely to be part of the condensed domain. This follows from the fact that the decrease of peak 4 was caused by the decomposition of carbonate and not by the decomposition of organic material (DTA, data not shown). All in all, condensed SOM appears to be more thermostable than amorphous SOM.

6.4.2 Pyrolysis-GC/MS. The results of the pyrolysis-GC/MS analysis are presented in Figure 2. In this figure Kralingen and Lemster Rien were not included as these samples were observed to be highly similar to Overschie and Assendelft, respectively. This similarity was also observed during TGA (Figure 1).

Figure 2 demonstrates that the pyrolysate of Assendelft (untreated) was dominated by the homologous series of *n*-alkenes/*n*-alkanes (▼, C_9-C_{32}). These straight-chain alkenes/alkanes are considered to be pyrolysis products of non-hydrolyzable aliphatic macromolecules such as cutan, suberan, and microbial polymers. *n*-Alkenes/*n*-alkanes are common in the pyrolysates of plant tissues and soil samples (*60-63*). The presence of non-hydrolyzable aliphatic macomolecules was confirmed by pyrolysis of the methylated samples (data not shown). Methyl esters and/or methyl esters of fatty acids were observed to be present in the pyrolysates of the methylated samples, which demonstrates the presence of suberin-derived moieties (C_{16}-C_{32} mono-, di-, and trihydroxy fatty acids and alkanols, predominant chain length C_{22}, C_{24}, and C_{26}) (*64*). Besides the alkenes/alkanes, also phenol, (2,3,4-)methylphenols, C_2-phenols, 4-vinylphenol (■), guaiacols (2-methoxyphenols, (○), and pristane (Pr) could be distinguished in the pyrolysate of Assendelft sediment. Of these products guaiacol can be regarded as a clear indicator of the presence of lignin (*65*). The other phenols may origin from lignin, but also from polyphenols or proteins (*66, 67*). The pyrolysate contained hardly any polysaccharide-derived products.

The oxidized Assendelft sample differed from the original sample. After oxidation the relative intensity of all aromatic and phenolic compounds decreased. Moreover, the relative abundance of the short-chain aliphatic products decreased in comparison with the abundance of the long-chain aliphatic products. The longer alkanes, especially C_{29} and C_{31}, were relatively well preserved. In addition, lignin became more oxidized. This was observed as an increase of 4-acetylguaiacol (not annotated) in comparison to non-oxidized isoeugenol. Overall, the guaiacols showed only a small decrease in intensity.

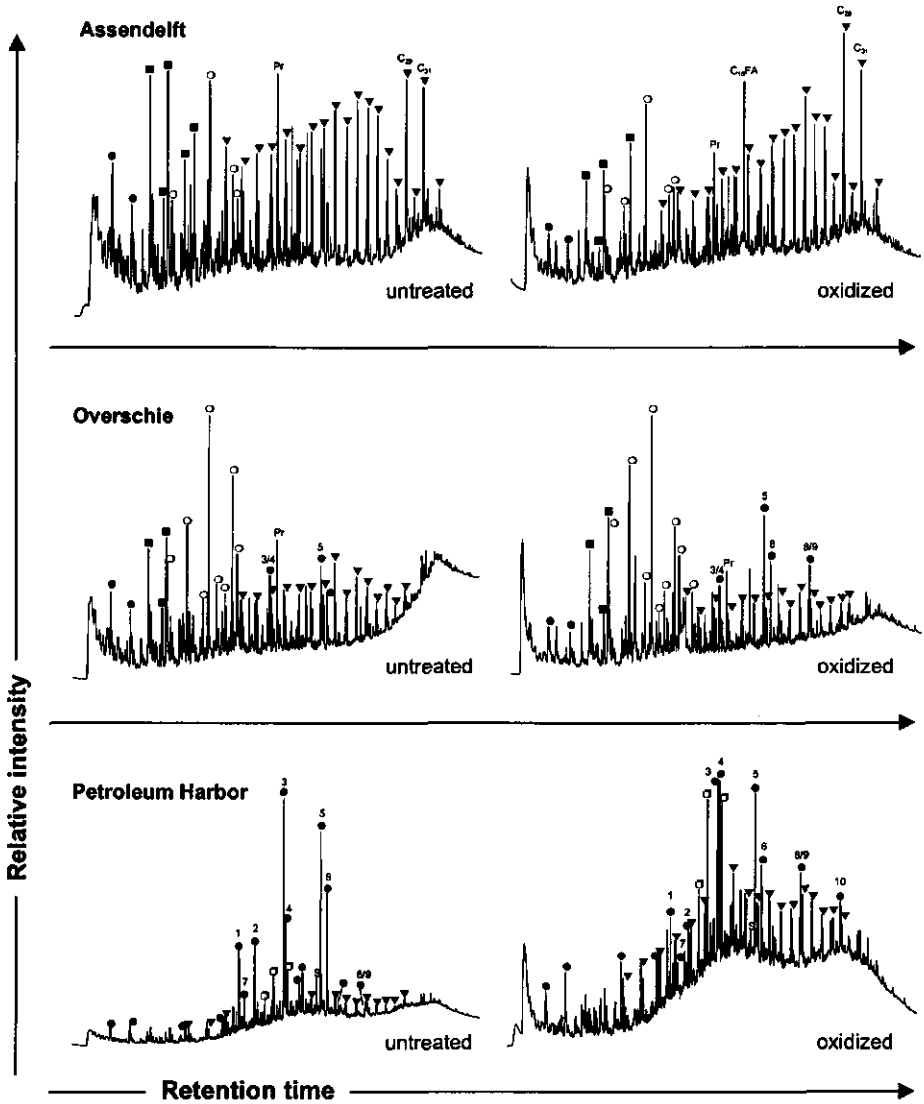

Figure 2. Pyrolysis-GC traces of untreated and oxidized samples. ○: (alkyl-)guaiacol or (alkyl-)syringol, ▼: n-alkene/n-alkane (doublet), ■: (alkyl-)phenol, ❑: branched alkane, S: sulfur, Pr: pristane, $C_{16}FA$: hexadecanoic acid, ●: (poly-)aromatic hydrocarbons, 1: acenaphthene, 2: fluorene, 3: anthracene, 4: phenanthrene, 5: pyrene, 6: fluoranthene, 7: dibenzofuran, 8: benz[*a*]anthracene, 9: chrysene, 10: benzo[*a*]pyrene.

The pyrolysate of Overschie had clear signals derived from toluene, styrene (●), phenol, methylphenol (■), guaiacols (○), pristane (Pr), several PAHs (●), and the homologous series of *n*-alkenes/*n*-alkanes (▼, C_9-C_{31}). In general, the composition of the oxidized sample was very similar to the composition of the untreated sample. The minor differences included a relative increase of guaiacol in comparison with (alkyl)phenols and a relative increase of benz[*a*]anthracene, chrysene, and benzo[*a*]pyrene. Also, the lignin-derived fraction became more oxidized.

The pyrolysate of Petroleum Harbor was dominated by polyaromatic compounds (●). The most distinct were acenaphthene, fluorene, anthracene, phenanthrene, pyrene, fluoranthene, and dibenzofuran. Other clear signals were derived from toluene, styrene, naphthalene, methylnaphthalene, benz[*a*]anthracene, chrysene, benzo[*a*]pyrene (●), sulfur (S), a few branched alkanes (❑), and a series of *n*-alkenes/*n*-alkanes (▼). Of these components the branched alkanes were likely to be derived from mineral oil, whereas the alkenes/alkanes were thought to be pyrolysis products of aliphatic biopolymers (confirmed by GC/MS analysis of extracted oil). It should be noted that most PAHs were evaporation products rather than pyrolysis products (*68*). They were not an integral part of the organic matter structure but an adsorbed contamination of the sample (Table 1).

The pyrolysate of the oxidized Petroleum Harbor sample showed a relative decrease of aromatic compounds and sulfur. The smaller aromatic compounds (toluene, styrene, naphthalene, methylnaphthalene), but also the larger ones (benz[*a*]anthracene, chrysene, benzo[*a*]pyrene) were less affected by oxidation than acenaphthene, dibenzofuran, fluorene, anthracene, phenanthrene, pyrene, and fluoranthene. The preservation of the larger aromatic compounds can be explained by their relatively strong sorption to SOM in comparison with the smaller aromatic compounds (*20*).

In summary, persulfate oxidation led to a reduction of aromatics in all samples, it affected lignin without really reducing its total amount, and it affected short-chain aliphatics in samples with relatively fresh organic material (Assendelft, Lemster Rien). Long chain aliphatics were well-preserved. In samples with more weathered organic material (Overschie, Kralingen) all *n*-alkenes/*n*-alkanes were well-preserved. Our findings correspond partly with the results of Saiz-Jimenez (*69, 70*), who, upon persulfate oxidation, observed a disappearance of proteins and polysaccharides, recalcitrance of the homologous series of aliphatic hydrocarbons, and a drastic reduction of lignin. Clearly, the major difference with our results is the removal of lignin. This removal of lignin may be explained by the extreme conditions applied by Saiz-Jimenez (140 °C, 2 h) in comparison with the conditions applied in this study (70 °C, 3 h).

For the further interpretation of the results, it should be noted that the pyrolysates in Figure 2 represent only a fraction of the SOM in the samples. Therefore, the results can not be used to obtain quantitative information on the structure of SOM as a whole and they do not allow a direct comparison with the results of TGA and NMR. Nevertheless, the changes in the pyrolysates upon persulfate oxidation provide new information on the composition of the amorphous and condensed SOM domains. It can be concluded that recalcitrant aliphatic

biopolymers are likely to be a part of the condensed SOM domain. These biopolymers can origin from plant and microbial biomass and are very stable in weathered organic material. For lignin it is not clear whether it is part of the amorphous or condensed domain. Although lignin was hardly removed by persulfate oxidation, it became progressively oxidized. Based on this and on the results of others (*69, 70*) the contribution of lignin to condensed SOM domains may be questioned.

6.4.3 CPMAS ^{13}C-NMR. The results of NMR analysis are presented in Figure 3 and Table 2. Figure 3 shows the basic NMR spectra (fingerprints) which were uncorrected for signal decay during measurement. In this figure the spectra of Kralingen and Lemster Rien were not included as they were highly similar to the spectra of Overschie and Assendelft, respectively. Table 2 gives the relative contribution of the chemical shift regions to the total NMR signal. Data in Table 2 were corrected for signal decay.

Table 2. Contribution of chemical shift regions to the total NMR signal. Data corrected for signal decay during the time of contact (1 ms).

Chemical shift range (ppm)[a]	**Relative contribution to NMR signal (%)**[b]									
	Assendelft		Kralingen		Lemster Rien		Overschie		Petroleum H.	
	Orig.	*Ox.*	*Orig.*	*Ox.*	*Orig.*	*Ox.*	*Orig.*	*Ox.*	*Orig.*	*Ox.*
0-50: alkyl C	32	37	26	27	28	38	21	20	34	39
50-110: O-alkyl C	36	32	29	25	31	27	30	28	26	21
110-160: aromatic C	18	18	27	30	22	19	32	36	25	25
160-220: carboxyl C	14	13	18	18	20	16	17	17	15	15

[a] 0-50 ppm includes terminal methyl groups and methylene groups in saturated rings and chains; 50-110 ppm includes methoxyl groups, carbohydrate C, C-N of amino acid, higher alcohols, C-6 of syringyl units of lignin; 110-160 ppm includes aromatic C-H, C-C, COR, and CNR (R: non-specified structure), guaiacyl C-2, lignin C-6, olefinic C; 160-220 ppm includes carboxyl C, carbonyl C, and amide C. [b] Orig.: original (non-oxidized) sample; Ox.: sample oxidized with persulfate.

The spectra in Figure 3 exhibit a range of peaks and shoulders which are usually derived from aliphatic C (30, 33 ppm), methoxyl and amino acid C (55 ppm), O-alkyl C (62, 72, 105 ppm), aromatic C (130 ppm), phenolic C (150 ppm), carboxyl and amide C (173 ppm), and carbonyl C (195 ppm) (*71, 72*). In Figure 3 the assignment of the peaks to the different carbon forms is slightly complicated by the occurrence of spinning side bands. Spinning side bands are secondary peaks that occur on both sides of the mother peak and contain part of the signal intensity. In our spectra, spinning side bands of aromatic C were of particular importance. They were likely to be responsible for the peaks at 195 ppm (carboxyl region) and 55 ppm (O-alkyl region). These spinning side bands have to be taken into account when interpreting the spectra.

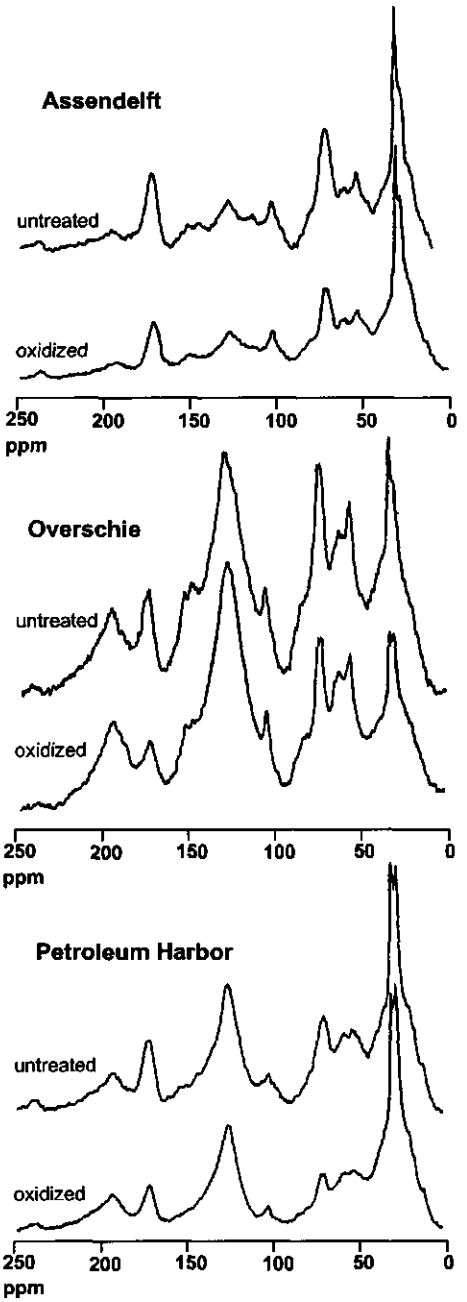

Figure 3. CPMAS ^{13}C-NMR spectra of untreated and oxidized samples.

Comparison of the spectra of the untreated samples shows that Assendelft and Lemster Rien differed dramatically from Kralingen and Overschie. The spectra of Assendelft and Lemster Rien were dominated by signals derived from aliphatic C, O-alkyl C, and carboxyl C, while Kralingen and Overschie samples also exhibited relatively high signals derived from aromatic C. The relatively high amount of aromatic C in Kralingen and Overschie was accompanied with a relatively low amount of aliphatic C. The spectra of Petroleum Harbor showed properties of the two extremes.

Persulfate oxidation lead to changes in the NMR spectra. In the O-alkyl region the 72 ppm peak decreased compared to the 55 ppm peak and the 62 ppm peak. In the carboxyl region the carboxyl (173 ppm) peak decreased compared to the carbonyl peak (195 ppm). Table 2 shows that the amount of O-alkyl C decreased in all samples, whereas the amount of alkyl C increased in all samples except Overschie. The amount of aromatic C decreased in Lemster Rien, increased in Kralingen and Overschie, and remained constant in Assendelft and Petroleum Harbor. The amount of carboxyl C decreased in Assendelft and Lemster Rien and remained constant in all other samples. Discussing the data in Table 2 it should be kept in mind that the peaks at 55 ppm and 195 ppm were derived from aromatic C. Therefore, part of the signal intensity in the O-alkyl and carboxyl regions has to be attributed to aromatic C. Correcting the data by addition of the intensity of the spinning side bands to the intensity of the mother signal of aromatic C (110-160 ppm) an attempt was made to obtain a more realistic indication of the distribution of carbon over its chemical forms. Correction was performed for Overschie and Petroleum Harbor and the results are presented in Table 3.

Table 3. Contribution of chemical shift regions to the total NMR signal. Data corrected for spinning side bands of aromatic C.

Chemical shift range (ppm)[a]	Relative contribution to NMR signal (%)			
	Overschie		Petroleum H.	
	Orig.[b]	*Ox.*[b]	*Orig.*	*Ox.*
0-50: alkyl C	21	20	34	39
50-110: O-alkyl C	19	15	18	12
110-160: aromatic C	54	62	41	43
160-220: carboxyl C	6	4	7	6

[a]0-50 ppm includes terminal methyl groups and methylene groups in saturated rings and chains; 50-110 ppm includes methoxyl groups, carbohydrate C, C-N of amino acid, higher alcohols, C-6 of syringyl units of lignin; 110-160 ppm includes aromatic C-H, C-C, COR, and CNR (R: non-specified structure), guaiacyl C-2, lignin C-6, olefinic C; 160-220 ppm includes carboxyl C, carbonyl C, and amide C. [b]Orig.: original (non-oxidized) sample; Ox.: sample oxidized with persulfate.

Combining all information in Figure 3, Table 2, and Table 3, two general characteristics of the amorphous and condensed SOM domains may be formulated: (*i*) condensed domains contain less O-alkyl C than amorphous domains, and (*ii*) condensed domains contain less carboxyl C than amorphous domains. These characteristics imply that condensed domains are less polar than amorphous domains (polarity expressed as the sum of O-alkyl and carboxyl C). Apart from

the general characteristics, the composition of the amorphous and condensed domains appears to be sample specific. Condensed domains in samples with relatively undecomposed SOM (Assendelft, Lemster Rien) were enriched in aliphatic C. Condensed domains in samples with relatively weathered and partly coal like organic matter (Kralingen, Overschie) were enriched in aromatic C.

All in all, the compositional changes upon persulfate oxidation, as measured by CPMAS ^{13}C-NMR, were in accordance with compositional changes upon humification. Humification generally leads to a decrease of O-alkyl C that is attended with an increase of aliphatic C and an increase/decrease of aromatic C, depending on the origin and age of the organic matter (*73, 74*).

6.4.4 Amorphous and Condensed Organic Matter Domains. The results of TGA, pyrolysis-GC/MS, and CPMAS ^{13}C-NMR revealed that some general characteristics of the amorphous and condensed SOM domains can be derived from changes in the SOM composition upon persulfate oxidation. Apart from these general characteristics, the composition of the domains appeared to be sample specific.

In general, it may be concluded that condensed SOM domains are more thermostable than amorphous domains. They are relatively low in O-alkyl and carboxyl C and they are likely to contain only small amounts of labile components like carbohydrates, peptides, and fatty acids. As a result, condensed SOM domains are less polar than amorphous domains. This low polarity is in accordance with several investigations which reported a negative correlation between SOM polarity and the affinity of SOM for HOCs (K_{oc}) (*23-31*).

Condensed SOM was enriched in aromatic C in some samples, but not enriched in aromatic C in others. This observation is in accordance with the results of Kile *et al.* (*31*) who found a poor correlation between aromaticity and the affinity of SOM for HOCs. This poor correlation indicates that there is a poor correlation between aromaticity and the degree of SOM condensation. The above seems to be in conflict with the excellent correlation between aromaticity and K_{oc} values reported for sorption of HOCs to humic acids, fulvic acids, and dissolved organic matter (*23, 26, 32-35*). However, it should be noted that humic and fulvic acids are operationally defined SOM fractions which are not necessarily similar to SOM as a whole. In general, humic and fulvic acids are less aliphatic and more aromatic than bulk samples (*75, 76*).

For the individual samples, major differences were observed between Assendelft and Lemster Rien on the one hand and Kralingen and Overschie on the other. Experiments demonstrated that the relatively undecomposed SOM in Assendelft and Lemster Rien was highly aliphatic, relatively susceptible to persulfate oxidation, and less thermostable than SOM in other samples. Condensed domains in the undecomposed SOM were enriched in aliphatic C, which originated from aliphatic biopolymers. This finding is in accordance with the recalcitrance of aliphats reported by others (*69, 70, 77*) and with the high aliphatic content that can be observed in humin fractions (*77, 78*).

The relatively weathered SOM in Kralingen and Overschie was more aromatic than the organic matter in Assendelft and Lemster Rien. It was relatively thermostable, resistant to persulfate oxidation, and it contained coal-like material which decomposed at very high temperatures. Condensed domains in the weathered SOM were enriched in aromatic C, which may have originated from (weathered) lignin and coal-like material.
Altogether, the compositional changes of SOM upon persulfate oxidation appeared to be similar to the compositional changes upon humification, which supports the conclusion that humified SOM is more condensed than the original material (*3, 27, 29, 30*).

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CHAPTER 7

Amorphous and Condensed Soil/Sediment Organic Matter Domains: 2. The Effect of Extraction, Hydrolysis, and Oxidation on PAH Concentrations and PAH Bioavailability

Chiel Cuypers, Jasperien de Weert, Tim Grotenhuis, Wim Rulkens

Abstract

In a recent conceptual model soil/sediment organic matter (SOM) has been described as a polymeric material consisting of amorphous and condensed domains. It has been suggested that readily bioavailable PAHs are sorbed in amorphous domains, while poorly bioavailable PAHs are sorbed in condensed domains. In the present study, the composition of the amorphous and condensed domains was investigated for 2 sediment samples. The samples were split in two parts, one part was bioremediated, and both the bioremediated and the non-bioremediated parts were subjected to 9 different chemical treatments (extraction, hydrolysis, oxidation). After chemical treatment, PAH concentrations were measured and the bioavailability of the residual PAHs was determined using solid-phase extraction. To obtain information on the composition of the amorphous and condensed domains, experimental results on the effect of the chemical treatments on PAH concentrations and PAH bioavailability were combined with literature information on the effect of the chemical treatments on the SOM structure. It appeared that condensed SOM domains are primarily situated in the humin fraction of SOM. This indicates that they have a relatively high C content, a relatively low O content, a relatively high degree of condensation, and a relatively low acidity. Accordingly, they are likely to be less polar than amorphous domains. Carbohydrates, proteins, fatty acids, free alkanes, fulvic acids, and humic acids are likely to be associated with the amorphous SOM domains. Apart from these general characteristics, the composition of the SOM domains appeared to be highly sample specific.

7.1 Introduction

In PAH contaminated soils and sediments, organic matter is the principal sorbent of PAHs if it is present above trace levels (organic carbon content > 0.02-0.1 %) (*5, 6*). Soil/sediment organic matter (SOM) has been described as the whole of the organic material in soils and sediments, including litter, microbial biomass, water-soluble organics, stabilized organic matter (humus), and plant residues in varying stages of decomposition (*1*). Except in the litter zone, the bulk of SOM is believed to consist of humic substances that bear little physical and chemical resemblance to their precursor biopolymers (*1, 2*).

To be able to study the structure and properties of SOM a lot of effort has been put into the development of wet-chemical methods for the extraction and degradation of SOM (*1, 3, 4*). The general purpose of these methods is either to separate organic matter from inorganic material or to degrade organic matter into structural sub-units. Separation of organic matter is normally applied as a first step in the investigation of SOM properties. Alkali (0.1-0.5 N NaOH) is a popular extractant, although milder extractants like sodium pyrophosphate, organic complexing agents, dilute acids, and organic solvents have been used also (*1*). Degradation of SOM by hydrolysis, oxidation, or reduction has been applied to characterize the chemical structure of humic substances. The primary objective of the degradation methods is to produce simple components, representative of the main structural units in the humic macromolecule. Ideally, the products obtained would provide information allowing the reconstruction of the chemical structure of SOM (*1*).

For the interpretation of sorption data, a conceptual model has been put forward in which SOM is represented as a three-dimensional entanglement of macromolecules which consists of highly amorphous (soft, rubbery) and relatively condensed (hard, glassy) domains (*2, 7-11*). It has been hypothesized (and verified) that sorption of hydrophobic organic contaminants in amorphous domains is linear, fast, and completely reversible, whereas sorption in condensed domains is non-linear, slow, and hysteretic (*2, 8, 10, 11*). In accordance, it may be reasoned that PAHs sorbed in amorphous organic matter are readily bioavailable, whereas PAHs sorbed in condensed organic matter are poorly bioavailable. Here, bioavailability is defined as the ability to desorb from the solid phase into the aqueous phase, within the time-frame of an experiment. Evidence for the existence of amorphous and condensed SOM domains has been published recently (*12-17*), but little information has been reported on the chemical composition of SOM domains, so far.

The aim of the present study was to elucidate some chemical characteristics of the SOM domains. To this purpose we investigated the effect of nine different wet-chemical extraction, hydrolysis, and oxidation methods on the concentration and bioavailability of PAHs in two sediments. The experimental data were combined with literature information on the effect of the wet-chemical methods on the SOM structure. The methods applied have been extensively described in soil science literature and are known to extract/degrade specific SOM structures. Using this literature information in combination with the experimental data, information was obtained on the structure of amorphous and condensed SOM domains. If wet-chemical treatment

affected poorly bioavailable PAHs this gave information on the structure of the condensed SOM domains. If it affected only readily bioavailable PAHs this gave information on the structure of the amorphous domains.

To be able to distinguish between readily and poorly bioavailable PAHs the sediment samples were split in two parts: one part was subjected to wet-chemical treatment directly, the other part was subjected to wet-chemical treatment after removal of bioavailable PAHs by bioremediation. Samples, both original and bioremediated, were subjected to the following wet-chemical treatments: extraction with 0.1 N HCl, 0.025 M H_2SO_4, 0.1 N NaOH, and 0.5 N NaOH; hydrolysis with hot water, 6 N HCl, and 5 N NaOH; oxidation with 2 N HNO_3 and 4 % $KMnO_4$. After treatment, PAH concentrations were measured in all samples; the bioavailability of PAHs was measured in the bioremediated samples, using a solid-phase extraction technique which removes the bioavailable PAHs with Tenax-TA. Solid-phase extraction was able to demonstrate whether PAHs that were poorly bioavailable in the bioremediated samples had become more readily bioavailable after chemical treatment. A schematic outline of the experiments is presented in Figure 1.

This study was intended as a first screening of the wet-chemical methods. It was chosen not to investigate methods that involved extraction with organic solvents.

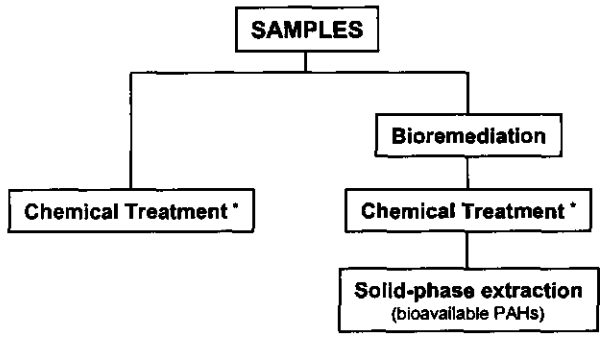

Figure 1. *Schematic outline of the experiments; residual PAH concentrations were measured after each treatment step in the diagram.* *Chemical treatment refers to extraction with 0.1 N HCl, 0.025 M H_2SO_4, 0.1 N NaOH, and 0.5 N NaOH; hydrolysis with hot water, 6 N HCl, and 5 N NaOH; oxidation with 2 N HNO_3 and 4 % $KMnO_4$.

7.2 Background

In this section, the effect of the different wet-chemical treatments on the composition of SOM is discussed. Moreover, the effect of wet-chemical treatment on the sorption and bioavailability of hydrophobic organic contaminants (HOCs) is addressed. Literature information on this second topic is largely limited to data on the sorption of spiked HOCs to isolated organic matter fractions (humic and fulvic acids) and data on the sorption of HOCs to chemically treated (extracted, hydrolyzed, oxidized) soil and sediment samples.

7.2.1 Acid and Alkaline Extraction. We studied the extraction of samples with alkaline solutions (0.1 and 0.5 N NaOH) and acidic solutions (0.1 N HCl and 0.025 M H_2SO_4).

NaOH extraction has been widely used as a method for the recovery of organic matter from soils and sediments. The solubility of humic substances in alkali is believed to be caused by the disruption of bonds holding organic material to inorganic soil components and by the conversion of acidic components to their soluble salt forms (*1*). Effectively, alkaline extraction provokes the dissociation of acid groups with solubilization of small organic molecules. At the same time, an increase of the surface charge of the organic colloids modifies the macromolecular structure in such a way that colloids become distended with an opening of internal voids (*18*). It should be noted that under alkaline conditions autoxidation of organic constituents may occur in contact with air. Besides, other chemical changes may occur in alkaline solution, including condensation between amino acids and the C=O groups of reducing sugars or quinones (*1*). Schnitzer and Skinner (*19*) stated that dilute solutions of alkalis may hydrolyze polysaccharides and nitrogenous compounds. As a general rule, extraction with 0.1-0.5 N NaOH leads to recovery of approximately two-thirds of the soil organic matter (*1*).

The organic matter that is extracted with alkali is operationally defined as the humic and fulvic acid fraction; the residue is generally referred to as the humin fraction (*1*). This humin fraction contains macromolecules with a high molecular weight, a high C content, a low O content, and a low acidity in comparison with the humic and fulvic acid fraction. The humin fraction consists of (*i*) humic acids intimately bound to mineral matter, (*ii*) highly condensed (humified) material with a high C content, (*iii*) fungal melanins, or (*iv*) parafinic substances (*1*). The humin fraction can be divided into three subfractions by the so-called MIBK fractionation method: a bound humic acid fraction, a bound lipid fraction, and an inorganic residue (*20, 21*). Hydrophobic organic contaminants tend to sorb stronger to the humin than to humic and fulvic acid fraction, which is reflected in their organic carbon-water distribution coefficients (K_{oc}) (*2, 22-25*). In accordance, it has been demonstrated by Schlebaum (*61*) that desorption of HOCs (pentachlorobenzene) from humin is much slower than desorption from humic acid. Furthermore, humin has been observed to be an important sink for organic contaminants (*18, 26, 27*). Xie *et al.* (*21*) showed that the bound residues of pesticides were mainly associated with the bound-humic acid and the lipid fraction in the humin samples they investigated. The actual distribution among the three humin sub-fractions appeared to be related to the nature of the contaminant.

In comparison with NaOH extraction, extraction with mineral acids has received little attention. Stevenson (*1*) stated that very little organic matter can be extracted from soil with dilute mineral acids, except for acid mixtures containing HF. Nonetheless, Schnitzer and Skinner (*19*) observed that chemical characteristics of a 0.1 N HCl extract were practically identical to the characteristics of a 0.5 N NaOH extract. Beyer and coworkers (*28-30*) applied extraction with 0.025 M H_2SO_4 within the framework of a sequential extraction procedure. They reported that H_2SO_4 extracted a so-called mobile fulvic acid fraction, consisting of sugar and starch. According to Schnitzer and Khan (*3*), treatment with dilute mineral acids dissolves metals,

hydrous oxides, and hydrated silicate minerals associated with humic substances, leading to the dissolution of fulvic acids. Extraction with dilute mineral acids has been applied as a pretreatment before the extraction of humic materials with NaOH. This pretreatment is thought to lead to the desorption of humic substances fixed on sesquioxides, free silicic acid, and clay minerals, and to the removal of carbonates and exchangeable bases (*4*).

7.2.2 Hydrolysis. Hydrolytic procedures are effective in removing proteins, carbohydrates, and other aliphatic constituents from crude humic preparations. They have been used primarily to "purify" humic acids prior to chemical and physical characterization. The residue from hydrolysis is sometimes termed the backbone or core of the humic macromolecule (*1*). We studied three types of hydrolytic procedures: hydrolysis with hot water, acid hydrolysis (6 N HCl), and alkaline hydrolysis (5 N NaOH). It should be noted that the HCl and NaOH solutions used for hydrolysis are more concentrated than the solutions used for acid and alkaline extraction.

Hydrolysis with hot water has been studied by several researchers (*31-34*). It has been reported to extract polysaccharides, polypeptides, and small amounts of relatively simple phenolic acids and aldehydes (*32-33*). Furthermore, hot water has been observed to set free alkanes and fatty acids, which were adsorbed on humic materials (*33*). Some ether soluble materials, like p-hydroxybenzoic acid, protocatchuic acid, vanillic acid, and vanilin can also be extracted (*3*). In general, hydrolysis with hot water is less drastic than acid hydrolysis and extracts less amino acids and phenols (*3*). Shin *et al.* (*31*) observed that hot water extraction resulted in a slight increase of the K_{oc} values of DDT in a muck soil and a sandy loam; extraction of a clayey soil decreased K_{oc} values.

Acid hydrolysis has been extensively studied and is by far the most popular hydrolysis method (*1, 18, 32, 34-42*). Acid hydrolysis allows the rupture of heteroatomic bonds (ester, ether, amide) and gives an indication of the chemical stability of the humic compounds, of their structure, and of their degree of condensation. The hydrolyzable fraction corresponds with the most labile fraction, the non-hydrolyzable fraction represents the nucleus (*35*). Acid hydrolysis is believed to remove protein derivatives, carbohydrates, and adsorbed materials (*1, 36, 37*). Besides, it has been demonstrated to reduce the lignin content of humic materials (*37*). The solubilized products include proteins, peptides, amino acids, sugars, uronic acids, phenols, pigmented substances, and metals (*3, 36*). Acid hydrolysis can be used to quantify labile and recalcitrant pools of organic matter and thus provides a rapid method to estimate short-term C and N mineralization (*39, 42*). In accordance, acid hydrolysis has been found to reduce the susceptibility of organic material to mineralization (*40*). It may increase the affinity of organic matter for pesticides, leading to an increased K_{oc} value in hydrolyzed organic matter in comparison to the original material (*34*).

Alkaline hydrolysis has been applied by several groups (*1, 33, 40, 43*). It is known to cleave C-O bonds and was found to be relatively specific, although not very efficient, for the degradation of structural phenolic components (*33*). Alkaline hydrolysis liberates a variety of phenolic

substances, presumably without degrading the central core of the molecule. In addition, it removes adsorbed aliphatics and N-compounds, e.g. proteinaceous constituents (*1, 33*).

7.2.3 Oxidation. We investigated permanganate (4 % $KMnO_4$) and nitric acid (2 N HNO_3) oxidation. Permanganate oxidation of humic materials has been observed to produce small amounts of aliphatic mono-carboxylic and di-carboxylic acids, as well as benzenecarboxylic acids. The benzenecarboxylic acids originate from rings unsubstituted by oxygen, but repeatedly substituted by carbon atoms (*3*). Permanganate oxidation of methylated substances has been used to elucidate the main structural features of complex materials like coal, lignin, and wood. Permanganate oxidation of methylated SOM produces phenolic acids, benzenecarbolxylic acids, and aliphatic dicarboxylic acids (*44*). The benzenecarboxylic acids are generally thought to be formed from oxidation of aliphatic side chains, some of which might be involved in linking together aromatic units, and/or from the destruction of aromatic rings containing oxygen. The aliphatic carboxylic acids may arise from oxidation of straight-chain compounds or labile ring systems (*1*).

HNO_3 oxidation was studied by Hansen and Schnitzer (*45*). They applied HNO_3 oxidation to find identifiable products that could not be obtained by $KMnO_4$ oxidation. The resistant fraction after oxidation with HNO_3 or $KMnO_4$ can be related to high contents of compact polyaromatic rings, as was revealed in studies on the oxidation of coal (*46*).

7.3 Materials and Methods

7.3.1 Samples. The sediment samples used in this study were dredged from the 1st Petroleum Harbor in Amsterdam and from a waterway near Assendelft, The Netherlands.

Assendelft sediment (AD) had a dry matter (dm) content of 41 % (w/w), an organic matter content of 13.3 % (w/w), and a pH of 7.1. The dry matter consisted of 79 % sand, 11 % silt, and 10 % clay. The sediment contained 122 mg/kg PAHs (16 EPA).

Petroleum Harbor sediment (PH) had a dry matter content of 44 % (w/w), an organic matter content of 9.7 % (w/w), and a pH of 7.8. The dry matter had the following composition: sand 64 %, silt 15 %, and clay 21 %. The sediment was contaminated with PAHs (2036 mg/kg, 16 EPA) and mineral oil (13,600 mg/kg). Thermogravimetric analysis in Chapter 6 demonstrated that both AD and PH sediment hardly contained any high surface area carbonaceous material (soot/coal).

Dredged samples were homogenized, passed through a 2-mm sieve, and stored at 4 °C in the dark until use. A fraction of the samples was bioremediated to remove bioavailable PAHs (see Figure 1). To that aim, wet sediment (750 g dm) was mixed with 5 L of an aqueous mineral medium (0.3g/L NH_4NO_3, 0.1 g/L $MgSO_4 \cdot 7H_2O$, 0.1 g/L $CaCl_2 \cdot 2H_2O$, 40 mg/L KH_2PO_4, 160 mg/L K_2HPO_4, 5 mg/L $FeCl_3$) and transferred into 10 L containers at room temperature. The slurry was aerated and continuously stirred to keep particles in suspension. After 25 days of intensive treatment the slurry was transferred into 1 L Schott bottles (600 mL/L). The bottles were placed in a rotary tumbler at 30 °C and mixed at 50 rpm. The oxygen concentration in the

headspace was measured regularly and maintained at a level >10 % (vol.) by flushing with air. When the oxygen consumption became negligible (1-2 weeks) bioremediation was terminated. Slurries were centrifuged, decanted, and stored at 4 °C in the dark.

Biodegradation reduced the amount of 3-4 ring PAHs (target compounds in this study) from 70 to 51 mg/kg in AD sediment and from 1238 to 307 mg/kg in PH sediment (amounts represent the sum concentrations of 8 individual PAHs). It was assumed that the residual 3-4 ring PAHs were poorly bioavailable.

7.3.2 PAH Analysis. Samples were mixed with acetone and water up to an acetone/water ratio of 4:1 (v/v) and a solid/liquid ratio of 1:10 g/mL. The resulting slurry was sonicated for 15 min (Retsch UR 2) and shaken at room temperature for 1 h (150 rpm, Gerhardt Laboshaker). Extracts were centrifuged (5 min, 13,000g) and analyzed for PAHs by HPLC. It has been demonstrated that over 95% of a PAH contamination can be extracted from soils and sediments with the method described above (*47*). This was verified for AD and PH samples. The amount of PAHs (3-4 rings) removed by acetone extraction was similar to the amount of PAHs removed by microwave extraction (N-methyl-2-pyrrolidinone, 1 h, 130 °C) and soxhlet extraction (dichloromethane, 8 h).

PAHs (20µL of extract) were separated on a reverse-phase C18 column (Vydac 201TP54, 5µ) with external guard column (Vydac 102GD54T, 5µ) using a mixture of acetonitrile and water as an eluens. The separation was performed at a constant flow of 1 mL/min, varying the acetonitrile/water ratio between 1:1 and 99:1 (v/v) (0-5 min, 1:1; 5-20 min, linear increase from 1:1 to 99:1; 20-40 min, 99:1; 40-45 min, linear decrease from 99:1 to 1:1; 45-50 min, 1:1). PAHs were detected by UV absorbance at 254, 264, 287, 300, 305, and 335 nm (Gynkotek UVD 340S). We analyzed the following 3 and 4 ring PAHs: phenanthrene [PHE], anthracene [ANT], fluoranthene [FLT] (3 ring PAHs), pyrene [PYR], benz[*a*]anthracene [BaA], chrysene [CHR], benzo[*b*]fluoranthene [BbF], and benzo[*k*]fluoranthene [BkF] (4 ring PAHs).

7.3.3 Chemical Treatment. Samples (original and bioremediated) were subjected to the following wet-chemical treatments: extraction with 0.1 N HCl, 0.025 M H_2SO_4, 0.1 N NaOH, and 0.5 N NaOH; hydrolysis with hot water, 6 N HCl, and 5 N NaOH; oxidation with 2 N HNO_3 and 4 % $KMnO_4$. After chemical treatment, the solids were separated from the liquid phase and extracted with acetone (acetone/water) to determine the residual PAH concentrations. A part of the solids of the bioremediated samples was subjected to solid-phase extraction (with Tenax) to determine the bioavailability of the residual PAHs. Chemical treatment was carried out in duplicate for the original samples and in triplicate for the bioremediated samples.

0.1 N HCl extraction: Method adapted from Schnitzer and Skinner (*19*). Wet sediment (9 g dm) was weighed into a 250 mL Schott bottle which contained 80 ml of a 0.1 N HCl solution. The bottle was closed and shaken end-to-end for 18 h (225 mpm). After 18 h the slurry was centrifuged and solids were extracted with acetone and Tenax. The experiment was performed at room temperature.

0.025 M H_2SO_4 extraction: Method based on the 2nd step of a sequential extraction procedure proposed by Beyer (*29*). Wet sediment (16 g dm) was weighed into a 250 mL Schott bottle which contained a 0.025 M H_2SO_4 solution (AD: 96 mL, PH: 48 mL). The bottle was closed and placed in a rotary tumbler at 20 °C. After 6 h of mixing (50 rpm) the slurry was centrifuged and solids were extracted with acetone and Tenax.

0.1 N NaOH extraction: Method based on the 3rd step of a sequential extraction procedure proposed by Beyer (*29*). Sediment samples were first subjected to 0.025 M H_2SO_4 extraction as described above. After H_2SO_4 extraction, samples were washed with demineralized water (12.5 mL/g), centrifuged, and washed with demineralized water again. Washed samples (8 g dm) were weighed into 250 mL Schott bottles which contained a 0.1 N NaOH solution (AD: 48 mL, PH: 32 mL). Bottles were placed in a rotary tumbler at 20 °C and mixed for 24 h (50 rpm). After 24 h samples were centrifuged and the alkaline extraction was repeated. After the second alkaline extraction the solids were extracted with acetone and Tenax.

0.5 N NaOH extraction: Method adapted from Rice and MacCarthy (*20*). Wet sediment (7 g dm) and a 0.5 N NaOH solution (700 mL) were weighed into a 1 L Schott bottle. The headspace of the bottle was filled with N_2 gas. The bottle was closed and shaken end-to-end for 24 h (225 mpm). After 24 h samples were centrifuged and solids were extracted with acetone and Tenax.

Hydrolysis with hot water: Method adapted from Neyroud and Schnitzer (*33*). Wet sediment (3 g dm) and demineralized water (AD: 4.7 mL/g, PH: 5 mL/g) were weighed into a Teflon microwave tube. The headspace was filled with N_2 gas, the tube was closed, and it was heated for 3 h (170 °C) in a microwave oven (CEM, MDS-2100). After 3 h the tube was allowed to cool down for 30 min. Then, the slurry was centrifuged and solids were extracted with acetone and Tenax.

6 N HCl hydrolysis: Method adapted from Jahnel *et al.* (*41*). Wet sediment (8 g dm) and a 6 N HCl solution (AD: 656 mL, PH: 480 mL) were weighed into 1.5 L glass containers. The containers were covered with a glass lid (to prevent excessive evaporation but allowing the release of overpressure) and placed in a ventilated oven at 110 °C. After 15 h the slurry was allowed to cool down for 30 min. The slurry was centrifuged and solids were extracted with acetone and Tenax.

5 N NaOH hydrolysis: Method adapted from Jakab *et al.* (*43*). Wet sediment (4 g dm) and a 5 N NaOH solution (AD: 4.2 mL/g, PH: 3 mL/g) were weighed into a Teflon microwave tube. The headspace was filled with N_2 gas, the tube was closed, and it was heated for 3h (170 °C) in a microwave oven (CEM, MDS-2100). After 3 h the tube was taken from the microwave and allowed to cool down for 30 min. Then, the slurry was centrifuged and solids were extracted with acetone and Tenax.

2 N HNO_3 oxidation: Method adapted from Hansen and Schnitzer (*45*). Wet sediment (8 g dm) was weighed into a glass destruction tube. A 2 N HNO_3 solution (40 mL) was added slowly and the mixture was refluxed gently for 30 h. After 30 h the slurry was allowed to cool down for 30 min. Then, the slurry was centrifuged and solids were extracted with acetone and Tenax.

4 % $KMnO_4$ oxidation: Method adapted from Ortiz de Serra and Schnitzer (*44*). Wet sediment (4 g dm) and a 4 % $KMnO_4$ solution (AD: 140 mL, PH: 100 mL) were weighed into a glass destruction tube. The mixture was refluxed for 8 h, after which the slurry was centrifuged, solids were rinsed with demineralized water (80 mL), and the slurry was centrifuged again. The solids were extracted with acetone and Tenax.

7.3.4 Solid-Phase Extraction. Solid-phase extraction (SPE) was applied to remove readily bioavailable PAHs from the bioremediated samples which had been subjected to chemical treatment. Residual concentrations after SPE were a measure of the amount of PAHs that were poorly bioavailable. The SPE method was adapted from Bonten *et al.* (*48*) and Cornelissen *et al.* (49). Extractions were carried out in 50 mL separatory funnels, which were filled with wet sediment (2-3 g dm), 40 mL of a 0.01 M $CaCl_2$ solution, 20 mg $HgCl_2$, and 2 g of Tenax-TA adsorbent. The separatory funnels were shaken end-to-end at room temperature at such a speed that the slurry and Tenax beads were well mixed. The slurry was shaken for 72 h, during which the Tenax was refreshed 4 times to keep the aqueous phase free of PAHs. After 72 h the soil suspension was separated from the Tenax, the suspension was centrifuged, and the solids were extracted with acetone. In total, 9 g of Tenax-TA was used for extraction (3×2 g and 2×1.5 g). On a weight basis, this was 23-46 times the amount of organic matter in the sediment samples. Given the fact that the affinity of PAHs for Tenax is approximately similar to the affinity for organic carbon (*49*) this excess should be sufficient for the desorption of (practically) all readily bioavailable PAHs.

7.4 Results

The effect of chemical treatment on PAH concentrations and PAH bioavailability is graphically represented in Figures 2-5. We distinguish three possible effects:

(i) A decrease of PAH concentrations in non-bioremediated samples. Such a decrease is observed as a difference between bars 1 and 2 (bar 2 < bar 1). PAHs involved are expected to be readily bioavailable, primarily.

(ii) A decrease of PAH concentrations in bioremediated samples. Such a decrease is observed as a difference between bars 3 and 4 (bar 4 < bar 3). A decrease indicates that chemical treatment affected the poorly bioavailable PAHs.

(iii) An increase of PAH bioavailability. This is observed as a difference between bars 3 and 5 (bar 5 < bar 3). If bar 5 is lower than bar 3 it indicates that chemical treatment changed the structure of the organic matter in such a way that a fraction of the poorly bioavailable PAHs became more readily bioavailable. This fraction could be removed by SPE with Tenax.

7.4.1 Acid Extraction. The results of acid extraction are presented in Figure 2. This figure shows that the effect of acid extraction on PAH concentrations and PAH bioavailability was similar in the experiments with 0.1 N HCl and 0.025 M H_2SO_4. Both extraction methods were capable of removing a certain amount of PAHs from the non-bioremediated AD and PH samples (bar 2 < bar 1). PAH concentrations in the bioremediated samples were not affected (bar 3 ≈ bar 4). To determine the effect of acid extraction on PAH bioavailability, residual PAH concentrations after SPE were compared with residual concentrations after biodegradation. Comparison shows that acid extraction slightly increased PAH bioavailability in AD samples (bar 5 < bar 3), whereas bioavailability in PH samples was unaffected (bar 5 ≈ bar 3).

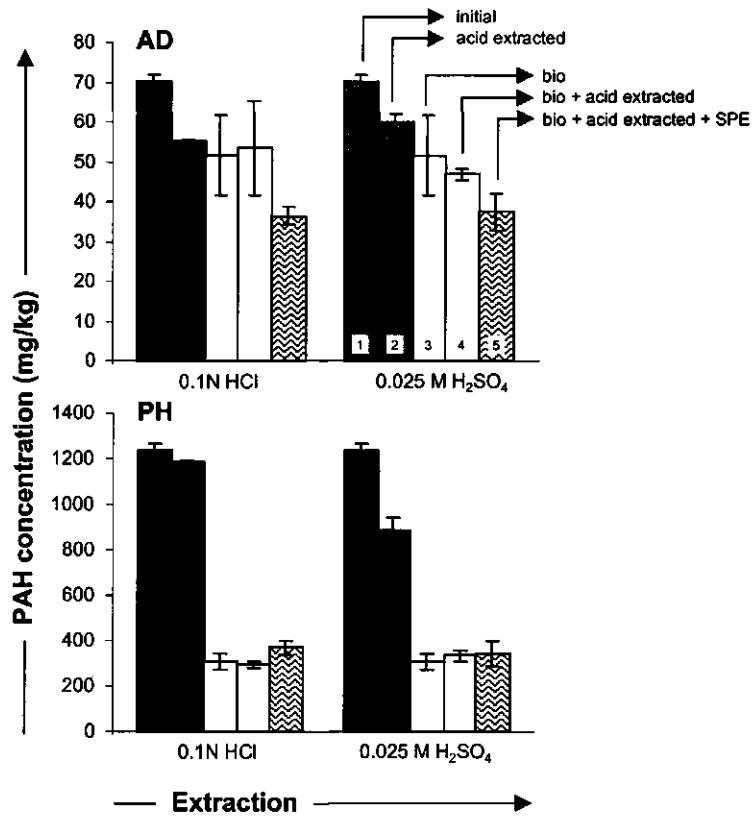

Figure 2. PAH concentrations in AD and PH sediment, before and after extraction with 0.1 N HCl and 0.025 M H_2SO_4. Bars represent PAH concentrations in non-bioremediated samples (*initial*), non-bioremediated samples after acid extraction (*acid extracted*), bioremediated samples (*bio*), bioremediated samples after acid extraction (*bio + acid extracted*), and bioremediated samples after successive acid and solid-phase extraction (*bio + acid extracted + SPE*). Error bars represent standard deviations, except for "*acid extracted*" samples (non-bioremediated) where they represent duplicate measurements.

7.4.2 Alkaline Extraction. Figure 3 shows that the effect of alkaline extraction on PAH concentrations and PAH bioavailability was almost similar in the experiments with 0.1 N and 0.5 N NaOH. Alkaline extraction lead to the removal of a certain amount of PAHs in the non-bioremediated samples, while PAHs in the bioremediated samples were unaffected by alkaline extraction. Alkaline extraction of AD sediment enhanced PAH bioavailability, while PAH bioavailability in PH sediment was not affected. It should be noted that the results of alkaline extraction in Figure 3 are rather similar to the results of acid extraction in Figure 2, although the effect of alkaline extraction on PAH concentrations and PAH bioavailability was somewhat more pronounced.

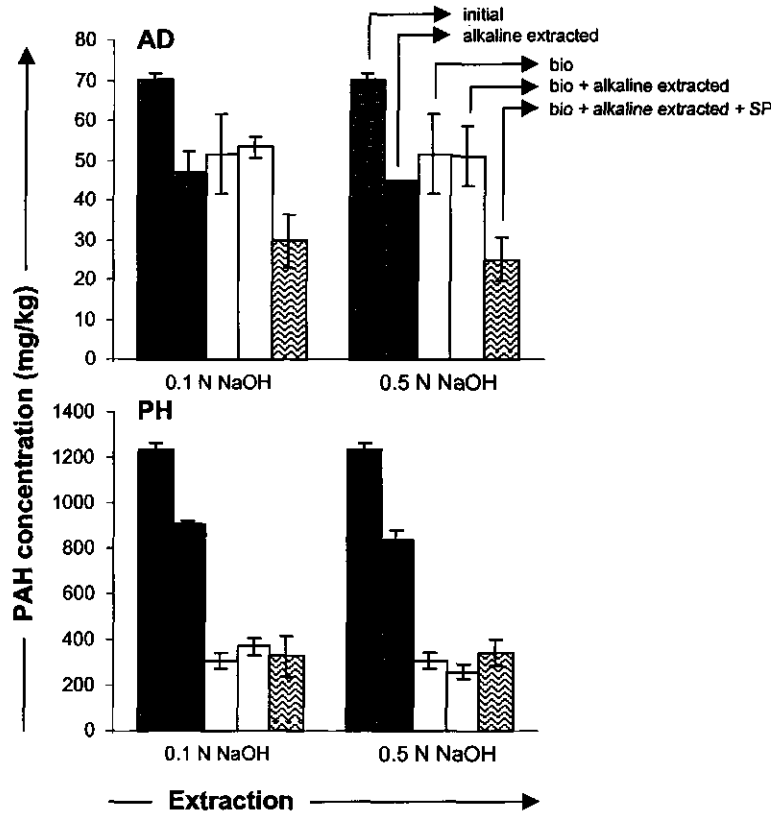

Figure 3. PAH concentrations in AD and PH sediment, before and after extraction with 0.1 N NaOH and 0.5 N NaOH. Bars represent PAH concentrations in non-bioremediated samples (*initial*), non-bioremediated samples after alkaline extraction (*alkaline extracted*), bioremediated samples (*bio*), bioremediated samples after alkaline extraction (*bio + alkaline extracted*), and bioremediated samples after successive alkaline and solid-phase extraction (*bio + alkaline extracted + SPE*). Error bars represent standard deviations, except for "*alkaline extracted*" samples (non-bioremediated) where they represent duplicate measurements.

7.4.3 Hydrolysis. The results of the hydrolysis experiments are presented in Figure 4. This figure shows that PAH concentrations in non-bioremediated samples were significantly reduced by hydrolysis, except in the hot water hydrolysis of AD sediment. Clearly, the reduction of PAH concentrations was strongest in the experiments with 5 N NaOH. This alkaline hydrolysis also reduced PAH concentrations in bioremediated samples, while hot water hydrolysis and acid hydrolysis were unable to reduce PAH concentrations in bioremediated samples. Bioavailability of PAHs was seriously enhanced in all hydrolysis experiments. The bioavailability enhancement was highest in the experiments with hot water and 5 N NaOH, which were both performed at 170 °C (acid hydrolysis: 110 °C).

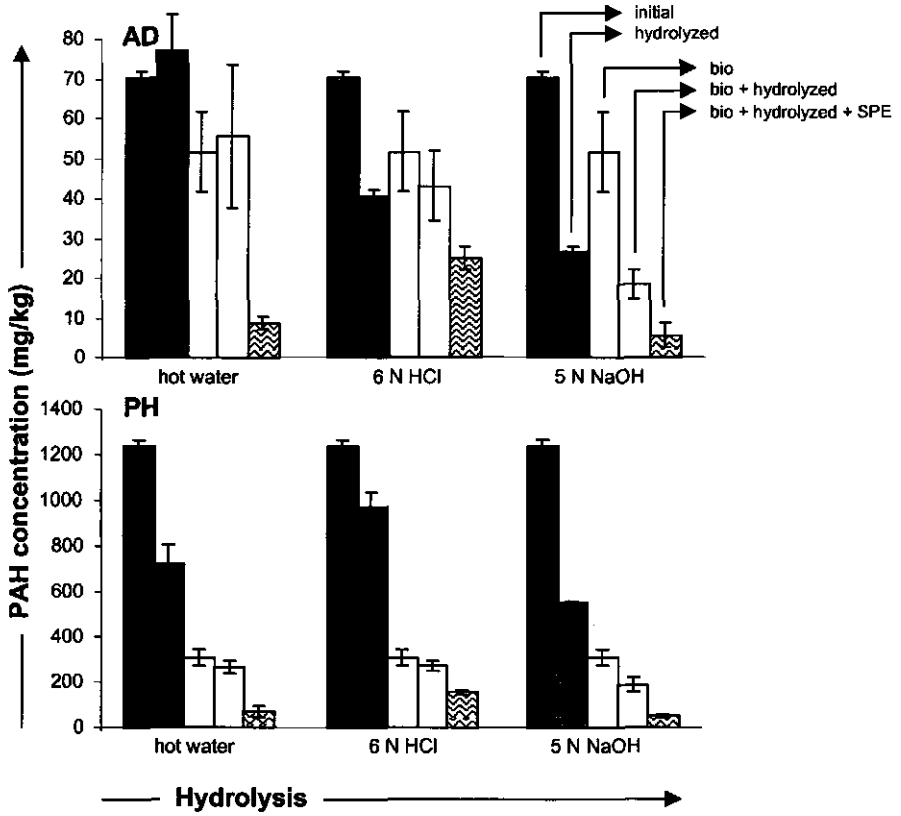

Figure 4. PAH concentrations in AD and PH sediment, before and after hydrolysis with hot water, 6 N HCl, and 5 N NaOH. Bars represent PAH concentrations in non-bioremediated samples (*initial*), non-bioremediated samples after hydrolysis (*hydrolyzed*), bioremediated samples (*bio*), bioremediated samples after hydrolysis (*bio + hydrolyzed*), and bioremediated samples after successive hydrolysis and solid-phase extraction (*bio + hydrolyzed + SPE*). Error bars represent standard deviations, except for "*hydrolyzed*" samples (non-bioremediated) where they represent duplicate measurements.

7.4.4 Oxidation. The results of the oxidation experiments in Figure 5 show that oxidation with 2 N HNO_3 and 4 % $KmnO_4$ strongly reduced PAH concentrations, both in non-bioremediated and bioremediated AD and PH samples. Residual PAHs after 2 N HNO_3 oxidation of AD sediment were partly bioavailable, indicating that oxidation resulted in bioavailability enhancement. In the other experiments, PAH removal was so high that conclusions with respect to the effect of oxidation on PAH bioavailability could not be drawn.

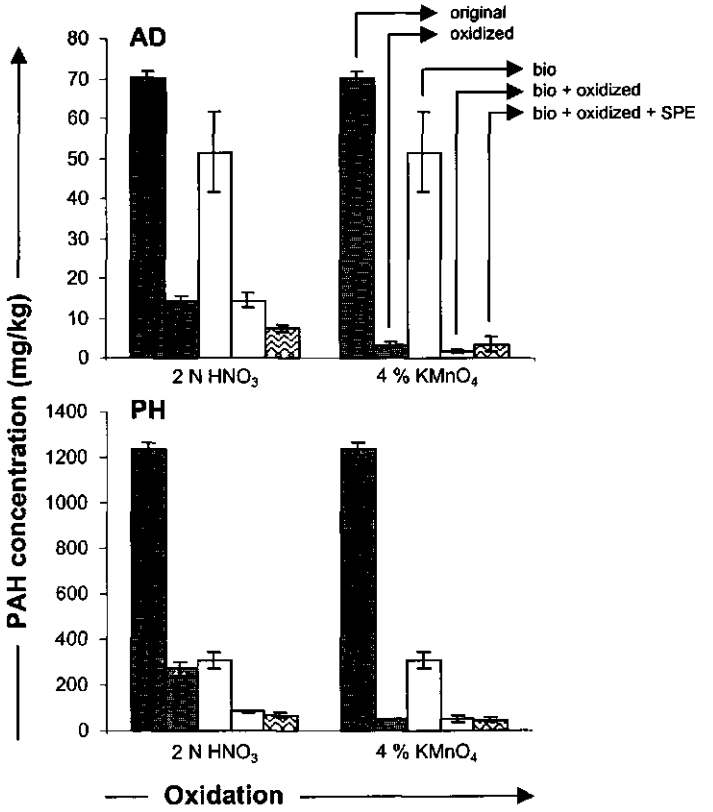

Figure 5. PAH concentrations in AD and PH sediment, before and after oxidation with 2 N HNO_3 and 4% $KMnO_4$. Bars represent PAH concentrations in non-bioremediated samples (*initial*), non-bioremediated samples after oxidation (*oxidized*), bioremediated samples (*bio*), bioremediated samples after oxidation (*bio + oxidized*), and bioremediated samples after successive oxidation and solid-phase extraction (*bio + oxidized + SPE*). Error bars represent standard deviations, except for "*oxidized*" samples (non-bioremediated) where they represent duplicate measurements.

7.5 Discussion

Table 1 summarizes the effect of chemical treatment on PAH concentrations and PAH bioavailability. The effect of treatment on readily bioavailable PAHs was derived from the experiments with non-bioremediated samples, the effect on poorly bioavailable PAHs from the experiments with bioremediated samples.

Table 1. Effect of extraction, hydrolysis, and oxidation on PAH concentrations and PAH bioavailability.

	Decrease readily bioavailable PAHs		**Decrease poorly bioavailable PAHs**		**Increase PAH bioavailability**	
	AD	*PH*	*AD*	*PH*	*AD*	*PH*
Extraction						
HCl (1N)	+	+	0	0	+	0
H_2SO_4 (0.025M)	+	+	0	0	+	0
NaOH (0.1N)	+	+	0	0	++	0
NaOH (0.5 N)	+	+	0	0	++	0
Hydrolysis						
Hot water	0	+	0	0	++	++
HCl (6N)	+	+	0	0	++	++
NaOH (5N)	+	+	+	+	++	++
Oxidation						
HNO_3 (2N)	+	+	+	+	++	(++)
$KMnO_4$ (4%)	+	+	+	+	(++)	(++)

0: PAH concentration/bioavailability not affected; +: decrease PAH concentration or increase PAH bioavailability <30%; ++: increase PAH bioavailability >30%; (++): almost all PAHs oxidized, obscures conclusion on PAH bioavailability.

Table 1 shows that chemical treatment reduced the amount of readily bioavailable PAHs in all experiments, except in the experiment in which AD sediment was hydrolyzed with hot water. In comparison, the amount of poorly bioavailable PAHs was reduced only by alkaline hydrolysis and by oxidation. This illustrates that poorly bioavailable PAHs could only be extracted by rather drastic treatments, while readily bioavailable PAHs could be extracted with relatively mild methods.

In contrast to the effect of wet-chemical treatment on PAH concentrations, which appeared to be practically similar in AD and PH sediment, the effect of wet-chemical treatment on PAH bioavailability was clearly different for the two sediments. Table 1 demonstrates that PAH bioavailability in AD sediment was enhanced by all treatments, while PAH bioavailability in PH sediment was only affected by hydrolysis and oxidation. This difference between AD and PH sediment is likely to result from a difference in organic matter composition. The composition of AD and PH sediment has been extensively described in Chapter 6. Roughly, it can be stated that organic matter in AD sediment was younger, more aliphatic, less resistant to thermal treatment, and less resistant to persulfate oxidation than organic matter in PH sediment. Moreover, PH sediment contained a considerable amount of oil. We observed that this oil was (partly) present

in a separate phase (NAPL) which formed sticky particles. We expect that the PAHs in these particles were readily bioavailable and that the differences between AD and PH sediment were not caused by the presence of oil. However, the exact role of the oil remains unclear.

In the Background section we discussed the different wet-chemical methods, addressing their effect on the composition of soil/sediment organic matter. Here, we combine this information with the results presented in Figures 2-5 and Table 1.

It appears from the extraction experiments that the mobile fulvic acids which were extracted with dilute mineral acids and the humic and fulvic acids which were extracted with alkaline solutions all contained a certain amount of readily bioavailable PAHs. Poorly bioavailable PAHs were not extracted with dilute mineral acids and alkaline solutions. They remained in the residue after extraction. The above illustrates that the poorly bioavailable PAHs were primarily associated with the humin fraction. This humin fraction has a higher C content and a lower O content than humic and fulvic acids and is less polar (*1*). The preference of the poorly bioavailable PAHs for the humin fraction is in correspondence with the work of others (*2, 22-25*), who demonstrated that sorption of hydrophobic organic contaminants to humin is much stronger than sorption to humic and fulvic acids.

Despite the fact that acid and alkaline extraction did not lead to the removal of poorly bioavailable PAHs, extractions did result in an enhancement of PAH bioavailability in AD sediment. Apparently, the removal of fulvic and humic acids modified the residual organic matter in such a way that the PAHs which were originally poorly bioavailable became more readily bioavailable after extraction. This increase of bioavailability could be caused by the opening of internal voids. Opening of voids may be due to the removal of metals by acid extraction or due to the increase of surface charge by alkaline extraction. Both modifications lead to a distension of the organic matter structure. In general, the effect of alkaline extraction on PAH bioavailability in AD sediment was stronger than the effect of acid extraction. This is in correspondence with the more extensive removal of humic material by alkaline extraction (*1, 4*). PAH bioavailability in PH sediment was not affected by acid and alkaline extraction. This indicates either that the residual organic matter was not seriously affected by the removal of humic and fulvic acids, or that the poorly bioavailable PAHs in PH sediment were sorbed in a principally different manner than in AD sediment. The absence of an effect of alkaline extraction on the bioavailability in PH sediment is in correspondence with the data of Carroll *et al.* (*50*), who observed no effect of a caustic extraction on the bioavailability of PCBs in a sediment sample. An important conclusion that may be drawn from the above is that a wet-chemical treatment can have a different effect on samples taken from a different origin.

The hydrolysis experiments demonstrated that there is a marked difference between hot water hydrolysis, acid hydrolysis, and alkaline hydrolysis. Hot water hydrolysis is generally considered the milder of the three methods (*3*), which is reflected in the inability to extract readily bioavailable PAHs from AD sediment. Hot water can extract polysaccharides, polypeptides, and small amounts of relatively simple phenolic acids and aldehydes. Acid hydrolysis is more drastic and has been reported to extract more amino acids and phenols than

hot water (*3*). Acid hydrolysis was capable of removing readily bioavailable PAHs from AD and PH sediment, but it was unable to remove poorly bioavailable PAHs. Apparently, the nucleus that remains after acid hydrolysis presented an important structure for the binding of organic contaminants. This finding is supported by the work of Gerstl and Kliger (*34*), who observed an increase of pesticide K_{oc} values after acid hydrolysis. The nucleus that remains after hydrolysis is believed to have a higher degree of condensation than the labile organic matter. Saiz Jimenez and de Leeuw (*37*) showed that the organic matter that is resistant to acid hydrolysis is similar to the organic matter that is resistant to persulfate oxidation. Taking in mind that persulfate oxidation is capable of removing readily bioavailable PAHs, while leaving poorly bioavailable PAHs unaffected (*51*), this confirms the importance of the condensed nucleus for sorption of poorly bioavailable PAHs. The exact composition of the nucleus is expected to vary with the source and age of the organic material (*53*).

Alkaline hydrolysis was shown to remove both readily and poorly bioavailable PAHs. It is known to cleave C-O bonds, but is relatively ineffective for degrading aromatic structures (*33*). Apparently, the C-O bonds form an important link in the recalcitrant nucleus that remains after acid hydrolysis. The central (aromatic) core that is unaffected by alkaline hydrolysis still contains poorly bioavailable PAHs.

Table 1 and Figure 4 show that hydrolysis seriously enhanced PAH bioavailability in AD and PH sediment. Although bioavailability enhancement may have been caused by the breakdown of organic matter, an important factor must have been the high temperature at which hydrolysis was performed. Comparing residual concentrations in Figure 4, it appears that the enhancement of bioavailability by acid hydrolysis was relatively low compared to the enhancement of bioavailability by hot water hydrolysis and alkaline hydrolysis. Acid hydrolysis was performed at 110 °C, while hot water hydrolysis and alkaline hydrolysis were both performed at 170 °C. If it is kept in mind that hot water hydrolysis yielded a less extensive degradation of organic matter than alkaline hydrolysis it may be reasoned that the high temperature was the main reason for the enhancement of PAH bioavailability by hydrolysis. This suggestion is supported by the work of Carroll *et al.* (*50*), who observed that heat treatment resulted in a similar enhancement of bioavailability as was achieved by a combination of heat treatment and the application of a caustic solution. Moreover, Bonten and coworkers (*48, 52*) demonstrated that PAH bioavailability could be seriously enhanced by high-temperature treatment.

Oxidation with HNO_3 and $KMnO_4$ led to an extensive degradation of the organic material, including parts of the condensed nucleus. As a result, both readily and poorly bioavailable PAHs were oxidized. Interpretation of the results in terms of bioavailability enhancement was difficult as the residual organic matter contained only small amounts of PAHs which were generally poorly bioavailable. This residual organic material is believed to consist of material that has a high content of compact polyaromatic rings (*46*).

7.6 Conclusions

The results of the extraction, hydrolysis, and oxidation experiments allow some general conclusions on the characteristics of the amorphous and condensed SOM domains.

Extractions demonstrated that the condensed SOM domains are situated in the humin fraction of SOM. This is in correspondence with the strong sorption of organic contaminants to humin, in comparison with sorption to humic and fulvic acids (*2, 22-25*). Given the characteristics of humin (*1*), it may be reasoned that condensed organic matter has a relatively high C content, a relatively low O content, a relatively high degree of polymerization, and a relatively low acidity. Condensed organic matter thus is less polar than expanded organic matter, which is in accordance with a previous study (*53*) and with several investigations which reported a negative correlation between SOM polarity and the affinity of SOM for hydrophobic organic contaminants (*22, 23, 54-60*). Acid hydrolysis demonstrated that condensed SOM domains are situated in a so-called nucleus, which is chemically stable and resistant to bacterial mineralization. The changes in SOM caused by acid extraction, alkaline extraction, and acid hydrolysis are generally considered to be analogous to those caused by natural diagenesis processes. This corresponds with the idea that diagenetically altered SOM is more condensed than the original organic material (*7, 23, 53, 58, 59*).

Our experiments further demonstrated that carbohydrates, proteins, fatty acids, free alkanes, fulvic acids, and humic acids, which can be removed by extraction and hydrolysis, do not contain poorly bioavailable PAHs. As a consequence, it seems likely that these substances are primarily associated with amorphous SOM domains. Still, it was shown for AD sediment that removal of the above mentioned substances could lead to an enhancement of PAH bioavailability. This indicates that, although the extracted substances do not contain poorly bioavailable PAHs, they can contribute to the stability of the condensed SOM domains.

Extrapolating the experimental results to situations in the field we may formulate an hypothesis on the possibility that PAHs, in practice, are mobilized by the dissolution of humic and fulvic acids. Based on our data, it may be reasoned that the dissolution of humic and fulvic acids is likely to mobilize some readily bioavailable PAHs. However, mobilization of poorly bioavailable PAHs seems to be highly unlikely, especially if it is considered that conditions in the field are much less drastic than the conditions applied in our experiments. Therefore, we hypothesize that poorly bioavailable PAHs, which remain in soil or sediment after bioremediation, are unlikely to be mobilized by the dissolution of humic material. This implies that the risk of spreading of PAHs by the dissolution and transport of humic material becomes limited after bioremediation.

7.7 References

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CHAPTER 8

General Discussion

8.1 Introduction

The study presented in this thesis had two main objectives: (1) the development of rapid laboratory methods for the prediction of PAH bioavailability in soils and sediments; and (2) the investigation of the composition of amorphous and condensed soil/sediment organic matter (SOM) domains. The first topic is addressed in Chapters 2-5, the second topic in Chapters 6 and 7.

Three methods were investigated for the prediction of PAH bioavailability: persulfate oxidation, cyclodextrin extraction, and surfactant extraction. These methods were compared with solid-phase extraction (SPE) and biodegradation, which were used as reference methods for the estimation of bioavailability and the potential for bioremediation, respectively. It was demonstrated that persulfate oxidation and cyclodextrin extraction are good methods for the prediction of PAH bioavailability; surfactant extraction seriously overestimates bioavailability. When compared to SPE, persulfate oxidation appears to be relatively rapid (3 h). Cyclodextrin extraction exhibits rather similar extraction kinetics as SPE, extraction taking one day up to several days for an accurate prediction of PAH bioavailability. Altogether, it can be concluded that persulfate oxidation currently provides the most rapid validated method for the prediction of PAH bioavailability in soils and sediments. The discussion in the second paragraph of this chapter will focus on the practical application of persulfate oxidation.

The composition of the amorphous and condensed SOM domains will be discussed in the third paragraph of this chapter. To elucidate the compositional characteristics, we studied the effect of persulfate oxidation on the composition of SOM. Besides, we studied the effect of wet-chemical extraction, hydrolysis, and oxidation on the concentration and bioavailability of PAHs. Some general characteristics of the amorphous and condensed SOM domains could be derived, although the composition appeared to be highly sample specific. Implications are discussed.

8.2 Practical Application of Bioavailability Prediction Methods

In Chapter 1 of this thesis it was suggested that a method that can measure the bioavailability of PAHs in soils and sediments can be used for the prediction of the extent of PAH removal during bioremediation and for the prediction of the exposure of animals and plants to PAHs. In the present chapter, these applications will be discussed

8.2.1 Prediction of Bioremediation Potential. Bioavailability is often considered to be the most important factor affecting the outcome of a bioremediation operation (*1*). This implies that a characterization method that can predict PAH bioavailability can often be used to predict the extent of PAH degradation during bioremediation. An important advantage of the (physical/chemical) bioavailability prediction methods is that they are considerably more rapid than the conventional biodegradation methods for the prediction of the bioremediation potential. Accordingly, they may serve as a rapid alternative. In the present paragraph we will focus on the practical application of persulfate oxidation.

Considering the application of persulfate oxidation for the prediction of the bioremediation potential of PAH contaminated soils and sediments, it should be kept in mind that the outcome of a bioremediation operation does not only depend on the bioavailability of PAHs but also on factors like toxicity, environmental conditions, and the presence of a microbial community capable of PAH degradation (*2*). In practice, the latter two factors can be controlled to a certain extent, leaving bioavailability and toxicity as the key parameters influencing bioremediation. All in all, this implies that persulfate oxidation alone, can not always give an accurate prediction of the success of a bioremediation operation. Therefore, it is suggested that persulfate oxidation and biodegradation experiments are combined in an integrated characterization strategy. Such a strategy should enable a rapid and reliable prediction of the bioremediation potential. An example of an integrated characterization strategy is presented in Figure 1.

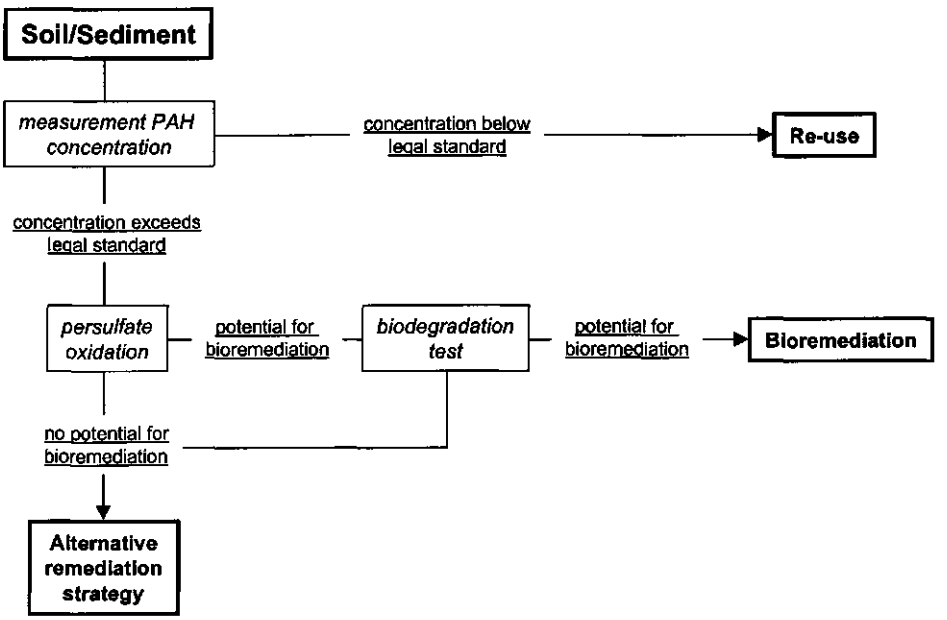

Figure 1. Characterization strategy to assess the bioremediation potential of PAH contaminated soils and sediments.

Persulfate oxidation has a central role in the characterization scheme in Figure 1. Oxidation is applied in all situations in which PAH concentrations exceed the legal standards for PAHs in soils or sediments. If persulfate oxidation indicates that bioavailability is too low to allow satisfactory bioremediation, no further characterization tests have to be performed and alternative remediation strategies must be considered (see Chapter 1). If persulfate oxidation indicates that bioavailability allows satisfactory PAH degradation, an additional biological characterization test has to be applied to rule out other inhibitory factors like toxicity. The

advantage of the strategy proposed is that no time-consuming biological tests have to be carried out if persulfate oxidation demonstrates that bioavailability is insufficient to allow cost-effective bioremediation. Moreover, the biological tests may be considerably shorter than conventional biodegradation tests if toxicity has to be established only. Toxicity limitations may be assessed in relatively short biological tests in which the conditions for biodegradation are optimized and an active microbial enrichment culture is added. However, if the biological test should give an indication of the bioremediation potential in unchanged samples, for example to assess the potential for natural attenuation, no nutrients or inocula may be added and the biological tests are likely to be as lengthy as the conventional biological tests in which bioavailability limitations have not been ruled out.

Characterization is complete after finishing the biological tests. If the biological tests indicate that bioremediation is limited by other factors than bioavailability, alternative remediation options have to be considered. If the biological tests indicate that bioremediation is feasible, a specific bioremediation set-up can be chosen. In practice, this choice for a specific bioremediation set-up is dictated by several location-specific conditions. These conditions will not be discussed in this chapter as this falls outside the scope of this thesis.

It should be noted that characterization protocols as proposed in Figure 1 have not been the standard in The Netherlands so far. However, we think that such protocols will become increasingly important in the near future, as the Dutch soil and sediment remediation policy has recently undergone some significant changes. In the new policy risk reduction has become a central issue (Chapter 1, *3*). This means that bioremediation is likely to become an increasingly attractive alternative for the clean-up of PAH contaminated soils and sediments. Moreover, there is a serious need for simple and rapid protocols for the assessment of ecotoxicological risks. In such protocols bioavailability prediction can play an important role, as is illustrated below.

8.2.2 Assessment of Ecotoxicological Risks. If we consider the application of persulfate oxidation for the assessment of the risks of a PAH contamination, a similar strategy as in Figure 1 can be followed. In such a strategy, persulfate oxidation can serve as a rapid method for the determination of PAH bioavailability, which indicates whether PAHs form a potential risk to the environment. If a considerable amount of PAHs are readily bioavailable, PAHs do form a potential risk and persulfate oxidation can be followed by a biological experiment (bioassay) to further substantiate the risk. These bioassays are of crucial importance because the actual risk that is posed by a PAH contamination does not only depend on the bioavailability of PAHs but also on factors like uptake and biotransformation (*4*).

The application of persulfate oxidation as a first screening method in risk assessment implies the assumption that only readily bioavailable PAHs can form a risk. Accordingly, it suggests that poorly bioavailable PAHs do not form a risk, even if soil or sediment is taken up by ingestion. This assumption is supported by experimental data in the literature, demonstrating that the uptake of PAHs by earthworms is strongly reduced if PAHs become less bioavailable (*5-7*). Besides, the results in Chapter 7 demonstrated that poorly bioavailable PAHs remain strongly

bound to the solid matrix, even when samples are subjected to extreme pH conditions. This observation indicates that it is unlikely that poorly bioavailable PAHs are mobilized in the digestive tract of animals. Therefore, we conclude that primarily readily bioavailable PAHs form a risk to the environment, which implies that bioavailability prediction methods can be used as a first and rapid screening step of an integrated risk assessment framework. To be able to decide whether the bioavailability measurements should be followed by biological tests, bioavailability has to be translated to risks. Target levels have to be formulated to indicate what concentrations of (bioavailable) PAHs are acceptable.

Of course, the application of bioavailability prediction methods is not restricted to soils and sediments contaminated with PAHs. For soils and sediments contaminated with other organic pollutants, a similar approach can be followed. In many cases solid-phase extraction may serve as a bioavailability prediction method (Chapter 5, *8*). Solid-phase extraction can be integrated in a characterization scheme that is similar to the one presented in Figure 1.

8.3 Composition of Amorphous and Condensed Soil/Sediment Organic Matter Domains

In Chapter 1 it was stated that the current information on the composition of amorphous and condensed SOM domains is rather scarce. The main sources of information are studies describing the correlation between the affinity of SOM for hydrophobic organic contaminants (K_{oc}, organic carbon normalized linear sorption coefficient) and properties describing the SOM composition. For interpretation, it has to be assumed that K_{oc} values express the degree of SOM condensation. High K_{oc} values are believed to indicate a high degree of condensation. Obviously, this is a rather indirect way of studying the composition of the amorphous and condensed SOM domains. Therefore, two different approaches were followed in the present thesis: (*i*) In Chapter 6 the composition of SOM was studied before and after removal of amorphous SOM by persulfate oxidation. Although it was not clear whether amorphous SOM could be completely removed by oxidation, it was sure that at least a part of the amorphous SOM was oxidized. Since condensed SOM was unaffected, the organic matter remaining after oxidation could be considered "more condensed". (*ii*) In Chapter 7 we studied the effect of wet-chemical treatment (extraction, hydrolysis, oxidation) on PAH concentrations and PAH bioavailability. To obtain information on the composition of the SOM domains the experimental data were combined with literature information on the effect of wet-chemical treatment on the structure of SOM.

Combining Chapters 6 and 7 and the information from studies relating sorption properties to compositional characteristics, the following overview of composition related characteristics of amorphous and condensed SOM domains was composed:

1. Condensed SOM is less polar than amorphous SOM. Polarity is expressed as (O-alkyl C + carboxyl C)/(alkyl C + aromatic C) (Chapter 6) or as O/C, (O+N)/C, and O/H atomic ratios (*9-17*). High ratios indicate high SOM polarity.
2. Labile components like carbohydrates, peptides, fatty acids, and free alkanes are likely to be associated with amorphous SOM (Chapters 6 and 7).
3. No clear relationship exists between the aromaticity of SOM and the degree of SOM condensation (Chapter 6, *17*). This is in contrast with studies describing a positive correlation between aromaticity and K_{oc} values for sorption to humic acids, fulvic acids, and dissolved organic matter (*9, 12, 18-21*);
4. Condensed SOM is situated in the humin fraction, indicating that it has a relatively high C content, a relatively low O content, a relatively low acidity, and a relatively high degree of polymerization (Chapter 7). Humic acids and fulvic acids are primarily associated with amorphous SOM. The above corresponds with sorption studies indicating that the affinity of organic contaminants for humic acids, fulvic acids, and humin decreases in the following order: humin > humic acids > fulvic acids (*11, 12, 14, 18-20, 22, 23*).
5. Diagenetic transformation of SOM increases the degree of SOM condensation (*13, 15, 16, 24*)
6. Coal and soot are specific condensed facies with a high affinity for hydrophobic organic contaminants (Chapter 6, *23, 25-29*).

Apart from these general characteristics, the composition of the amorphous and condensed domains appears to be highly sample specific (Chapters 6 and 7). For example, it was found in Chapter 6 that the condensed domains in samples with relatively undecomposed SOM were enriched in aliphatic C, while the condensed domains in samples with relatively weathered SOM were enriched in aromatic C. The above illustrates that the amorphous and condensed SOM domains are not likely to be formed by one unique chemical structure.

Altogether, it can be concluded that condensed SOM, as described in Chapter 1, represents a collection of apolar organic structures with a high affinity for hydrophobic organic contaminants. These structures are likely to consist of aliphatic and aromatic parts of complex organic macromolecules and of discrete aromatic facies formed by coal or soot particles. Amorphous SOM is likely to consist of the polar structures of the complex organic macromolecules and of a wide variety of low molecular weight organic compounds.

In addition to these chemical aspects, the physical arrangement of the organic structures has to be considered. Clearly, an open organic structure with flexible/mobile organic macromolecules will exhibit different sorption behavior than a rigid structure in which the mobility of the macromolecules is restricted. Overall, the sorption behavior of SOM is likely to be governed by

a combination of physical and chemical aspects. It remains unclear how the two are related and to what extent sorption is governed by physical conformation. In future research, both the chemical composition and the physical conformation have to be addressed in order to obtain a more fundamental understanding of the sorption processes governing bioavailability of organic pollutants in soils and sediments.

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SUMMARY

Polycyclic aromatic hydrocarbons (PAHs) constitute a group of priority pollutants which are produced in high amounts by natural and anthropogenic sources. Generally, soils and sediments serve as a sink for PAHs, leading to the accumulation of PAHs at contaminated sites. The presence of PAHs at contaminated sites is of increasing environmental concern because of the toxicity of PAHs, leading to adverse effects on humans, animals, plants, and ecosystems as a whole.

In the last decade, bioremediation has been frequently used for the clean-up of PAH contaminated soils and sediments. However, despite the common use and cost-effectiveness of bioremediation, it is generally observed that a residual fraction remains undegraded even when optimal biodegradation conditions have been provided. In many cases the recalcitrance of this residual fraction is caused by a limited bioavailability of the PAHs, resulting from strong sorption to the matrix.

In the past years, much effort has been put into the development of simple laboratory methods for the prediction of PAH bioavailability. Such methods may be applied for the prediction of the bioremediation potential and for the assessment of ecotoxicological risks. The methods that have been developed so far rely on the biodegradation or (solid-phase) extraction of readily bioavailable PAHs. Although operational, these methods can be quite laborious and time-consuming. Therefore, a need exists for more simple and rapid alternatives. The main objective of the present dissertation was to study and develop such alternative methods for the prediction of PAH bioavailability. This topic was addressed in Chapters 2-5.

As an integrated part of this study, it was aimed to expand the current knowledge on the relationship between PAH bioavailability and the structure of soil/sediment organic matter (SOM). In a recent conceptual model SOM has been represented as an entanglement of macromolecules which consists of amorphous and condensed domains. PAHs sorbed in the amorphous domains are considered to be readily bioavailable, while PAHs in the condensed domains are considered to be poorly bioavailable. So far, the information on the composition of the amorphous and condensed domains has been rather scarce. In Chapters 6 and 7 it was attempted to elucidate some of the compositional characteristics of the domains.

Chapter 1 contains a literature overview addressing the following topics: (*i*) structure and properties of PAHs, (*ii*) remediation of PAH contaminated soils and sediments, (*iii*) processes affecting the bioavailability of PAHs in soils and sediments, and (*iv*) measurement of PAH bioavailability in soils and sediments.

In Chapter 2, persulfate oxidation was introduced as a potentially rapid method for the prediction of PAH bioavailability in soils and sediments. It was hypothesized that persulfate is capable of oxidizing amorphous SOM as well as the readily bioavailable PAHs that are sorbed in it. We studied the effect of oxidation temperature (50-121 °C), oxidation time (1-24 h), and persulfate to organic matter ratio (2.9-28.9 g/g) on the extent of PAH removal by oxidation. Residual PAH concentrations after oxidation were compared with residual concentrations after 21 days of biodegradation in an optimized slurry reactor. Persulfate oxidation at 50 and 70 °C gave a good indication of the PAH removal by bioremediation, but only if the oxidation time was sufficiently long (≥ 24 and 3 h, respectively) and the persulfate to organic matter ratio was sufficiently high (≥ 5.8 g/g). Oxidation at 90 and 121 °C overestimated PAH bioavailability.
Two methods were selected for further investigation: a 3 h oxidation at 70 °C and a 2 h oxidation at 95 °C ($S_2O_8^{2-}$/OM ratio 12 g/g). These methods were tested on 3 field samples containing an aged PAH contamination. It was demonstrated that the residual PAH concentrations after 3 h of oxidation at 70 °C were similar to the residual concentrations after 21 days of biodegradation. A 2 h oxidation at 95 °C overestimated biodegradation. This overestimation could not be attributed to a thermal enhancement of PAH bioavailability. It was likely to result from a more extensive oxidation of organic matter at 95 °C.

The persulfate oxidation method that was developed in Chapter 2 (3 h; 70 °C; $S_2O_8^{2-}$/OM 12 g/g) was validated in Chapter 3. Validation was performed in a study with 14 historically contaminated soil and sediment samples, containing an aged PAH contamination. The residual PAH concentrations after persulfate oxidation were compared to the residual concentrations after 21 days of biodegradation in an optimized slurry reactor. It was observed that the residual PAH concentrations after persulfate oxidation corresponded well to the residual PAH concentrations after biodegradation. Persulfate oxidation of samples that had first been subjected to biodegradation yielded only limited additional PAH oxidation. This implies that oxidation and biodegradation removed approximately the same PAH fraction. Solid-phase extraction was performed on two of the 14 samples. The results of solid-phase extraction and persulfate oxidation were compared. It was demonstrated that PAH removal during 3 h of oxidation was approximately similar to PAH removal during 72 to 264 h of solid-phase extraction. Altogether, it was concluded that persulfate oxidation provides a good and rapid method for the prediction of PAH bioavailability.

In Chapter 4, the application of cyclodextrins and surfactants for the prediction of PAH bioavailability in soils and sediments was investigated. Two sediment samples were extracted

with aqueous solutions of hydroxypropyl-β–cyclodextrin (HPCD) and Triton X-100. The removal of PAHs during extraction was compared with the removal of PAHs during biodegradation and solid-phase extraction. It was demonstrated that HPCD extracted primarily readily bioavailable PAHs, while Triton X-100 extracted both readily and poorly bioavailable PAHs. Moreover, HPCD did not affect the degradation of PAHs in the biodegradation experiments, while Triton X-100 enhanced the biodegradation of low molecular weight PAHs. It was concluded that HPCD extraction may provide a good method for the prediction of PAH bioavailability. Triton X-100 extraction is unfit for the prediction of PAH bioavailability. The enhancement of bioavailability by Triton X-100 is likely to be due to the interaction of Triton X-100 with the organic matrix.

In Chapter 5, solid-phase extraction and persulfate oxidation were investigated for the prediction of total petroleum hydrocarbon (TPH) bioavailability. One sediment sample and two soil samples were subjected to solid-phase extraction, persulfate oxidation, and biodegradation, after which hydrocarbon removal was compared. A short solid-phase extraction (168 h) provided a good method for the prediction of the extent of TPH degradation in an optimized slurry reactor (84 d). Solid-phase extraction slightly underestimated the degradation of readily biodegradable hydrocarbons, whereas it slightly overestimated the degradation of poorly biodegradable hydrocarbons. Persulfate oxidation appeared to be unfit for the prediction of TPH bioavailability. Persulfate was unable to oxidize hydrocarbons with a high ionization potential. Moreover, hydrocarbons that were affected by persulfate were likely to be transformed rather than completely oxidized. Despite this inability to predict TPH bioavailability, it was demonstrated that persulfate oxidation could be applied for the prediction of PAH bioavailability, even in the presence of a considerable amount of petroleum hydrocarbons.

In Chapter 6, the composition of amorphous and condensed soil/sediment organic matter (SOM) domains was investigated for 1 soil sample and 4 sediment samples. These samples were oxidized with persulfate to remove amorphous SOM, before and after which the composition of SOM was studied by thermogravimetric analysis, pyrolysis-GC/MS, and CPMAS ^{13}C-NMR. Comparison of the SOM composition before and after oxidation showed that condensed SOM is more thermostable and less polar than amorphous SOM. Condensed SOM is relatively low in O-alkyl C and carboxyl C and it is likely to contain only small amounts of labile organic components (carbohydrates, peptides, fatty acids). Apart from these general characteristics, the composition of the condensed and amorphous domains appeared to be highly dependent on the origin and nature of the SOM investigated. Condensed domains in relatively undecomposed SOM were enriched in aliphatic C, whereas condensed domains in relatively weathered SOM were enriched in aromatic C. Altogether, the compositional changes upon persulfate oxidation were similar to the compositional changes upon humification, which supports the idea that weathered SOM is more condensed than the original material.

In Chapter 7, the composition of the amorphous and condensed SOM domains was investigated for 2 sediment samples. The samples were split in two parts, one part was bioremediated to remove bioavailable PAHs, and both the bioremediated and the non-bioremediated parts were subjected to 9 different chemical treatments: extraction with 0.1 N HCl, 0.025 M H_2SO_4, 0.1 N NaOH, and 0.5 N NaOH; hydrolysis with hot water, 6 N HCl, and 5 N NaOH; oxidation with 2 N HNO_3 and 4 % $KMnO_4$. After chemical treatment, PAH concentrations were measured and the bioavailability of the residual PAHs was determined using solid-phase extraction. To obtain information on the composition of the amorphous and condensed domains, experimental results on the effect of the chemical treatments on PAH concentrations and PAH bioavailability were combined with literature information on the effect of the chemical treatments on the SOM structure. It appeared that condensed SOM domains are primarily situated in the humin fraction of SOM. This indicates that they have a relatively high C content, a relatively low O content, a relatively high degree of condensation, and a relatively low acidity. Accordingly, they are less polar than amorphous domains. Carbohydrates, proteins, fatty acids, free alkanes, fulvic acids, and humic acids are likely to be associated with the amorphous SOM domains. Apart from these general characteristics, the composition of the SOM domains appeared to be sample specific.

In Chapter 8, the practical application of persulfate oxidation was discussed. A characterization strategy was proposed in which persulfate oxidation is combined with biological experiments for the prediction of the bioremediation potential of PAH contaminated soils and sediments. A similar strategy was proposed for the assessment of ecotoxicological risks. The combination of persulfate oxidation and biological experiments was considered to be beneficial as it allows a more rapid characterization than can be achieved by biological experiments alone.
A second topic that was addressed in Chapter 8 is the composition of amorphous and condensed SOM domains. It was concluded that condensed SOM represents a collection of apolar organic structures with a high affinity for hydrophobic organic contaminants. These structures are likely to consist of aliphatic and aromatic parts of complex organic macromolecules and of discrete aromatic facies formed by coal or soot particles. Amorphous SOM is likely to consist of the polar structures of the complex organic macromolecules and of a wide variety of low molecular weight organic compounds. It was suggested that, in future research, both the chemical composition and the physical conformation of SOM domains have to be adressed in order to obtain a more fundamental understanding of the sorption processes governing bioavailability of organic pollutants in soils and sediments.

SAMENVATTING

Biobeschikbaarheid van polycyclische aromatische koolwaterstoffen in bodems en sedimenten:

Voorspellen van biobeschikbaarheid en karakterisering van organisch-stof-domeinen

Polycyclische aromatische koolwaterstoffen, ofwel PAKs, vormen een belangrijke groep van verontreinigingen die kunnen worden geproduceerd door diverse natuurlijke en antropogene bronnen. Van nature hebben PAKs een grote affiniteit voor bodems en sedimenten, hetgeen leidt tot de ophoping van PAKs in de vaste matrix en de vorming van vervuilde lokaties. Vanwege de toxiciteit van de PAKs zijn deze vervuilde lokaties een directe bedreiging voor de omgeving. De toxiciteit zorgt voor negatieve effecten op mensen, dieren en planten, en kan zo het functioneren van complete ecosystemen beïnvloeden.

In de afgelopen jaren is veelvuldig gebruik gemaakt van biologische reiniging voor het schoonmaken van met PAKs vervuilde bodems en sedimenten. Helaas komt het dikwijls voor dat een deel van de PAKs achterblijft na biologische reiniging, zelfs wanneer er alles aan is gedaan om de biologische reiniging zo optimaal mogelijk te laten verlopen. In veel gevallen kan het achterblijven van een dergelijke restconcentratie worden toegeschreven aan de beperkte biobeschikbaarheid van een deel van de PAKs. Deze beperkte biobeschikbaarheid wordt veroorzaakt door sterke sorptie aan de matrix. De sorptie resulteert erin dat de PAKs onbereikbaar zijn voor de micro-organismen die voor afbraak moeten zorgen.

Het probleem dat hierboven is geschetst heeft geleid tot een aanzienlijke onderzoeksinspanning, gericht op het ontwikkelen van laboratoriummethoden voor het voorspellen van biobeschikbaarheid. Dergelijke methoden zouden kunnen worden ingezet voor het voorspellen van het reinigingsperspectief, maar ook voor het vaststellen van mogelijke ecotoxicologische risico's. Tot dusverre zijn methoden ontwikkeld die zijn gebaseerd op biologische afbraak van biobeschikbare PAKs of op solid-phase-extractie van biobeschikbare PAKs. Hoewel deze methoden operationeel zijn, zijn ze helaas nogal bewerkelijk en vereist een nauwkeurige voorspelling van de biobeschikbaarheid een aanzienlijke hoeveelheid tijd. Daarom is er behoefte aan alternieve karakteriseringsmethoden die sneller en eenvoudiger zijn. De belangrijkste doelstelling van het onderhavige promotieonderzoek was het bestuderen en ontwikkelen van dergelijke snelle en eenvoudige methoden. Dit onderwerp beslaat de hoofdstukken 2 t/m 5.

Het tweede deel van het onderzoek was gericht op de relatie tussen de biobeschikbaarheid van PAKs en de structuur van de organische stof (OS) in bodems en sedimenten. In een recent conceptueel model is OS voorgesteld als een kluwen macromoleculen die bestaat uit amorfe en

gecondenseerde domeinen. Deze domeinen hebben een verschillende affiniteit voor PAKs. PAKs zijn relatief zwak gebonden aan amorfe OS en relatief sterk gebonden aan gecondenseerde OS. Dienovereenkomstig is verondersteld dat PAKs die zijn gesorbeerd in amorfe domeinen biobeschikbaar zijn, terwijl PAKs die zijn gesorbeerd in gecondenseerde domeinen niet biobeschikbaar zouden zijn. Tot dusverre is er weinig bekend over de chemische samenstelling van de amorfe en gecondenseerde OS-domeinen. Daarom is in de hoofdstukken 6 en 7 een poging gedaan om enige eigenschappen van de structuur van deze domeinen te beschrijven.

Hoofdstuk 1 bevat een literatuuroverzicht met de volgende onderwerpen: (*i*) structuur en eigenschappen van PAKs, (*ii*) reiniging van bodems en sedimenten vervuild met PAKs, (*iii*) processen die de biobeschikbaarheid van PAKs in bodems en sedimenten beïnvloeden, (*iv*) het meten van de biobeschikbaarheid van PAKs in bodems en sedimenten.

In Hoofdstuk 2 is een nieuwe methode geïntroduceerd voor het voorspellen van de biobeschikbaarheid van PAKs. Deze methode is gebaseerd op oxidatie van OS met persulfaat. Uitgangspunt van de methode is dat persulfaat in staat is om selectief amorfe OS te oxideren. Dit zou kunnen leiden tot een selectieve oxidatie en verwijdering van biobeschikbare PAKs.
In een parameterstudie is het effect van de oxidatietemperatuur (50-121 °C), de oxidatietijd (1-24 uur) en de persulfaat/OS-ratio (2,9-28,9 g/g) op de mate van PAK-verwijdering onderzocht. De PAK-concentratie na oxidatie is daarbij vergeleken met de PAK-concentratie na een biologische reiniging (21 dagen in een geoptimaliseerde bioreactor). Uit deze vergelijking bleek dat de PAK-verwijdering door middel van oxidatie bij 50 en 70 °C goed overeenkwam met de PAK-verwijdering door middel van biologische reiniging, indien de oxidatietijd lang genoeg (≥ 24 respectievelijk 3 uur) en de persulfaat/OS-ratio hoog genoeg was (≥ 5,8 g/g). Oxidatie bij 90 en 121 °C gaf een overschatting van de biobeschikbaarheid.
Uiteindelijk zijn twee methoden geselecteerd voor verder onderzoek: een oxidatie van 3 uur bij 70 °C en een oxidatie van 2 uur bij 95 °C ($S_2O_8^{2-}$/OS 12 g/g). Deze methoden zijn getest op monsters van drie locaties, alle met een verouderde PAK-vervuiling. Uit het onderzoek bleek dat de restconcentratie PAK na 3 uur oxideren bij 70 °C overeenkwam met de restconcentratie PAKs na 21 dagen biologische reiniging. Een oxidatie van 2 uur bij 95 °C gaf een overschatting van de biobeschikbaarheid. Nader onderzoek wees uit dat deze overschatting niet kon worden toegeschreven aan een thermische verhoging van de biobeschikbaarheid: overschatting was veeleer te wijten aan een verregaandere aantasting van de OS bij 95 °C.

In Hoofdstuk 3 is de oxidatiemethode gevalideerd die is omschreven in Hoofdstuk 2 (3 uur, 70 °C, $S_2O_8^{2-}$/OS 12 g/g). De validatie is uitgevoerd met 14 bodem- en sedimentmonsters, alle vervuild met een verouderde PAK-verontreiniging. De resultaten toonden aan dat de restconcentratie PAKs na oxidatie goed correspondeerde met de restconcentratie na 21 dagen biologische reiniging in een geoptimaliseerde slurryreactor. Daarnaast bleek dat oxidatie van

monsters die eerst waren onderworpen aan biologische reiniging slechts tot geringe additionele PAK-verwijdering leidde. Dit geeft aan dat oxidatie en biodegradatie dezelfde PAK-fractie verwijderen. Ter referentie is een solid-phase-extractie uitgevoerd op 2 van de 14 monsters, waarna het resultaat is vergeleken met het resultaat van de persulfaatoxidatie. Het bleek dat de PAK-verwijdering tijdens een solid-phase-extractie van 72-264 uur overeenkwam met de PAK-verwijdering gedurende een oxidatie van 3 uur. Zo is in Hoofdstuk 3 aangetoond dat persulfaatoxidatie een geschikte en snelle methode is voor het voorspellen van de biobeschikbaarheid van PAKs in bodems en sedimenten.

In Hoofdstuk 4 is onderzocht of cyclodextrines en surfactants kunnen worden toegepast voor het voorspellen van de biobeschikbaarheid van PAKs. Twee sedimentmonsters zijn geëxtraheerd met waterige oplossingen van hydroxypropyl-β-cyclodextrine (HPCD) en Triton X-100. De PAK-verwijdering gedurende extractie is vergeleken met de PAK-verwijdering gedurende biologische reiniging en solid-phase-extractie. De resultaten toonden aan dat de HPCD-oplossing in staat was om selectief biobeschikbare PAKs te extraheren, terwijl de Triton X-100-oplossing zowel biobeschikbare PAKs als niet-biobeschikbare PAKs extraheerde. Daarnaast bleek de toevoeging van HPCD in biologische experimenten niet te leiden tot een toename van de biologische PAK-afbraak. De toevoeging van Triton X-100 leidde wel tot een toename van de PAK-afbraak. Er is daarom geconcludeerd dat extractie met HPCD een kansrijke methode is voor het voorspellen van de biobeschikbaarheid van PAKs, terwijl extractie met Triton X-100 ongeschikt is voor het voorspellen van de biobeschikbaarheid van PAKs. Triton X-100 veroorzaakte een toename van de biobeschikbaarheid, hetgeen kan worden verklaard door de interactie van Triton X-100 met de organische matrix.

In Hoofdstuk 5 zijn persulfaatoxidatie en solid-phase-extractie onderzocht voor het voorspellen van de biobeschikbaarheid van petroleumverbindingen. Deze methoden waren al geschikt gebleken voor het voorspellen van de biobeschikbaarheid van PAKs. Eén bodemmonster en twee sedimentmonsters zijn onderworpen aan solid-phase-extractie, persulfaatoxidatie en biologische reiniging, waarna de verwijdering van de totale hoeveelheid petroleumverbindingen (TPV) met deze methoden is vergeleken. Uit de experimenten bleek dat een korte solid-phase-extractie (168 uur) een goede indicatie gaf van de langetermijnafbraak van TPV in een geoptimaliseerde slurryreactor (84 dagen). Solid-phase-extractie onderschatte de afbraak van goed afbreekbare petroleumverbindingen enigszins, terwijl het de afbraak van slecht afbreekbare petroleumverbindingen enigszins overschatte. In tegenstelling tot solid-phase-extractie bleek persulfaatoxidatie ongeschikt voor het voorspellen van de biobeschikbaarheid van petroleumverbindingen. Persulfaat was niet in staat om verbindingen met een hoge ionisatiepotentiaal te oxideren. Bovendien werden de verbindingen die wel door persulfaat werden omgezet niet volledig afgebroken. Ondanks deze complicaties was persulfaatoxidatie wel in staat de biobeschikbaarheid van PAKs te voorspellen, ook in de aanwezigheid van een aanzienlijke hoeveelheid petroleumverbindingen.

In Hoofdstuk 6 is de samenstelling van amorfe en gecondenseerde organisch-stof-domeinen onderzocht. Hiertoe zijn vijf monsters, één bodemmonster en vier sedimentmonsters, onderworpen aan een oxidatie met persulfaat ter verwijdering van amorfe OS. Voor en na persulfaatoxidatie is de OS-samenstelling geanalyseerd met behulp van thermogravimetrische analyse, pyrolyse-GC/MS en CPMAS ^{13}C-NMR. Vergelijking van de samenstelling van de OS voor en na oxidatie leerde dat OS in de gecondenseerde domeinen thermostabieler en minder polair is dan OS in de amorfe domeinen. Gecondenseerde OS bevat relatief weinig O-alkyl C, carboxyl C en labiele componenten zoals suikers, eiwitten en vetzuren. Buiten deze algemene karakteristieken bleek de samenstelling van de amorfe en gecondenseerde domeinen sterk af te hangen van de oorsprong en de aard van de OS. Gecondenseerde domeinen in relatief vers, onaangetast materiaal bleken rijk aan alifatische koolstof. De gecondenseerde domeinen in sterk verweerd organisch materiaal bleken rijk aan aromatische koolstof. Al met al zorgde persulfaatoxidatie voor veranderingen in de OS-samenstelling die overeenkomen met de veranderingen die doorgaans optreden als gevolg van humificatie. Dit onderschrijft de algemeen geldende opvatting dat gehumificeerd/verweerd organisch materiaal meer gecondenseerd is dan het moedermateriaal.

In Hoofdstuk 7 is een andere methode toegepast voor het bestuderen van de samenstelling van de amorfe en gecondenseerde organisch-stof-domeinen. Twee sedimentmonsters zijn elk gesplitst in 2 delen, waarna 1 deel biologisch is gereinigd ter verwijdering van de biobeschikbare PAKs. Het andere deel is opgeslagen. Na biologische reiniging is zowel het biologisch gereinigde deel als het ongereinigde deel onderworpen aan negen verschillende chemische behandelingen: extractie met 0,1 N HCl, 0,025 M H_2SO_4, 0,1 N NaOH en 0,5 N NaOH; hydrolyse met heet water, 6 N HCl en 5 N NaOH; oxidatie met 2 N HNO_3 en 4 % $KMnO_4$. Na chemische behandeling zijn de PAK-concentraties bepaald en is de biobeschikbaarheid van de PAKs gemeten met behulp van solid-phase-extractie. Teneinde informatie te verkrijgen over de samenstelling van de OS-domeinen, zijn de resultaten met betrekking tot het effect van de chemische behandelingen op de concentraties en de biobeschikbaarheid gekoppeld aan literatuurinformatie over het effect van de verschillende chemische behandelingen op de structuur van het organisch materiaal. Uit de interpretatie bleek dat gecondenseerde OS-domeinen vooral te vinden zijn in de huminefractie van OS, hetgeen impliceert dat deze domeinen een relatief hoog C-gehalte en een relatief laag O-gehalte hebben. In overeenstemming hiermee zijn de gecondenseerde OS-domeinen minder polair dan de amorfe OS-domeinen. Suikers, eiwitten, vetzuren, vrije alkanen, fulvozuren en humuszuren moeten worden geassocieerd met amorfe OS. Buiten de bovengenoemde algemene karakteristieken bleek dat de samenstelling van de OS-domeinen monsterspecifiek is.

In Hoofdstuk 8 is het onderzoek in een breder kader geplaatst. Het eerste deel van de discussie in Hoofdstuk 8 is toegespitst op het in de praktijk toepassen van laboratoriummethoden voor het voorspellen van de biobeschikbaarheid van PAKs. Persulfaatoxidatie is hierbij als uitgangspunt

genomen. Er is een karakteriseringsstrategie voorgesteld waarin persulfaatoxidatie wordt gecombineerd met een microbiologische karakterisering voor het vaststellen van het reinigingsperspectief van met PAKs vervuilde bodems en sedimenten. Een soortgelijke strategie is voorgesteld voor het bepalen van de ecotoxicologische risico's van PAK-verontreinigingen. De combinatie van persulfaatoxidatie en biologische experimenten is gunstig omdat dit een snellere en efficiëntere karakterisering van met PAKs vervuild materiaal mogelijk maakt.

Het tweede deel van de discussie richt zich op de samenstelling van amorfe en gecondenseerde OS-domeinen. Er wordt geconcludeerd dat de gecondenseerde OS-domeinen bestaan uit apolaire organische structuren met een hoge affiniteit voor hydrofobe organische verontreinigingen. Deze structuren bestaan waarschijnlijk uit alifatische en aromatische delen van complexe organische macromoleculen en uit discrete kool- en roetdeeltjes. Amorfe OS-domeinen bestaan waarschijnlijk uit de polaire delen van complexe organische macromoleculen en uit tal van labiele organische moleculen met een laag molecuulgewicht. In toekomstig onderzoek zou, naast de karakterisering van de chemische samenstelling, ook de karakterisering van de fysieke conformatie van de organische moleculen voldoende aandacht moeten krijgen. Verdere bestudering van de organisch-stof-domeinen is van belang om een beter beeld te krijgen van de sorptieprocessen die ten grondslag liggen aan de biobeschikbaarheid van organische verontreinigingen.

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Chiel Cuypers
Wageningen, 6 juli 2001

CURRICULUM VITAE

Michiel Pierre Cuypers, geboren op 7 september 1972, behaalde in 1990 het Atheneumdiploma aan de Philips van Horne Scholengemeenschap te Weert. Vanaf 1990 studeerde hij Milieuhygiëne aan de toenmalige Landbouwuniversiteit te Wageningen, waar hij in 1996, na een afstudeervak Milieutechnologie en een stage aan het Institute of Landscape Ecology te České Budějovice, zijn studie afsloot met een afstudeervak Bodemscheikunde. In de periode 1996-2000 werkte hij als AIO bij de Sectie Milieutechnologie van Wageningen Universiteit. De vruchten van het AIO-schap zijn (deels) gepresenteerd in dit proefschrift. Vanaf september 2001 werkt dr. Cuypers als medewerker chemie en ecotoxicologie aan het Rijksinstituut voor Integraal Zoetwaterbeheer en Afvalwater-behandeling te Lelystad.

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