USE OF NITRATE TO STIMULATE THE BIODEGRADATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN ANOXIC MARINE SEDIMENT

by

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

(Department of Civil Engineering)

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

July 1997

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Date JULY 31/97

Abstract

Bottom sediments in waters near industrial areas and cities are frequently contaminated with polycyclic aromatic hydrocarbons (PAH). This observation is of particular concern because some PAH are proven carcinogens and mutagens, and cancerous lesions in bottom fish have been correlated with high PAH concentrations in sediment. In the Vancouver, B.C. area, a number of severely impacted sites have been identified.

This research was carried out to investigate the feasibility of using nitrate to stimulate the biodegradation of PAH in anoxic marine sediments. It is based on the assumption that the factor that most often limits microbial activity in sediments that receive a high organic load is the lack of available electron acceptors. Oxygen, usually the most efficient electron acceptor, is only sparingly soluble in water and may not be provided to the sediment at a sufficient rate to meet the demand. Nitrate can be used as an alternative electron acceptor by denitrifying microorganisms, is very water soluble, and yields relatively innocuous gaseous products which can leave the system upon reduction.

In laboratory experiments, nitrate addition stimulated the loss of some added PAH in False Creek sediment incubated under anoxic conditions for up to 20 weeks. The smaller, less hydrophobic compounds were degraded more quickly than the higher molecular weight compounds, and benzo(a)pyrene, a 5-ring, highly carcinogenic PAH, was not degraded under the test conditions. The half lives of the low molecular weight compounds ranged from approximately 4 to 13 weeks. When they were degraded, the half lives of the high molecular weight compounds were from 16 to 70 weeks. PAH degradation was inhibited when nitrate was provided as calcium nitrate, probably as a result of calcium phosphate precipitation leading to phosphate limitation.

Since PAH degradation seemed to be related to the solubility and probable bioavailability of the PAH, two degradation experiments were carried out which included a measure of the "available" fraction using False Creek sediment and one with sediment taken near the ESSO refinery. In this availability measure, called reverse semipermeable membrane device (rSPMD) extraction, the sample is sealed in a polyethylene tube and dialyzed against a solvent. The contaminant must desorb from the solid phase and diffuse through the tubing to reach the solvent, thus only the available fraction is measured. Degradation of the more readily

available added PAH occurred more rapidly than that of endogenous material. The PAH degradation rates were higher in the ESSO sediment than in the False creek sediment. The high molecular weight compounds were also more readily available as measured by rSPMD from the ESSO sediment.

The likelihood of successful bioremediation at these two sites was examined. *In situ* nitrate addition is unlikely to be effective at the False Creek site because degradation of the larger compounds is too slow. Furthermore, the metal concentrations at the site could exert toxic effects even after harmful organics are removed. PAH biodegradation at the ESSO site would likely be accomplished more quickly and with a lower nitrate dose, however benzo(a)pyrene could also persist there. Bioremediation in a slurry phase reactor under denitrifying conditions might be more successful since the process can be optimized to favour desorption of the pollutants.

Table of Contents

Page

List of Tables

Page

List of Figures

Page

Acknowledgments

My experience at UBC has been enriched by a great number of people and I'm pleased to have the opportunity to thank some of them here. Without the help and support of my friends, colleagues and mentors this time would have been much less fruitful and rewarding.

Grateful thanks are due to my supervisor, Dr. Ken J. Hall, for his guidance and support. His unflagging interest in the "Big Picture" is always a source of inspiration. Also, thank you to my committee members: Jim W. Atwater, Dr. Eric R. Hall, Dr. J. Tom Beatty and Dr. Bill Ramey for useful and insightful input through many lengthy committee meetings.

I owe a great debt of gratitude to Susan Harper, Paula Parkinson and Jufang Zhou, not only for excellent technical assistance and advice, but for their friendship. Susan was a reliable source of information on any subject I could toss her way, Paula helped me out of countless chemical conundrums and Jufang ran more of my samples than I would like to remember. All three made working in the lab something to look forward to.

I also want to thank Eileen Rawling and Alan Werker for reading my thesis, but more importantly, for late night conversations, great food, shop talk, getaways, perspective, ribbing, and wisdom. I can't imagine what this would have been like without many friends in the same boat, so cheers to the other grad students who have come and gone while I was completing this work.

I would also like to express my gratitude to my family who have been very supportive, especially in not asking (very often) when I was going to finish and get a real job. Finally, thanks to Farahad Dastoor, my partner, for always doing just the right thing.

I would like to dedicate this thesis to the memories of my father, Bob MacRae and grandfather, W.D. Porter.

List of Abbreviations

ACE	Acenaphthene
ACY	Acenaphthylene
AEP	Available for equilibrium partitioning
ANT	Anthracene
APHA	American Public Health Association
ATAD	(ATAR) Autothermal thermophilic aerobic digestor/reactor
AVS	Acid volatile sulphide
BAA	Benz(a)anthracene
BAP	Benzo(a)pyrene
B.C.	British Columbia
BOD	Biochemical Oxygen Demand
C_{aq}	Concentration in the aqueous phase
C_s	Concentration in the solid phase
CHR	Chrysene
CMC	Critical micelle concentration
COV	Coefficient of variation (100*St Dev/mean)
CSO	Combined sewer overflow
DMSO	Dimethyl sulphoxide
DNRA	Dissimilatory nitrate reduction to ammonia
DOM	Dissolved organic matter
EC_{50}	Effective concentration 50 (50% decrease in light production)
EDTA	Ethylenediamine tetraacetic acid
ESSO	Port Moody Arm sampling site near ESSO refinery
EPA	(USEPA) United States environmental protection agency
FCE	False Creek east basin (sampling site)
FLA	Fluoranthene
FLU	Fluorene
F_{oc}	Fractional organic carbon
gc-fid	Gas chromatography with flame ionization detection
GVRD	Greater Vancouver Regional District
GVSDD	Greater Vancouver Sewerage and Drainage District
HMW	High molecular weight
HPLC	High performance liquid chromatography
K_{oc}	Organic carbon-normalized partition coefficient
K_{ow}	Octanol-water coefficient
K_p	Partition coefficient (Cs/Caq)
L	Allowable error
LC_{50}	Lethal concentration 50 (50% death at that concentration)
LMW	Low molecular weight
MOELP	Ministry of environment, lands and parks
n	Number of replicates
NAP	Naphthalene
NAPL	Non-aqueous phase liquid

NOx	Nitrogen oxides (NO_2^-, NO_3^-)
NRC	National Research Council of Canada
NWRI	National Water Research Institute
O&G	Oil and grease
OC	Organic carbon
OMD	Organic matter diffusion
PAH	Polycyclic aromatic hydrocarbons
PHE	Phenanthrene
ppm	Parts per million
pretx	Pretreatment
PYR	Pyrene
r^2	Coefficient of correlation
rpm	Rotations per minute
rSPMD	Reverse SPMD
SPMD	Semipermeable membrane device
SRPD	Sorption-retarded pore diffusion
St Dev	Standard deviation
Surr	Extraction surrogate
t	T statistic
$T_{1/2}$	Half life (time taken for half the material to degrade)
TPAH	Total PAH (sum of measured PAH)
TSS	Total suspended solids
WTC	Wastewater Technology Centre
WTIC	Water Technology International Corporation

1. INTRODUCTION

1.1 Trace Pollutants in the Environment

Until relatively recently, pollution was viewed as significant only when the air, water or soil was visibly and severely affected. As a result, broad measures of pollution such as Biochemical Oxygen Demand (BOD), Total Suspended Solids (TSS), and Oil and Grease (O&G) have been used to assess the strength and likely impacts of wastes on the receiving environment. While these tests are useful indicators of severe and acute problems such as oxygen deficit, benthic burdens and even specific types of contamination or toxicity, they do not address the potential impacts of waste constituents that are active at much lower concentrations. With the growing realization of the effects of trace amounts of pesticides in the environment, which became widely recognized in the '60s, more attention has been focused on the impacts of trace contaminants on humans as well as ecosystems.

Polycyclic aromatic hydrocarbons (PAH) are the longest studied chemical carcinogens and have been of medical interest since the 1800s. They are produced both naturally and anthropogenically and are ubiquitous in the environment. Concentrations in soils and sediments worldwide have increased with the consumption of fossil fuels, leading to an increased burden of PAH contamination with time. Near anthropogenic sources, PAH concentrations in the environment may be very high. Although they may exert acute toxic effects on some organisms, the primary concern about PAH is in their potential to cause cancer or birth defects at relatively low concentrations, and the potential for an increase in the incidence of cancer as a result of environmental exposure (Mix, 1984; Hallett and Brecher, 1984).

Like many other hydrophobic contaminants that are relatively slow to biodegrade, the ultimate repository of this class of compounds is in the sediments of our waterways and oceans. Atmospheric contaminants are deposited directly or may reach the aquatic environment by runoff or leaching. Soil contaminants also reach the water by runoff, leaching and erosion, and some contamination occurs by direct discharge of waste effluents or petroleum spills to waters. In the aquatic environment, PAH are quickly adsorbed to suspended particles or organic matter and are ultimately deposited in the sediments (Mix, 1984; Hallett and Brecher, 1984).

Sediments act as both sinks and sources of organic pollutants. Bound residues are less available and hence less toxic to biota, but desorption from solids does occur, so even after contaminant input ceases, continued biological effects may be observed (McElroy et al., 1989). Sediment as a pollutant source is a major concern with respect to water supplies, fisheries, and recreation since the effects of chronic exposure to trace contaminants are difficult to measure or predict, and once steps are taken to remove contaminant sources, the biological problems may persist.

1.2 Bioremediation

Bioremediation can be defined simply as the use of organisms, primarily bacteria, to detoxify or destroy pollutants (Cookson, 1995). As such, it is not a new technology. Biological treatment of wastes has been carried out for centuries, however it has more recently been applied to the reclamation of polluted soils and groundwater in addition to the preemptive treatment of wastes before release. In all cases, bioremediation is an extension of the natural biodegradation or mineralization of organic compounds which completes the carbon cycle begun by atmospheric CO_2 fixation by photosynthetic organisms.

Bioremediation is brought about by supplying the factors that limit the rate or extent of biodegradation of pollutants. Possible limiting factors are:

(1) the lack of microorganisms that can bring about the required transformations

(2) the lack of nutrients or, more rarely, a trace element

(3) the lack of electron acceptors

(4) insufficient available substrate (either the contaminant or another primary substrate)

(5) toxicity

(6) temperature

(7) pH of the environment

Once suitable conditions are provided, organisms can usually destroy the contaminants and the risk or hazard is alleviated. The principal advantage of this approach over other methods such as incineration or landfilling is that the elimination of long-term liability by destruction of the pollutants is accomplished at relatively low cost (Cookson, 1995).

In sediments, the most common limiting factor is an adequate supply of a suitable electron acceptor. Oxygen, the electron acceptor that has the potential to yield the highest

amount of energy in degradation reactions, is only sparingly soluble in water. As a result, the rate of oxygen supply is usually far exceeded by its consumption rate, so alternative electron acceptors are used in progressively deeper sediments. In decreasing order of thermodynamic energy yield, the other electron acceptors typically used are: nitrate, Mn(IV), Fe(III), sulphate and carbonate. Fermentation, in which organic compounds act as both electron donor and acceptor, also occurs in the absence of oxygen (Jones, 1985).

The bioremediation of soils and groundwater has primarily been spurred on as a result of concerns over groundwater contamination and the environmental laws that make the polluter pay for cleanup. These laws have led to contaminant testing before real estate transactions are closed, so that the buyer does not assume liability for contamination at the site, and the seller may be indemnified. These incentives to clean up sites do not yet exist for sediment contamination, so fewer remediation options have been explored; however once sediments are extracted from the environment and dewatered, they can be treated in systems similar to those used in soil remediation. Some recent efforts have been made to study sediments, primarily through the Canadian-American cooperative effort to clean up the Great Lakes (Wardlaw, 1994).

In situ bioremediation is usually the most cost effective and least disruptive treatment option when it can be applied. It eliminates the need for handling, transportation and storage of hazardous material. For sediments, it also precludes the need for dredging, a process which can expose the water column biota to the pollutants, and in some cases reintroduces buried historical contamination to the active sediment strata. The logistics and expense of providing oxygen to sediments are likely to limit the use of this approach to remediation of sediments.

Nitrate, which can be used by many heterotrophic microorganisms in the absence of molecular oxygen, is highly soluble in water, does not undergo as many side reactions and yields relatively innocuous end-products upon reduction. Nitrate can therefore be used to make *in situ* sediment bioremediation a more economically attractive and technically feasible process, and has been used to treat PAH-contaminated freshwater sediments (Murphy et al., 1995). Recently, more attention has been focused on the use of nitrate as an alternative electron acceptor in petroleum-contaminated soil remediation because it can be provided more economically than oxygen, both *in situ* and in bioreactors (Norris, 1995).

Bioremediation is unlikely to be successful when the contaminants are strongly associated with the solid matrix. Adsorbed residues are less available to microorganisms for biodegradation, and might persist, despite otherwise optimal growth conditions. The bioavailability of the pollutants is therefore an important factor in determining whether or not bioremediation should be undertaken for a given sediment or soil.

The research described in this thesis was carried out to investigate the feasibility of using nitrate to stimulate the biodegradation of PAH in two marine sediments in the Vancouver area, namely: 1) the east basin of False Creek (FCE) and 2) near the ESSO refinery/storage and distribution facility in the Port Moody arm of Burrard Inlet (ESSO). Sediments from both sites have elevated levels of PAH as well as other pollutants. Murphy et al. (1995) found that PAH could be degraded in freshwater sediments with nitrate addition, but PAH degradation under denitrifying conditions in marine sediments has not been observed before. In addition to this *in situ* treatment, nitrate can be used to lower the operational costs of bioremediation in a reactor *ex situ.* The effects of PAH availability on degradation rates was studied to determine which, if either, of these approaches to bioremediation would most likely succeed at the test sites.

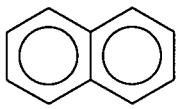
2. LITERATURE REVIEW

2.1 Polycyclic aromatic hydrocarbons (PAH)

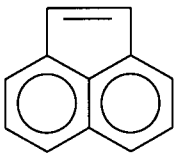
Polycyclic aromatic hydrocarbons (PAH) are also called polynuclear aromatic or polyaromatic hydrocarbons. They are organic compounds composed exclusively of carbon and hydrogen atoms, arranged in two or more fused aromatic rings, where fused means that at least two carbons are shared between two rings. Low molecular weight (LMW) PAH have two or three rings, and high molecular weight (HMW) PAH are made up of 4-7 rings. Some properties and the molecular structures of the PAH that were used in this research are shown in Table 2.1 and Figure 2.1, respectively.

Table 2.1 Properties of PAH
The abbreviations (abbr.) shown are those used in this thesis. Compounds with no proven carcinogenicity are marked -, +/- are weakly carcinogenic, + are carcinogenic and ++ are strongly carcinogenic. Solubility values were taken from Nagpal (1993) except Ace, from Pearlman et al. (1984), log octanol-water coefficients (K_{ow} = equilibrium concentration in octanol/concentration in water phase) are from Nagpal (1993).

PAH	Abbr.	MW	Carcino-genicity	Solubility (mg/L)	Log K_{ow}
Naphthalene	Nap	128	-	12-34	3.37
Acenaphthylene	Acy	152	-	3.4	4.07
Acenaphthene	Ace	154	-	2.4-7.4	3.98
Fluorene	Flu	166	-	0.8	4.18
Phenanthrene	Phe	178	-	0.435	4.46
Anthracene	Ant	178	-	0.059	4.50
Fluoranthene	Fla	202	-	0.260	4.90
Pyrene	Pyr	202	-	0.133	4.88
Benz(a)anthracene	BaA	228	+	0.011	5.63
Chrysene	Chr	228	+/-	0.0019	5.63
Benzo(a)pyrene	BaP	252	++	0.0038	6.06



Naphthalene



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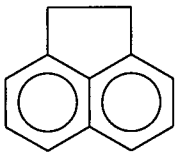
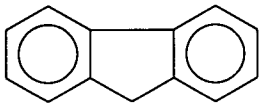
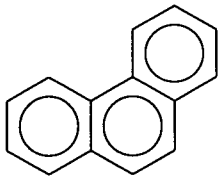


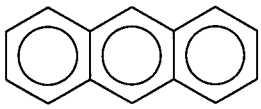
Acenaphthene



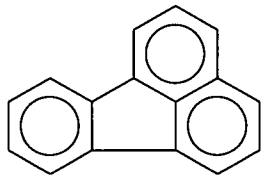
Fluorene



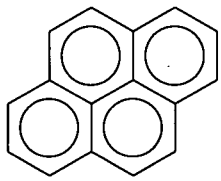
Phenanthrene



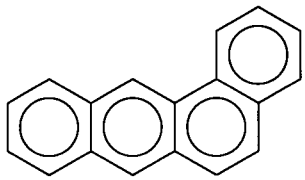
Anthracene



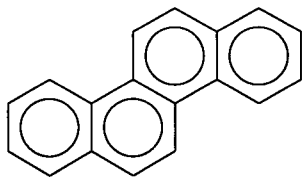
Fluoranthene



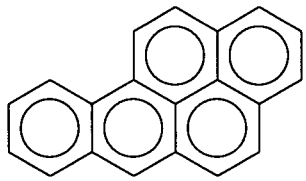
Pyrene



Benz(a)anthracene



Chrysene



Benzo(a)pyrene

Figure 2.1 PAH ring structures

These compounds can undergo three different types of chemical reactions: electrophilic substitutions, oxidations and reductions. The latter two reactions destroy the aromaticity of the ring. PAH are of environmental concern because some are known carcinogens, while others are acutely toxic or have other chronic effects (Hallet and Brecher, 1984; Neff, 1979).

2.1.1 Formation

PAH are formed in three ways: by pyrolysis, diagenesis of sedimentary organic material, or biosynthesis. Direct biosynthesis of PAH by microorganisms and plants has been postulated, although formation of PAH in this manner is controversial. It has been determined that PAH are produced by biological reduction of aromatic structures such as lignin (Nagpal, 1993; Neff, 1979). PAH are also produced during the diagenesis of fossil fuels at low to moderate (100-300 °C) temperatures over a long period of time (Albers, 1995).

Most PAH are formed by pyrolysis. At high temperatures, aliphatic C-C or C-H bonds are broken to form free radicals which recombine to form aromatics. Aromatic rings are stable at 400-800 °C in the chemically reducing environment at the centre of most flames (Hallet and Brecher, 1984). At higher temperatures and over longer reaction times there tends to be less alkylation of PAH since side chains are less stable than aromatic structures. All combustion reactions of organic compounds produce PAH except when flammable gases are well mixed with air. The specific profile of compounds produced depends on the conditions and the composition of the organic material being burned. For example, crude oils and crank case oil typically contain relatively higher proportions of phenanthrene and alkylated phenanthrenes. Combustion reactions usually produce more of the higher molecular weight compounds, with fluoranthene and pyrene in roughly equal proportions. Gasoline exhaust, however, is usually enriched in pyrene and coronene (Albers, 1995; Mix, 1984).

2.1.2 Sources

PAH are produced both naturally and anthropogenically, and are ubiquitous in the environment. Natural sources include forest and prairie fires, volcanic eruptions, oil seeps, and biosynthesis. The PAH content of fossil fuels varies considerably. Crude oils contain 0.2-7% PAH. Higher density oils have higher PAH contents. Between 70-75% of the carbon in coal is

in aromatic form, mostly as 6-membered rings with some 5-membered rings (Nagpal, 1992; Albers, 1995).

Anthropogenic sources account for the elevated PAH levels near industries and populated centres. All activities or industries that exploit combustion reactions or use fossil fuels as feedstocks produce PAH. Some examples of industrial sources are coke, carbon black, coal tar pitch, asphalt, acetylene, aromatic solvent, synthetic alcohol, and charcoal production, coal gasification and liquefaction, and petroleum cracking. PAH are also generated by agricultural burning, home wood-burning appliances (fireplaces and wood stoves), and residential heating. Vehicle exhaust is another significant source. Direct PAH intake by humans can be through cigarette smoke or the consumption of smoked foods such as ham, bacon, smoked fish or grilled foods (Albers, 1995; Mix, 1984; Hallet and Brecher, 1984; National Research Council of Canada (NRC), 1983).

2.1.3 Routes to the aquatic environment

Atmospheric fallout is the main source of PAH in remote areas. PAH formed in combustion reactions become associated with particulate matter. If the particle size is small, the PAH may travel hundreds of kilometers before being deposited. Much of this material reaches the aquatic environment in precipitation, although dry deposition is also possible. Larger particles are usually deposited much closer to the source. Up to 1% of fly ash is made up of relatively large carbonaceous particles with a honeycomb-like structure of very high surface area. This material has a higher affinity for PAH than the bulk of the ash, and contains about 2/3 of the PAH formed (Schure and Natusch, 1983, Hallet and Brecher, 1984). Thus, most of the PAH released into the air are deposited close to the source.

Reduction of aromatic compounds by microorganisms can contribute to the PAH load in sediments when an aromatic substrate is available. This tends to give rise to a fairly simple mixture of PAH at the site in the absence of other PAH sources. Submarine oil seeps are important sources of petroleum contamination to the oceans, both locally and globally (Mix, 1984).

Creosote has long been used to preserve wooden structures that contact water. Such structures are important local sources of PAH contamination to the aquatic environment. However, in global terms, the overall contribution of PAH to the environment from these

structures is small (Mix, 1984). Waste effluents from many industries and smelting operations contain high PAH concentrations and are discharged directly into the aquatic environment. Domestic wastewaters also contain PAH. The HMW PAH content of sewage treatment plant effluents has been observed to increase with rainfall after a dry period. This pattern is likely due to the flushing of street dust into the system. Phenanthrene, a three ring PAH, did not correlate well with the HMW compounds. It most likely comes from another source, such as undegraded fuel oil (Mix, 1984).

Surface runoff from land is a major source of PAH to the aquatic environment. The PAH component of road runoff comes from the wear and leaching of asphalt, wear of tires containing carbon black and from condensation of vehicle exhaust. Spilled petroleum products can also reach the aquatic system from the land in runoff. The other major routes to waterways and the ocean are from the oil industry; in oil spills, operational releases, and during exploration and extraction offshore (Mix, 1984; Hallet and Brecher, 1984; Neff, 1979).

2.1.4 Fate in the aquatic environment

PAH in the aquatic environment can either exist in the dissolved form, or associated with high molecular weight dissolved organic matter (DOM), particulates, or biota. Removal from the system may result from photooxidation, volatilization, chemical oxidation or biotransformation. Photooxidation is important primarily in clear and shallow waters. Volatilization is only significant for LMW PAH that are not bound to DOM or solids, and chemical oxidation is of minor importance in natural waters. Biological metabolism by microorganisms or higher organisms is usually the major route of PAH removal from aquatic systems (Mix, 1984).

PAH tend to adsorb rapidly to the organic component of suspended solids because they are not very soluble in water. These PAH-contaminated particles (or contaminated particles that reach the water by direct deposition or runoff), then settle to the bottom. Once in the bottom sediment, PAH are less subject to photooxidation due to the lack of light. Microbial degradation is also limited by the low supply of oxygen in water. Small amounts of sediment-associated PAH can be reintroduced into the water column by leaching and biological activity, but the HMW compounds especially tend to persist in sediments. Typically, concentrations in sediments and biota decrease with distance from the source (NRC 1983; Mix, 1984). Table 2.2

shows the ranges of PAH concentrations observed in sediments with and without significant levels of pollution. In some cases the impacted sediments have over 1000 times more PAH than the reference sediments. Levels at extremely polluted sites, such as some "hot spots" in Hamilton Harbour, Ontario, can have total PAH concentrations of over 2000 mg/kg dry weight (Murphy et al., 1995).

Table 2.2 PAH concentrations in clean and impacted sediments
Concentrations are given as mg/kg dry weight. In the Impact column, "No" means the site was reported to be clean and "Yes" indicates the site was impacted by human activity. References are: 1 Nagpal, 1993; 2 Bennett and Cubbage, 1992; 3 Barrick et al., 1988; 4 Goyette and Boyd, 1989; 5 Boyd and Goyette, 1993. LMW PAH are Nap, Acy, Ace, Flu, Phe, Ant; HMW are Fla, Pyr, BaA, Chr, BaP plus benzo(b) fluoranthene, benzo(k)fluoranthene, benzo(g,h,i)perylene, dibenz(a,h)anthracene and indeno(1,2,3-cd)pyrene except in the Estevan Sound sample where LMW are Phe, Ant and HMW are BaA, Chr, benzofluoranthenes and triphenylene. Blanks (-) indicate that no value was given.

Site	Impact	LMW	HMW	Total	Ref.
	Fresh water sites				
New York Lakes	No	0.02-0.04	0.06-0.22	-	1
Lake Washington	Yes	3.2-190	25-990	28-1200	2
	Marine sites				
Puget Sound, WA	No	.003-.055	.022-0.14	-	3
Puget Sound, WA	Yes	.001-630	.008-3200	-	3
Estevan Sound, B.C.	No	.003-0.01	.003-.016	.027-.068	1
Burrard Inlet:					
Outer Harbour	Yes	0.31-0.79	1.13-3.04	1.45-3.83	4
Inner Harbour	Yes	0.32-7.51	0.71-14.03	1.03-17.42	4
Pt. Moody Arm	Yes	0.71-5.64	2.23-31.97	2.94-36.73	4
False Creek	Yes	0.70-18.7	2.4-61.5	3.1-80.2	5

The water phase concentration of PAH is also affected by the presence of DOM. Although uptake of PAH by aquatic organisms is hindered by association with DOM, an equilibrium between the truly dissolved and DOM-associated phases is reached more rapidly than between the sediment and water phases. Thus the PAH "solubilized" by association with DOM can become available more readily than when they are bound to sediment (McElroy et al., 1989; Maxin and Kogel-Knabner, 1995).

Uptake of PAH by most aquatic organisms is from the water phase, although particulate food may be an important exposure route for some filter feeders. Even those organisms that are exposed to PAH by feeding on contaminated particles acquire contaminants at a greater rate from the water phase (Landrum, 1989). The overall order of uptake is thus water > food > sediment (Nagpal, 1993).

2.1.5 Toxicity

The toxicity of a pollutant to a particular organism depends on the concentration of the contaminant at the target organ or biological system. In higher organisms the tissue concentration depends on the relative uptake and depuration rates. In the case of PAH, accumulation typically correlates with lipid content, reproductive status, behaviour and the ability to metabolize PAH (McElroy et al., 1989). Bioconcentration usually occurs such that the tissue concentration in exposed organisms is much higher than the water concentration, however higher organisms can metabolize PAH. Metabolism has been observed in polychaete worms, crustaceans and mollusks, although at much lower levels than in fish and higher animals (NRC, 1983). Biomagnification, in which organisms higher on the food chain acquire greater burdens of pollutant through the consumption of contaminated food, does not seem to occur. This is likely to be due to the ability of higher organisms to rapidly metabolize and excrete PAH (McElroy et al., 1989; NRC, 1983). Typically the PAH concentration is highest in the sediment, medium in biota and lowest in the water column.

Table 2.3 shows the tissue/sediment and tissue/water ranges for PAH with 3, 4 and 5 rings.

Table 2.3 PAH tissue to sediment and water ratios
Tissue to sediment ratios were taken from McElroy et al., 1989, and tissue to water ratios from Nagpal, 1993.

	Number of Rings		
	3	4	5
Tissue/Sediment	0.02-0.05	0.08-0.5	0.04-0.2
Tissue/Water	10-1000	10-670	10-2560

The acute toxicity of PAH is thought to be primarily due to membrane-level effects (Sikkema et al., 1995). Association of PAH with membranes causes changes in membrane

fluidity and permeability. Fluidity changes affect protein movement and function, the formation of multicomponent systems, and can alter specific protein-membrane lipid interactions. Permeability changes can dissipate proton or other electrolyte gradients across the membrane which are required for energy transduction and transmembrane transport. It can also lead to leaking of cellular components and the establishment of futile cycling (Sikkema et al., 1995). Short-term lethal concentrations (LC_{50}) to invertebrates are in the 0.3-5.6 parts per million (ppm) range. The egg and larval stages are more sensitive than juveniles and adults. For fish, 24-96 hour LC_{50} values range from 1-100 ppm, although the juvenile and larval stages are more susceptible. Simultaneous exposure to UV radiation and PAH increases the toxicity of these compounds, endangering young fish which usually reside in shallow waters (Albers, 1995; Nagpal, 1993). For HMW PAH, the lethal concentration is usually higher than the aqueous solubility of the compounds, however they can have sublethal or chronic effects. Typically, sublethal effects are observed in the parts per billion (ppb) range.

The greatest concern about PAH is that some of the 4-6 ring PAH are proven carcinogens, mutagens and teratogens (compounds which interfere with the normal development of embryos). In fact, benzo(a)pyrene was the first chemical to be linked to cancer when the high incidence of some cancers in chimney sweeps was investigated. Ironically, the cellular biochemistry that is required to metabolize PAH for excretion also renders some of them mutagenic. Cytochrome P_{450}-like monooxygenases form reactive epoxides at certain ring positions which can bind to biological macromolecules like DNA and protein (Cerniglia, 1984; Hallet and Brecher, 1984; Neff, 1979). In the aquatic environment, correlations have been drawn between the incidence of tumours or neoplastic disorders in resident bivalve mollusks, salamanders and fish, and sediment PAH concentrations or chronic exposure to PAH (Nagpal, 1993; Schiewe et al., 1991; Malins et al., 1988; Mix, 1984). In Burrard Inlet, idiopathic neoplastic and preneoplastic liver lesions in bottom fish were correlated with sediment PAH concentrations (Goyette and Boyd, 1989).

PAH have been observed to have many other effects. In plants, 2-3 ring PAH can interfere with cell division or even cause death. Sublethal effects on invertebrates include changes to reproduction, embryo or larval development, emergence, respiration rate, heart rate, and blood chemistry. Lesion formation may also be observed. In fish, reptiles and amphibians cancerous and non cancerous tumours have been observed as well as reproductive effects.

Birds have not been studied very intensively, but embryo effects have been observed in exposed eggs. In mammals, lethal and acute toxic effects, immune responses and cancerous and non-cancerous neoplasms occur (Albers, 1995; White, 1986).

2.2 Biodegradation of PAH

Until the mid 1980s, it was generally believed that PAH could not be degraded in the absence of oxygen. The only known mechanisms for the catabolism of aromatic hydrocarbons involved the incorporation of hydroxy substituents onto the ring structure through the action of mono- or dioxygenases. (Cerniglia, 1984).

Most of the early research on the metabolic pathways of PAH degradation was performed in mammalian tissues to better understand the links to cancer. It was later found that PAH could be transformed by fungi, yeast and bacteria. Typically, transformations carried out by higher organisms render the PAH more hydrophilic to facilitate excretion. In bacteria, transformations usually lead to the incorporation of degradation products or mineralization of the PAH. In some cases dead-end products may be formed (Cerniglia, 1984).

In mammalian systems, the initial transformations result in the addition of hydroxyl groups to the ring structure by cytochrome P450 monooxygenases. These reactions yield *trans*-dihydrodiols, which can be further metabolized or conjugated prior to excretion (see Figure 2.2). Fungi also use monooxygenases to add hydroxy residues to adjacent carbons in the aromatic ring, and similarly give rise to *trans*-dihydrodiols (Cerniglia, 1984). Some fungi can use extracellular enzymes such as lignin peroxidase to oxidize higher molecular weight PAH, but such reactions may be inhibited by the presence of soil (Muncnerova et al., 1994; Davis et al., 1993).

2.2.1 Aerobic degradation of PAH by bacteria

In contrast to the mammalian and fungal systems, most bacteria that are capable of degrading PAH aerobically use a pathway that involves the incorporation of both atoms of molecular oxygen onto a ring by a dioxygenase, giving rise to a *cis*-dihydroxy product. This reaction is carried out by a multicomponent enzyme system in which the terminal oxygenase is an iron-sulphur protein. There are some exceptions to this rule. *Trans*-dihydroxy degradation products have been detected in culture extracts from some bacteria fed HMW PAH (Smith,

1994). Both the dioxygenase and monooxygenase degradation pathways for naphthalene are shown in Figure 2.2.

Most of the research on the degradation pathways of PAH has been carried out on LMW compounds. The research on HMW PAH is largely limited to the initial attack on the ring with the rest of the pathway being predicted rather than proven (Li et al., 1996a, 1996b). Nevertheless, some patterns have emerged about both HMW and LMW PAH degradation. The first stage in the degradation of aromatic hydrocarbons is the preparation of the ring for cleavage. This involves the removal of substituents, if present, to yield a central aromatic substrate. Then, one or two hydroxyl residues are added to adjacent carbons in the ring to destabilize the aromatic structure prior to cleavage by another dioxygenase (Hopper, 1991; Harayama and Timmis, 1992). Cleavage of the ring may occur by the *ortho* (cleavage between the hydroxy groups) or *meta* (cleavage adjacent to the pair of hydroxy groups) pathway. HMW PAH tend to undergo ring cleavage by the *meta* pathway, and are degraded one ring at a time (Smith, 1994).

The naphthalene degradation pathway is typically plasmid encoded and the ability to degrade PAH can be acquired and lost with relative ease in the environment. Regulation of gene expression is usually by induction by the substrate (PAH) or metabolites (Yen and Serdar, 1988). Uptake of naphthalene has been shown to be passive, and unrelated to the presence of the degradative plasmid (Smith, 1994). Genes for the degradation of phenanthrene and anthracene have also been shown to be plasmid encoded. The pathways show some similarity to those for naphthalene degradation, and in some but not all cases, may share enzymes (Sanseverino et al., 1993).

In the marine environment, hydrocarbon degradation is thought to be carried out primarily by bacteria. As with other substrates, PAH degradation by bacteria is optimal within a certain concentration range. At very low levels, the rate of degradation is low due to substrate limitation. At high concentrations, the rate of degradation may be depressed by oxygen or nutrient limitation, or by toxicity of the substrate (Leahy and Colwell, 1990).

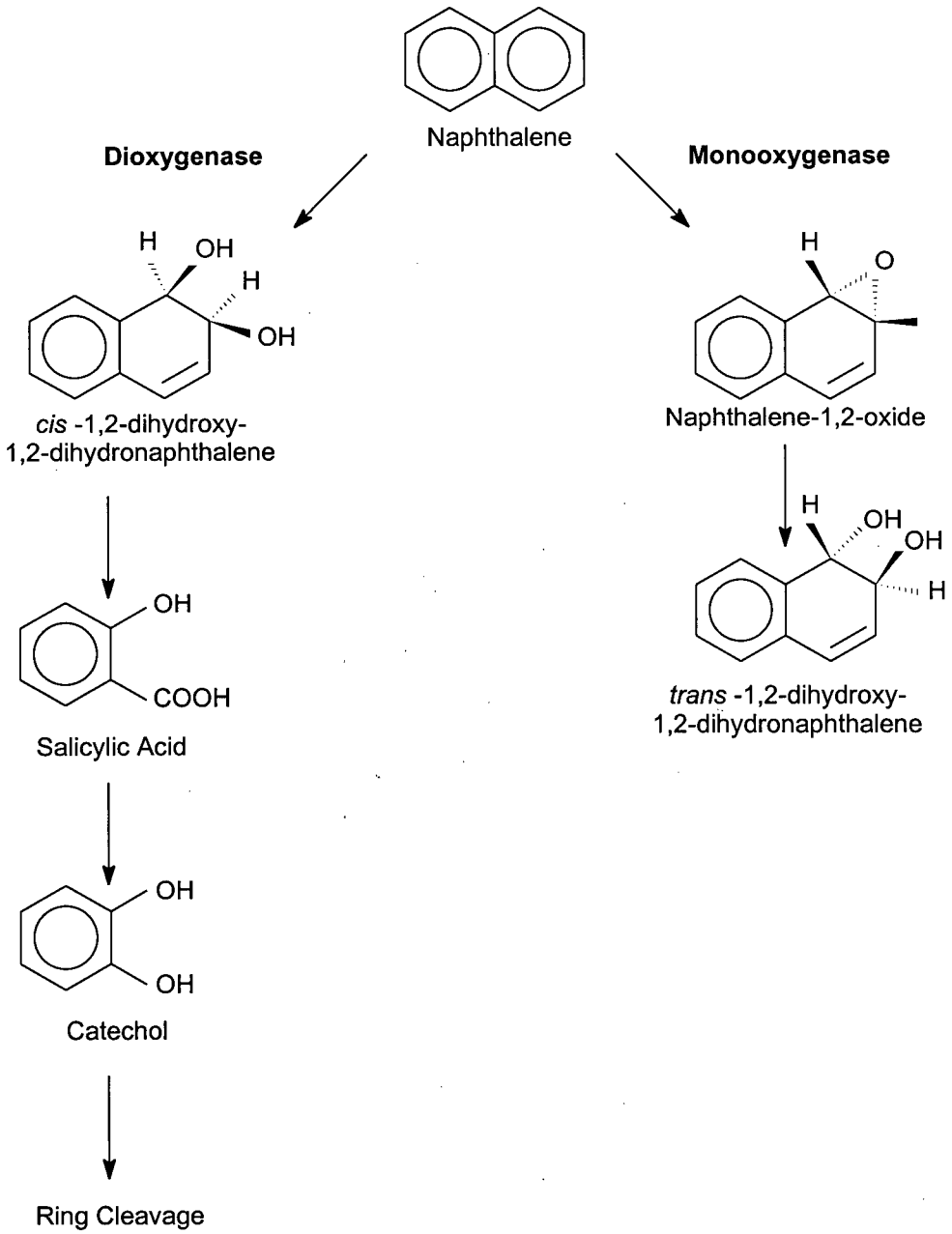


Figure 2.2 Aerobic PAH metabolism
Both the dioxygenase (left) pathway usually used by bacteria, and monooxygenase (right) used by fungi and higher organisms are shown. It is the epoxide formed from some PAH by monooxygenases that can react with biological macromolecules to cause mutations (adapted from Cerniglia, 1991).

Substrate interactions have been observed in some cases. Bauer and Capone (1988) found that the degradation rates of PAH were similar regardless of whether they were added separately or as part of a mixture. In contrast, Millette et al. (1995) found that the presence of p-cresol inhibited phenanthrene mineralization, and Stringfellow and Aitken (1995) observed that phenanthrene degradation was inhibited by naphthalene, methylnaphthalene and fluorene. Inhibition may be due to toxicity, substrate preferences, use of a common transport mechanism, or a common oxidase. Conversely, in a soil system, the half lives of PAH were shorter when complex mixtures (creosote or oil refinery waste) were added to the soil than when PAH were added singly or in a synthetic mixture containing only PAH. Since the difference in half lives was most pronounced for some of the HMW compounds, the authors suggested the effect could have been due to cometabolism (Keck et al., 1989). Alternatively, there could have been increased bioavailability due to other chemical constituents or biological factors such as surfactants in the complex wastes.

2.2.2 Anoxic degradation of monoaromatic compounds

The research on the biochemistry of anaerobic degradation of aromatics to date deals only with monoaromatic hydrocarbons. Since the late nineteen eighties, there have been numerous reports of the degradation of monoaromatic hydrocarbons under denitrifying (e.g., Major et al., 1987; Kuhn et al., 1988; Gersberg et al., 1991; Hutchins et al. 1991; Hutchins, 1991, Ball and Reinhard, 1996) and sulphate reducing (e.g., Beller et al. 1992; Edwards et al., 1992, Ball and Reinhard, 1996) conditions. Aromatic compounds with oxygenated substituent groups can also be degraded under fermentative, methanogenic, and anaerobic photometabolic conditions (Fuchs et al., 1994).

Similar principles hold true for anaerobic and aerobic metabolism of aromatic substrates: a diverse substrate pool must be converted to a number of central intermediates which can be channeled through common degradative pathways, and the aromatic structure of the substrate must be destabilized before cleavage. Although there are some facultative anaerobes that use oxygenases to introduce hydroxy residues onto the aromatic ring, reductive pathways seem to be more common. This indicates a very different mechanism of degradation under anaerobic conditions (Fuchs, et al., 1994).

Carboxylations, anaerobic hydroxylations or thioester formations are carried out to produce activated central intermediates. Central intermediates can be attacked by reductases to yield aliphatics which are converted to central metabolites. Reduction of the central intermediate is strongly endergonic. One of the following conditions is probably required to overcome the high energy requirement of this reaction:

(1) a four electron reduction,

(2) a two electron reduction accompanied by hydration of the diene or some other energetically favourable reaction,

(3) two sequential two-electron transfers where the intermediate is not free, or

(4) a partial dearomatization of the ring before the reduction so the diene is resonance stabilized by functional groups (Fuchs, et al., 1994; Evans and Fuchs, 1988; Berry et al., 1987).

The degradation pathway for Benzyol-CoA is shown in Figure 2.3 as an example of aromatic ring cleavage under anaerobic conditions.

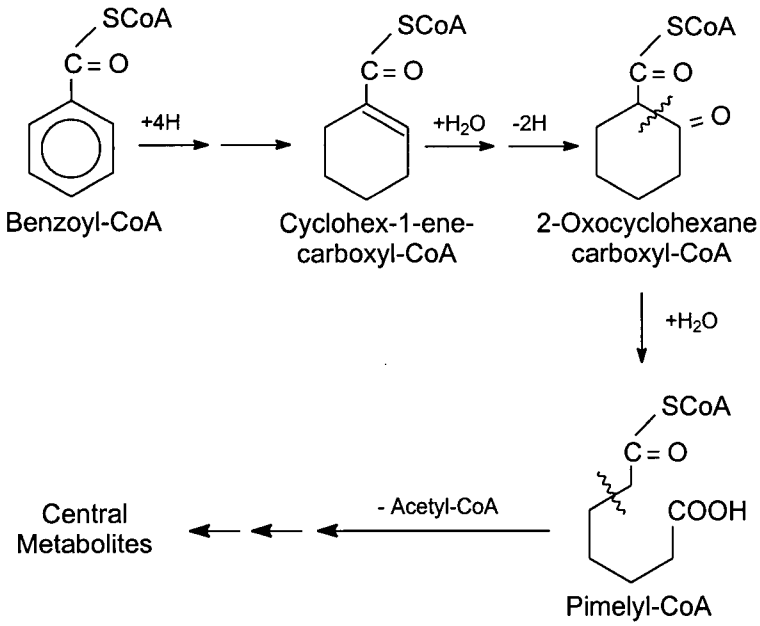


Figure 2.3 Anaerobic ring cleavage
Benzyol-CoA degradation is shown. The thioester is formed first to destabilize the ring structure. Adapted from Fuchs, et al., 1994.

2.2.3 Anoxic degradation of PAH

Thermodynamically, the degradation of PAH under denitrifying conditions should yield nearly as much energy as the degradation under aerobic conditions (MacFarland and Sims, 1991). The ability of a given population to do so, however, depends on the ability of the microorganisms to harness the energy released in the chemical breakdown of the substrate. The growth yield of microorganisms using simple substrates under denitrifying conditions can be 40% less efficient than under aerobic conditions, despite similar theoretical energy release as determined by half cell reactions (Stouthammer et al., 1982). Predictions of degradability based on thermodynamics should be viewed as an upper ideal since no predictions about the rates at which such reactions occur can be made using the half cell reactions.

There have been numerous cases in which the degradation of PAH was not observed under denitrifying conditions (e.g., Bauer and Capone, 1985; Nielsen and Christensen, 1994). Whether these failures were a result of some other limitation (inadequate microbial population with the ability to degrade PAH under the test conditions, nutrient limitations or toxicity) or insufficient time for the reactions to get underway is not clear. In recent years there have been a few reports in which PAH were shown to degrade in the absence of molecular oxygen. These studies are described below.

In 1988, Mihelcic and Luthy reported on the degradation of naphthalene, acenaphthene and 1-naphthol under denitrifying conditions. They incubated soil-water slurries with media containing PAH concentrations between 1-10 mg/L. Once the reversibility of PAH adsorption to the soil was established, loss of parent compound and nitrate were measured. Carbon dioxide evolution from the oxidation of soil natural organic carbon was also measured. The conditions of the experiment were such that readily oxidizable soil organic content was similar to that of the added PAH. Degradation of PAH was faster under aerobic conditions than denitrifying conditions, but naphthalene and acenaphthene were both degraded under conditions of nitrate reduction. With nitrate present in excess, the ammonia and nitrite levels did not rise, and the sulphate concentration remained stable.

They found nitrate reduction began immediately but degradation of PAH occurred only after a lag phase, which increased with the length of time the soil had been stored prior to use. The lag phase was eliminated by using soils that had previous exposure to the test substrates and conditions. Several observations were made regarding the factors that affected degradation

of PAH: the nitrate concentration had to be in excess of requirements for PAH degradation since soil organic matter was more easily degraded than PAH, a sufficiently large and active PAH-degrading population had to be established, and the PAH had to be readily desorbed from the soil. The maximum rate of naphthalene degradation, established in a later experiment using acclimated soil from which most of the readily usable organic content had been depleted, was $9x10^{-3}$ mg/g•day. This rate was dependent upon the soil to water ratio, since the aqueous concentration of PAH decreased as more soil was added to the system (Mihelcic and Luthy, 1991).

Al-Bashir et al. (1990) studied the degradation of naphthalene in soil-water slurries at much higher concentrations than in the Mihelcic and Luthy studies described above. Two soils were used: one was a mixture of pristine (90%) and contaminated (10%) soils, and the other was 100% contaminated soil. Radiolabelled substrates were used and CO_2 formation was monitored over the 160 day incubation period. The temperature was maintained at 35 ^{o}C. Very high nitrate concentrations were added (3300 mg N/L) and the concentration of PAH added to the system was 50, 200 and 500 mg/kg for the clean soil and 200 mg/kg for the contaminated soil. After an 18 day adaptation period, the maximum rate of mineralization when 50 ppm naphthalene was added was 1.3 ppm/day. In the 200 and 500 ppm additions, the maximum rate was 1.8 ppm/day for the first 50 ppm, and then the rate decreased exponentially. Similar rapid degradation was observed in the contaminated soil initially, but it slowed down earlier and proceeded more slowly than in the pristine soil. The authors speculated that lower aqueous phase naphthalene concentration, a rapid increase in pH, toxicity, or substrate interactions could account for the lower degradation rate in the contaminated soil.

In another study (Leduc et al., 1992), the degradation of acenaphthylene, acenaphthene, fluorene and anthracene added to soil-water systems at 100 mg/kg soil dry weight was examined. Nitrate was added at 813 mg N/L and loss of PAH from the soil was monitored. Samples were agitated continuously in one set of experiments, and 3 times/day for 20 seconds at 120 rpm in the second set. Zero order kinetics was assumed and the reactions were followed for 120 days. No degradation of PAH was observed under methanogenic or sulphate reducing conditions. PAH were degraded in the presence of oxygen or nitrate, but more quickly in the former case. The authors also observed large abiotic losses in the aerated samples, presumably

due to volatilization. The biodegradation rates ranged from 0.29-0.37 ppm/day under denitrifying conditions.

The above experiments were all carried out on soil/water slurries. The properties of soil organic matter are slightly different than those of bottom sediments. Polluted soils and sediments also have different adsorptive characteristics than pristine ones (Kile et al., 1995). The organic matter composition will therefore affect PAH degradation, since the degradation rate is often limited by desorption.

Langenhoff et al. (1996) conducted an investigation using 60 mL flow-through sediment/soil/sludge columns under various redox conditions. Media containing naphthalene, benzene and toluene were pumped through the columns at 3.5 mL/hr and the effluents were monitored for up to 520 days. Under denitrifying conditions using 140 mg N/L, naphthalene was consistently removed when benzoate was also present in the media. By the end of the experiment, up to 70% of the influent naphthalene was removed upon passage through the column. In this case all the naphthalene (3.2 mg/L) was added in the water phase. There was no information on the column material (solids concentration and organic content), and the nitrate concentration in the effluent was not monitored. They speculated that the benzoate might have acted as an inducer, primary substrate, or electron donor in the reduction of the aromatic ring.

Research has been done on PAH degradation in freshwater sediments by Murphy et al. (1995). In laboratory experiments conducted using sediment from the Dofasco Boatslip in Hamilton Harbour, which contained more than 2000 mg/kg dry weight total PAH, the addition of nitrate caused a 68% loss of total PAH in 197 days. All of the measured PAH (the same as those in Figure 2.1 plus benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(ghi)perylene, dibenzo(a,h)anthracene, and indeno(1,2,3-cd)pyrene) were degraded at approximately equal rates, so there was no enrichment of the larger compounds after treatment. Laboratory experiments using sediments from other sites in the Toronto area gave 78 and 82% decreases in PAH after 150 and 44 days respectively, when calcium nitrate and a proprietary organic amendment were added.

In *in situ* field applications of nitrate, the degradation rate was much slower than in the laboratory experiments (52% loss of total PAH in the part of the test area that was efficiently treated with four applications over two years). However, PAH degradation was only observed

after the last treatment which included the addition of the organic amendment with the nitrate. Sampling was undertaken in the spring after a fall treatment, so temperature conditions were probably not optimal for degradation. Other reasons for slower PAH degradation in the field include lower than expected nitrate concentrations due to dilution, sinking of the dense salt solution, and higher than predicted nitrate consumption due to degradation of methane formed in the sediment below the treated area.

The most surprising factor to note in the research carried out by Murphy et al. is that the HMW and LMW PAH were degraded equally well. This is unusual since the degradation rates of PAH usually correlate with their solubility in water (see Wild and Jones, 1993; Figure 5.1). Since the HMW PAH are less soluble, they are usually degraded more slowly than the LMW compounds.

2.2.4 Population effects

Many researchers have noted that PAH degrade faster and with shorter or no observable lag periods in soils and sediments that have had previous exposures to PAH. This can be attributed to the establishment of a sizable community of microorganisms that can use the PAH as growth substrates (e.g., Herbes and Schwall, 1978; Heitkamp et al., 1987). However, an increased PAH degrading community does not necessarily correlate with a larger overall heterotroph population (Massie et al., 1985). It also appears that some microorganisms that use one or more PAH as carbon and energy sources can transform other PAH by cometabolism (e.g., Weissenfels et al., 1991). In a mixed population this can lead to the complete mineralization of the cometabolized compound, since other organisms in the environment may use the products of the cometabolic transformations.

In pure culture, some organisms that can mineralize PAH have been found to produce transient intermediates which are degraded fully when the parent compound has disappeared. This effect is less pronounced, but may still be observable in mixed cultures, and is most apparent when the PAH is supplied at high concentration. (Mueller et al., 1989; Guerin and Jones, 1988).

Environmental conditions also affect the ability of microorganisms to degrade PAH. Communities that are chronically exposed to PAH are usually adapted to or tolerant of the conditions they usually encounter. Estuarine microorganisms can efficiently degrade PAH

under a wide array of salt regimes. Microorganisms that are usually only exposed to fresh or sea water are adversely affected by high or low salt concentrations, respectively (Kerr and Capone, 1992). Similar effects would be expected for other parameters such as temperature and pH.

2.3 Availability of organic contaminants

The degradability of PAH and other compounds is not solely limited by the ability of microorganisms in the environment to use them. Substrate availability is also an important factor. In aquatic environments, hydrophobic pollutants usually become associated with sediments where they can become highly enriched. Since uptake of contaminants by animals and microorganisms is generally from the aqueous phase, sediment-contaminant interactions are important determinants of toxicity and degradability. The correlation between PAH degradation and aqueous solubility has been reported by a number of researchers (e.g., Wild and Jones, 1993; Boldrin et al., 1993; Bossert and Bartha, 1986). Cullen et al. (1993) found that the degradation of solid phenanthrene was slower than when substrate was added in acetone solution or solubilized with the surfactant TWEEN. In a study by Weissenfels et al. (1992), PAH that were strongly bound by soil particles or XAD2 resin were not degraded, and elutriates of the soil were not toxic. PAH were degraded in another soil to which PAH were weakly bound, and elutriates from the latter soil were toxic in a MICROTOX assay. Thus degradability and toxicity of the PAH were linked to their availability.

The degradation of these and other hydrophobic compounds, or their introduction into the food web, therefore depends on the chemistry and physics of adsorption to and desorption from the solid phase.

2.3.1 Association of hydrophobic contaminants with sediments

The rate of dissolution of hydrophobic organics depends on the difference between the equilibrium concentration and the actual concentration in the pore water which surrounds the sediment particles. The surface area between the particulate and aqueous phases is also important (Sikkema et al., 1995). The mineral component of sediment acts as an adsorbent which is particularly important in the fate of charged or polar molecules. Hydrophobic compounds partition into the organic matter associated with the sediment. Chiou et al. (1979)

demonstrated that PAH associated with sediment organic matter, and Karikhoff et al. (1979) and Means et al. (1980) showed that PAH sorption to sediments and soils varied with the organic content of the solid phase and inversely with the solubility of the compound. The PAH degradation rate also decreased with increasing soil organic content in experiments carried out by Manilal and Alexander (1991).

The composition of the organic material is also important in determining the fate of hydrophobic compounds. Kile et al. (1995) studied the characteristics of the organic carbon content (OC) of soils and sediments from a large number of sites in China and North America. They found that the OC normalized partition coefficients (K_{oc}) for their test compounds, carbon tetrachloride and 1,2-dichlorobenzene, were remarkably similar among uncontaminated soils and among clean sediments. Sediment OC was more hydrophobic than soil OC. They speculated that the more polar components of soil OC might be carried away in the water phase when they reach the aquatic environment. Sites contaminated with hydrocarbons or chlorinated hydrocarbons had very high K_{oc} values, so at equal organic contents, much more material was associated with the solid phase in polluted sites than in pristine locations. Indeed, Chin and Gschwend (1992) found that there was a preferential association of PAH with the oily residue on polluted soils or sediments compared with natural organic carbon or humic material found in uncontaminated samples.

2.3.2 Effect of non aqueous phase liquids

Non aqueous phase liquids (NAPLs) are often present in soils that have been contaminated with solvents from leaking underground storage tanks, for example. The solvent remains as a discrete phase rather than dissolving in the aqueous phase. Other contaminants may partition into the NAPL, decreasing their aqueous concentrations, and hence their availability for degradation. Sometimes microorganisms are efficient at scavenging material from NAPLs, but only if the NAPL is non-toxic, not degraded preferentially, and does not associate too strongly with the substrate (Effroymson and Alexander, 1995). The oily residues on polluted soils or sediments might more accurately be considered NAPLs than soil or sediment organic carbon, since the nature of the residue is not the same as that of natural organic matter. Residual (weathered) oil is roughly 10X more effective as a partition medium than natural organic matter (Morris and Pritchard, 1994).

Effroymson and Alexander (1994) carried out experiments on phenanthrene degradation in the presence of NAPLs. They determined phenanthrene partition rates out of NAPLs in abiotic systems, and mineralization rates in experiments with bacterial isolates or enrichment cultures in water-NAPL systems. In the absence of soil, the mineralization rate was higher than the abiotic partition rate, indicating that the bacteria were able to increase the partitioning rate out of the NAPL or to take up the substrate directly from the NAPL. When soil was added to the system, the mineralization rate decreased to below the spontaneous dissolution rate. Since the organic content of the soil was low and nutrients had been supplied in the media, the authors speculated that the decrease in degradation rate might have been due to attachment of the bacteria to soil where diffusion is limited, inhibition of biosurfactant production, binding of biosurfactant to the soil, grazing by protozoa, or some other soil-associated inhibition that slowed growth.

In experiments carried out at low aqueous phenanthrene concentrations (Effroymson and Alexander, 1995), the rate of degradation fell below the spontaneous dissolution rate, and was much lower than was predicted from losses at higher concentrations. The authors concluded that there is a threshold dissolution rate that is necessary for maintenance of microbial activity, and at concentrations near that threshold, the rate of degradation is decreased. The presence of a NAPL, when the contaminant concentration is low, can thus lower the aqueous concentration below the threshold so the actual degradation rate is much lower than would be predicted from research done at higher aqueous concentrations.

2.3.3 Characteristics of desorption

A number of investigators have found that the loss of organic contaminants from soils or sediments is biphasic: a period of rapid dissipation is followed by longer and slower release. In the first stage, volatilization, leaching, and biodegradation occur rapidly. In the second stage, these processes are limited by transport from the solid matrix (Beck et al., 1995). Slow diffusion within particles may be due to entrapment in pores, hydrogen bonding, and multiple bonds per contaminant molecule with the natural organic matter (Hatzinger and Alexander, 1995; Pignatello and Xing, 1995).

Two different theories have been proposed to explain this limited transport stage: sorption-retarded pore diffusion (SRPD) and organic matter diffusion (OMD). In both cases,

the diffusion rate is limited by the nature of the interactions between the contaminant and its environment. In the SRPD mechanism, contaminant diffusion is impeded by interactions with the surfaces and the ordered water in the film layer adjacent to the surfaces. In the OMD theory, the contaminant is thought to be dissolved in the (solid phase) organic matter and diffusion is limited by interactions with this medium. The bulk of the evidence with hydrophobic contaminants points to the latter mechanism, however both kinds of interactions are likely to occur, and in environments with little organic matter, SRPD may play a more important role (Pignatello and Xing, 1995).

The form of the limitation will affect what can be done to increase the desorption rate. If SRPD limits desorption, then the rate can be increased by grinding the material to decrease the size of the particles. In the case of OMD, the rate of diffusion can be increased by swelling the natural organic matter. This can be achieved by altering the pH, raising the temperature, and changing the hydrophobicity of the medium with surfactants or cosolvents. Brusseau et al. (1991) found that the addition of methanol to the water phase, which lowered the polarity of the medium, increased the rate of diffusion of PAH out of soil organic matter once a critical cosolvent level was reached.

In research carried out by Cornelissen et al. (1997) desorption from the solid phase was better described by a three compartment model. Fast desorption occurred first, followed by slow desorption, then very slow desorption. The fraction in each compartment depended on the hydrophobicity of the sorbate and the contact time between the sorbate and the sorbent. Desorption rates were greatly influenced by temperature. The long-term behaviour of a pollutant in a given matrix could therefore be determined rapidly by conducting experiments at high temperatures. The decrease in availability with prolonged contact has been observed by many researchers (e.g., Sandoli et al., 1996; Carmichael et al., 1997).

2.3.4 Effects of pore water characteristics

Organic contaminants in the dissolved, adsorbed, and occluded states have been discussed above. They may also be incorporated into micelles or colloidal suspensions (see Figure 2.4). Contaminants associated with dissolved organic matter (DOM) or colloidal material are less available for degradation or to exert toxic effects than in the truly dissolved phase (McElroy et al., 1989; Volkering et al., 1995). Desorption from the pseudo-dissolved

(DOM) phase is not affected by slow desorption mechanisms, so this represents a more readily exchangeable contaminant pool than that associated with the solid phase.

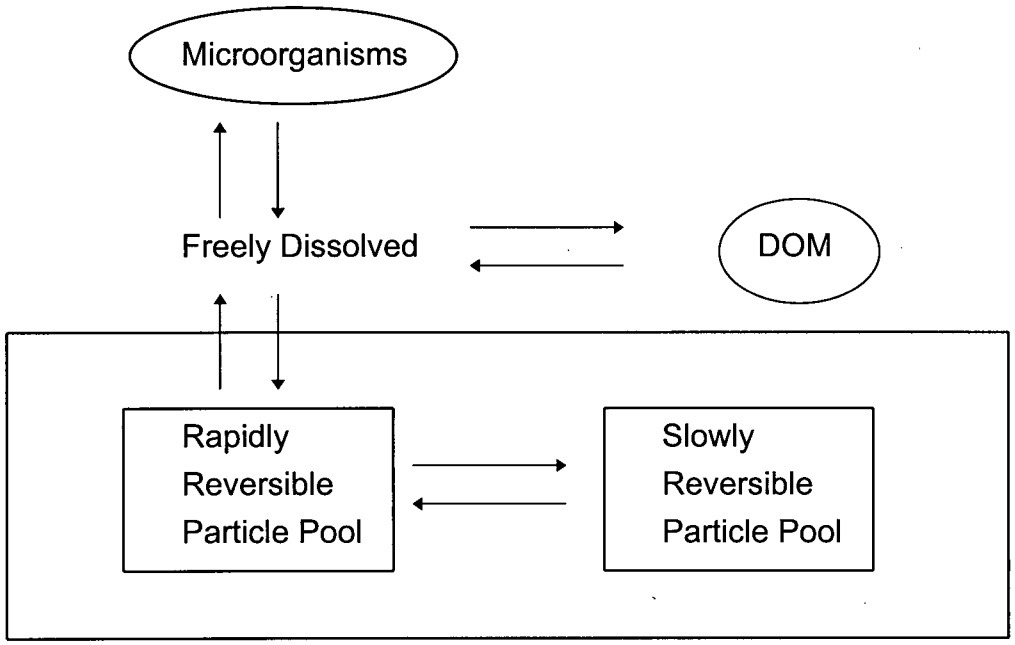


Figure 2.4 PAH partitioning
This figure shows the various phases where PAH can be found. Dissolved organic matter (DOM) may be humic material, marine colloidal material, surfactant micelles or other emulsions. The slowly reversible particle pool may be the portion of PAH associated with soot (which could also be the very slowly reversible pool), or material that has been in contact with the sediment long enough to form multiple interactions.

In 1984, Wijayaratne and Means published two studies on the effects of the presence of marine colloidal material on the partitioning of organic contaminants. These marine colloids are made of carbohydrate-protein matrices with crystalline clay and trace metals, and are 70 to >90% organic, depending on the time of year. In the first study, a higher percentage of pesticide was found in the pseudo-aqueous phase when colloidal material was present than in the absence of such material. In the second study, they established that naphthalene and anthracene partitioning into colloidal material was 10 times higher than into sediment organic material on an organic carbon basis. They speculated this effect might be due to organic matter occlusion by the mineral component of the sediment. In contrast to these results, Luers and ten Hulscher (1996) observed similar affinities of DOM and particulate organic matter for PAH. In their experiments, the DOM was prepared by concentrating leachate from the soil, so the

composition should have been similar to that of the soluble component of the soil organic matter. The difference in affinity observed by Wijayaratne and Means (1984) might therefore have been due to differences in the composition of the DOM and the particulate organic matter in their system. Maxin and Kogel-Knabner (1995) also observed that the composition of the DOM affected PAH binding.

Hegeman et al. (1995) also observed that the concentration of PAH in the aqueous phase increased in the presence of colloidal and dissolved organics. Furthermore, they determined that the influence of salt concentration on the apparent partition coefficient (and the extrapolated true partition coefficient which reflects the truly dissolved fraction), was small. Luers and ten Hulscher (1996) found that as the temperature increased, PAH association with the DOM decreased.

Organic contaminants can associate with humic materials in several ways. Most are reversible, weak interactions (e.g., hydrogen bonding), but in the presence of light, oxidants or aerobic alkaline conditions, they may be covalently bound. In these cases, they essentially become part of the humus (Senesi, 1992, Bollag et al., 1992).

2.3.5 Surfactant effects

Surfactants, or surface active agents are amphipathic molecules; that is, they are made up of a hydrophobic and a hydrophilic moiety. This characteristic allows them to form micelles in which a stable pseudo-hydrocarbon phase is contained within a hydrophilic exterior. Micelles are formed at or above the critical micelle concentration (CMC) which is characteristic of each surfactant. Below the CMC, surfactant monomers are dissolved and tend to migrate to interfaces or surfaces, lowering the interfacial or surface tension. Above the CMC, surfactant monomers exist in equilibrium between the micelle and dissolved phases. Hydrophobic compounds can partition into micelles and increase the apparent concentration in the water phase above the aqueous solubility (Rouse et al., 1994).

Biosurfactants are sometimes produced by microorganisms. These are typically high molecular weight complexes of polysaccharide, fatty acids and proteins which might originate in the outer membrane or from capsule material. The production of biosurfactants has been connected with growth on water-insoluble substrates, however, biosurfactants are not always formed, nor are they exclusively produced in the presence of such substrates (Hommel, 1994).

Guerin and Boyd (1992) found that of the two microbial isolates they studied, only one was capable of using sorbed naphthalene as a substrate. This organism might have produced a biosurfactant to make the material available. Alternatively, it might have been able to move along the surface of the particles in response to local elevated levels of substrate (chemotaxis). Stringfellow and Aitken (1994) also studied a microorganism with a hydrophobic cell surface that could mineralize phenanthrene. This organism colonized phenanthrene crystal surfaces and decreased the surface tension of the media, but the authors were unable to isolate a surfactant from the culture media.

Surfactants have been added to soils and sediments to increase the rate of biodegradation because they can improve the availability of pollutants. For example, Mueller et al. (1990) found that the rate of fluoranthene degradation by a bacterial isolate was limited by the substrate's solubility, but was increased upon surfactant addition. However, the impact of surfactant addition on availability and degradability is not always straightforward. Sometimes the addition of surfactants can be inhibitory due to toxic effects of the contaminant or the surfactant, usually at the membrane level. Preferential use of the surfactant as a carbon source, or inhibition of surface contact between the microorganism and the substrate (when that is required), can also hinder biodegradation (Tiehm, 1994; Rouse et al., 1994). In some cases surfactants may sequester the substrate inside micelles, lowering the aqueous concentration that is available for biodegradation (Mihelcic et al., 1993). When the substrate itself is toxic, surfactant addition above the CMC can lower the true aqueous concentration to a level that is nontoxic to the microorganisms while maintaining it in a pool that is easily exchangeable with the water phase (Volkering et al., 1995).

Some researchers have stated that the increased contaminant concentration in the water phase by partitioning into micelles is the mechanism by which surfactants stimulate biodegradation. Stimulation of biodegradation has also been observed at concentrations below the CMC, so they must also act by some other mechanism. Surfactant monomers probably facilitate transport at the interface by lowering the surface tension (Aronstein and Alexander, 1993; Tiehm and Fritzsche, 1995; Liu et al., 1995). This hypothesis is supported by research using surfactants bound to solids as well as free surfactant to separate the sequestering function from the facilitated interfacial transport function. Degradation was much higher in samples exposed to free surfactant (Yeom, 1996).

2.3.6 Source effects on PAH availability

PAH availability is further complicated by the differences that stem from the source of contamination, or mechanism of PAH formation. In 1984, Readman et al. found that PAH desorption from samples did not agree with their calculated or experimental partition coefficients. PAH were highly enriched in the solid phase. They concluded that a fraction of the material was occluded. Since that material was unavailable for exchange, the PAH in the system did not reach equilibrium. They found that the high PAH concentration was associated with large, low density particles, as opposed to the smaller size fraction with large surface areas, a result which goes contrary to sorption theory. A fraction of these larger particles had the characteristics of charcoal or charred organic material. Larger PAH, which are typically formed during combustion, were relatively enriched in this larger particle size fraction. Schure and Natusch (1983), found that the carbonaceous fraction of fly ash, which was only 1% of the total ash, had a higher affinity for PAH than the bulk ash. In fact, about 2/3 of the PAH formed in the combustion process was associated with that high surface area carbonaceous fraction. The occluded material observed by Readman et al. (1984) might have been associated with this kind of particulate material.

In 1995, McGroddy and Farrington found that marine colloidal material increased the PAH concentration in pore water as expected, but the concentration was not always predictable. In samples from one site, the PAH concentration associated with the solid phase was much higher than predicted. PCBs from the same samples behaved exactly as expected. In another study, McGroddy et al. (1996) carried out desorption experiments in the laboratory and compared their results to sediment and pore water samples taken from the field. Again, PAH from the field samples did not behave as predicted. From these results they concluded that the source of the PAH must have affected their availability. They speculated that PAH from petroleum products are more readily available than soot-associated PAH formed during combustion. Based on their findings, they proposed the use of a factor called the AEP (Available for Equilibrium Partitioning) to predict the behaviour of PAH in sediments. Their theory of PAH occlusion in particulates formed during combustion is supported by work done on gas phase desorption of PAH from soot (Rounds et al., 1993).

Gustafsson et al. (1997) found that desorption of PAH was well predicted by a model which included a separate phase for soot-associated PAH. The soot fraction was measured by

removal of the more labile humic fraction and the inorganic carbon prior to CHN elemental analysis. The partition coefficient was assumed to be similar to that of activated carbon (which is higher than that of natural organic matter). The strong association between PAH and soot could also help to explain the results of a study by Paine, et al. (1996). The environmental impacts of PAH in sediments near an aluminum smelter in Kitimat Arm, B.C. were minimal, despite high sediment PAH concentrations. Much of the PAH contamination came from wet scrubbers on the site and might have been associated with soot.

2.4 Sediments

For many organic contaminants, sediments become the primary repository in the aquatic environment, and contaminants from land or the air often reach the aquatic environment by runoff, leaching, or precipitation. Hydrophobic contaminants are usually quickly adsorbed by particulate matter which eventually settles, so that the bottom sediments have much higher pollutant concentrations than the overlying water column. The sediment may then become a principal source of contaminants to the aquatic food web (Landrum and Robbins, 1990; Burton, 1992). Thus, sediments are important as sources and sinks of contaminants.

Typical organic pollutants in sediments include aliphatic hydrocarbons, monoaromatic hydrocarbons, PAH, chlorinated pesticides, phenols, PCBs, dioxins and dibenzofurans. Some of these are present due to their long term persistence, others are only found in significant concentrations near contaminant sources. Soluble or volatile compounds are not usually present at high concentrations in sediments except near their sources (Forstner, 1989). PAH are found at elevated levels near cities and industrial sources and tend to be persistent.

The biodegradation of organic substrates in sediments tends to be governed by the availability of electron acceptors because sediments are underwater and oxygen is only sparingly soluble. There is commonly a pattern of electron acceptor disappearance that corresponds to the order of free energy release from their reduction. Oxygen is used in the upper layer of sediment, followed by nitrate, Mn(IV), Fe(III), sulphate and carbonate as the dominant terminal electron acceptors in successively lower layers. Fermentation occurs irrespective of the redox potential, as long as oxygen is absent. The rate limiting, energetically expensive hydrolytic reactions occur most rapidly in the aerobic zone, but more and more compounds are being shown to degrade, albeit more slowly, in anaerobic systems.

Dissimilatory nitrate reduction to ammonia, which is a slightly more thermodynamically favourable reaction than sulphate reduction, can also occur in sediments. This will be discussed further in the section on denitrification (section 2.5.2). The succession of electron acceptors is most notable in sediments with a relatively low degradable organic content. In polluted sediments with a great deal of readily available organic material, the predominant metabolic systems are sulphate reduction in marine environments and methanogenesis in freshwater systems, with the rest of the electron acceptors consumed within a short distance from the surface (Jones, 1985).

The fate of contaminants depends also on their solubility and sorption to the solid phase. Cations interact strongly with negatively charged particles and organic content, while acids are repelled by them. Non-polar compounds interact with the organic component of the particles which may be weak for volatile compounds or strong for non volatiles (Forstner, 1989). Silt and clay are the main carriers of metals since they tend to be negatively charged and have large surface to volume ratios. The organic component of the particles sorbs organic contaminants and can form some metal complexes.

The duration of contact between contaminants and the sediment affects extractability and bioavailability. Long contact times result in lower availability. The rates of adsorption and desorption are also not equal. Desorption occurs at a lower rate due to the higher activation energy required (Carmichael et al., 1997; Pignatello and Xing, 1996). For PAH, desorption may be especially slow since the rate of desorption seems to be slower than for other organic compounds with similar octanol-water coefficients, or hydrophobicities (Landrum and Robbins, 1990). Alternatively, this observation could be related to the source of the material as discussed in section 2.3.6. Since most organisms take up contaminants from the aqueous phase, sediments lower the effective toxicity of contaminants. Sorbed materials are also more difficult to access by the microorganisms that can degrade them.

The complexity of the sediment environment and the interactions among the various phases makes it difficult to set adequate criteria for ecosystem protection based on the concentrations of contaminants in bulk sediments. For most organisms, the route of exposure is through the aqueous phase. However, some organisms ingest sediment particles and desorption of pollutants might be different inside the organisms than in the sediment environment. Furthermore, organisms that ingest sediments tend to take up the smaller size fractions which

are often enriched in both metal and organic contaminants. Contaminants rarely occur singly, which complicates the task of setting safe limits for specific chemicals in sediments.

Several different methods have been used to try to address some of these problems. They include measurements of interstitial contaminant concentrations, bulk sediment toxicity, equilibrium partitioning, and ecosystem approaches such as the "apparent effects threshold" and the "sediment quality triad" (Landrum and Robbins, 1990; Burton, 1992). Each method has advantages and disadvantages in terms of cost, reliability and reproducibility, so no single method has been agreed upon by the regulatory agencies.

The province of B.C. has chosen to set sediment quality criteria for fresh and marine water based on the equilibrium partitioning approach. The criteria for the PAH used in this research are given in Table 2.4. The previous interim criteria for B.C. which were based on the Puget Sound Benthic Apparent Effects Threshold (BAET) with a 1/10 factor for protection are also included in Table 2.4. New criteria were only established where sufficient data were available (Nagpal, 1993). The Environment Canada threshold effects levels (TEL) and probable effects levels (PEL), Puget Sound BAET, National Oceanic and Atmospheric Administration (NOAA) effects range (ER) 10th percentile (L) and median (M) values, and Washington Department of Ecology (DOE) sediment criteria (Nagpal, 1993) are also included for comparison. The BAET values (Barrick et al., 1988) were determined based on chemical and biological analyses of samples from Puget Sound and, strictly speaking, are specific to that area. The Environment Canada (Smith et al., 1995) and NOAA (Long et al., 1995) effects levels are based on an evaluation of all the toxicity data available in the literature that met certain requirements. Effects are minimal below the TEL and ERL values and probable above the PEL and ERM concentrations. The Washington DOE criteria are normalized to the sediment organic carbon content.

The wide range in sediment PAH concentrations shown in Table 2.4 illustrates the difficulty in determining "safe" PAH concentrations in sediment.

Table 2.4 Sediment quality criteria and effects levels for PAH
Concentrations are in mg/kg dry weight except Washington DOE which is in mg/kg organic carbon. B.C. criteria are given assuming a 1% OC content. These values must be multiplied by the sediment OC to give appropriate levels for that sediment. Where blanks appear, no criteria were set due to insufficient data. Abbreviations are explained in the text.

	B.C. Criteria		Old	Env. Canada			NOAA		Wash.
PAH	Marine	Fresh	B.C.	TEL	PEL	BAET	ERL	ERM	DOE
Nap	0.01	0.01	0.2	0.035	0.391	2.1	0.16	2.1	99
Acy	-	-	0.06	0.006	0.128	0.64	0.044	0.64	66
Ace	0.15	0.15	0.05	0.007	0.089	0.5	0.016	0.5	16
Flu	0.2	0.2	0.05	0.021	0.144	0.64	0.019	0.54	23
Phe	-	0.04	0.15	0.087	0.544	3.2	0.24	1.5	100
Ant	-	0.6	0.1	0.047	0.245	1.3	0.085	1.1	230
Fla	-	2	0.17	0.113	1.494	6.3	0.6	5.1	160
Pyr	-	-	0.26	0.153	1.398	>7.3	0.605	2.6	1000
BaA	-	0.2	0.13	0.075	0.693	4.5	0.261	1.6	110
Chr	0.2	-	0.14	0.108	0.846	6.7	0.384	2.8	110
BaP	0.06	0.06	0.16	0.089	0.763	6.8	0.430	1.6	99
LMW	-	-	0.5	-	-	6.1	0.552	3.16	370
HMW	-	-	1.2	-	-	>51	1.7	9.6	960
Total	-	-	-	-	-	-	4.022	44.79	-

2.5 Bioremediation of Contaminated Sites

Bioremediation is the use of organisms to destroy or detoxify environmental pollutants. Typically the process uses microorganisms, however plants have also been employed. Microorganisms, especially bacteria, are well suited to this work because of their diversity. Individually, bacterial species are very specific as a result of membrane selectivity, permeases, and enzyme synthesis and specificity. The huge numbers of environmental niches occupied and substrates utilized illustrates the immense diversity of biological function, even though individual species might succeed best under a narrow range of conditions (Tiedje, 1993).

Most of the research done on remediation has focused on soil and groundwater contamination, but many of the principles of soil remediation can also be applied to aquatic sediments. Between 1982 and 1992, 9% of the US Environmental Protection Agency (EPA) Superfund sites were treated using bioremediation; 5% at surface and 4% *in situ*. The rest were treated by other chemical and physical methods: solidification and stabilization (26%), off-site and on-site incineration (17% and 13%), soil vapor extraction (17%), thermal desorption (6%), soil washing (3%), *in situ* flushing (3%), dechlorination (2%), solvent extraction (1%) and others (<3%) were used (Cookson, 1995). The cost of physical removal, transport, and incineration can be more than ten times the cost of biological treatment, especially when *in situ* biological treatments can be used (Atlas, 1995), so bioremediation should be considered when cleanup options are explored.

Bioremediation has several compelling advantages. It can often be done on site, which eliminates the need to transport hazardous materials, and restricts contamination to a single area. These considerations have ramifications in terms of cost and liability, as well as the degree of site disruption. Typically, biological treatment is cheaper than chemical or physical treatments and results in the permanent elimination of the waste. The latter point means that the long term liability is also eliminated. Biological treatment can also be coupled to other treatments, if required or if combined treatment is cheaper. Public acceptance of bioremediation is quite high since it is an extension of a natural process.

The disadvantages of bioremediation are that not all chemicals are biodegradable, and it cannot be carried out on all sites, especially if there is a problem with toxicity. Extensive monitoring is required and individual site characteristics determine the requirements, so the process is "scientific intensive". There is also the potential for production of unknown toxic or persistent byproducts and there is a perception that the technology is unproven (Cookson, 1995). The length of time required to remediate a particular site will also influence the cost of the treatment. The cost of bioremediation can meet or exceed the cost of some conventional methods if the time required to meet legislated levels is too long (Davis et al., 1995).

2.5.1 Biodegradation by microorganisms

Microorganisms degrade organic pollutants because they can release and utilize the energy stored in the chemical structures of reduced compounds to grow and survive. The

oxidation of the pollutants, or substrates, must be coupled to the reduction of some other compound, the electron acceptor. The highest energy yield is obtained if oxygen is used as the final electron acceptor. In the absence of oxygen, other electron acceptors can be used in decreasing order of energy yield: nitrate, Mn(IV), Fe(III), sulphate or carbon dioxide. For degradation to occur, there must be organisms in the system that can degrade the pollutants of interest under the specific physicochemical conditions of the site (Cookson, 1995).

If the organic compound of interest is used as a primary growth substrate, the presence of that pollutant in the system will serve as an enrichment for the population that can use the substrate. The concentration of degrading enzymes should thus increase with time. In such cases, the compound is almost always mineralized, unless there is some other limitation such as insufficient electron acceptor, in the system (Cookson, 1995).

If the pollutant is present at a very low concentration, it might only be used as a secondary substrate. Under such circumstances, microorganisms that can degrade the target compound must use a different compound to fulfill their energy requirements. The rate of degradation will only increase if the population of interest grows as a result of the presence of the primary substrate. Compounds can also be degraded fortuitously by cometabolism. In this case, the relaxed specificity of an enzyme used for the degradation of a different, usually similar, compound can cause the biotransformation of a pollutant. The product of the initial transformation is not recognized by other enzymes required to produce central metabolites that can be used to produce energy for growth. Thus the organism does not derive any advantage from the transformation, and the presence of the pollutant does not select for the degraders. Cometabolism can cause a buildup of intermediates if other organisms in the system cannot degrade the product of the initial transformation (Cookson, 1995).

Microorganisms must not only compete effectively for electron donors and acceptors, but their other nutritional requirements must be met so that they can grow. Typically, an inadequate supply of phosphorus or nitrogen limits growth, however limitation of other elements such as potassium, calcium, magnesium, or iron, or the lack of organic growth factors like amino acids or vitamins can be responsible for growth inhibition. Microorganisms must also be able to cope with environmental conditions, phage infection, and predation by protozoa. When a site is being considered for bioremediation it is therefore important to consider the concentration and availability of the substrate(s), the presence of preferred substrates in the

matrix that might inhibit the degradation of the target compound, microbial activity, the availability of electron acceptors, the toxicity of the material, the nutrient status of the site, pH, temperature, osmolarity, moisture content, alkalinity, and metal concentrations. All of these factors can affect the rate of biodegradation and the success of the project (Cookson, 1995).

Natural selection of microorganisms capable of degrading pollutants at a contaminated site typically takes on the order of 1-2 years (Cookson, 1995). Microorganisms have been added successfully in bioremediation projects where: (1) the contamination is due to a recent incident or spill, (2) there is an extremely high pollutant concentration or high toxicity, (3) rapid cleanup is required, or (4) there is a very low, but unacceptable concentration of the target contaminant. The addition of microorganisms has been less successful and cost effective at chronically contaminated or older spill sites, and at sites containing many contaminants at moderate concentrations (Ritter and Scarborough, 1995). In the latter cases, microbial diversity is likely to be high, and competition from endogenous microorganisms will probably inhibit the added organisms (Tiedje, 1993).

2.5.2 Denitrification

Since oxygen is not very water soluble and is expensive to provide in many treatment systems, alternative electron acceptors have been used to stimulate biodegradation of pollutants when degradation was limited by the availability of electron acceptors. Nitrate reduction to gaseous products (called denitrification since it removes fixed nitrogen from the system) is the most energetically favourable of these electron acceptors. Typically, microorganisms that can exploit this energy generating mechanism also have other metabolic modes such as aerobic respiration, fermentation or photosynthesis (Jeter and Ingraham, 1981). Most denitrifiers are heterotrophic and nitrate reduction is tied to energy yielding electron transport (Knowles, 1982; Goering, 1985; Tiedje, 1988). Some autotrophs can also use nitrate to oxidize one-carbon compounds, hydrogen, manganese, iron or reduced sulphur compounds like sulphide and thiosulphate, so the presence of these compounds might exert a nitrate demand on the system (Korom, 1992).

Denitrification occurs in a stepwise series of reductions: $NO_3^- \Rightarrow NO_2^- \Rightarrow NO \Rightarrow N_2O \Rightarrow N_2$. Inhibition of denitrification occurs at low pH and in the presence of oxygen. In addition to lower overall denitrification rates, the relative proportion of nitrous oxide increases

due to the sensitivity of the nitrous oxide reductase. This is a problem because nitrous oxide is a greenhouse gas, however when the pH is above 7.3 nitrogen gas is the main product of denitrification. The presence of sulphide can also inhibit denitrification (Knowles, 1982; Goering, 1985; Brown, 1988).

Denitrification coupled to bacterial growth results in more than 80% of the nitrate or nitrite being converted to gaseous products. The rate of reduction is rapid and the process is not affected by ammonia or amino acid concentrations. Presumably some of the nitrate is used in the assimilatory pathway of reduction to ammonia to produce amino acids. The latter pathway is regulated by the ammonia and amino acid concentrations. Nitrate can also be reduced to ammonia in a dissimilatory pathway where ammonia is excreted. In this pathway, NADH is reoxidized using nitrite as an electron sink. This mechanism, in contrast to the assimilatory pathway, is not affected by the ammonia or amino acid concentrations. It is typically carried out by fermentors which can convert nitrate to nitrite (Tiedje, 1988). Whether nitrate is used for denitrification or dissimilatory nitrate reduction to ammonia (DNRA) depends on the balance between organic carbon and nitrate. When nitrate is present in excess, denitrification occurs and nitrogen is lost from the system in gas form. When nitrate is scarce relative to carbon, DNRA predominates and fixed nitrogen is conserved in the ecosystem (Tiedje, 1988; Koike and Sorensen, 1988; Brown, 1988; Caffrey, 1993; Bonin, 1996).

2.5.3 Solid phase bioremediation

Solid phase remediation is significantly different from liquid phase cleanup for a number of reasons. Materials handling obviously requires different techniques, and limitations exist for pumping and mixing. Soils and sediments are chemically and biologically heterogeneous and mass transfer rates for solutes and gases are limited due to interactions with the matrix. The degradation rate in solid systems may therefore be limited by the desorption rate. Analytical and monitoring problems may also be encountered due to the heterogeneity of the solid matrix. Biological treatment may be applicable to soils and sediments at higher pollutant concentrations than in liquid systems since adsorption of the pollutants leads to higher effective toxic concentrations (Cookson, 1995).

Bioremediation of soils is generally carried out *in situ* or by landfarming, composting, or in slurry bioreactors. *In situ* methods for soils have not been applied to sediments, however

similar *ex situ* methods may be used as for soils and sludges. The method of choice will depend on the mobility of the contaminants in the environment, the availability and concentration of the contaminants, time constraints, the risk factors associated with the site, and the cost.

Landfarming is usually used when there is little risk of contaminant migration and when sufficient land is available for the treatment. The solids are mixed, and may be amended with a bulking agent such as wood chips, sawdust, or sand to 10-30% of the contaminated soil. Nutrients are added, either by irrigation or in a single application at the time of amendment and mixing. The pH may also be modified by the addition of lime, alum or phosphoric acid. The site is prepared by laying down a clay or synthetic liner, followed by a base layer of sand with or without a gravel sublayer that usually includes a drainage system to collect leachate. The mixed and amended soil is then applied to the site to 30-45 cm depth, depending on the method of tilling. The site might be enclosed in areas of high precipitation, in low temperature conditions or when air emissions are a problem. Tilling is carried out on a regular basis to maintain aerobic conditions as well as to provide mixing.

The cost of landfarming can be broken down to the following (in US dollars): construction \$40/m^3, containment \$13/m^3, soil conditioning \$33/m^3, soil disposal \$10/m^3 and operation about \$25-50/m^3. The total cost ranges from \$65-150/m^3 (Cookson, 1995). In the case of sediment remediation, the cost of dredging and transportation would be added to this.

Composting can be carried out in windrows, static piles or in vessels. Soils and sediments must be treated differently from sewage sludges because they have lower organic contents. Bulking agents are added to improve the porosity of the matrix, and forced aeration or mixing is carried out to ensure aerobic conditions and to reduce odours. Sometimes the treatment is carried out in two stages: a high rate system followed by a second "polishing" or "curing" stage. For hazardous materials, a single stage is usually used to minimize exposure during handling (Cookson, 1995).

The windrow system is simple to operate and has the lowest cost, however it requires a lot of space, there is no air emissions control, and the degradation rate is climate-dependent. Contaminated material is mixed with a bulking agent and nutrients, and arranged in long rows (1.2-1.5 m in height and 3-3.5 m in width). These are mixed daily with front end loaders, rototillers or other mixing equipment.

The static pile system uses forced air which allows the construction of piles up to 6 m in height. Perforated aeration pipes are placed on an impervious surface for runoff collection, and are covered with the bulking agent for better air distribution. The contaminated material is piled on top and covered with more bulking agent for dust emission control. The aeration rate is usually controlled by the temperature, especially in sludge composting, since temperatures above 60 °C typically give rise to odour problems and may inhibit degradation. This method is intermediate between the other two composting methods in operation and capital costs, process and emissions control, space requirement and climatic dependency.

Composting in vessels requires sophisticated mixing equipment, but allows for the greatest control of the process. There are many possible configurations, including vertical and horizontal plug flow reactors, and agitated bed reactors. Typically, the conveyors present the major operational and maintenance problems. This approach requires the highest operational skill, capital and maintenance costs, but the requirement for space and dependence on climatic factors is low (Cookson, 1995).

Slurry treatment, in which the solid is mixed with water, allows greater process control. Solubilization of organic chemicals, contact between microorganisms and substrates, and distribution of nutrients, electron acceptors and substrates are all improved in slurry systems. The availability of the organics can also be improved by addition of surfactants. This optimized process is faster than composting or landfarming. The energy requirements are high for slurry treatment, and more materials handling may be required. Stone and rubble removal may be necessary, the liquids and solids must be separated after treatment, and the water has to be treated. Consequently, slurry phase treatment is more expensive than landfarming or composting, but it is still less expensive than incineration. Typical slurry treatment costs range from \$100-200/m^3, plus design, project administration, treatability studies (about 40%), transport, and disposal of the material (Cookson, 1995).

Slurry treatment is usually carried out as a batch process using lagoons, open vessels or closed systems. Mixing is provided to maintain suspended biomass and particulates, disperse contaminants, and maintain degradation at a maximum. Mixing can be carried out in stages if the contaminants require both aerobic and anaerobic treatment and the degree of mixing can be used to control toxicity, since adsorbed material is less toxic (Cookson, 1995).

2.5.4 Bioremediation of PAH

Bioremediation of PAH has been carried out in a number of systems, but primarily under aerobic conditions. PAH in the water phase are easily and rapidly degraded. For example, in a two-stage system, more than 99% of creosote constituents were removed from groundwater with 90-98% of the PAH destruction resulting from biodegradation (Mueller et al., 1993). In another case, liquid coal tar in the absence of solids was easily degraded with oxygen and nutrient addition (Findlay and Dooley, 1991).

Degradation of PAH associated with solids, however, is more problematic. In their 1993 review, Wilson and Jones stated that *in situ* bioremediation of soils contaminated with PAH had not been very successful. Solid phase treatments such as landfarming had only been effective for treatment of compounds with 3 rings or less in a reasonable length of time. This problem has since been observed by others. No PAH degradation was observed in a lab scale degradation experiment using contaminated soils supplemented with nutrients with or without manure supplementation or the addition of uncontaminated soil with a known population of PAH degraders. Added PAH were degraded rapidly in this system so degradation was not limited by toxicity or the lack of a suitable microbial population or nutrients, but by the availability of the PAH in the soil (Erickson et al., 1993). The addition of a solid phase oxidant (Tremaine et al., 1994) or compost which presumably contained fungi that could produce extracellular enzymes (Mahro et al., 1994), improved the biodegradation of PAH in solid phase systems, but still, degradation was slow and incomplete. Degradation of the higher molecular weight compounds is particularly slow (Mueller et al., 1991).

Slurry phase reactors have met with more success, presumably because there is more control over the process so desorption can be maximized (Linz et al., 1991; Wilson and Jones, 1993). Joshi and Lee (1996) found that PAH degradation in shake flasks with added microorganisms and mineral media, which is similar to a slurry phase reactor, was much faster than in their static system. In a slurry bioreactor treating a highly contaminated soil, 95% of the PAH content was removed in two weeks before leveling off at a total PAH concentration of 500 ppm. Further treatment of this soil would probably be required for it to be used as fill material (Lewis, 1993).

Even in bioreactors, the rate of degradation of HMW PAH is slower than for the LMW compounds. The addition of Fenton's reagent, a combination of peroxide and ferrous iron, has

been shown to chemically oxidize HMW PAH preferentially (Gauger et al., 1991; Kelley et al., 1991). The suggestion has been made that it be used before or after biological treatment to improve HMW PAH removal (Lewis, 1993).

All of the above results suggest that the availability of PAH is the controlling factor in their biodegradation. The potential for the use of anaerobic degradation in bioreactors is great, since treatment duration will probably have to be long, and operation of a reactor in the anaerobic mode would likely be cheaper than maintaining aerobic conditions.

2.5.5 Bioremediation using nitrate as electron acceptor

There has been increasing interest in the use of alternative electron acceptors for bioremediation in recent years. This interest has been spurred by the technical difficulties and costs associated with providing sufficient oxygen to contaminated aquifers in order to stimulate biodegradation of organic contaminants in the subsurface. Typically the rate of biomass formation is lower when other electron acceptors are used, so there tends to be less clogging of aquifer material, which has been observed with oxygen addition (Norris, 1995). Nitrate does not produce precipitates (such as ferric hydroxide formation when Fe (III) is used), and when complete denitrification occurs the products are non-toxic (contrary to the sulphide produced in sulphate reduction).

In petroleum bioremediation, nitrate has been used to stimulate biodegradation of monoaromatics with some success (e.g., Batterman and Meier-Lohr, 1995; Hutchins et al., 1995; Batterman et al, 1994). The primary reasons cited for the choice of nitrate as electron acceptor were to decrease cost and in one case because clogging of the aquifer had made the use of oxygen impossible. The decision to use an alternative electron acceptor will depend on whether the contaminants of interest are degradable under the provided redox conditions, the cost of delivering the electron acceptor, the length of time required to meet regulated levels, and other regulatory conditions (Norris, 1995). Nitrate concentrations, for example, must be limited in groundwater used for drinking water. The cost of nitrate is less than half that of peroxide, which is often used as an oxygen source. Furthermore, nitrate is involved in far fewer side reactions, is highly soluble in water and is not adsorbed by soil particles, and so it is more efficiently used (Norris, 1995).

2.5.6 Bioremediation of sediments

Less attention has been focused on the bioremediation of sediments than of soils, although lake sediment remediation to control nutrient cycling has been undertaken, and an intensive research effort has been made to try to find effective and cost efficient ways of treating contaminated sediment in the Great Lakes of North America. In efforts to control nutrient cycling, the focus has primarily been to prevent the release of phosphate, often the limiting nutrient, from lake sediment to control eutrophication. Conventionally, this is achieved by the addition of aluminum salts to precipitate the phosphate. The RIPLOX procedure is an *in situ*, biologically mediated method of achieving the same goal. Calcium nitrate is added to the sediment to stimulate denitrification and the oxidation of organics, which lowers the oxygen demand. Ferric chloride is added to remove hydrogen sulphide and form an insoluble iron hydroxide floc which traps phosphate. This method has been effective at maintaining adequate oxygen levels in the hypolimnion of lakes 10 years after treatment (Cooke et al., 1993).

The Great Lakes effort has been aimed at the reduction of pollutant concentrations in sediments severely affected by anthropogenic activities. Several pilot scale treatments were tried on sediments from the Great Lakes in studies co-sponsored by the Canadian government, in addition to many more fully funded bench scale assays. The high percentage of fines makes sediments difficult to oxygenate and to handle on a large scale using soil remediation techniques, although technologies are improving, and a few of the pilot projects showed great promise for sediment bioremediation (Wardlaw, 1994).

In three of four slurry treatment systems the results obtained were extremely variable and therefore inconclusive. In these cases (IGT, SNC-Lavalin, WasteStream) the authors attributed increases in the concentrations of pollutants in the treated sediments to analytical problems and the production of surfactants which improved the extractability of the contaminants, or were measured as target compounds. In the fourth slurry treatment system (Silt NV) a very rigourous extraction was used and the results were more consistent. They achieved a 94% reduction in total PAH in 108 days (LMW 99%, HMW 82% lost) (Wardlaw, 1994).

Another slurry phase treatment system was operated by the Wastewater Technology Centre (WTC) as an autothermal thermophilic aerobic reactor (ATAD or ATAR) system in

which domestic sewage sludge was used as a cosubstrate to boost heat production. Although the reactor was too small to self-generate the required heat, it was effective at removing 97% of the PAH in the sediment using an 8 day hydraulic retention time (HRT). Only 30% of the HMW compounds were degraded at this rate, however a longer retention time might further reduce those compounds if the levels remain above the required criteria (Wardlaw, 1994).

One landfarming type operation was used by GRACE Dearborn in which the sediment was applied to a prepared bed, amended with a proprietary nutrient and organic supplements, and tilled regularly. The moisture content of the soil was also maintained by covering the operation with a greenhouse and by manual spraying. The greenhouse also moderated the temperature in winter. The treatment effectively removed about 90% of the PAH over the course of one year (again with LMW PAH losses being the greatest), compared to 50% loss of PAH in control tilled and untilled sediments. Several difficulties were encountered while handling the wet sediments, primarily because the sediments could not support the weight of the tilling/mixing equipment material until it had dried to >50% solids. Dewatering or different mixing equipment would likely have to be used in full scale operations of this kind (Bucens et al., 1996; Wardlaw, 1994).

The only *in situ* method used was the NWRI/Golder process in which nitrate, and later nitrate plus an organic amendment, were injected into sediment to stimulate biodegradation of organics by denitrifiers. After two years, about 50% of the PAH were degraded, with roughly equal disappearance of LMW and HMW material. Degradation only occurred after the final application which included the organic amendment with the nitrate. The major advantages of this approach are the ease of application, lack of sediment handling, and low cost (Murphy at al., 1993; Wardlaw 1994). The cost of treatment in Canadian dollars ranges from about $25-35/m^3 for the first treatment and increases to a maximum of about $75/m^3 depending on the number of treatments required. No further costs associated with dredging, transport, dewatering, pretreatment or disposal are incurred (Jay Babin, Golder Associates, personal communication).

Some concerns remain about the depth of treatment achieved and the fate of metals when using this last approach. The process treats only the top 15 cm of sediment, and more research will be needed to determine if this is sufficient to make the sediments safe. Since the material is not physically removed, metals may remain in the treated sediment and could have

deleterious effects. The process does not, however, result in the suspension of dredge material in the water column, or risk uncovering historical contamination that might be buried at the site (Murphy et al., 1995; Wardlaw, 1994). In some cases, surface sediments after dredging have been more contaminated than before the operation (Murphy et al., 1995).

2.6 Test sites

Two sites in Burrard Inlet near Vancouver, British Columbia were chosen for this research based on chemical and biological data from the literature. One was in the east basin of False Creek (FCE), the other just off the IOCO petroleum storage and distribution terminal in Port Moody (ESSO). Analyses have been carried out on sediments and biota from both of these areas to determine the level of contamination and to identify sites that have been negatively impacted. The primary inputs to False Creek are from combined sewer overflows (CSOs) (GVRD, 1990; EVS Consultants et al., 1996). Historically, PAH were discharged from various industries: lumber-related industries and manufacturers, gas works, power plants, metal works, and machine shops to name a few (Burkinshaw, 1984). Until 1955, most of the domestic sewage and industrial process water produced in the surrounding area was discharged directly to False Creek without treatment. The ESSO site is mainly affected by stormwater runoff (GVRD, 1990; EVS Consultants et al., 1996) and septic tank effluent from the storage and distribution facility. Discharge of process effluent from the refinery at the site was permitted until 1991. Process effluent was then diverted away from Burrard Inlet and into the GVSDD system until cessation of refining operations in 1995 (Ministry of Environment, Lands and Parks, 1996, 1991, 1987).

The impact of various pollutants on several areas, including False Creek and Port Moody arm, was evaluated in the Burrard Inlet Environmental Improvements Action Plan (GVRD, 1990). An abbreviated list of the compounds that were reviewed and the severity of their associated effects is shown below (Table 2.5). "Suspected severe" listings are assigned to areas for which no biological or chemical data were available but for which there were known inputs at levels that would be expected to give rise to deleterious biological effects. Both sites are severely affected by PAH pollution (GVRD, 1990).

Biological effects were assessed for Port Moody Arm by Goyette and Boyd (1989). They found that crabs collected near the ESSO refinery, as well as from False Creek, had

elevated tissue PAH levels. The incidence of non-parasitic, non-infectious lesions in the livers of bottom fish was very high near the refinery, and the abundance of benthic infauna was low. The incidence of neoplastic and preneoplastic liver lesions in English sole was correlated with sediment PAH concentrations.

Table 2.5 Impacts of selected sediment quality parameters in False Creek and Port Moody Arm
For samples with no available biological data but known inputs, suspected (susp.) impacts are reported, + denotes some impacts, ++ severe impacts and - no impact. Both sites are severely impacted by PAH pollution (GVRD, 1990).

Pollutant	False Creek	Port Moody Arm
PAH	++	++
Hydrocarbons	++	++
Copper	++	+
Lead	++	++
Zinc	++	++
Cadmium	++	++
Chromium	++	-
Arsenic	++	+
Nickel	++	-
Mercury	++	-
Hydrogen Sulphide	++	++
Suspended Solids	++	++
Cyanide	susp. ++	+
Faecal Coliforms	++	++
Sediment Toxicity	++	++
Lesions	susp. ++	++
Benthic Diversity	susp. ++	++

The impact of pollution at these two sites made them likely candidates for this research. To improve the environmental quality of these areas, remediative action is required, so a determination of the effectiveness of nitrate addition is important. The different compositions of pollutants at the two sites was useful because possible differences in remediation could be

assessed: False Creek was affected by a complex mix of contaminants, whereas ESSO was primarily affected by a single source.

Source control is the best way to control pollution, but it will take a long time for these sediments to return to a non-toxic state, even if all inputs are stopped immediately. Therefore, the historical burden of toxic material must be removed. In False Creek, it is unlikely that input from combined sewer overflows will cease for a long time. Separation of Vancouver's domestic sewage and storm runoff lines is not expected to be completed for 60 years, although, steps are now being taken to reduce the number and volume of CSO discharges to the waterways around Vancouver (GVSDD, 1996).

At the ESSO site, refinery process effluent is no longer discharged to Port Moody Arm although stormwater from the site still reaches Burrard Inlet. With less PAH input from the storage and distribution terminal as it is presently operated, a single, successful remediation effort to remove past pollution might be sufficient to rehabilitate the site.

3. METHODS AND MATERIALS

3.1 Experimental Procedure

Preliminary experiments were undertaken to find a suitable monitoring method for PAH in False Creek sediment and to determine the number of replicates that would be required to observe differences in PAH concentration between control and treated samples. Once established, this cleanup method was used throughout for both FCE and ESSO sediments, for whole sediment and SPMD extracts.

A series of experiments was carried out to determine if the addition of nitrate could stimulate the biodegradation of PAH in marine sediment. Sediments were spiked with PAH to facilitate the chemical analysis and to ensure that trends could be observed reliably. Reaction vessels were prepared with sediment, artificial sea water and phosphate. Half of the reaction vessels received nitrate, while the other half did not (the latter samples are called "control" samples in this thesis). After various incubation periods, reaction vessels were removed and the aqueous phases were tested for nitrate, phosphate, and ammonia. Acid volatile sulphide, toxicity and PAH analyses were carried out on the sediment phases.

Sediments in the early experiments were analyzed only at the end of the incubation period, and control and treated sample results were compared. These were done to see if the addition of nitrate would affect the PAH concentration in the sediment. Together, these are called single time point experiments (section 4.2). Once it was established that nitrate addition stimulated PAH loss, samples were taken at time intervals to study the rate of degradation. These are called multiple time point experiments (sections 4.3, 4.5.1). The effects of nitrate source (section 4.3.2), PAH spike level (section 4.3.2), and ammonia addition (section 4.5.1) were investigated.

The availability of PAH in the sediments was an important consideration since this research was carried out primarily on spiked samples. Two methods were used to investigate the availability of spiked and endogenous PAH: conventional and reverse SPMDs (section 4.4). Reverse SPMDs were then used to monitor PAH availability in a degradation experiment

(section 4.5.1). The final experiments were carried out on spiked and unspiked samples (sections 4.5.2, 4.5.3) from False Creek and Port Moody Arm.

3.2 Sediment sampling

3.2.1 Sampling sites

Samples were taken from two sites in the Vancouver, B.C. area: the east basin of False Creek adjacent to Science World, and offshore from the ESSO petroleum storage and distribution terminal in the Port Moody Arm of Burrard Inlet (Figure 3.1). False Creek sediment was used for the majority of this research..

False Creek is a 200 m wide by 5.3 km long arm of English Bay that is completely surrounded by the city of Vancouver. The water at the site is of lower salinity than full strength sea water (10-23 $^{o}/_{oo}$ at the sampling site used here) and the sediment is affected by street runoff, CSO discharge, and groundwater from the adjacent land. Much of the surrounding land was infilled and until recently the area was largely industrial (GVRD, 1990). The water depth at the sampling site was approximately 7 m.

Port Moody Arm is a shallow basin roughly 300 to 1000 m wide by 6.3 km in length with relatively poor water circulation. The salinity of the water at the sampling site ranged from 15-20 parts per thousand and the water depth was approximately 15 m. Inputs of pollutants to the basin are mainly through industrial and stormwater discharges, although there are two combined sewer overflows that empty into the basin (Goyette and Boyd, 1989). At the sampling site used in this work, the refinery is likely to be the main PAH source.

3.2.2 Sampling

Surface sediment samples were taken using an Ekman dredge. Sediment was removed from the sampler with a metal spoon and transferred to plastic bags. At the laboratory, the material was passed through a 2 mm sieve and stored at 4 ^{o}C in amber glass jars until use. Sediments for the degradation experiments were not stored for longer than 24 hours before setting up the experiment.

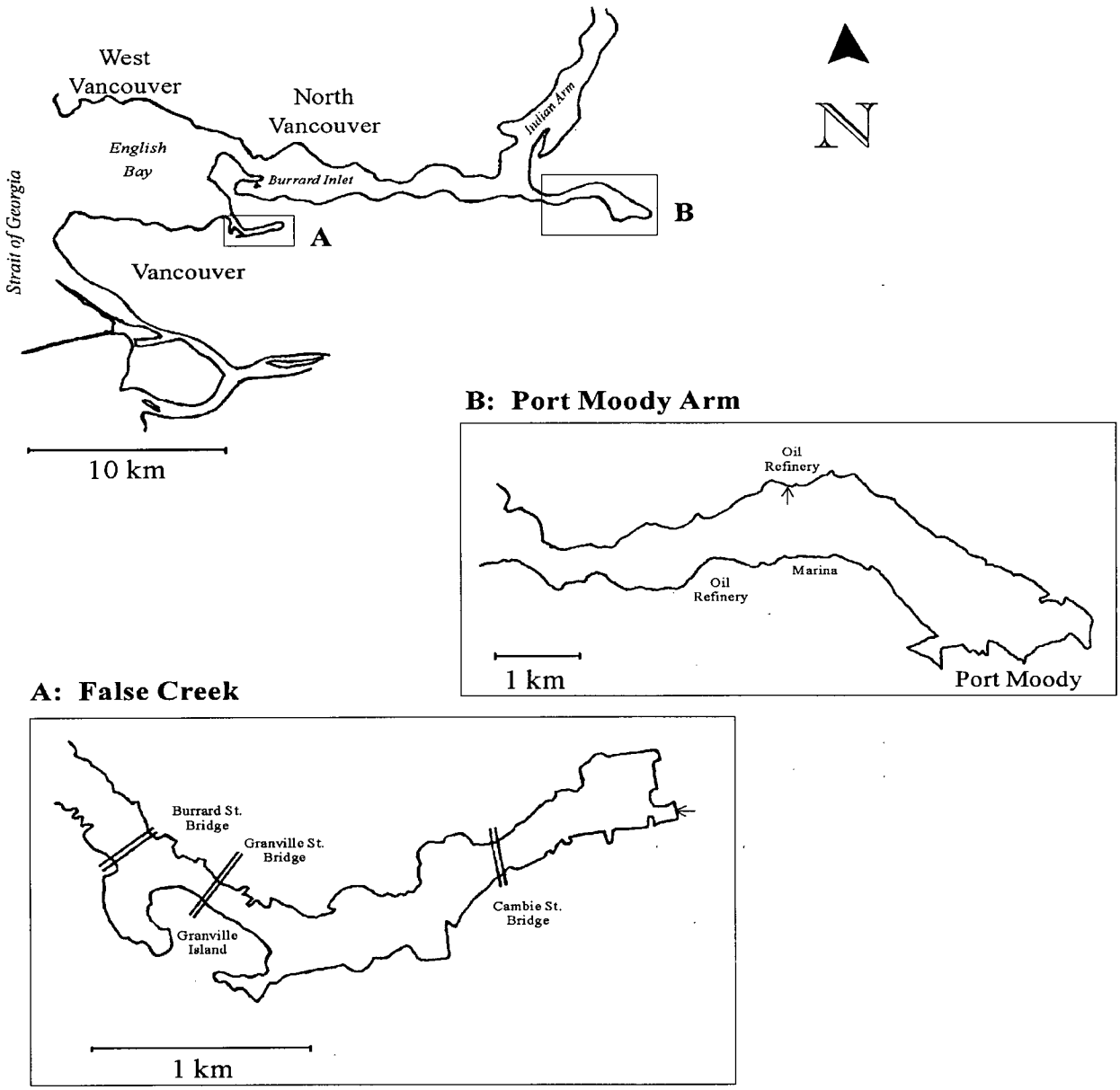


Figure 3.1 Sediment sampling sites
The top map shows Burrard Inlet and boxes A and B are expansions of False Creek and Port Moody Arm, respectively. Sampling sites are marked with arrows in the expanded maps.

3.2.3 Sediment characteristics

Dry weight and loss on ignition were determined as the reaction vessels were being set up. Sediment was added to tared aluminum dishes, dried for 24 hours at 104 ^{o}C, then fired in a muffle furnace at 550 ^{o}C for at least 1 hour (2540 G, APHA, 1995). Some samples were also air dried and sieved to give size fraction information (> 2 mm, > 0.5 mm, > 0.25 mm, > 0.125 mm, > 0.063 mm fractions: see Appendix A).

3.3 Reaction vessels

3.3.1 Reaction vessels

The experiments were carried out using sealed flasks (sections 4.2.2 and 4.2.3), 250 mL ICHEM amber bottles with Teflon lined lids (VWR, Brisbane, CA) (section 4.3.1) or 125 mL HYPOVIAL serum bottles with Teflon/silicone TUFBOND septa (Pierce, Rockford, Illinois) (sections 4.3.2, 4.5.1, 4.5.2, and 4.5.3). The amounts of sediment, water, spike and other stocks added in each experiment are described in Table 3.1. Control samples were identical to the nitrate-treated samples, except nitrate was not added. All experiments were conducted at room temperature (approximately 22-25 ^{o}C), and the samples were shaken, briefly, once daily.

In the High/Low experiment (section 4.3.2) and the Unspiked False Creek sediment experiment (section 4.5.2), resazurin (1 mg/L) was added to show that oxygen was not present in the reaction vessels after that present in the headspace and aqueous phase were used up This occurred very quickly in False Creek sediment due to the rapid reactions between oxygen and reduced compounds such as hydrogen sulphide, and possibly some of the more labile organics. The resazurin in the nitrate-treated samples turned pink, and the controls were colourless within one day. After the colour change, the positive pressure in the nitrate-treated flasks (due to the formation of nitrogen gas) demonstrated that no gas exchange occurred between the reaction vessels and the air. In the control samples, the production of hydrogen sulphide also indicates that oxygen was not present.

3.3.2 Spike material

PAH were added to the sediments to achieve a high enough concentration to trace the losses of individual compounds over time. The intent was to add as little PAH as possible while adding sufficient material to simplify the analysis and ensure that the concentrations didn't go below the detection level during the incubation. Stock solutions (approximately 1 mg/mL in toluene) were made up for each of the following PAH: naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fla), pyrene (Pyr), benz(a)anthracene (BaA), chrysene (Chr) and benzo(a)pyrene (BaP) (Supelco, Bellafonte, PA). These were added to bentonite that had been ashed for one hour at 550 ^{o}C or to ashed False Creek sediment, and the solvent was allowed to evaporate. The spiked solid was then mixed well and added to the reaction vessels. Some PAH losses occurred during spike preparation, especially of the LMW compounds, likely as a result of volatilization during the solvent evaporation step which took from 2-3 days. Preliminary experiments were carried out with toluene solutions of the PAH added directly to the reaction vessel.

Table 3.1 Reaction vessel contents
Experiments are identified by the Results section in which they are described. The stock solutions are described in Section 3.3.3, the sea salts solution was made up to 15 parts per thousand, except in the preliminary single time point experiments where the concentration was 20 parts per thousand. The spike concentration is the approximate concentration of each PAH added per gram of clay or ashed sediment (some losses, especially of the LMW PAH, occurred during solvent evaporation while the spike material was prepared). Asterisks indicate the number of times the stock (phos. in phosphate stock, amm. is ammonia stock) was added after time=0. Ammonia was added to the ESSO sediment at 3 weeks only. No nitrate was added to controls.

Experiment	Results section	sea salts (mL)	trace elem. (mL)	phos. (mL)	nitrate (mL)	amm (mL)	wet sed. (g)	spike (g)	Conc. spike (mg/g)
Air pretx/pre	4.2.2	1800	18	4	-	-	200	-	0.05
Air pretx/post	4.2.2	1000	10	2	10	-	50+5	5	0.05
NO3 pretx/pre	4.2.2	1800	18	4	20	-	200	-	0.05
NO3 pretx/post	4.2.2	1000	10	2	10	-	50+5	5	0.05
No pretx	4.2.3	100	1	0.1	1	-	10	1	0.1
Kinetics	4.3.1	190	1.9	0.38	.2***	-	30	1	0.1
High/Low	4.3.2	100	0.5	0.1*	1**	-	10	0.5	0.1 or 0.5
Ammonia/Avail	4.5.1	100	0.1	0.1**	1*	0.1*	10	0.5	0.5
FCE Unspiked	4.5.2	100	1	0.1**	1*	-	10	0.5	0.5
ESSO	4.5.3	100	1	0.1*	1	0.1*	10	0.5	0.2

3.3.3 Stock solutions

All stocks were made up with either distilled or deionized, organic-free (MilliQ HPLC grade or AlphaQ) water. Artificial sea water was made to either 15 or 20 parts per thousand with food grade sea salt. The trace mineral stock solution contained 500 mg EDTA, 200 mg $FeSO_4.7H_2O$, 10 mg $ZnSO_4.7H_2O$, 3 mg $MnCl_2.2H_2O$, 2 mg $NiCl_2.6H_2O$, 3 mg $Na_2MoO_4.2H_2O$ per liter of water (Pfennig and Lippert, 1966). Calcium and sodium nitrate solutions contained 50 g N/L and were diluted in the experimental flasks approximately 1/100 to give a final concentration of about 500 mg N/L. The phosphate (1000X) stock solution was 0.5 M Na_2HPO_4-KH_2PO_4 (pH 7.0). The sodium chloride solution contained the same concentration of sodium as the sodium nitrate stock (3.57 M) and was added to the controls at the same rate as the nitrate solution to the test reaction vessels. The ammonia stock solution (1000X) was 2 M NH_4Cl. The quantities of stock solutions added in each experiment are given in Table 3.1. Stock solutions were added to the reaction vessels after the start of the experiments using a syringe and needle through the septum. In sections 4.5.1, 4.5.2 and 4.5.3 the stock solutions were purged with N_2 gas before addition to the reaction vessels.

3.3.4 Separation of sediment and supernatant

Samples were taken for acid volatile sulfide (AVS) and reverse semipermeable membrane device (rSPMD) analyses before the vessels were opened by withdrawing material through the septum with a 16 or 18 gauge needle and syringe. The contents of the reaction vessels were then transferred to 250 mL glass centrifuge bottles and centrifuged at 2000 RPM for 15 minutes in a Beckman GS-6 benchtop centrifuge. Small (100-300 mg) samples of the sediment were taken for MICROTOX toxicity testing and the rest was frozen at -20 °C prior to drying. The supernatants were handled as described in the section on inorganic chemical analyses.

3.4 PAH analysis

3.4.1 Glassware

All glassware that was used in the extraction and cleanup procedures was washed with phosphate-free laboratory detergent and water, rinsed, baked at 440 °C/1 hr and rinsed with the solvent used in the procedure. After use the glassware was again rinsed with solvent. All solvents were obtained from Fisher Scientific and were pesticide grade or better.

3.4.2 Sediment extractions

Frozen sediments were dried under vacuum in a Speedivac freeze dryer (Edwards High Vacuum Ltd., Crawley, Sussex) One gram samples were extracted three times with 50 mL (95:5) methylene chloride:acetone for one hour on a wrist action shaker in 250 mL amber bottles with Teflon lined lids. After each extraction the solvent was decanted through a Whatman #4 filter into a round bottom flask. The combined extracts were concentrated on a rotary evaporator (Buchi, Switzerland or Buchler, Fort Lee, NJ) to approximately 1-2 mL and transferred to 15 mL screw cap tubes with Teflon lined lids. The flasks were rinsed with two small washes of solvent to recover all of the extracted material. The extracts were stored at -20 °C until they could be cleaned up. Later samples also had 0.25 g copper powder added to the extraction bottles to bind sulfur, and 20 μg 9-chloroanthracene as extraction surrogate.

3.4.3 Silica gel cleanup

In preliminary experiments, extracts were purified by a silica gel column chromatography method (USEPA SW-846 Method 3630B, 1986). The silica gel (Fisher Scientific, Fair Lawn, NJ) was activated at 130 °C for 16 hours. Slurries containing 10 g silica gel in dichloromethane were poured into 1 cm diameter columns. One to 2 cm anhydrous sodium sulfate was added to the top, and the columns were pre-eluted with 40 mL pentane to remove the dichloromethane. The extraction solvent (dichloromethane) was replaced with 1 mL cyclohexane under a stream of nitrogen gas. This solution was applied to the top of the column, followed by two 1 mL cyclohexane washes and the column was eluted with 25 mL of pentane. This eluate contained the aliphatic fraction and was discarded. The aromatic fraction was eluted with 25 mL of (4:6)

dichloromethane:pentane, collected in a round bottom flask and concentrated by rotary evaporation.

3.4.4 Dimethyl sulphoxide cleanup

The extraction solvent (dichloromethane) was replaced with approximately 5 mL of pentane under a stream of nitrogen. The pentane extracts were extracted three times with 5 mL dimethyl sulphoxide (DMSO) (Spectranalyzed, Fisher Scientific, Fair Lawn, NJ) and the combined DMSO extracts were transferred to a separatory funnel containing 30 mL Milli-Q HPLC grade, AlphaQ or solvent washed water. The DMSO-water phase was extracted three times with 45 mL pentane. The pooled pentane extracts were washed with organic-free water and concentrated by rotary evaporator (Natusch and Tomkins, 1978). The final extracts were made up in 1 mL isooctane or toluene with 20 ppm 1-chloroanthracene as internal standard.

3.4.5 Gas chromatography

The extracts were analyzed on a Hewlett Packard 5890 Series II gas chromatograph with a flame ionization detector (gc-fid) on a J&W Scientific DB-5 fused silica capillary column (internal diameter 0.32 mm, length 30 m, film thickness 0.25 μm). Alternatively, a Supelco SPB-5 column with similar characteristics was used. One μL splitless injections were made using an HP 7673 automatic sampler The carrier gas was helium, with a flow rate of approximately 20 cm/sec. at 290 ^{o}C and the column head pressure was 9 psi. The peaks were confirmed by running the samples on a J&W Scientific DB-1 fused silica capillary column (internal diameter 0.32 mm, length 30 m, film thickness 0.25 μm). Agreement of the results obtained using the two different columns indicated that the peaks were attributed correctly. The detector gases were air (400 mL/min.) and hydrogen (30 mL/min.) and the makeup gas to the detector was nitrogen (20 mL/min.). The temperature programmes for the columns are listed in Table 3.2. The detection limits for PAH in this matrix ranged from approximately 0.1 to 0.5 μg/g dry weight of sediment, depending on the size of the compound (larger PAH have higher detection limits) and the concentration factor. Concentrations were determined by comparing the areas of the peaks generated by the HP 3396 Series II integrator to standards.

Table 3.2 Temperature programmes for gc/fid

Column	DB-5/SPB-5	DB-1
Initial Temperature	80 °C	80 °C
Time at Initial Temp.	1.5 min.	1.5 min.
Ramp A	15 °C/min.	15 °C/min.
Final Temp. A	150 °C	150 °C
Final Time A	0.5 min.	0.5 min.
Ramp B	5 °C/min.	5 °C/min.
Final Temp. B	315 °C	310 °C
Final Time B	10 min.	2 min.
Detector Temp.	325 °C	320 °C
Injector Temp.	315 °C	310 °C

3.5 Semipermeable membrane devices (SPMDs)

3.5.1 Pre-extraction of tubing

Low density polyethylene layflat tubing (2.54 cm tubing width, 53.6 μm thickness) from Cope Plastics Inc. was cut to 117 cm lengths. Up to 12 lengths of tubing were added to 1 L of pentane for 24 hours for pre-extraction. The tubes were hung to dry for 15 minutes in the fume hood. Dry tubing was stored in closed amber bottles at room temperature. Once pre-extracted, the tubing and SPMDs were only handled using polyethylene gloves.

3.5.2 Conventional SPMDs

SPMDs were constructed on an acetone washed pane of glass or piece of aluminum foil. One milliliter of triolein (Sigma, St. Louis, MO) was inserted into a length of pre-extracted tubing, about 10 cm from the end. The end was then heat sealed with an Impulse Sealer (Dea Lun Co., Taiwan, P.R.C.) and the triolein spread down the length of the tubing using a small

plastic roller. The other end was then heat sealed at approximately 10 cm from the end, avoiding air bubbles when possible. The ends were sealed 2-3 times to be sure they were secure and the ends brought together, one turned 180° to form a Mobius strip, and heat sealed together.

Triolein is sensitive to both air and light and was stored at -20 °C in amber vials.

3.5.3 Exposure of conventional SPMDs

SPMDs were exposed to 10 g of sediment and 210 mL of 1.5 $^{o}/_{oo}$ sea salts solution in dichloromethane-washed 250 mL Mason jars with foil-lined lids. These were placed on an end-over-end tumbler at 10 rpm for 24 hours. Longer exposures to that amount of sediment caused damage to the membrane. After exposure, the SPMDs were removed from the jars and washed under running tap water to remove any adhering material. They were then washed sequentially with denatured alcohol and acetone to dry the membranes prior to dialysis. The sealed ends of the tubing were kept above the solvent by slipping the end through a slit in a piece of aluminum foil and attaching a dialysis tubing clip to the seal above the slit. SPMDs were dialyzed against 150 to 200 mL of pentane for 24 hours. After dialysis the membranes were discarded and the pentane was concentrated by rotary evaporation. These extracts were cleaned up by the same DMSO method as the sediment extracts (section 3.4.4), except the solvent replacement step was omitted.

3.5.4 Reverse SPMDs

These were constructed similarly to the conventional SPMDs except sediment rather than triolein, was added to the tubing. Five mL of the reaction vessel contents (except 10 mL of unspiked sample) were taken by syringe with a 16 or 18 gauge needle through the septum and injected into the tubing. The end was heat sealed, the material spread along the length of the tubing and the other end was sealed. In this case both ends were sealed 5 times since the seals were to be exposed directly to the extraction solvent, pentane. Each reverse SPMD was placed in a 250 mL amber screw top bottle with a Teflon lined lid. One hundred and fifty mL of pentane were added as well as 20 µg of 9-chloroanthracene (surrogate) and the bottles were placed on an end-over-end tumbler for 24 hours at 10 rpm. After exposure, the solvent was removed and concentrated, then cleaned up by the DMSO method.

3.6 Inorganic chemical analyses

3.6.1 Reaction vessel liquid phase chemistry

The pH of the supernatant samples were measured using a Beckman ϕ44 pH meter. The pH was then adjusted to 3.0 and the samples filtered using Whatman #4 filters and refrigerated (4 ^{o}C) until they could be analyzed. Ammonia, nitrate/nitrite (NO_x) and soluble orthophosphate concentrations were determined using a Lachat Quikchem AE automated ion analyzer. The methods used were the Automated Phenate method (4500-NH_3 H), the cadmium reduction method (4500-NO_3^- F) and the ascorbic acid reduction method (4500-P F) respectively (APHA, 1995). Concentration ranges for all three nutrient analyses were 0.05 - 20 mg of N or P/L. Samples with concentrations higher than 20 mg/L were diluted.

3.6.2 Acid volatile sulfide (AVS)

This analysis was carried out on a custom-made apparatus with four tandem reaction trains (see Figure 3.2). Essentially, the apparatus consisted of 50 mL three-neck round bottom flasks: one neck was stopped with a butyl rubber stopper through which samples and reagents were injected by syringe, one was used for the gas input and the third lead to the sodium hydroxide traps for sulfide through bent 1 mL pipettes inserted through rubber stoppers on two side arm tubes connected in series.

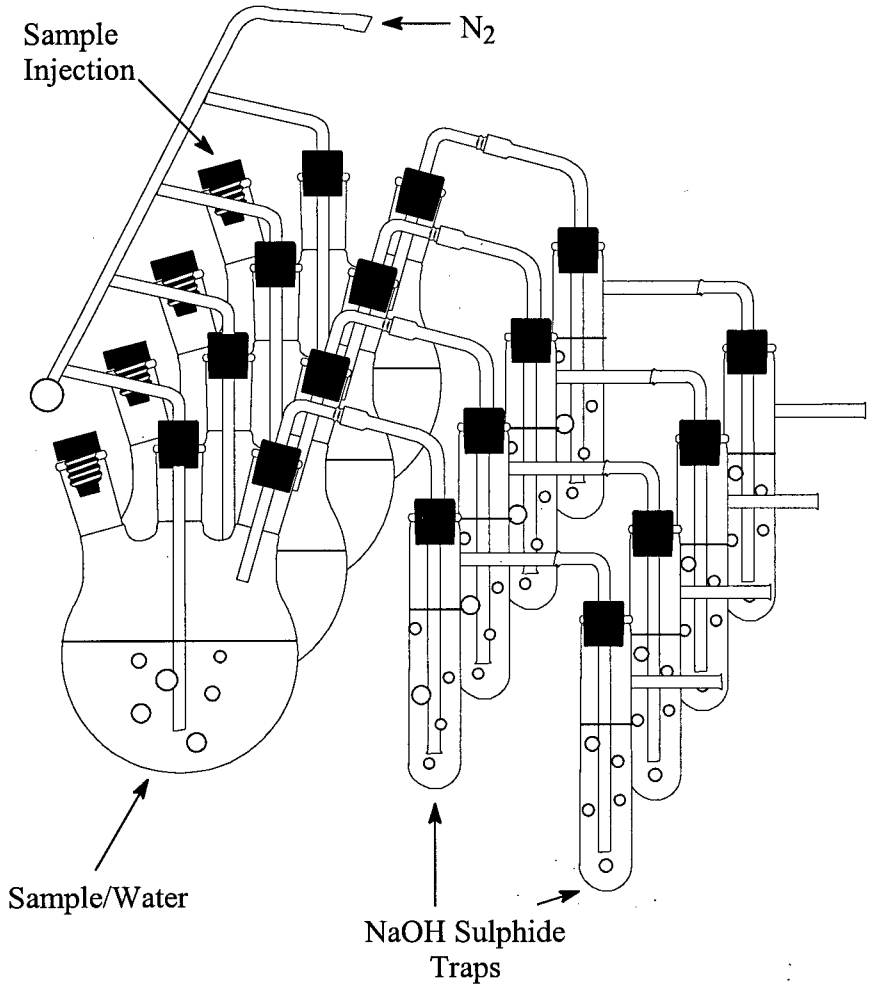


Figure 3.2 AVS apparatus

Ten mL of 0.5 M NaOH were added to each of the traps and 18 (nitrate-treated samples) or 19 (control samples) mL distilled water were added to the flasks. The system was purged with nitrogen gas for 10 minutes. One (control) or 2 (nitrate-treated) mL of sample collected by syringe from the unopened reaction vessels were added to the flasks and purged for a further 10 minutes. Four mL of 6M HCl was added to each flask and the flasks were bubbled with nitrogen for 30 minutes. For nitrate-treated samples the contents of the first NaOH trap were used, and for untreated samples, the two NaOH traps were combined. The sulfide concentration in the traps was determined by the methylene blue method (Allen et al. 1991). The detection limit for 2 mL

reaction vessel material (testing only the contents of the first trap) was approximately 0.16 μmol/g dry weight.

3.7 MICROTOX toxicity

The toxicity of the samples was measured using the MICROTOX Solid Phase Test (MICROBICS Corporation, Carlsbad, CA). The test was carried out using 300 mg wet weight of centrifuged sediment in 3 mL of solid phase test diluent and a two fold dilution series, 150 mg sediment per 3 mL diluent and a two fold dilution series, or 200 mg sediment in 2 mL diluent and a 4 fold dilution series, depending on the toxicity of the sediment. Light emission was measured using the MICROBICS M500 Toxicity Analyzer, and the software programme used to calculate the EC_{50} was MICROTOX version 6 (MICROBICS Corp., 1992). Results are expressed on a wet weight basis.

3.8 Statistical analyses

Most of the statistical analyses including unpaired, one tail T tests and linear best fits by the least squares method were carried out using SYSTAT for Windows Version 5.0 (SYSTAT, Inc., Evanston, Illinois). Figures that show nonlinear fits including the equations of the lines and the correlation coefficients (r^2 values) were generated using GRAPHER Version 1.23 (Golden software, Golden, Colorado).

4. RESULTS

4.1 Cleanup method development for PAH

4.1.1 Comparison of cleanup procedures

PAH in solvent extracts of False Creek sediment could not be quantified directly by gas chromatography using the flame ionization detector due to the large amount of interfering material present in the samples. A cleanup procedure was therefore needed to separate the PAH from other components of the extracts. Two methods were used: the silica gel cleanup procedure and the dimethyl sulphoxide (DMSO) method. These are both described in the Methods section 3.4.

To compare the two cleanup methods, 72 g of air dried sediment were extracted using hexane:acetone (1:1). This sample was made up to 50 mL. Each cleanup was carried out using 4 mL of this extracted sediment matrix spiked with 1 mL of a 100 ppm naphthalene, phenanthrene, fluoranthene and pyrene mixture.

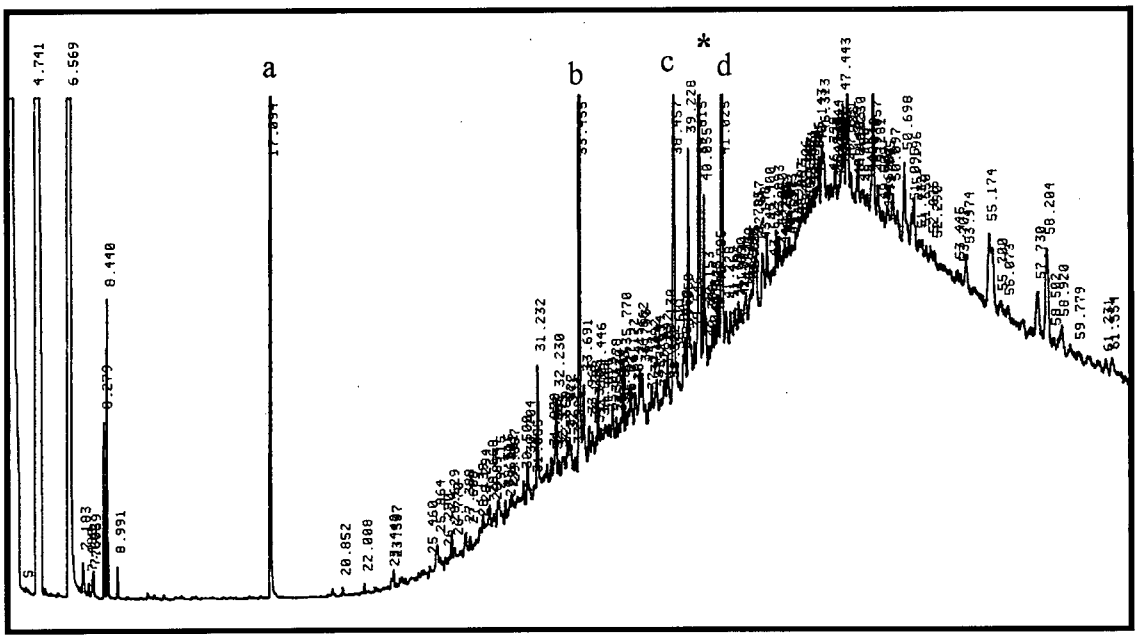
For the DMSO method, washing with acid, base, or acid followed by base (1 N NaOH or H_2SO_4) before the cleanup was also investigated. Washing did not seem to affect the number of interfering peaks in the chromatograms, but caused some losses of PAH, so it was not subsequently used.

A comparison of the chromatograms of the final extracts using these two methods shows that there was less interfering material in the DMSO-cleaned samples than in the silica gel-cleaned samples (Figure 4.1)

Inspection of the recoveries of the spiked PAH (naphthalene, phenanthrene, fluoranthene and pyrene) using each method shows that the silica gel recoveries were slightly higher than those achieved using the DMSO method for fluoranthene and pyrene (Table 4.1). Both methods yielded lower recoveries of naphthalene than of the larger compounds.

Since there were fewer interferences in the DMSO cleaned extracts, the results using this method were more precise, even though the overall recoveries of some PAH were lower, reflecting a slightly lower accuracy.

Silica Gel Cleanup



DMSO Cleanup

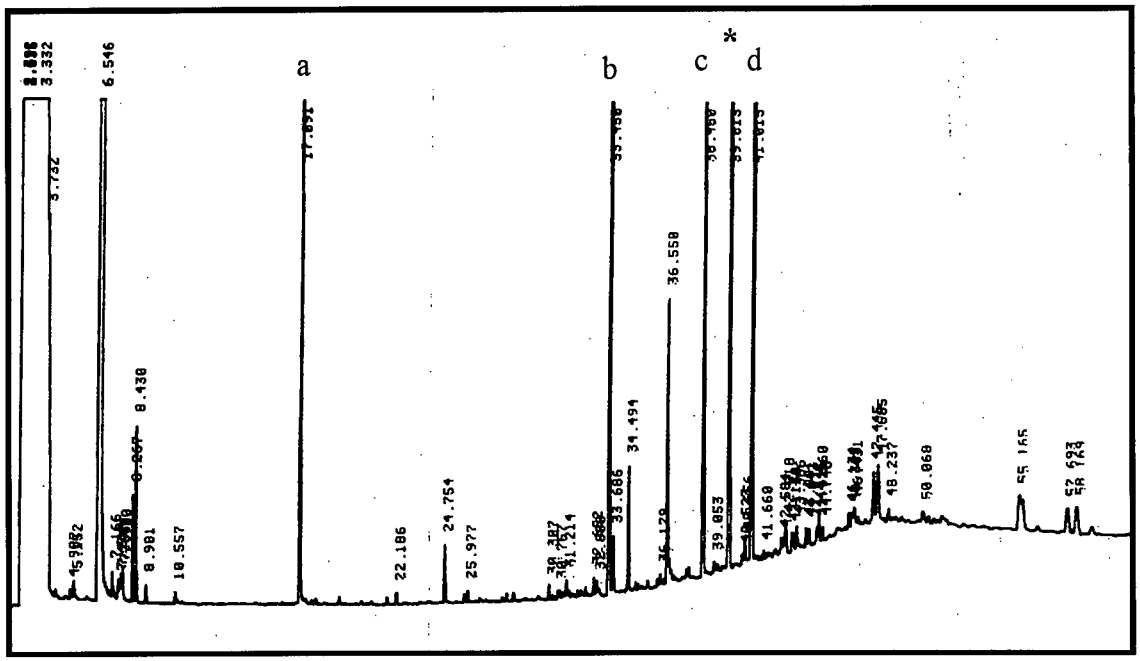


Figure 4.1 Comparison of cleanup procedures
Chromatograms of False Creek sediment. extracts cleaned up by the silica gel (top) and DMSO (bottom) methods. Both samples contained the same amount of matrix material and were spiked with naphthalene (a), phenanthrene (b), fluoranthene (c) and pyrene (d); the large peak at 38.45 minutes marked with an asterisk is the internal standard, 1-chloroanthracene.

Table 4.1 PAH recoveries (%) using silica gel and DMSO cleanup methods
Average values and ranges of duplicate analyses are shown.

PAH	Silica Gel	DMSO
Naphthalene	62.1 (55-69)	60.4 (41-79)
Phenanthrene	78.7 (70-87)	85.9 (85-87)
Fluoranthene	92.3 (92-93)	83.5 (83-84)
Pyrene	95.1 (93-97)	82.8 (82-84)

4.1.2 Investigation of DMSO cleanup procedure

Several experiments were conducted to determine whether the recoveries of PAH obtained using the DMSO method were consistent and precise. In an experiment using the naphthalene, phenanthrene, fluoranthene and pyrene spike and a dichloromethane extraction of wet sediment, the recovery of naphthalene (67%) was marginally higher than from the hexane:acetone extract (Table 4.1), but the other PAH recoveries were slightly lower (79, 75 and 77%). The higher relative recovery of naphthalene might be due to lower losses in the initial concentration step since dichloromethane is more volatile than hexane.

Using a spike mixture that contained eleven PAH at three different levels, the PAH recoveries agreed well with previous experiments, regardless of the amount of spike that was added. The spread of the data expressed as coefficient of variation (% standard deviation/mean) was less than 10% (see Table 4.2). This shows that the method gave precise results, even if the recoveries of some compounds are not complete. The narrow spread of the data was desirable for the comparative work done in the degradation experiments.

The recoveries of the low molecular weight PAH in subsequent experiments were always much lower than was found here. In these initial experiments, the spike was added in solvent at the time of extraction. In subsequent experiments, the sediments were spiked with clay to which PAH had been added by mixing the clay with a PAH solution in toluene and allowing the solvent to evaporate. The experimental sediments were also freeze dried after the addition of the spike material. Since extracts of the spiked clay alone gave similar recovery

patterns, it seems that low molecular weight PAH were lost when the solvent was allowed to evaporate from the spike matrix, and to a lesser extent, from samples dried prior to extraction.

Table 4.2 Percent recovery of PAH using the DMSO cleanup method
Recovery is the average percent recovery from duplicates at all three spike levels, and COV is 100*standard deviation/mean.

PAH	1 mL	2 mL	4 mL	Recovery	COV
Nap	61	57	62	60	6.0
Acy	72	76	79	76	4.6
Ace	72	73	77	74	5.5
Flu	74	77	81	77	5.6
Phe	80	82	85	82	5.1
Ant	78	79	84	80	6.0
Fla	80	83	84	82	4.8
Pyr	81	83	84	83	4.5
Baa	81	81	86	83	8.8
Chr	82	83	87	84	11.7
Bap	75	77	76	76	3.9

The DMSO cleanup procedure was used in the degradation experiments because it removed more of the interfering material in the False Creek sediment extract matrix. Since the experiments were designed to compare the differences between treated and control sediments, the precision of the results was judged to be more important than achieving the highest recovery of material. For consistency, the DMSO method was used in the experiment on ESSO sediment.

4.1.3 Analytical variability of the extraction/cleanup procedure

To determine the number of replicates required to resolve a 20% difference between the means of two (treatment and control) groups, a variability experiment was carried out using the False Creek sediment collected for the pretreatment experiment described in section 4.2.2. Four replicate samples of spiked and unspiked sediment were incubated with artificial sea water for one week. After that time the samples were centrifuged and the sediments were extracted,

cleaned up by the DMSO method, and analyzed as usual by gc-fid. The results were tabulated, the means and standard deviations were calculated and the required number of samples (n) was determined using the following relationship (Ellersieck and La Point, 1995):

$$n = t^2 (St\ Dev)^2 / L^2$$

where t is the T statistic with 3 degrees of freedom, confidence limit 0.05; St Dev is the standard deviation and L is the allowable error (20% of the mean). The T statistic for 3 degrees of freedom at the 95% confidence level for a one tail test is 2.353. The relationship becomes:

$$n = (2.353)^2 (St\ Dev)^2 / (0.2*average)^2$$

For this comparative work, the relevant question is whether the nitrate-treated samples have less PAH than the control samples, so a one tail T test is appropriate.

The results for sediment samples that were not spiked are shown in Table 4.3. Spiked sediment results are presented in Table 4.4. In both cases, four replicates were used for the analysis.

Table 4.3 Number of replicates required: No Spike
The number of unspiked sediment replicates required to resolve a 20% difference in means is given in the Replicates column for a one tail T test at the 95% confidence level (n=4). The average and standard deviation values are given in µg/g dry weight. Compounds that were not detected are listed as n/d.

PAH	Average	St Dev	Replicates
Naphthalene	n/d	n/d	n/d
Acenaphthylene	n/d	n/d	n/d
Acenaphthene	0.15	0.08	36
Fluorene	0.27	0.08	14
Phenanthrene	1.03	0.45	26
Anthracene	0.31	0.11	19
Fluoranthene	1.44	0.20	3
Pyrene	1.46	0.23	4
Benz(a)anthracene	1.06	0.15	3
chrysene	1.67	0.33	6
Benzo(a)pyrene	0.59	0.08	3
Total PAH	8.06	1.17	7

Table 4.4 Number of replicates required: Spiked
The number of spiked sediment replicates required to resolve a 20% difference in means is given in the Replicates column for a one tail T test at the 95% confidence level (n=4). Average and standard deviation values are given in µg/g dry weight.

PAH	Average	St Dev	Replicates
Naphthalene	0.41	0.56	259
Acenaphthylene	0.76	0.13	5
Acenaphthene	2.49	0.62	9
Fluorene	5.87	0.80	3
Phenanthrene	9.33	2.97	14
Anthracene	6.27	0.75	2
Fluoranthene	14.26	0.96	1
Pyrene	12.10	1.13	2
Benz(a)anthracene	12.10	1.13	2
chrysene	13.19	2.17	4
Benzo(a)pyrene	7.67	0.43	1
Total PAH	84.47	11.26	3

The variability of the results in the unspiked samples was higher than the spiked samples, particularly for those PAH that were present at low concentrations. This is a result of the size of the peaks relative to background "noise" and the heterogeneity of the samples. Six replicates were used in later experiments which should have been able to resolve differences of less than 20% for most PAH, and a 30% difference for phenanthrene in spiked samples. The spread of the phenanthrene replicates in other experiments was not always as broad as was observed here (e.g., see Figure 4.10: COV at time = 0 for the control samples was 11% compared to 32% in Table 4.4).

4.1.4 Supernatant PAH Concentrations

The supernatant liquids were extracted in the spiked variability sediments to see how much of the PAH was present in the water phase. The relatively high organic content of the sediment (9.6% loss on ignition), should prevent partitioning of PAH into the water phase.

Table 4.5 shows the percent of total recovered PAH in the supernatant and the expected % in the water phase from the relationship:

$$\log K_p = \log K_{ow} + \log f_{oc} - 0.21 \qquad \text{(Karickhoff et al., 1979)}$$

where K_p is the partition coefficient (C_s/C_{aq}), K_{ow} is the octanol-water partition coefficient and f_{oc} is the fractional organic carbon content. The latter was estimated to be half of the loss on ignition (9.6%) for this sediment, or 4.8%. The concentrations of PAH in the aqueous phase were higher than expected, particularly for the HMW compounds. Even if the actual sediment organic carbon content was only 2.4%, the expected concentration of acenaphthene, for example, in the water phase would be 1.76 mg/L (46% of the observed value). Deviations from the expected results could be a reflection of interference at the low levels of PAH encountered, better recoveries from the supernatant phase since these fractions were not cleaned up, and the influence of a colloidal phase in the sediment pore water. The amount of material in the water phase was small compared to that in the sediment phase, so supernatant samples were not extracted in later experiments. PAH that are not included in Table 4.5 were not detected in the water extracts.

Table 4.5 Concentration of PAH in the aqueous phase: expected and experimental results
Concentrations are in mg/L. K_p is calculated from the equation given by Karickhoff et al., 1979, the fractional organic content is taken as 4.8%, log K_{ow} values were taken from Nagpal, 1993. The expected concentration in the supernatant is $100*(C_s/K_p)$ since K_p = conc. in solid phase/conc. in aqueous phase.

PAH	experimental conc. in aq. phase	log K_{ow}	K_p	expected conc. in aq. phase
acenaphthylene	2.0	4.07	348	0.22
acenaphthene	3.8	3.98	283	1.02
fluorene	3.0	4.18	448	1.29
phenanthrene	3.8	4.46	853	1.09
anthracene	3.5	4.50	936	0.69
fluoranthene	5.4	4.90	2350	0.61
pyrene	4.1	4.88	2244	0.54

4.2 Single time point experiments

4.2.1 Preliminary PAH degradation experiments

Several preliminary experiments were carried out that helped clarify a few elements which were important in the design of subsequent experiments. The PAH levels in unspiked False Creek sediment were quite low, so the gc peaks were sometimes hard to identify, and subject to interference by co-eluting compounds. Consequently the spread of the data among replicates was quite high (also see variability of unspiked samples, Table 4.3). Spiked samples were therefore used to try to overcome the effects of these factors. The samples were spiked with clay or ashed sediment to which PAH had been added, rather than spiking with PAH dissolved in toluene to ensure that a solvent/PAH slick was not formed at the water surface, which could affect equilibration with the sediment. After collection, the sediment was also wet sieved through a 2 mm sieve to reduce the heterogeneity of the samples.

It was also clear from these early experiments that there was activity in the reaction vessels over the course of the experiment, but that it was not all related to the loss of PAH. Treated sediments were much lighter in colour than the controls, produced gas, and no longer smelled of sulphide. The loss of PAH was not ruled out, but there was no dramatic decrease in the PAH levels over the course of the experiments. It was therefore necessary to lengthen the incubation time or to reduce the amount of easily degraded (non-PAH) material in the sediment that might be degraded preferentially. Two experiments were carried out simultaneously to address these problems. In one experiment, sediment was bulk pre-treated with either air or nitrate to reduce the biodegradable content of the sediment before spiking and monitoring PAH loss. In the other experiment there was no pretreatment other than sieving. In both of these experiments, spiked clay was added to the sediment, the incubation was longer, and more replicates were used than in previous experiments. The results of these experiments are described in the following two sections. In all of the degradation experiments, control samples were included which were treated as the test samples were, except nitrate was not added.

4.2.2 Air or nitrate pre-treatment

In this experiment, 200 g of wet False Creek sediment was added to 2 L flasks that contained artificial sea water, trace elements and phosphate (see Table 3.1). Calcium nitrate was added to one flask which was then sealed. The other flask was aerated for 7 days with an air stone followed by mixing with an impeller. Light was excluded by covering the flasks with foil. This pretreatment was carried out for 4 weeks, then the sediments were centrifuged. Fifty gram samples of the pretreated sediment were placed into flasks containing fresh sea salts solution, phosphate, 5 grams of freshly collected False Creek sediment, PAH-spiked clay and calcium nitrate (in the treated flasks). The flasks were sealed and incubated for 6 weeks in the dark at room temperature. MICROTOX toxicity and supernatant nitrate, phosphate, ammonia, and pH were measured after pretreatment and at the end of the experiment (Appendix B).

At the end of the experiment, regardless of the pre-treatment, the PAH concentrations in the nitrate-treated sediments were lower than in the control sediments (Figure 4.2). Therefore, the addition of nitrate stimulated the transformation of PAH in spiked False Creek sediment. In the air pretreatment experiment, the differences in concentration between treatments for acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene and chrysene were statistically significant at the 95% confidence level. The losses ranged from 83% for fluorene to 28% for pyrene. With the nitrate pretreatment, the losses were only from 64% (acenaphthene) to 12% (benz(a)anthracene), and the difference was not statistically significant for pyrene, which was higher in the nitrate-treated flask. Acenaphthylene was not detected in any of the sediments. While a peak eluted at the naphthalene retention time (shown on Figure 4.2 as naphthalene) it is likely that this was not naphthalene, since in later experiments in which the peaks were confirmed using the DB-1 column, the results using the two columns did not agree. A number of peaks were recorded near that retention time, and the temperature ramp at the start of the programme was quite steep, which could have resulted in inadequate separation of the early-eluting compounds. The absence of naphthalene from the system could be due to losses during spike preparation and sample handling prior to analysis.

The differences between the results of the two pretreatments can be explained by examining the control sediments. The PAH concentrations in the control sediments that had been pretreated with nitrate were lower than in the air pretreated sediments. It is likely that

there was some nitrate carry-over to the control flasks after pretreatment since the nitrate concentration did not decline to zero over the 4 week period. Still, the sediment that received nitrate a second time had lower PAH concentrations than in the control, indicating that the nitrate in the control flask had become limiting during the second incubation. Assuming that the nitrate concentration in the sediment was the same as in the supernatant (84 mg N/L) at the end of the pretreatment, and the volume of sediment added was approximately 50 mL, 4.2 mg NO_3-N/L would have been carried over to the control. Assuming complete oxidation of the PAH, a roughly equal mass of PAH could potentially have been consumed. There was approximately 0.4 mg PAH less than expected in the nitrate pre-treated control, so the presence of residual nitrate from the pretreatment could easily account for the losses. The rest of the nitrate would have gone to the oxidation of other material in the control sediment.

In both pretreatment experiments, the nitrate consumed per g dry weight of sediment was less than was observed in experiments without pretreatment to reduce the degradable organic matter.

Table 4.6 shows the difference in nitrate consumption in the experiments described above and the following single incubation experiment with no pretreatment. The difference indicates that much of the easily degraded material present in the sediments was degraded in the first incubation period. With the air pretreatment, the difference is less pronounced. There were indications that the environment in the air pretreatment flask was not completely oxic since it was darker in colour (presumably due to the presence of ferrous sulfide) than in the nitrate pretreatment sediment and had a higher toxicity at the end of the first incubation (Appendix B), so the degradation of some of the sediment organic matter might have been electron acceptor-limited.

Table 4.6 Nitrate consumption in single time point experiments

Pretreatment	Incubation time (weeks)	sediment dry weight (g)	Nitrate used (mg N)	mg N/g dry wt sediment
Air	6	30.83	76	2.5
Nitrate	6	28.5	15.2	0.5
None	7	4.36	31	7.1

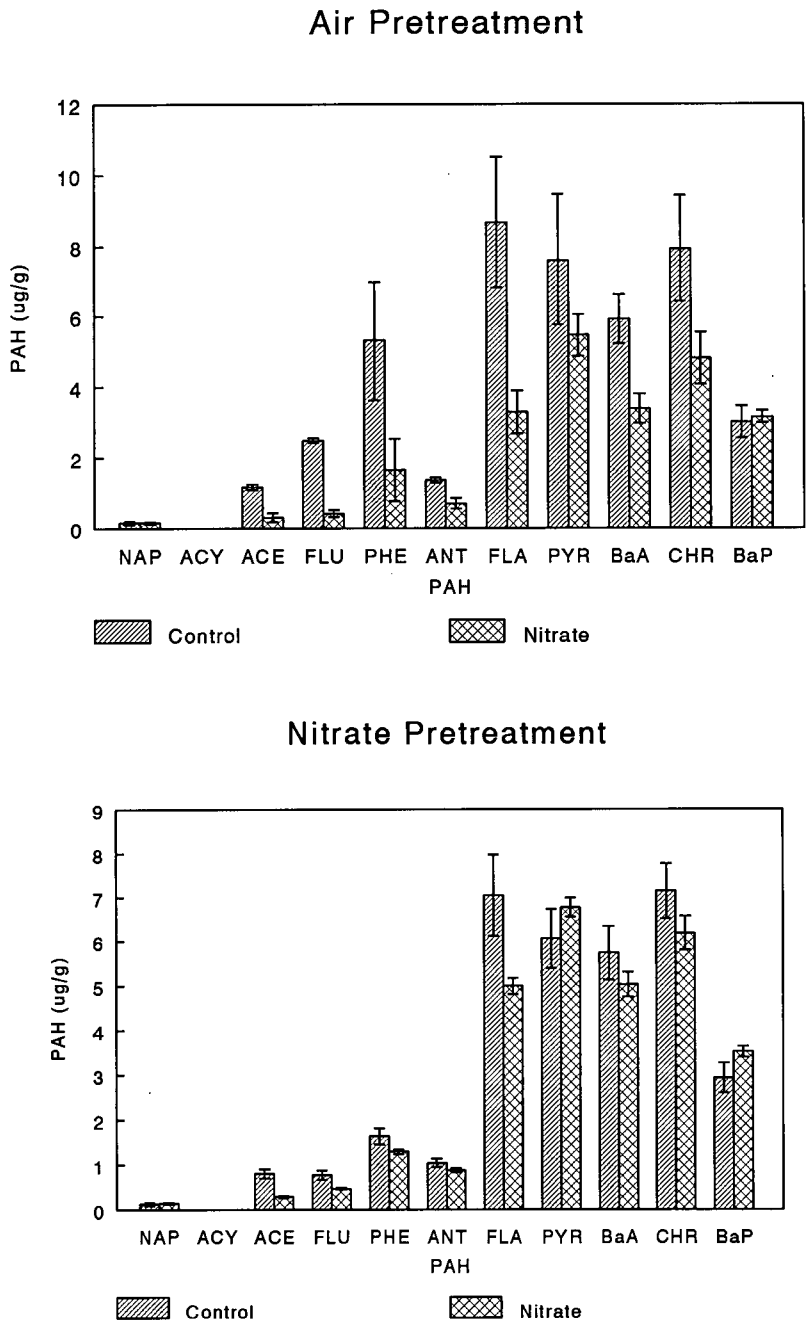


Figure 4.2 PAH concentrations at the end of the Pretreatment experiment
The top panel shows the PAH concentrations in control (no nitrate added) and nitrate-treated sediments pretreated with air, and the bottom panel shows those pretreated with nitrate. In both cases, the Benzo(a)pyrene and naphthalene concentrations were not diminished with nitrate treatment. In the nitrate pretreated sediments, pyrene degradation was not stimulated by nitrate addition. All other differences between control and treated sediments were significant at the 95% confidence level (n=5).

4.2.3 No pretreatment

Five replicates of each treatment were prepared with 10 g fresh False Creek sediment (4.36 g dry weight, 9.57% loss on ignition), spiked clay, sea salts solution, trace elements, phosphate and calcium nitrate in the treated samples. After incubation for seven weeks at room temperature, the phosphate concentrations were lower in the treated flasks than in the controls. The total amount of nitrate consumed in the test flasks was 31 mg as N, which corresponds to 7.1 mg N/g dry weight. At the end of the incubation, the MICROTOX toxicity of the treated sediment was approximately ten times less than that of the control sediment (the EC_{50} of the nitrate-treated sample was 22578 ppm, compared to 2260 ppm for the control samples; Appendix C).

Figure 4.3 shows the PAH concentrations in nitrate-treated and control sediments. All the PAH concentrations except naphthalene, acenaphthylene and benzo(a)pyrene were significantly lower in the nitrate-treated sediment extracts than in the control extracts. PAH losses ranged from 84% for fluorene to 4% for acenaphthylene. As in the previous experiment, the results recorded as naphthalene might have been another compound, since this (small) peak was not confirmed using the DB-1 column. These results indicated that pretreatment was not necessary for PAH degradation to occur under denitrifying conditions.

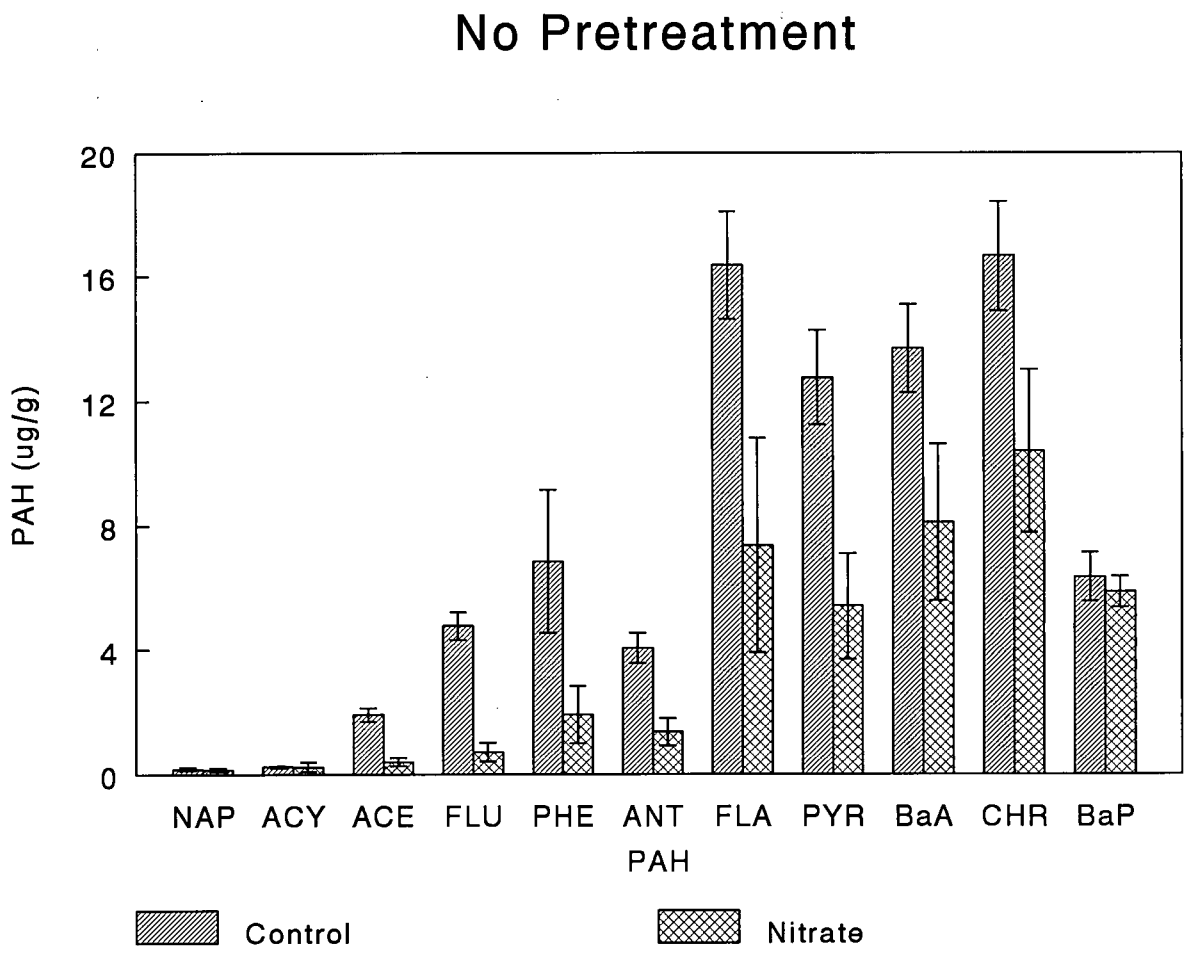


Figure 4.3 PAH concentrations after 7 weeks: no pretreatment
The differences between nitrate-treated and control sediment PAH concentrations are significant at the 95% confidence level except for naphthalene, acenaphthylene and benzo(a)pyrene (n=5).

4.3 Multiple time point PAH degradation experiments

Once it had been established that PAH could be degraded under denitrifying conditions, the kinetics of this degradation in False Creek sediment could be determined. In the following experiments, identical reaction vessels were prepared so that samples could be extracted at various times. Supernatants were analyzed for pH, ammonia, NO_x and ortho-phosphate. The sediments were tested for acid volatile sulphide concentration, MICROTOX toxicity and PAH content.

4.3.1 Kinetics experiment

In this experiment, 250 mL amber bottles were prepared with 30 g False Creek sediment (14.5 g dry weight, 9% loss on ignition), PAH-spiked clay, sea salt solution, trace elements, phosphate and calcium nitrate in the nitrate-treated vessels. Samples were taken at 0, 1, 2, 4, 8, 12 and 16 weeks. A sample containing no sediment and a killed control (autoclaved on 3 successive days, and containing approximately 225 mg N/L calcium nitrate) were also included in this experiment. Unspiked sediments were evaluated at 0, 8 and 16 weeks as well (Data in Appendix D).

The ammonia levels in the nitrate-treated samples declined to near zero from 2 mg N/L in the first week, then gradually rose to 10 mg N/L by week 4 and 14 mg N/L by week 12 before falling to near 0 mg N/L by the end of the experiment. The control samples started at a similar level and gradually rose to 13 mg N/L where it remained to the end (Figure 4.4).

Phosphate concentrations in the water phase of the autoclaved and nitrate-treated samples were low initially and dropped to near zero after one week. Since this decline also occurred in the autoclaved sample, it was probably due to precipitation of phosphate with calcium from the calcium nitrate (Figure 4.4). The blank (no sediment) and control (no nitrate) supernatants both contained constant levels of phosphate throughout the experiment, of 32 and 20 mg P/L respectively.

Nitrate (measured as NOx, but nitrite levels were not significant) in the blank, autoclaved and control sediment reaction vessels remained constant throughout the experiment at approximately 0, 230 and 0 mg N/L respectively. In the nitrate-treated samples, the nitrate level dropped quickly in the first 2 weeks from 450 to 95 mg N/L. Approximately 225 mg N/L

was added at 3 weeks, and by 4 weeks the level had dropped to 115 mg N/L. Nitrate was added at 6 and 12 weeks (Figure 4.4). The rate of nitrate use slowed over the course of the experiment, but the total amount added was 250 mg of N, and the total amount consumed was approximately 193 mg N. On a dry weight basis this corresponds to 13.3 mg N/g dry sediment over the 16 weeks. At 8 weeks the nitrate consumption was 11 mg N/g dry weight. Although direct comparisons to the numbers in Table 4.6 can't be made, since the incubation times were different, there was more nitrate consumed per mass of sediment in this experiment than in the single time point experiments. This was expected for the pretreated samples since much of the easily degraded material would have been consumed in the first incubation, but it was surprising for the samples that were not pretreated. It is interesting to note that nitrate use did not correspond to degradation of PAH. Nitrate use was lower, while PAH degradation was higher in the single time point experiment with no pretreatment than in this (Kinetics) experiment.

The acid volatile sulphide (AVS) concentration of the nitrate-treated sediment decreased from approximately 150 μmol/g dry weight to near zero in the first week. In the control sediments, there was a slight increase in AVS the first week as the sulfate from the added sea water was reduced, and then the level stayed fairly constant, barring the value at 12 weeks. This test was subject to large errors due to the size and heterogeneity of the samples. The toxicity of the sediments as measured by MICROTOX behaved quite similarly. There was a slight increase in the toxicity of the control sediments at the beginning of the experiment. In nitrate-treated samples, the toxicity decreased most rapidly in the first week, then more slowly until the fourth week after which the toxicity remained fairly constant (Figure 4.5).

The degradation of PAH with nitrate treatment in this experiment was much less pronounced than in the single time point experiments. The percent loss of PAH after 16 weeks of incubation ranged from 4-59%. This compares to 4-84% in the no pretreatment experiment after 7 weeks of incubation. Comparing the control and nitrate-treated samples in this experiment, only the differences in the naphthalene, acenaphthene, fluorene and phenanthrene concentrations were statistically significant at the 95% level. Figure 4.6 and 4.7 show the PAH Concentration (μg/g dry weight) vs. Time (Weeks) plots for some low and high molecular weight PAH, respectively. None of the PAH were degraded within the first 4 weeks. Only acenaphthene and fluorene seemed to continue to be degraded after 8 weeks, although

naphthalene and phenanthrene were significantly lower in treated than control sediments after 8 weeks. As in the single time point experiments, the naphthalene results are based on the integration of a small peak that eluted at the same time as the standard, but was not confirmed using the DB-1 column. This peak could be naphthalene, another compound that elutes at the same time, or a mixture of compounds. The absence of naphthalene could be due to losses during spike preparation and sample extraction and cleanup. None of the HMW PAH were degraded in this experiment. For each compound, the best first order fit for the data is included in the figures as well as the correlation coefficients. The fits were not very good, indicating that first order kinetics do not accurately describe PAH degradation in this experiment. This might have been due to phosphate limitation since the phosphate concentration rapidly dropped to near zero in the nitrate-treated samples.

The PAH concentrations in the autoclaved and blank samples remained roughly constant throughout the experiment, except that benzo(a)pyrene recoveries were low in all cases after the initial time point. The PAH concentrations in nitrate-treated sediment without the spike were not significantly different from the control concentrations at the end of the incubation period, although the spread of the data was quite wide.

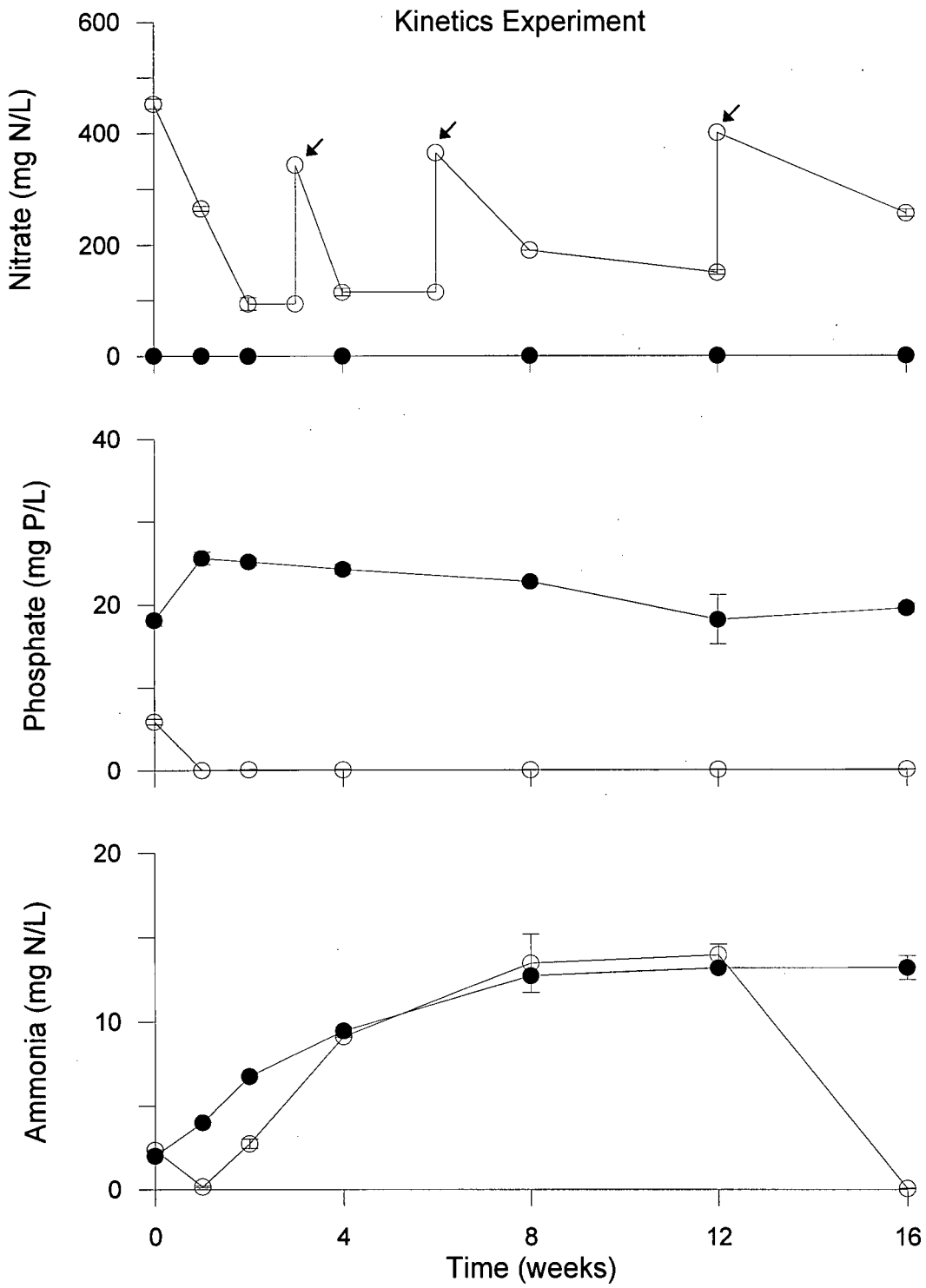


Figure 4.4 Nitrate, phosphate and ammonia concentrations: Kinetics experiment
Concentration (mg N or P/L) vs. time (weeks) plots for nitrate (top), phosphate (middle) and ammonia (bottom) in nitrate-treated (O) and control sample (●) supernatants (n=2). Arrows indicate when stock solutions were added.

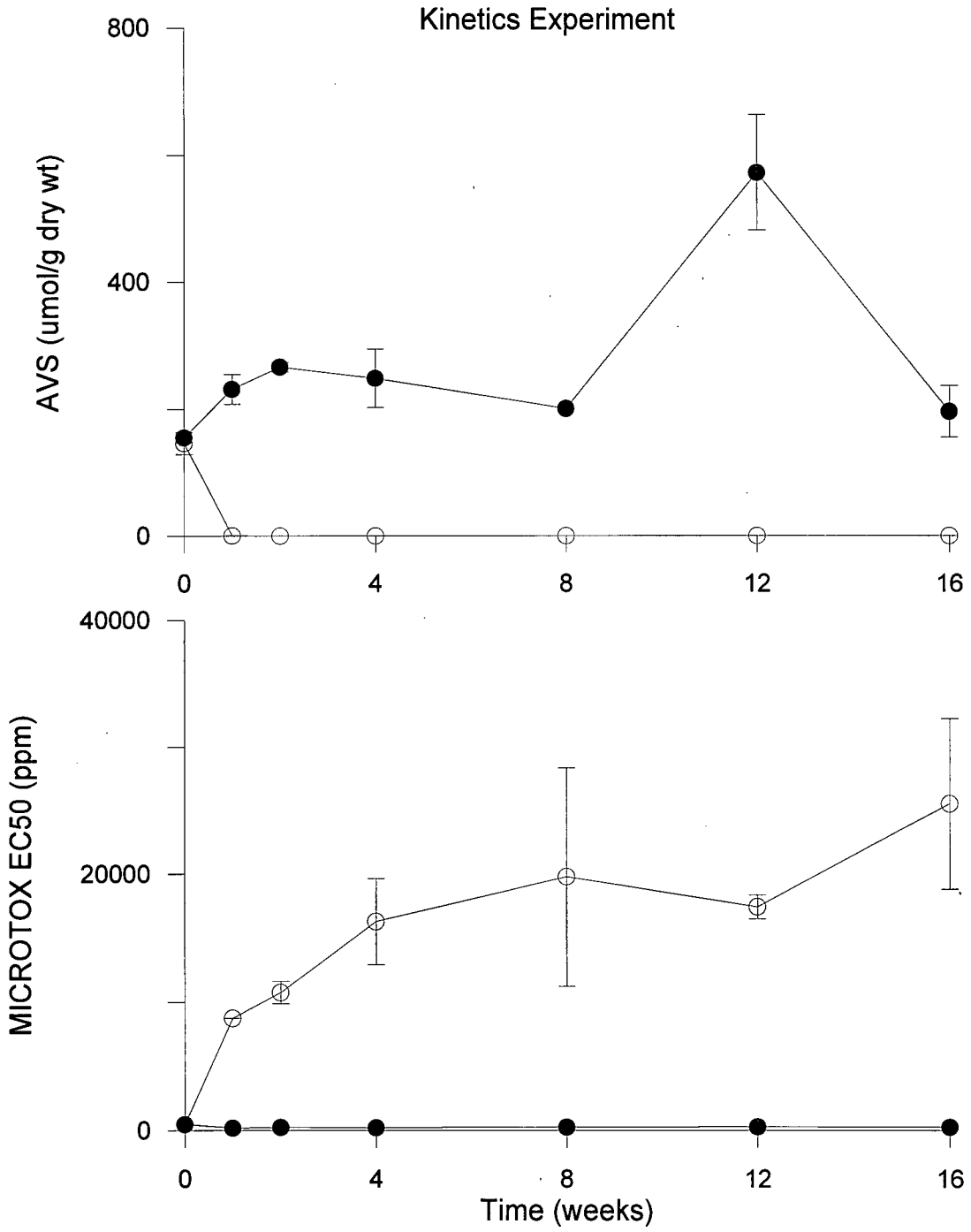


Figure 4.5 AVS and MICROTOX toxicity: Kinetics experiment
AVS is expressed as µmoles/g dry weight of sediment, and the MICROTOX EC_{50} is in parts per million. Note that a lower EC_{50} indicates a more toxic sediment. Control samples are ● and nitrate-treated samples are O (n=2).

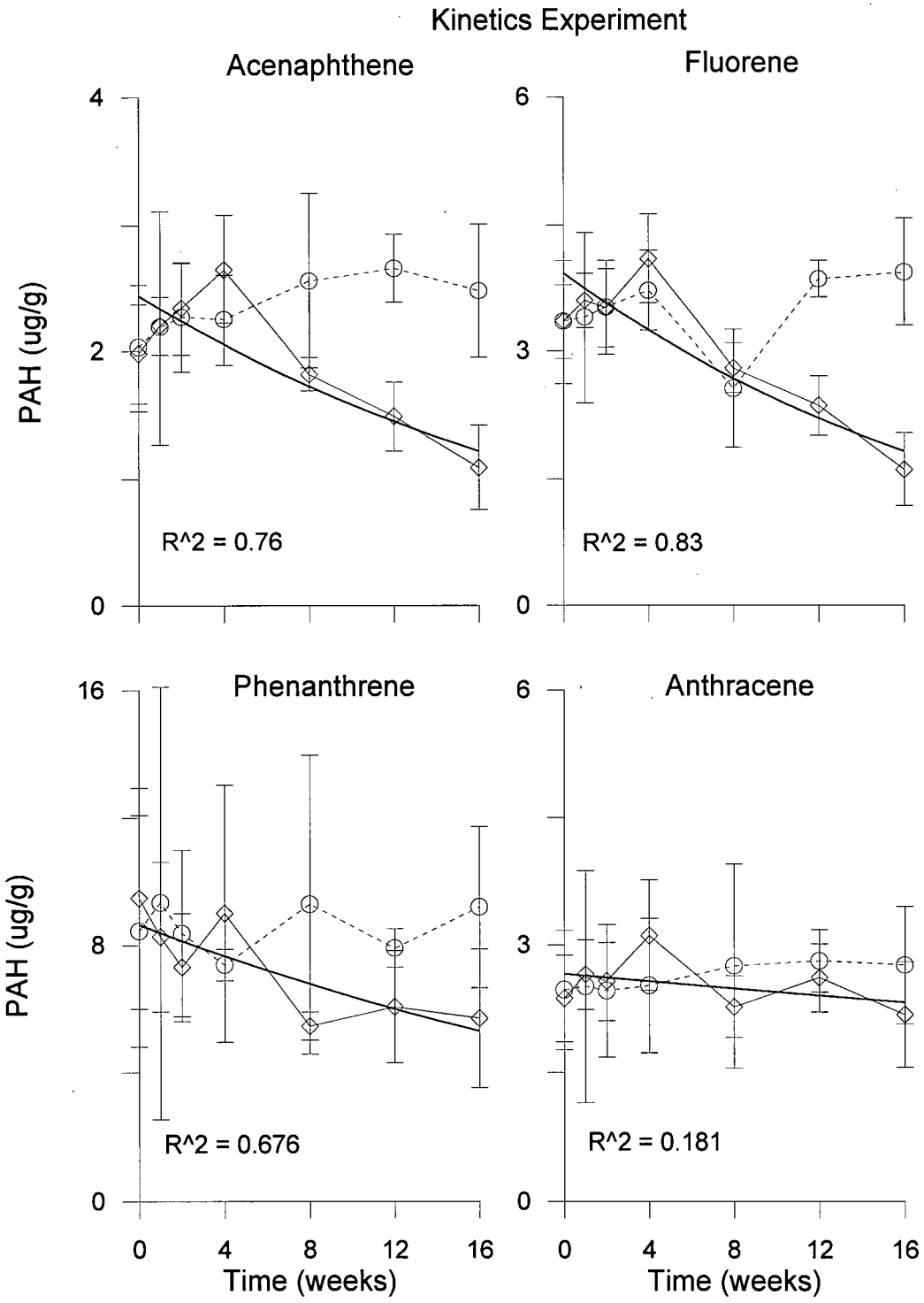


Figure 4.6 LMW PAH concentrations with time: Kinetics
PAH concentration (μg/g dry weight) vs. Time (Weeks) plots for, acenaphthene, fluorene, phenanthrene and anthracene for spiked sediments in the Kinetics experiment using calcium nitrate as nitrate source. At the end of the experiment, acenaphthene, fluorene and phenanthrene concentrations are significantly lower in the nitrate-treated samples (◊) than control samples (O) (95% confidence, n=6). Also included are the best fit first order degradation curves (thick lines) and correlation coefficients (r^2) for nitrate-treated samples.

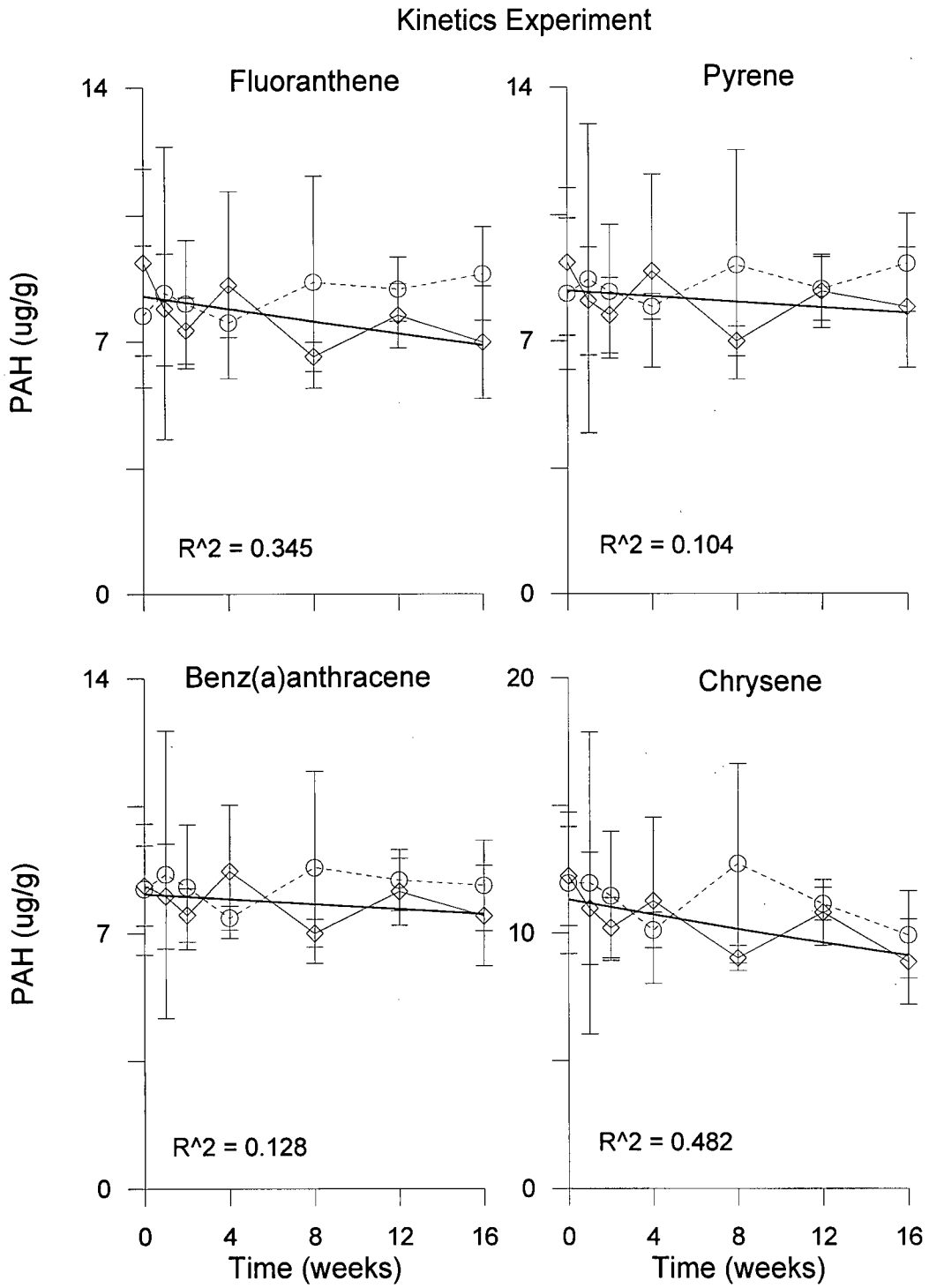


Figure 4.7 HMW PAH concentrations with time: Kinetics
PAH concentration (μg/g dry weight) vs. Time (Weeks) plots for fluoranthene, pyrene, benz(a)anthracene and chrysene for spiked sediments in the Kinetics 1 experiment using calcium nitrate as nitrate source. None of the differences between nitrate-treated (◊) and control (O) samples are significant at the 95% level (n=6). Also included are the best fit first order degradation curves (thick lines) and correlation coefficients (r^2) for nitrate-treated samples.

4.3.2 High and low spike experiments

At the end of the Kinetics experiment (section 4.3.1) there was some question as to whether the low degradation of PAH, was due to phosphate limitation, toxicity, or if a threshold PAH concentration was reached below which degradation would not occur. Therefore, an experiment was done with high and low levels of PAH added, in which all of these possibilities were addressed. Less sediment was added to each reaction vessel, nitrate was added as sodium nitrate to prevent the precipitation of phosphate, and two spike levels were used: one approximately the same as in the kinetics experiment (low level), and one about five times higher (high level).

Ten grams of sediment (3.64 g dry weight, 14.26% loss on ignition), sea salts, trace elements, phosphate and spiked clay (low or high PAH level) were added to serum bottles with Teflon lined lids. The treated sediments received sodium nitrate whereas the same molar amount of sodium chloride was added to the controls. More nitrate was added at 4 and 8 weeks, and phosphate was added at 8 weeks. The sediments and supernatants were analyzed at 0, 4, 8, 12 and 16 weeks (Data in Appendix E).

As in the Kinetics experiment, the sulphide concentration and toxicity of the treated sediment dropped within the first four weeks. Sulphide in the nitrate-treated sediments remained close to zero and the toxicity declined very slowly after the first rapid fall. The control sulphide concentration rose at the beginning of the experiment, then remained relatively constant. The toxicity of the control sediments also increased at the start of the experiment, then remained steady (Figure 4.8).

Ammonia concentrations in the reaction vessels followed a similar pattern as was observed in the previous experiment (Figure 4.9), except that the concentrations in the nitrate-treated bottles had dropped to near zero by week 12 in this experiment, rather than week 16.

Phosphate was not expected to precipitate in this experiment as it had previously because calcium was not added (except for the calcium in the sea salts and trace elements solutions). In the nitrate-treated sediments, phosphate in the supernatants dropped to <1 mg P/L within the first 4 weeks. More phosphate was added to all bottles at 8 weeks, after which the concentration in the treated bottles dropped more slowly and did not reach zero by the end

of the experiment. The phosphate levels remained constant in the control samples at approximately 15 mg P/L and 30 mg P/L after the second addition (Figure 4.9).

Nitrate was added to the treated sediments at 0, 4, and 8 weeks. Again, the rate of nitrate consumption slowed down over time (Figure 4.9). The total amount of nitrate added was 150 mg N, and the amount used by the end (16 weeks) was approximately 105 mg N. The PAH spike level in the reaction vessels did not affect nitrate consumption. Total nitrate use was 29 mg N/g dry sediment, or 404 mg N/g organic carbon. This consumption rate is higher than was observed in the Kinetics experiment, which could indicate that the use of sodium nitrate prevented phosphate limitation to the microorganisms that existed when calcium nitrate was used.

After 8 weeks of incubation, acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe) and anthracene (Ant) were lower in the treated samples than in the controls for both spike levels (Figure 4.10 and Figure 4.12). At the high spike level the differences between treated and control concentrations were statistically significant for acenaphthylene (Acy), pyrene (Pyr) and chrysene (Chr) as well. By week 16, all the tested PAH in the high spike samples, and all the PAH except benzo(a)pyrene (BaP), naphthalene (Nap) and Acy in the low spike samples were significantly lower in the treated sediments than in the controls. Nap and Acy in the low spike samples were present at low concentrations, and were below the detection limit in some nitrate-treated samples. BaP was not degraded in the low spike samples, and was only significantly different from controls at the last time point in the high spike samples. The spike level seems to have made little difference in the rates of degradation (see Table 4.7). The low molecular weight PAH were degraded more quickly than the larger, less soluble compounds. Phenanthrene, a three ring PAH, was degraded much more rapidly than anthracene which is exactly the same size, but is less soluble due to its ring configuration. The concentration versus time graphs for low and high molecular weight PAH receiving the high spike are shown in Figure 4.10 and Figure 4.11 respectively; the plots for sediments spiked with the lower PAH levels are in Figure 4.12 and Figure 4.13.

These results indicate that the main factor that inhibited PAH degradation in the low level Kinetics experiment (section 4.3.1) was probably phosphate limitation due to calcium phosphate precipitation when nitrate was added as the calcium salt.

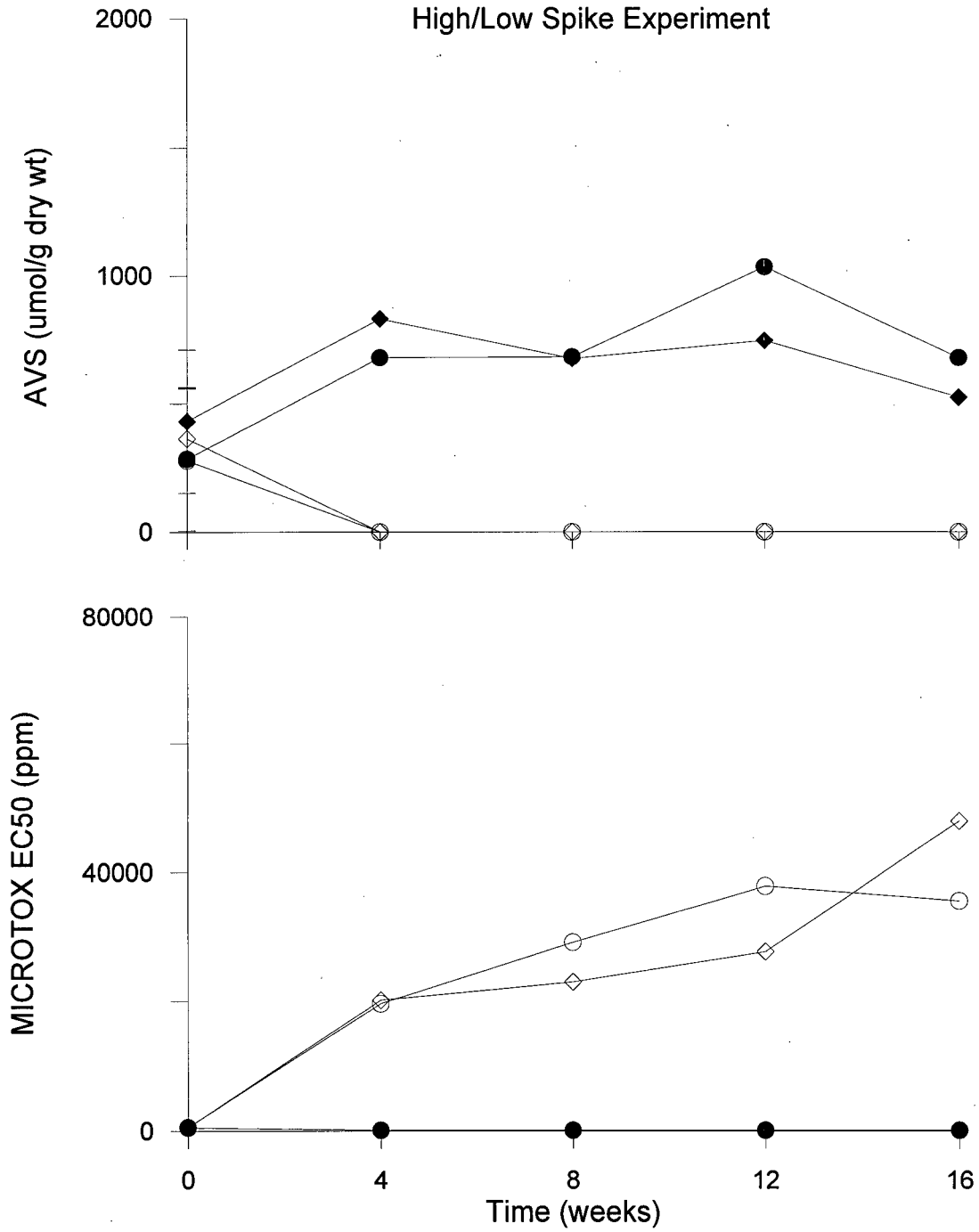


Figure 4.8 AVS and MICROTOX toxicity: High/Low experiment
AVS is expressed as μmoles/g dry weight of sediment, and the MICROTOX EC_{50} is in parts per million. Note that a lower EC_{50} indicates a more toxic sediment. Symbols are as follows: high spike nitrate-treated (O) and control (●); and low spike nitrate-treated (◊) and control (◆) samples.

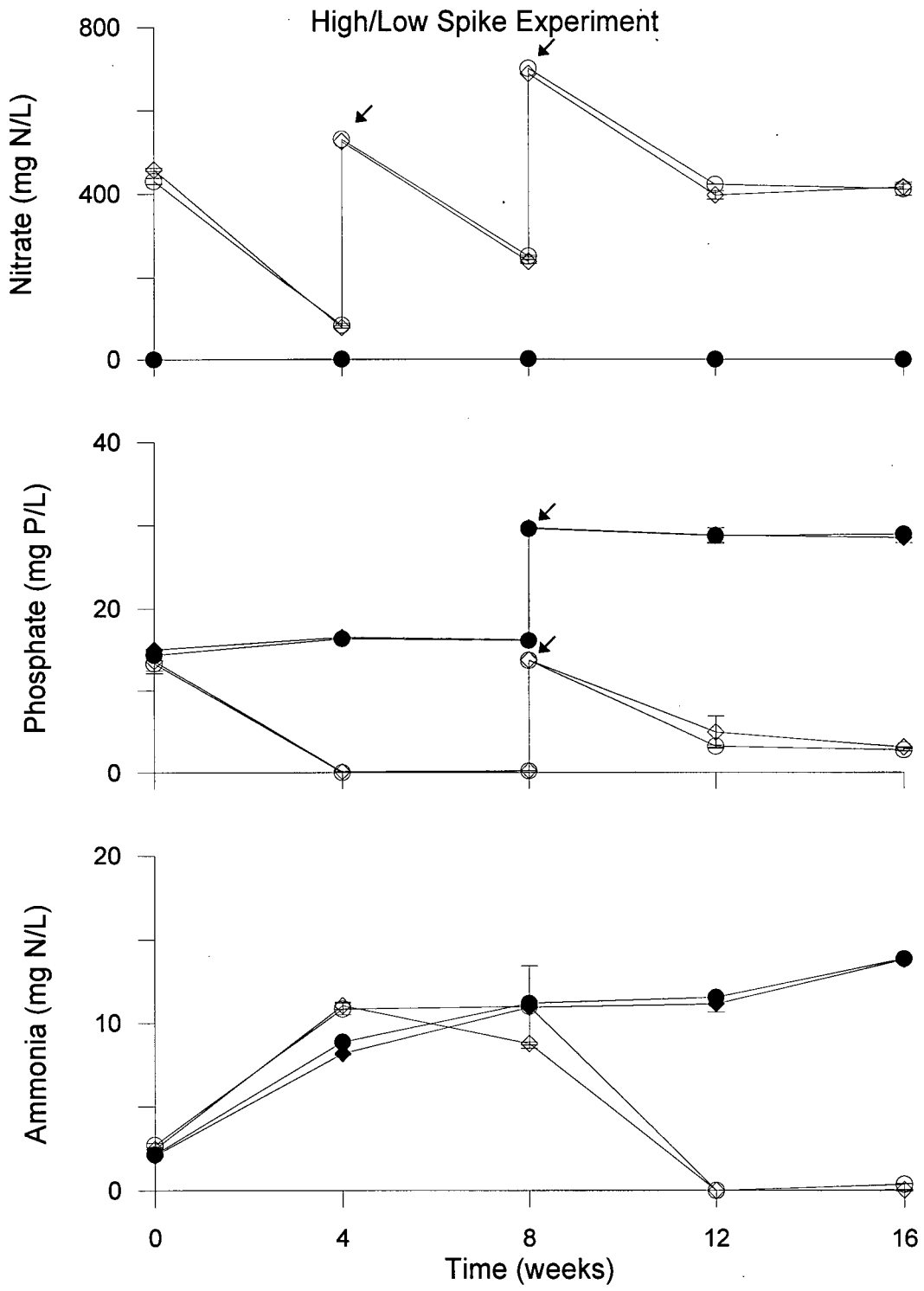


Figure 4.9 Nitrate, phosphate and ammonia concentrations: High/Low
Concentration (mg N or P/L) vs. time (weeks) plots for nitrate (top), phosphate (middle) and ammonia (bottom). Symbols are as follows: high spike nitrate-treated (O) and control (●); and low spike nitrate-treated (◊) and control (◆) samples. Arrows indicate when stock solutions were added.

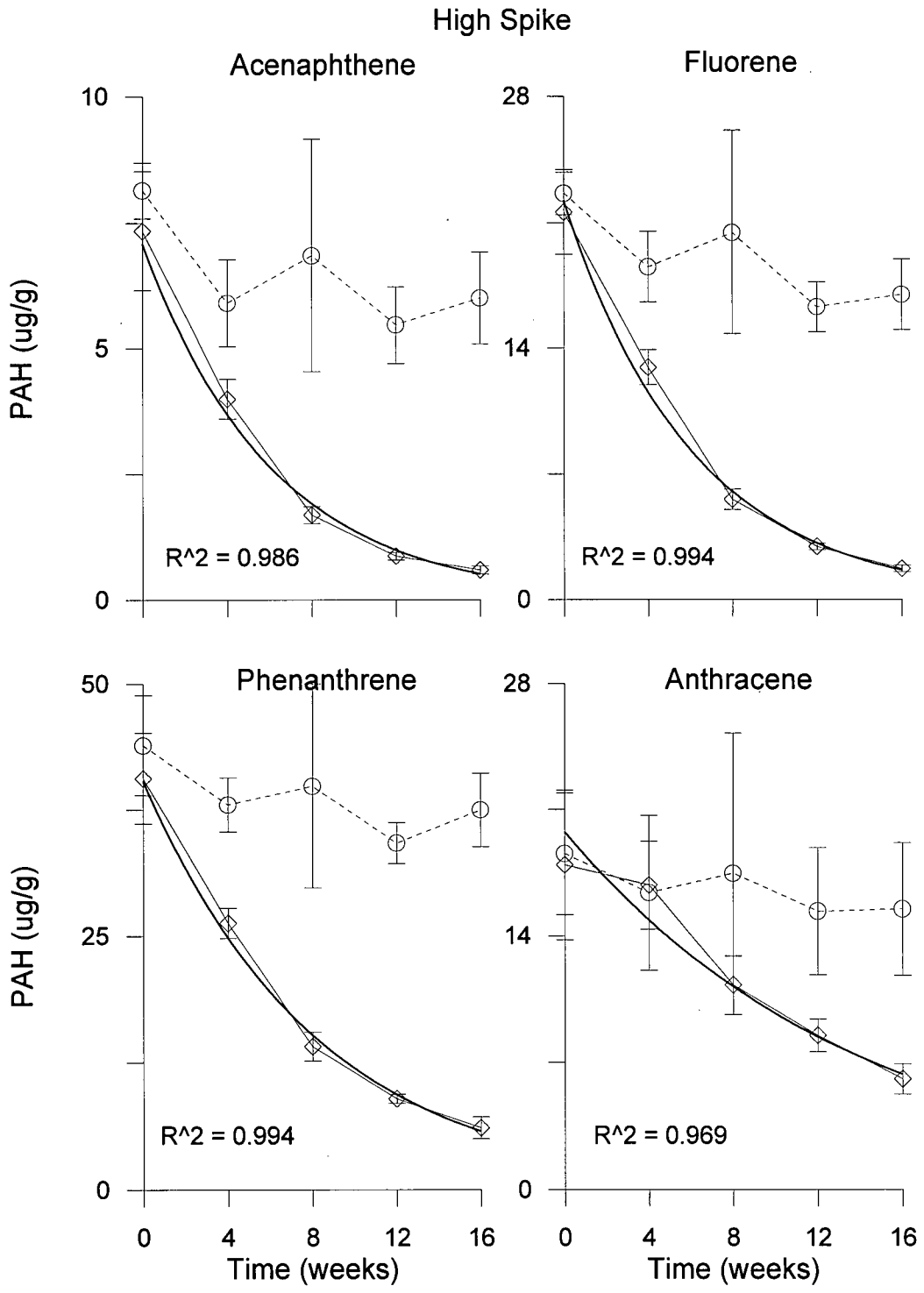


Figure 4.10 LMW PAH concentrations with time: high spike
Concentration (μg/g dry weight) vs. Time (Weeks) plots for acenaphthene, fluorene, phenanthrene and anthracene receiving the high level PAH spike. Control (O), and nitrate-treated (◊) samples are shown. Also included are the best fit first order degradation curves (thick line) and correlation coefficients (r^2) for nitrate-treated samples (n=6).

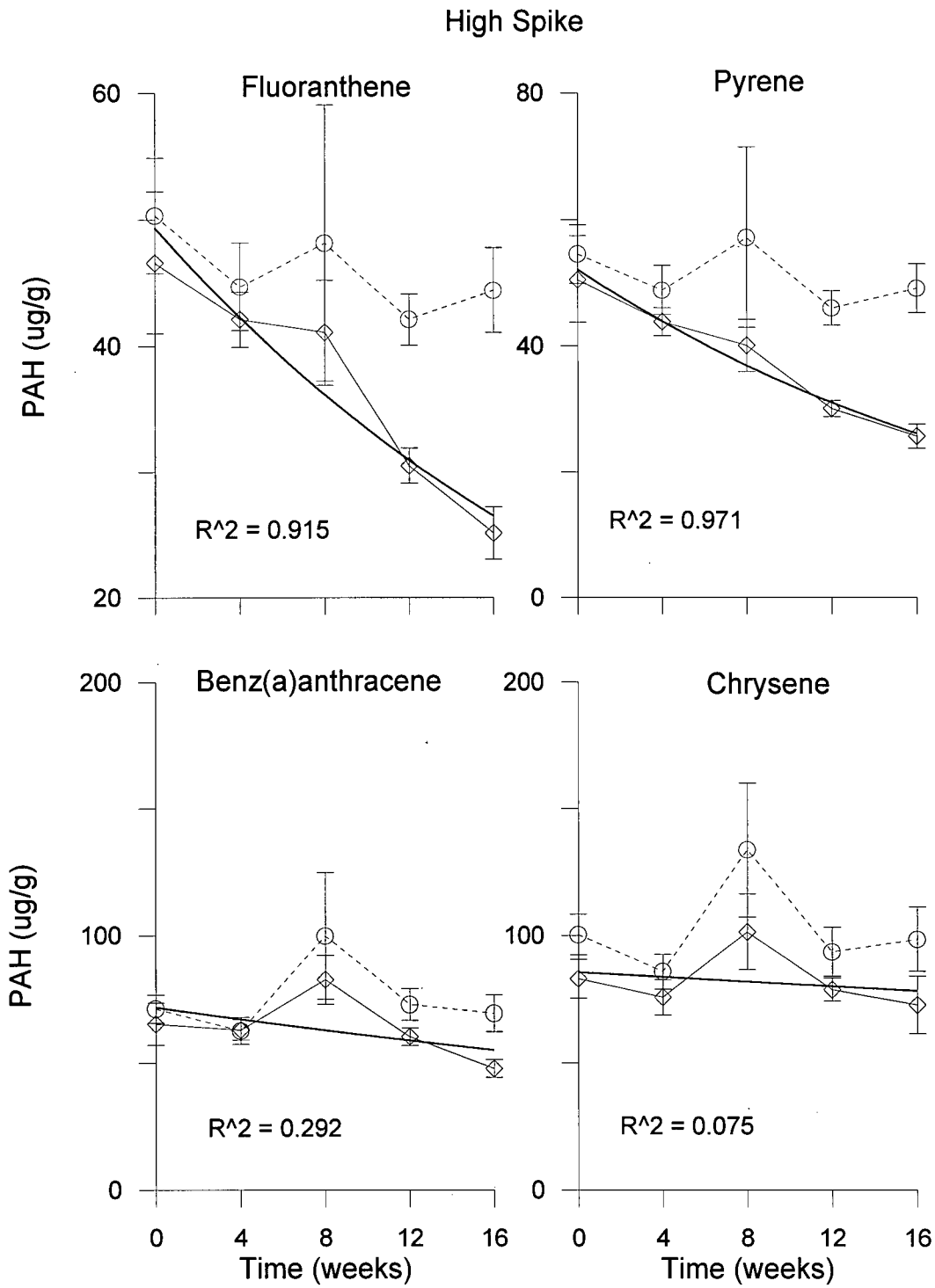


Figure 4.11 HMW PAH concentrations with time: high spike
Concentration (μg/g dry weight) vs. Time (Weeks) plots for fluoranthene, pyrene, benz(a)anthracene and chrysene receiving the high level PAH spike. Control (O), and nitrate-treated (◊) samples are shown. Also included are the best fit first order degradation curves (thick line) and correlation coefficients (r^2) for nitrate-treated samples (n=6).

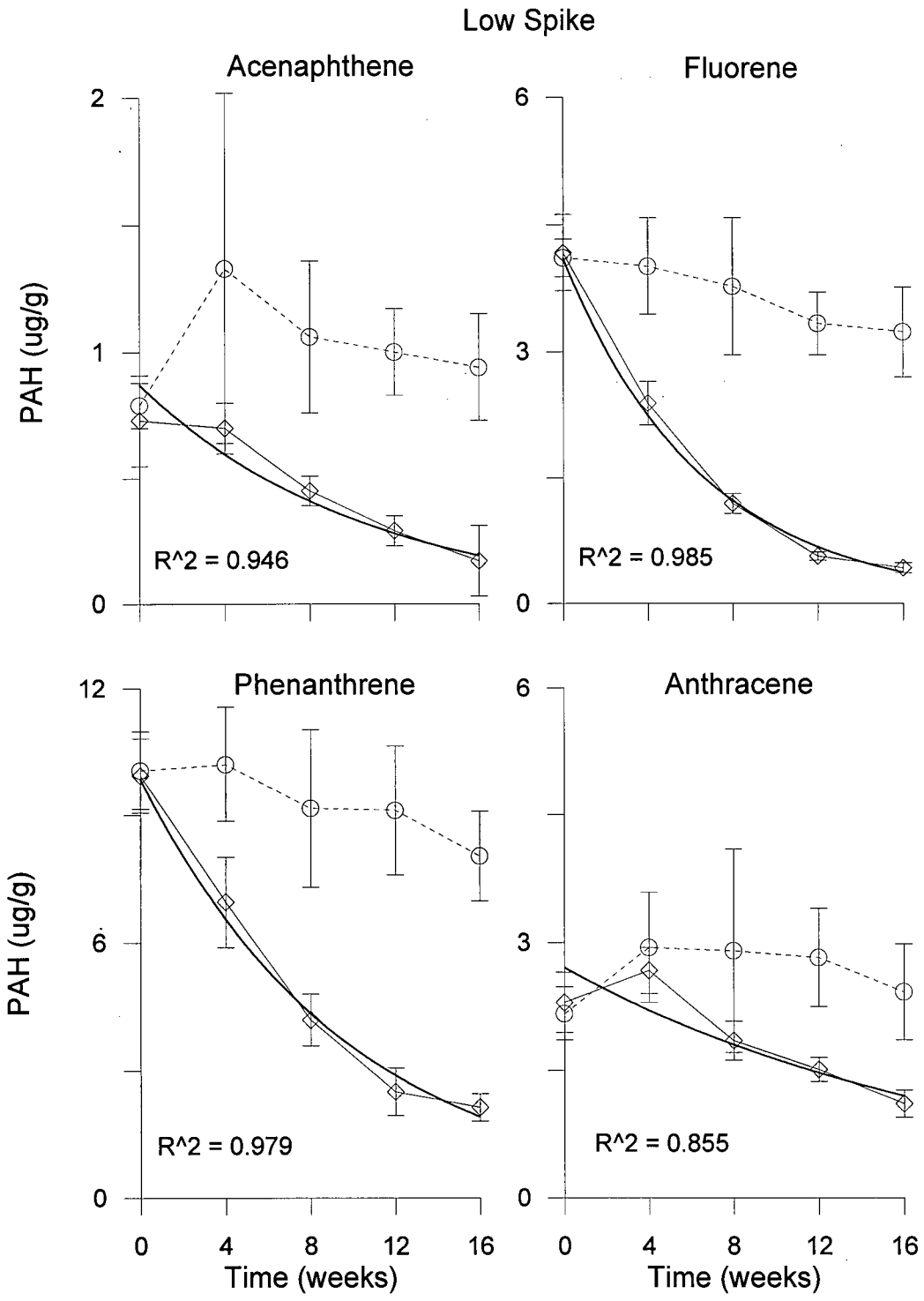


Figure 4.12 LMW PAH concentrations with time: low spike
Concentration (μg/g dry weight) vs. Time (Weeks) plots for acenaphthene, fluorene, phenanthrene and anthracene receiving the low level PAH spike. Control (O), and nitrate-treated (◊) samples are shown. Also included are the best fit first order degradation curves (thick line) and correlation coefficients (r^2) for nitrate-treated samples (n=6).

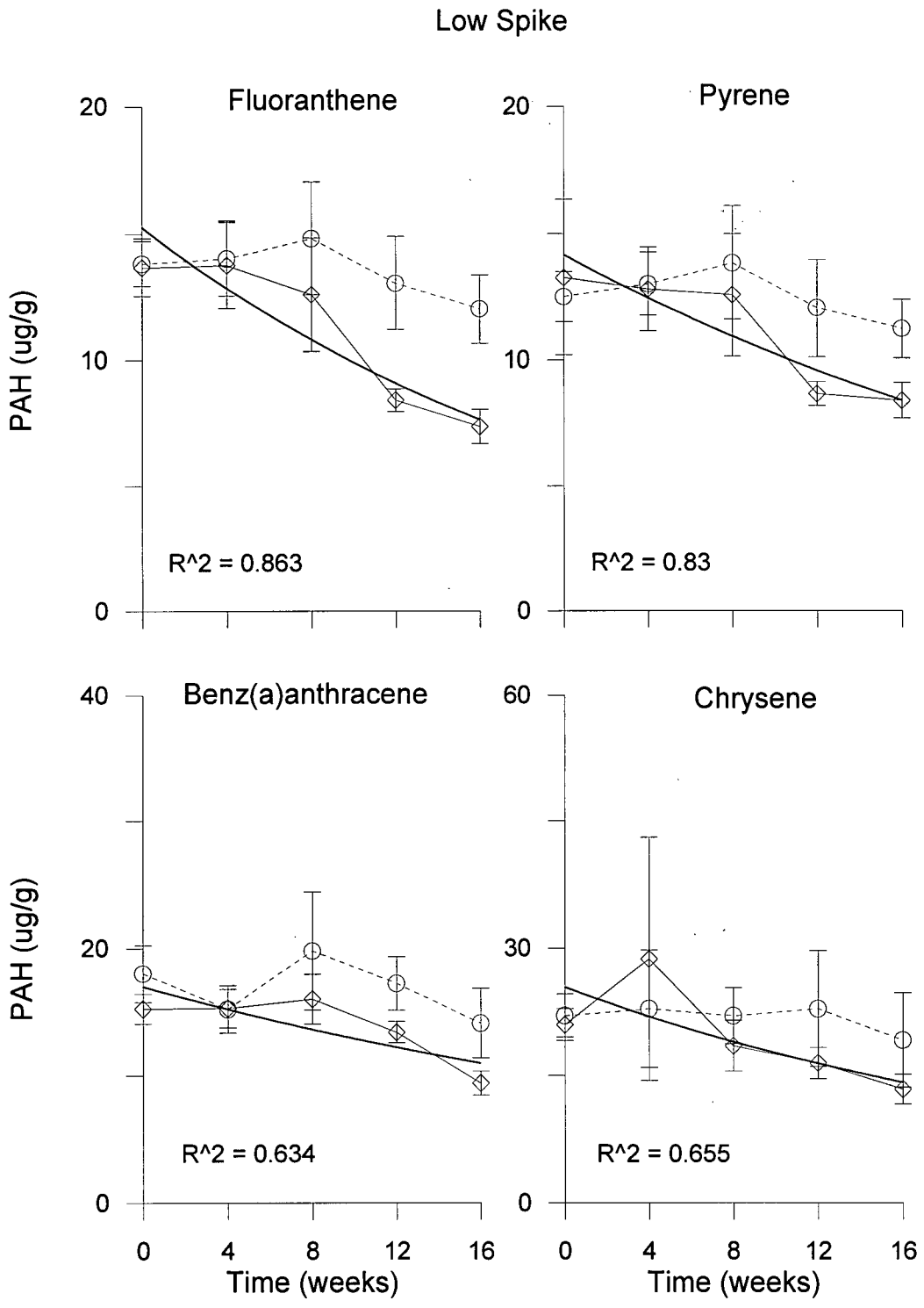


Figure 4.13 HMW PAH concentrations with time: low spike
Concentration (μg/g dry weight) vs. Time (Weeks) plots for fluoranthene, pyrene, benz(a)anthracene and chrysene receiving the low level PAH spike. Control (O), and nitrate-treated (◊) samples are shown. Also included are the best fit first order degradation curves (thick line) and correlation coefficients (r^2) for nitrate-treated samples (n=6).

By plotting the natural log of PAH concentration vs. time, the decay coefficients and half lives of PAH were calculated (Table 4.7). Also included is the r^2 value for correlation, which shows clearly that the fits are much better (closer to 1.0) for the LMW PAH than for the HMW compounds. This indicates that there is probably not an exponential decay of the larger molecules. This is confirmed by examination of Figure 4.10, Figure 4.11, Figure 4.12, and Figure 4.13, which show modest declines after an initial lag period. Benz(a)anthracene and chrysene did not seem to be degraded any further after 8 weeks relative to control samples.

Table 4.7 PAH degradation rates in the High/Low experiment
Decay rates (ln[μg PAH/g sed. dry wt.]/week), half lives (weeks) and correlation coefficients (r^2) are given for PAH spiked at two levels (high and low) in False Creek sediments treated with nitrate.

Spike Level	High			Low		
PAH	decay	$T_{1/2}$	r^2	decay	$T_{1/2}$	r^2
Acenaphthene	-0.162	4.28	0.971	-0.092	7.53	0.826
Fluorene	-0.163	4.25	0.985	-0.151	4.59	0.969
Phenanthrene	-0.122	5.68	0.975	-0.103	6.73	0.919
Anthracene	-0.070	9.90	0.855	-0.051	13.59	0.734
Fluoranthene	-0.039	17.77	0.812	-0.043	16.12	0.740
Pyrene	-0.043	16.12	0.871	-0.032	21.66	0.548
Benz(a)anthracene	-0.016	43.32	0.205	-0.027	25.67	0.517
Chrysene	-0.006	115.52	0.012	-0.034	20.39	0.363

4.4 Availability experiments

An important factor in the degradation of compounds in complex environments such as sediment is the availability of the substrate. Since in the High/Low Spike experiment the rate and extent of PAH degradation seemed to decrease with increasing molecular weight, it seemed important to investigate the availability of PAH in this sediment. Semi-permeable membrane devices (SPMDs) were used to study PAH availability. SPMDs are lengths of polyethylene tubing containing triolein, a major neutral lipid of fish. Small, hydrophobic compounds pass through the membrane material and become concentrated in the lipid phase relative to the concentration in the water (Huckins et al., 1990, 1993). Substrate uptake by microorganisms is usually via the aqueous phase, and so in these experiments, only material that can desorb from the sediment and is not associated with high molecular weight dissolved organic matter should be available for degradation. The polyethylene membrane used in SPMD construction excludes charged molecules and compounds with a molecular width of more than 10 angstroms. Therefore, SPMDs exclude all material that is associated with sediment particles or HMW dissolved organic matter, and take up small molecules that are truly dissolved or available (Huckins et al., 1990, 1993). SPMDs have been used successfully to monitor the available fraction of PAH in aquatic environments (Lebo et al., 1992). Numeric data for the availability experiments are presented in Appendix F.

4.4.1 Comparison of SPMD extracts of spiked clay with and without sediment

To see if the presence of sediment affected the ability of PAH to desorb from the solid phase, an experiment was carried out using 0.5 g PAH-spiked clay alone, or 0.5 g PAH-spiked clay mixed with 10 g wet False Creek sediment. Conventional SPMDs were prepared and exposed to these samples in amber bottles containing 400 mL sea salts solution for 24 hours. Both the SPMD extracts and the sediment (after exposure) extracts were analyzed for PAH and the results were compared to see the effect of the presence of the False Creek sediment.

In the absence of sediment, almost all of the PAH were extracted in the SPMD (Figure 4.14). Only small amounts of some HMW PAH were left associated with the solids. With the addition of False Creek sediment, less of the added material was extracted in the SPMD. The sediment therefore decreased the availability of PAH as evaluated by SPMD.

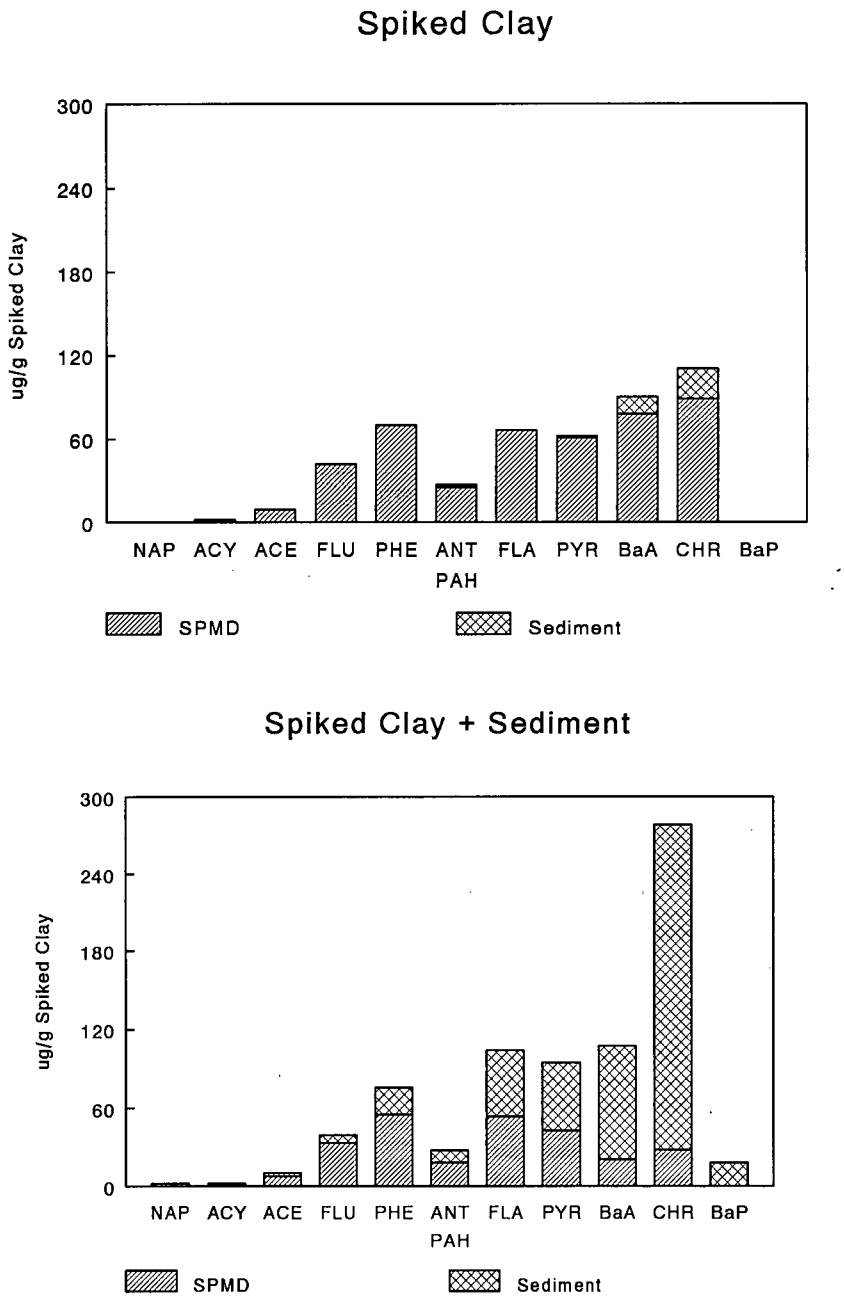


Figure 4.14 PAH availability from clay and clay plus sediment
PAH concentration (μg/g spiked clay) recovered in SPMD and solids extracts of clay alone (above) and in combination with False Creek sediment (below). The amount of material in the SPMD is indicated by the hatched (lower) bars and in the sediment by the cross-hatched (upper) bars

4.4.2 Effect of sediment organic content on PAH availability

To further investigate the effect of sediment organic content on the extractability of PAH by SPMDs, mixtures of freeze dried and ashed sediment were made, mixed with water in glass jars, and exposed to a series of SPMDs. These samples were spiked with PAH added to ashed sediment. The concentrations of PAH in the sediments and SPMDs from each treatment can't be compared directly because the False Creek sediment also contains some PAH. Results are expressed as a percentage of the total extracted. Figure 4.15 shows that the percentage of the extracted PAH in the first SPMD generally decreased with increasing organic content, and the percentage of PAH left in the sediment increased. This indicates that the PAH are associated more strongly to sediments with a higher organic content. Acenaphthene and fluorene seem to remain relatively constant rather than following this trend.

4.4.3 Comparison of availability of spiked and "endogenous" PAH

It is commonly noted that compounds added to environmental matrices seem to be more easily degraded than material that has been in place for some time (e.g., Hatzinger and Alexander, 1995). To compare the relative availabilities of the PAH in False Creek sediment and added material, conventional SPMDs were exposed to duplicate spiked and unspiked sediments. Sequential SPMDs were used to try to extract all of the PAH that could be desorbed from the sediment. The unspiked sediment was exposed to 9 SPMDs and the spiked sediment to 11. There were still small but detectable amounts of fluoranthene in the last SPMDs exposed to unspiked sediment. Fluoranthene, pyrene and chrysene were still detected in the last SPMD extracts of spiked sediment. In all cases, the sequential values seemed to approach a minimum. Table 4.8 shows the percentage of total PAH present in the first SPMD extract and in the sediment extract at the end of the experiment for both the spiked and unspiked sediments. The spiked samples released a higher percentage of the total PAH in the first SPMD, and a lower percentage was left in the sediment at the end of the experiment. This difference indicates that the endogenous material was more tightly bound to the sediment than the added PAH.

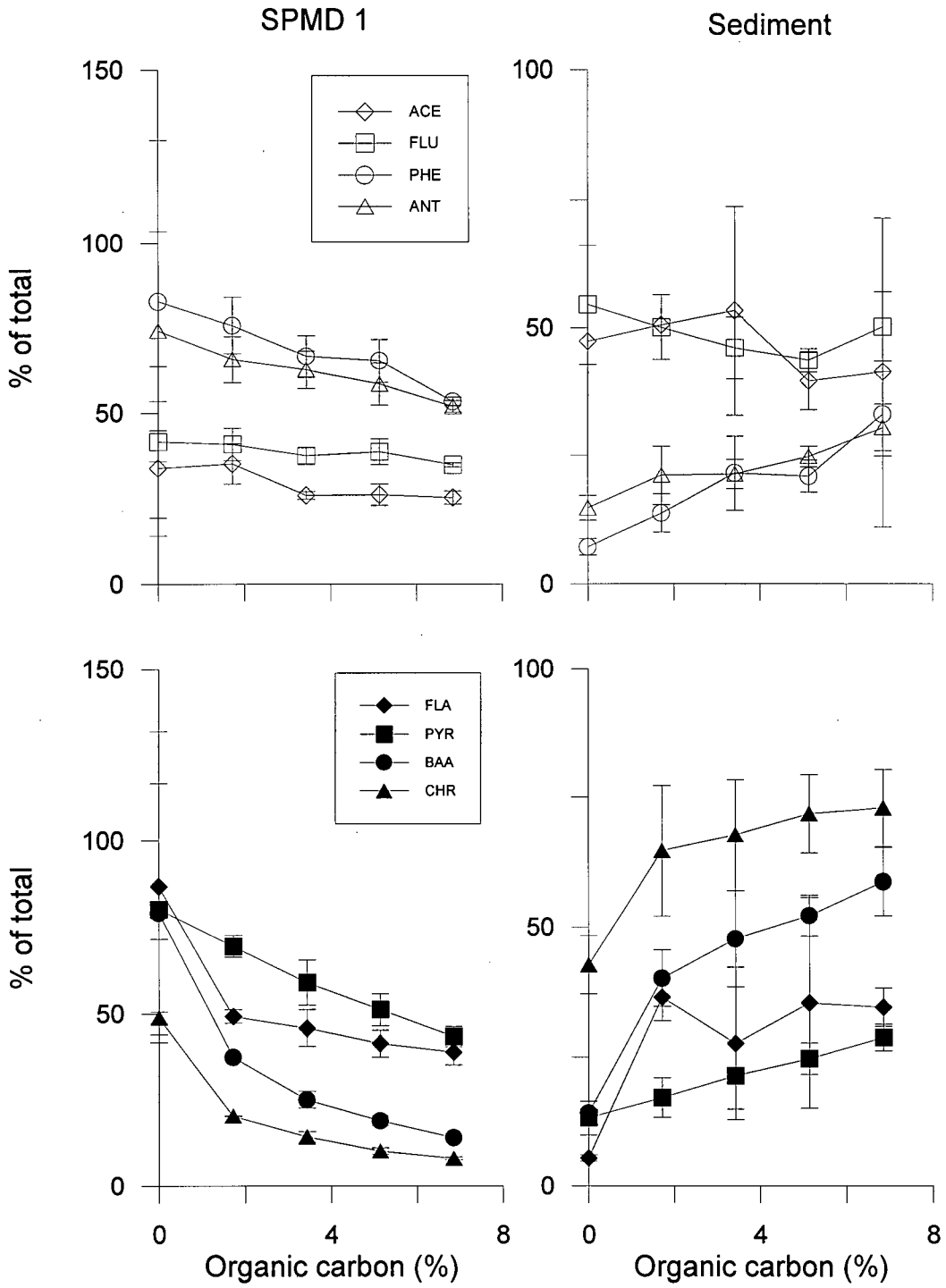


Figure 4.15 PAH in SPMD extracts of sediments with increasing organic content
SPMD extracts (n=2) are shown on the left, and sediment extracts (n=4) on the right. The LMW PAH are in the top panels and the HMW compounds in the lower panels. Individual compounds are listed in the figure.

Table 4.8 PAH availability in spiked and unspiked sediment
The percentage of total recovered PAH remaining in False Creek sediment after exposure to a series of SPMDs and in the first SPMD of the series are shown. A higher percentage left in the sediment indicates less availability for extraction by the SPMDs.

	Sediment		Sediment + Spike	
PAH	% in sediment	% in SPMD 1	% in sediment	% in SPMD 1
Acenaphthene	69	31	36	55
Fluorene	15	85	1	81
Phenanthrene	96	3	46	44
Anthracene	82	13	38	43
Fluoranthene	48	23	31	38
Pyrene	44	16	32	28
Benz(a)anthracene	80	3	50	15
Chrysene	88	6	38	17

The PAH concentrations in the sediments after exposure to the SPMDs were compared to see if the levels in the spiked sediments approached those in the unspiked samples. Similar results would indicate that essentially all of the added material was available. In Figure 4.16, the concentrations of some PAH in the unspiked sediment appear to be higher than in the spiked sediment. The error bars on the unspiked sediment are large because one of the three unspiked sediment (pooled) samples analyzed at the end had approximately double the PAH concentrations of the other two. Even without this unspiked sediment outlier, the PAH concentrations of the spiked and unspiked sediment were quite close. This indicates that all of the spiked material was probably available, although the less soluble HMW PAH associated more strongly with the sediments than did the LMW compounds (indicated by the smaller fraction present in the first SPMD). The persistence of some PAH in the sediment after sequential exposure to SPMDs indicates that a portion of the "endogenous" PAH was not available.

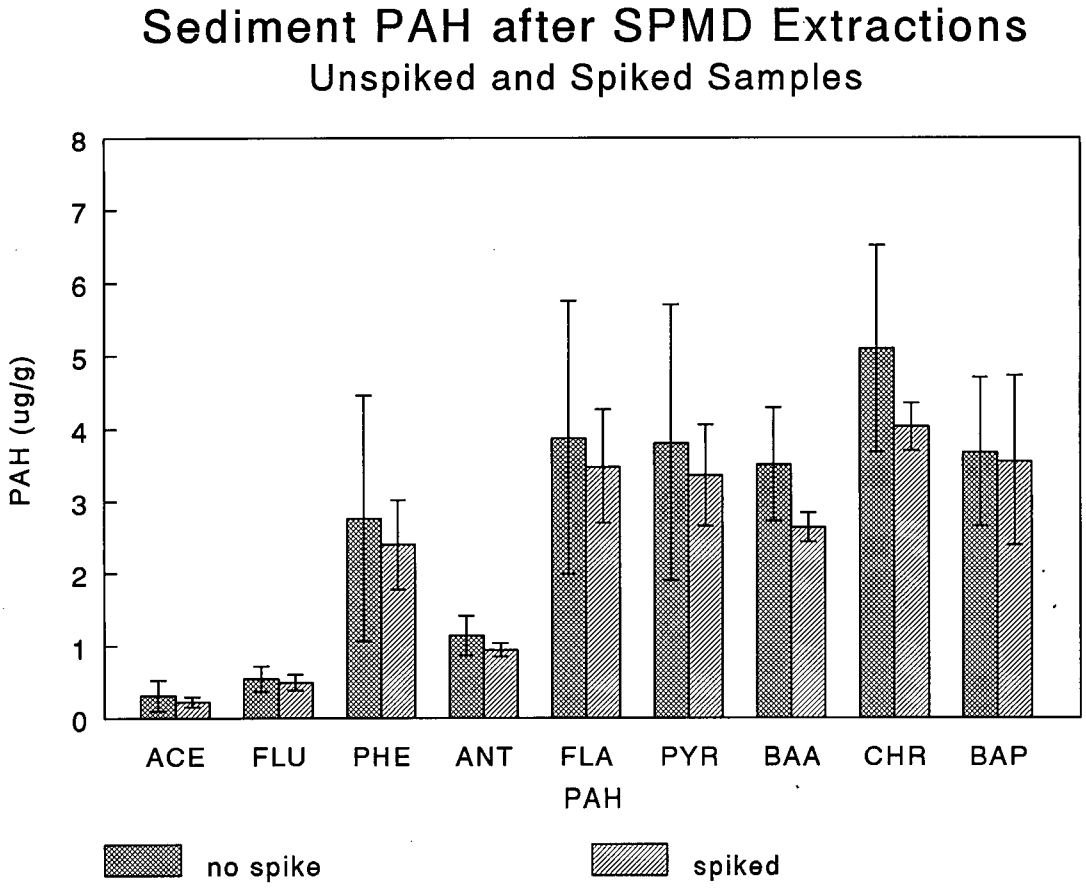


Figure 4.16 PAH concentrations left in spiked and unspiked sediments after exposure to a series of SPMDs The bars indicate averages of 3 replicates taken from the pooled sediments of duplicate treatments. The error bars indicate the standard deviation.

4.4.4 Reverse SPMD

Conventional SPMDs are expensive due to the cost of triolein, and serial exposure of spiked sediment to SPMDs did not achieve extinction (the point at which no more PAH could be detected in the SPMD extracts) even after exposure to 11 SPMDs. Therefore, a different method was devised. In reverse SPMDs (rSPMD), the sediment/water slurry is sealed inside polyethylene tubing, and the tube containing the sample is dialyzed against pentane. PAH extracted into pentane by this method would still have to desorb from the sediment and pass through the membrane to the pentane. This is expected to be a more stringent extraction since the amount of organic solvent into which the PAH partition from the aqueous phase is much greater in the rSPMD. Conventional SPMDs contain 1 mL of triolein compared to 150 mL of pentane outside the rSPMDs.

Several dialysis times were examined to see if an equilibrium extraction concentration would be reached. Reverse SPMDs were prepared and dialyzed against pentane for 2, 4, 8, and 24 hours. Equilibrium extraction appeared to be reached after 24 hours for most compounds, but not for some of the HMW PAH (benz(a)anthracene and chrysene). This experiment was repeated in triplicate, dialyzing for 4, 8, 24 and 48 hours. The fastest increases in extract PAH concentrations were observed in the first 8 hours (Figure 4.17), but the concentrations of most compounds continued to increase slowly, even after 24 hours. In further experiments, rSPMDs were dialyzed for 24 hours to provide sufficient contact time to approach the slow extraction phase, while preventing solvent damage to the tubing.

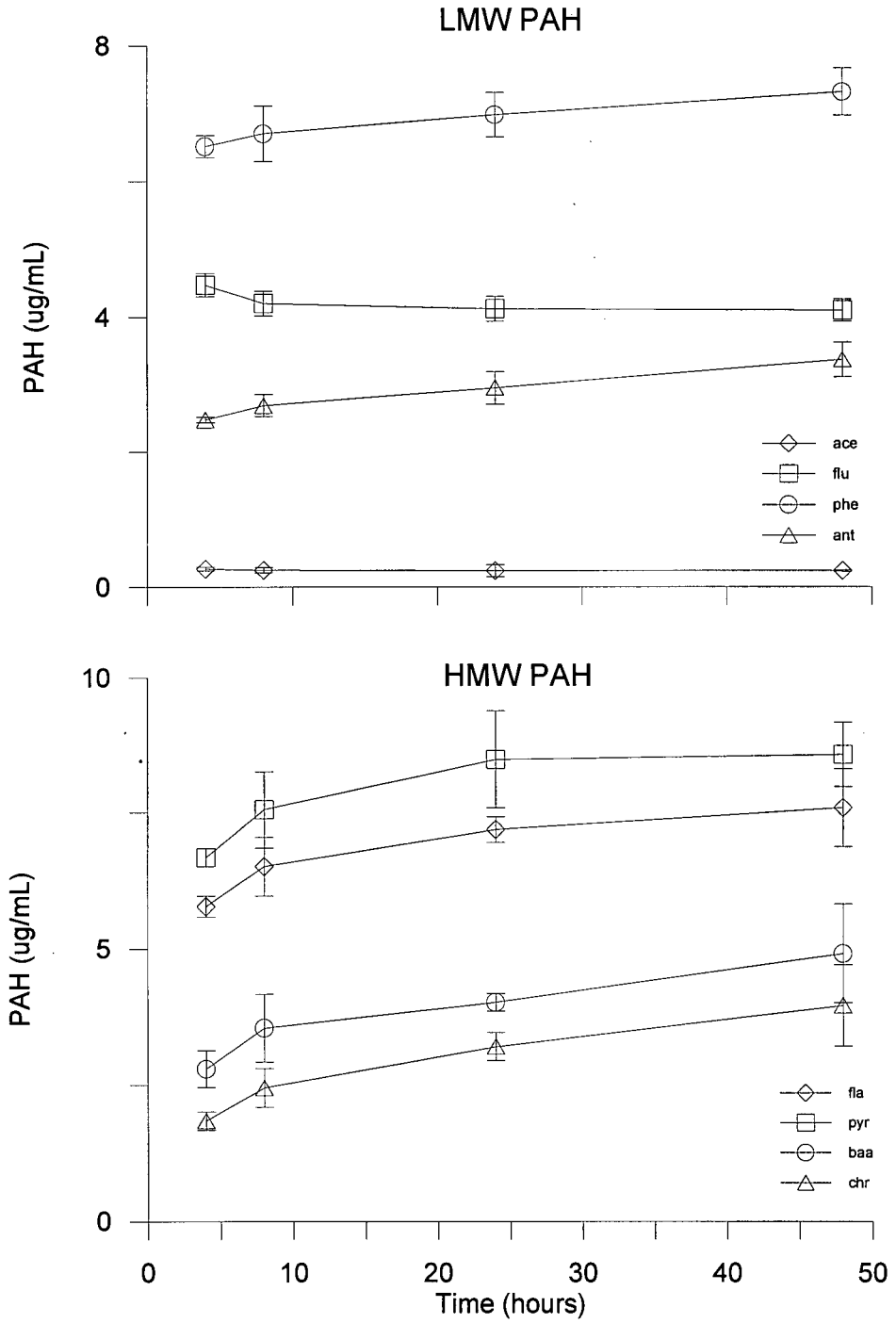


Figure 4.17 Effect of dialysis time on reverse SPMD extract concentration
Concentration (μg/mL extract) vs. Time (hours) plots for reverse SPMD extracts. The symbols represent the average of three determinations, and the individual compounds are listed on each graph.

4.4.5 Spiked and endogenous PAH availability determined by reverse SPMD

The objects of this experiment were to compare the availability of spiked and endogenous PAH using rSPMDs, and to compare the results of reverse and conventional SPMDs. Sediment/water samples were prepared, with and without a PAH spike, and rSPMDs were prepared containing either 5 or 10 mL of sample. These rSPMDs were dialyzed against pentane for 24 hours. The PAH concentrations (per g dry weight sediment) in the rSPMD extracts for the 5 and 10 mL samples were similar, although there was less spread in the 10 mL sample data for the unspiked sediment because the levels of PAH in the extracts were higher. The concentrations of PAH in the rSPMD and dialyzed sediment extracts using unspiked samples are shown in Figure 4.18. The "whole" sediment concentrations had been determined previously. The sum of the sediment and rSPMD values were usually higher than that found for the whole sediment extracts. This could either be a result of the analysis being done at another time (given that the values are quite low), or it could indicate that the extraction recoveries are better for the rSPMDs than for the sediment extracts. From the analyses of sediment and rSPMD extracts, it seems that only half of the PAH present in the False Creek sediment are available as measured by this method. Exceptions are fluorene, which was mostly extracted, and benzo(a)pyrene which was not extracted well. In spiked samples, higher percentages of the LMW compounds were present in the rSPMD extracts, indicating a higher degree of availability of these added compounds (Figure 4.19).

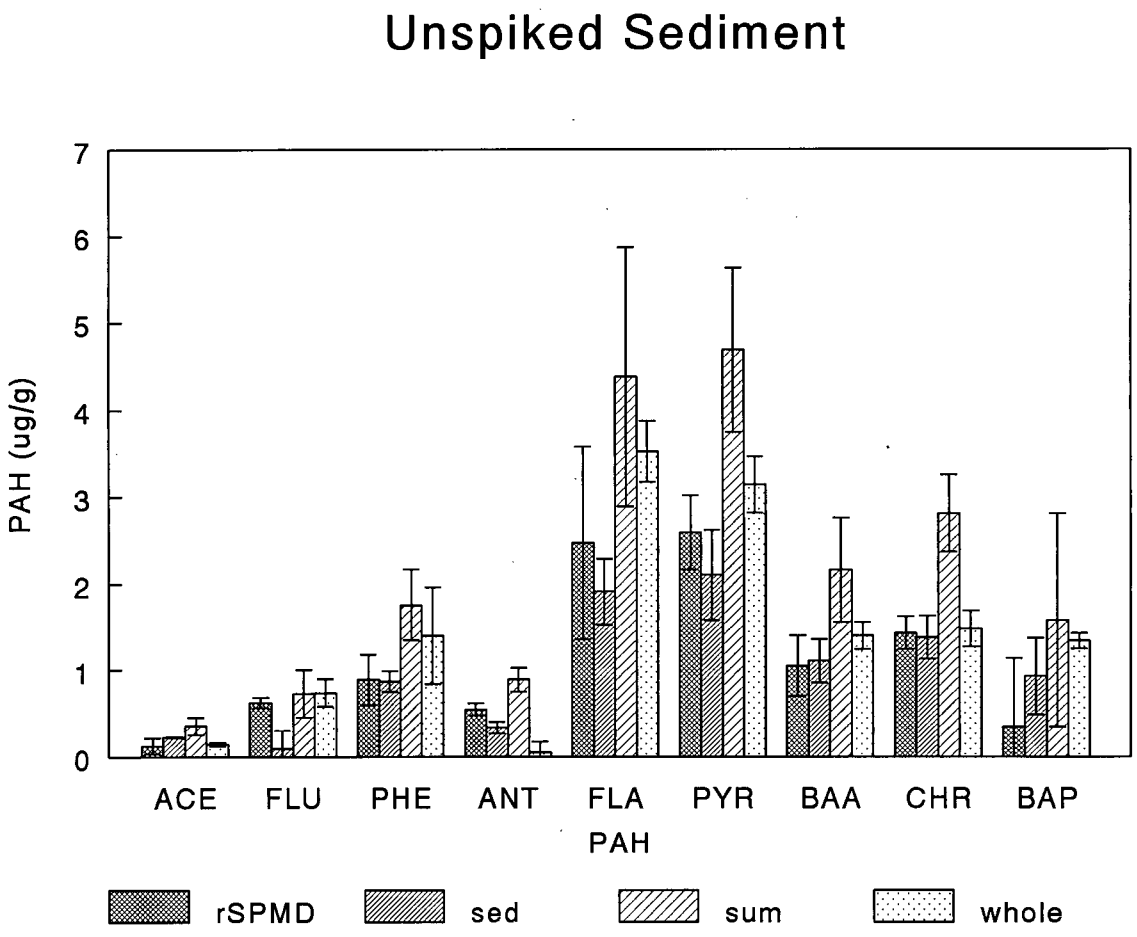


Figure 4.18 PAH concentrations in rSPMD and FCE sediment (exposed and whole) extracts
Abbreviations are as follows: "rSPMD" is reverse SPMD (10 mL sample), "sed" is the exposed sediment, "sum" is the sum of rSPMD and exposed sediment and "whole" is the concentration of PAH from sediment that had not been previously extracted by rSPMD. The error bars represent the standard deviation (n=4).

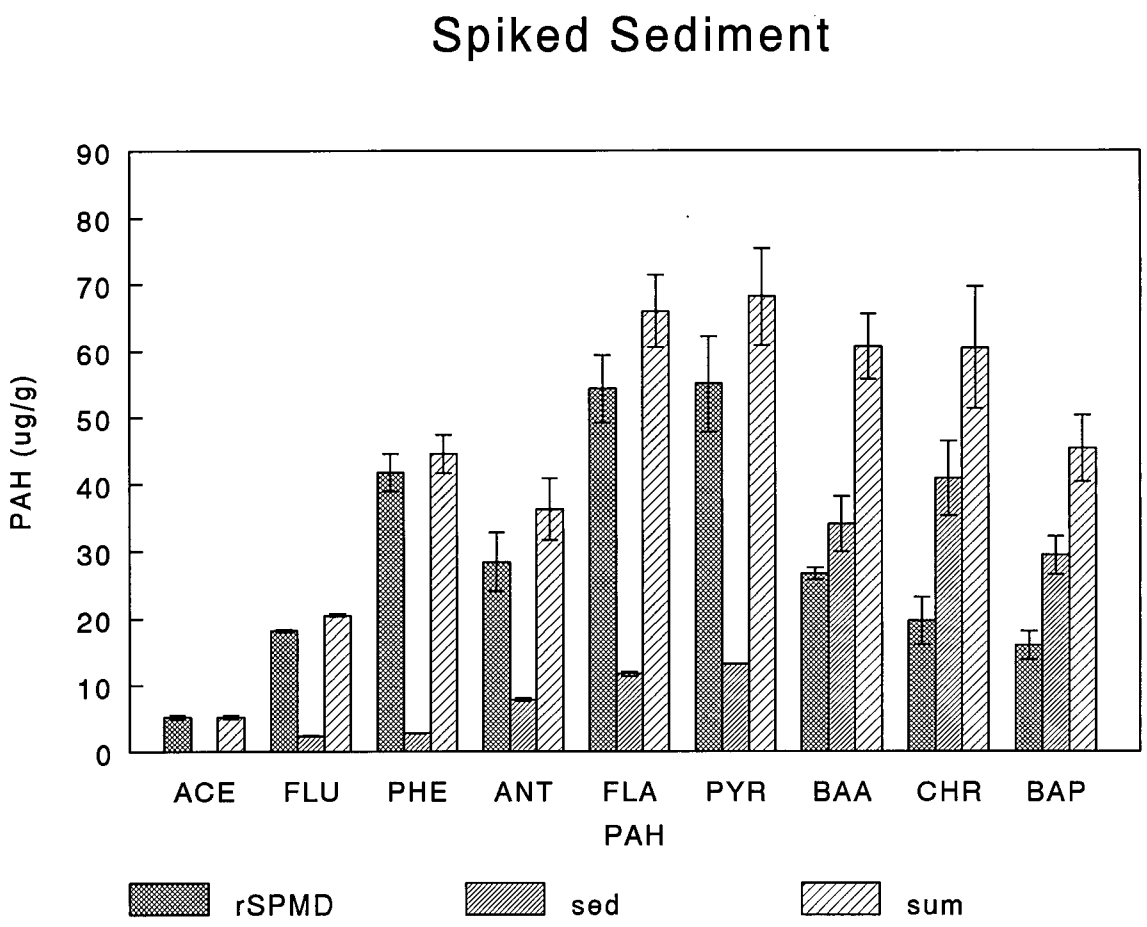


Figure 4.19 PAH concentrations in rSPMD and sediment extracts of spiked FCE sediment
Abbreviations are as follows: "rSPMD" is reverse SPMD extract (10 mL sample), "sed" is the concentration from sediment after exposure, and "sum" is the sum of rSPMD and exposed sediment. The error bars represent the standard deviation (n=4).

A comparison of the two estimates of "availability" is shown in Table 4.9. The results are expressed as percent of total measured PAH (sum of rSPMD and sediment after exposure) in the SPMD extract. For the conventional SPMDs, the percent in SPMD 1 from the comparison of availability of spiked and endogenous PAH experiment was used (section 4.4.3). Using both methods, the "available fraction" was generally higher for the spiked PAH than for the endogenous material, except for a few cases. It is also clear that the reverse SPMD is a more stringent extraction, since the fraction in the rSPMD extracts was always higher than in the conventional SPMD extracts.

Table 4.9 Comparison of reverse and conventional SPMDs
Both spiked and unspiked sediments are shown, "SPMD" values are for the conventional method, "rSPMD" for the reverse SPMD method. All values are given as percent of total measured in SPMD 1 or reverse SPMD extract.

	unspiked sediment		spiked sediment	
PAH	SPMD	rSPMD	SPMD	rSPMD
Acenaphthene	29	36	56	100
Fluorene	75	86	81	89
Phenanthrene	3	51	44	94
Anthracene	15	62	48	79
Fluoranthene	25	56	40	83
Pyrene	17	55	30	81
Benz(a)anthracene	0	49	5	45
Chrysene	8	51	8	34

4.5 Degradation experiments including availability measures:

4.5.1 Ammonia and Availability

The results of the High/Low Spike level experiment demonstrated that the rate and extent of degradation of PAH was dependent on the size of the compound. This raised the question of whether biodegradation was limited because the microorganisms in the sediment could not degrade HMW PAH under the experimental conditions, or the larger PAH were not available to them. Since the PAH in this system were added, they should initially have been in

an available pool. Over the course of the experiment, they could be rendered less available by occlusion in the sediment matrix. Were this to occur, the available (rSPMD) fraction in the control samples should decrease over time, relative to the sediment extracts. If the HMW PAH are not degraded because they are not present in the water phase at high enough concentrations but do not become occluded, their availability should remain constant in the controls. This would be indistinguishable from an inability of the microorganisms in the system to use these compounds.

Ammonia was added to one set of samples to make sure it didn't limit degradation later in the experiment, because the ammonia levels in the previous two experiments dropped to near zero toward the end of the incubation period.

Serum bottles were sealed containing 10 g of sediment (2.49 g dry weight, 17.79 % loss on ignition), sea salts solution, trace elements, phosphate, ammonia (for the ammonia-treated samples) and sodium nitrate (in nitrate-treated samples) or sodium chloride (in the controls). Samples were analyzed at 0, 4, 8, 12, 16, and 20 weeks. Addition of nutrients was required during the experiment (nitrate at week 4, phosphate at weeks 4 and 17, and ammonia at week 17). Sediments that had been autoclaved three times were also analyzed (Appendix G).

Nitrate was consumed rapidly in the first four weeks and then more slowly (Figure 4.20). A total of 100 mg N was added per bottle, and by the end, 54 mg had been used in the samples without ammonia, and 60 mg in the samples with ammonia added. The amount of nitrate used per gram of sediment was 21.7 and 24 mg N/g dry weight, respectively. The approximate nitrate consumption rates per gram of organic carbon in the sediments were 245 and 273 mg N/g OC.

Ammonia levels rose slowly in control samples that received no additional ammonia. In the nitrate-treated samples, ammonia fell to near zero at 16 weeks after an initial rise. Even with ammonia added, the levels in the nitrate-treated samples fell to 5 mg N/L at 16 weeks. Ammonia was reapplied at 17 weeks (approximately 30 mg N/L), and by week 20, the level had again fallen to 5 mg N/L. The ammonia was not consumed in the control samples (Figure 4.20).

Ortho-phosphate levels in the control samples remained at the added levels throughout the experiment, with or without ammonia addition (Figure 4.20). In the nitrate-treated samples, the phosphate levels dropped to near zero in the first 4 weeks. More phosphate was added and

the level again dropped to 1.5 mg P/L where it remained until more was added at week 17. Again some phosphate was consumed, and at the end of the experiment the concentrations were 6.4 and 7.8 mg P/L. When more stock nutrient solution was added, only the first (lower) symbol in Figure 4.20 is a measured value. The second value was deduced from the amount added.

As in past experiments, the AVS and toxicity in the treated sediments initially dropped rapidly, then remained roughly constant. In the control sediments, AVS and MICROTOX toxicity rose slightly then remained constant for the remainder of the experiment (Figure 4.21).

The PAH concentrations stayed roughly constant in the autoclaved samples (data not shown) as well as in the untreated controls. With nitrate addition, PAH with molecular weights of up to 202 (fluoranthene and pyrene) were degraded. Benz(a)anthracene might have been slightly transformed, but chrysene and benzo(a)pyrene were not degraded (nitrate-treated and control sediments are parallel). The concentration vs. time plots for acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene benz(a)anthracene and chrysene for samples without ammonia added are shown in Figure 4.22 and Figure 4.23. The results with ammonia addition (Figure 4.24, and Figure 4.25) were similar to those from samples without ammonia addition.

The availability of PAH as measured by reverse SPMDs is also shown in Figure 4.22 and Figure 4.23. For most PAH, all of the added PAH was "available" by this measure. Benz(a)anthracene and chrysene as well as benzo(a)pyrene were not completely available, and these HMW PAH were not degraded under the test conditions. For some of the smaller PAH, the "available" fraction measured by rSPMD seems to be higher than the "total" PAH. Since the recoveries using the two extraction procedures are not the same, direct comparisons of the results cannot be made.

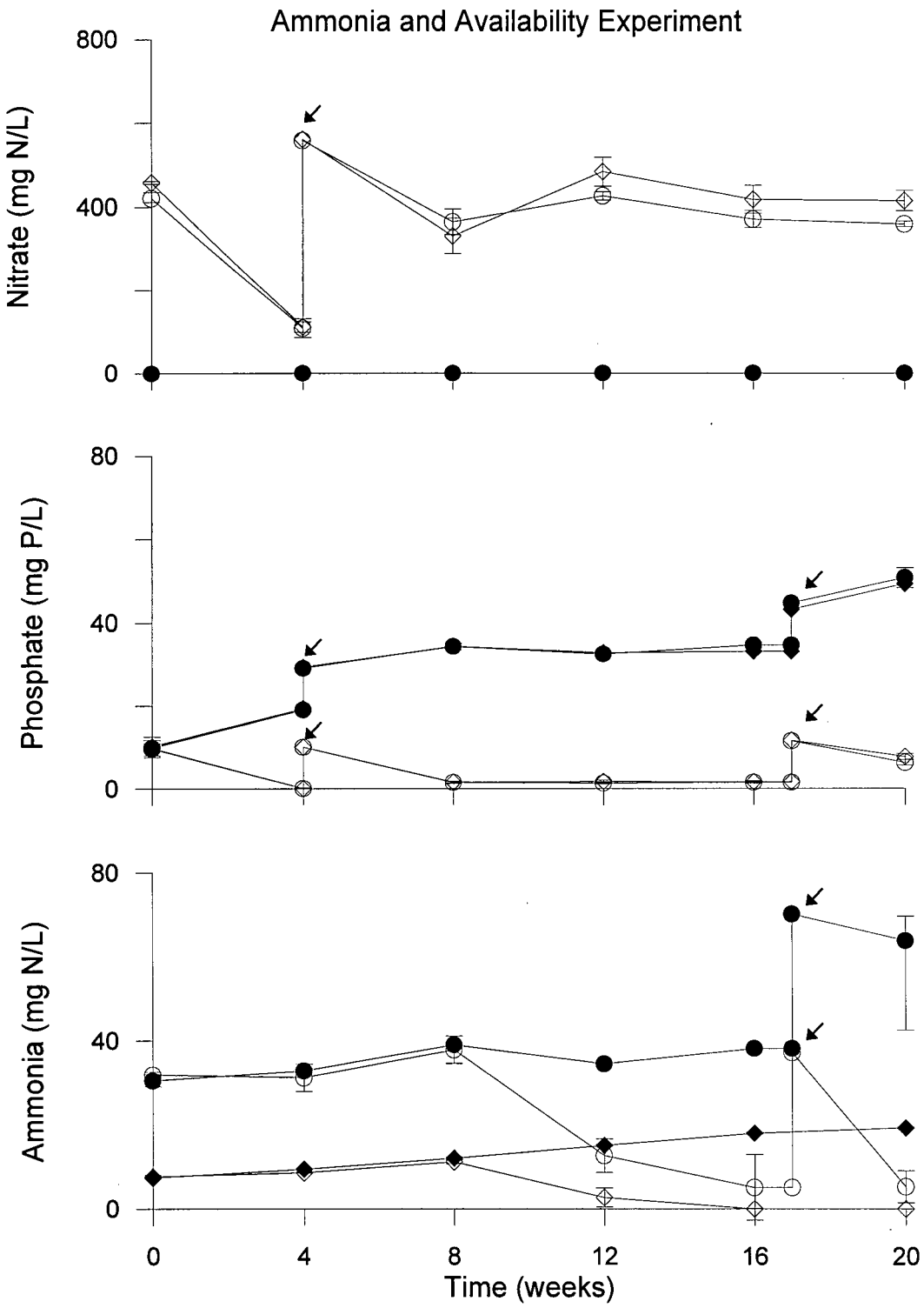


Figure 4.20 Nitrate, phosphate and ammonia concentrations: Ammonia and Availability experiment

The top panel shows nitrate concentration (mg N/L), the second orthophosphate (mg P/L) and the third ammonia (mg N/L). The symbols represent the average of 3 replicates for each treatment: no ammonia/nitrate-treated (◊), no ammonia/control (◆), ammonia/nitrate-treated (O), ammonia/control (●). Arrows indicate when stock solutions were added.

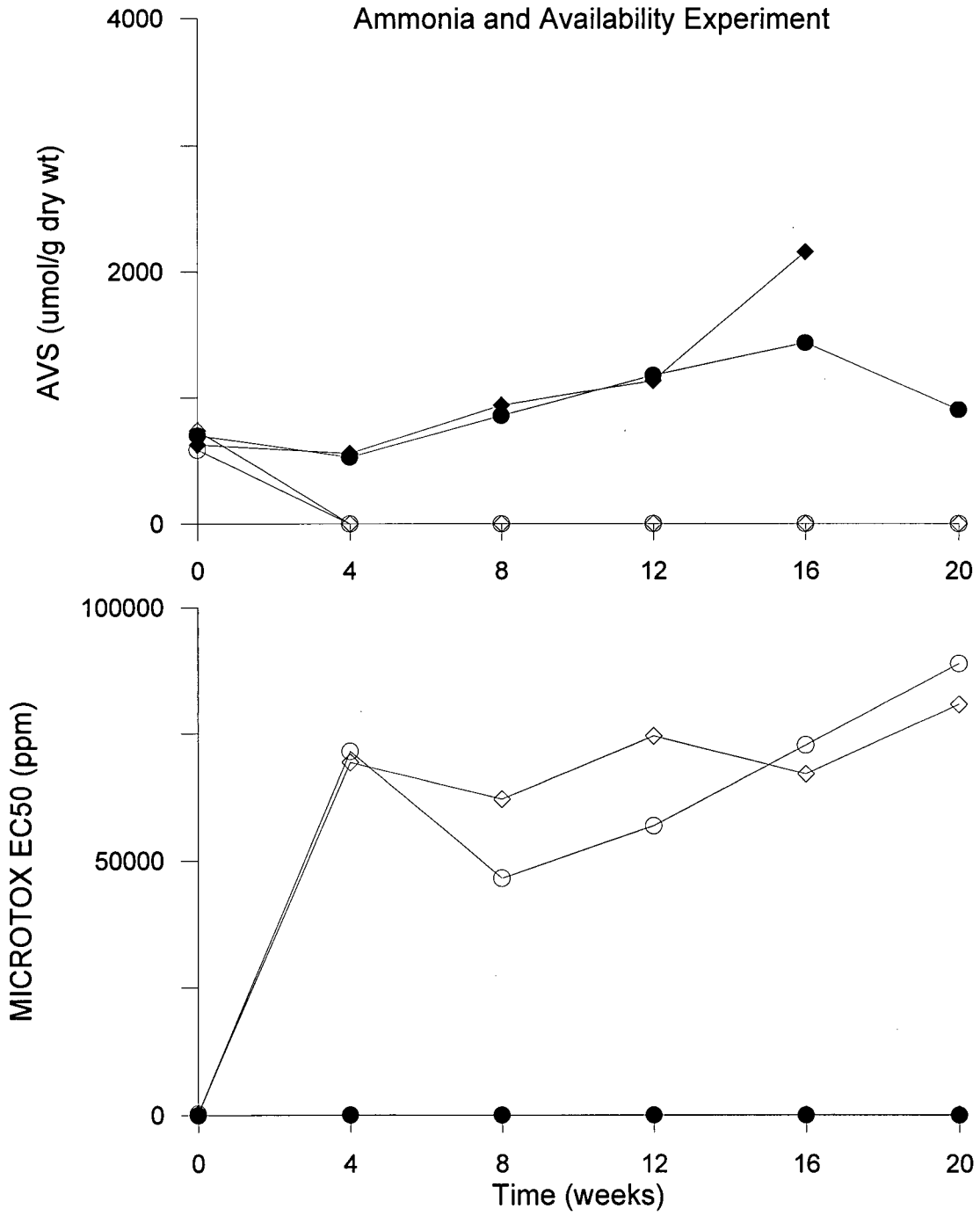


Figure 4.21 AVS and MICROTOX toxicity: Ammonia and Availability experiment
The top panel shows acid volatile sulphide (AVS) in μmoles/g dry weight, and the lower panel shows the MICROTOX EC_{50} values in ppm. Samples: no ammonia/nitrate-treated (◊), no ammonia/control (◆), ammonia/nitrate-treated (O), ammonia/control (●).

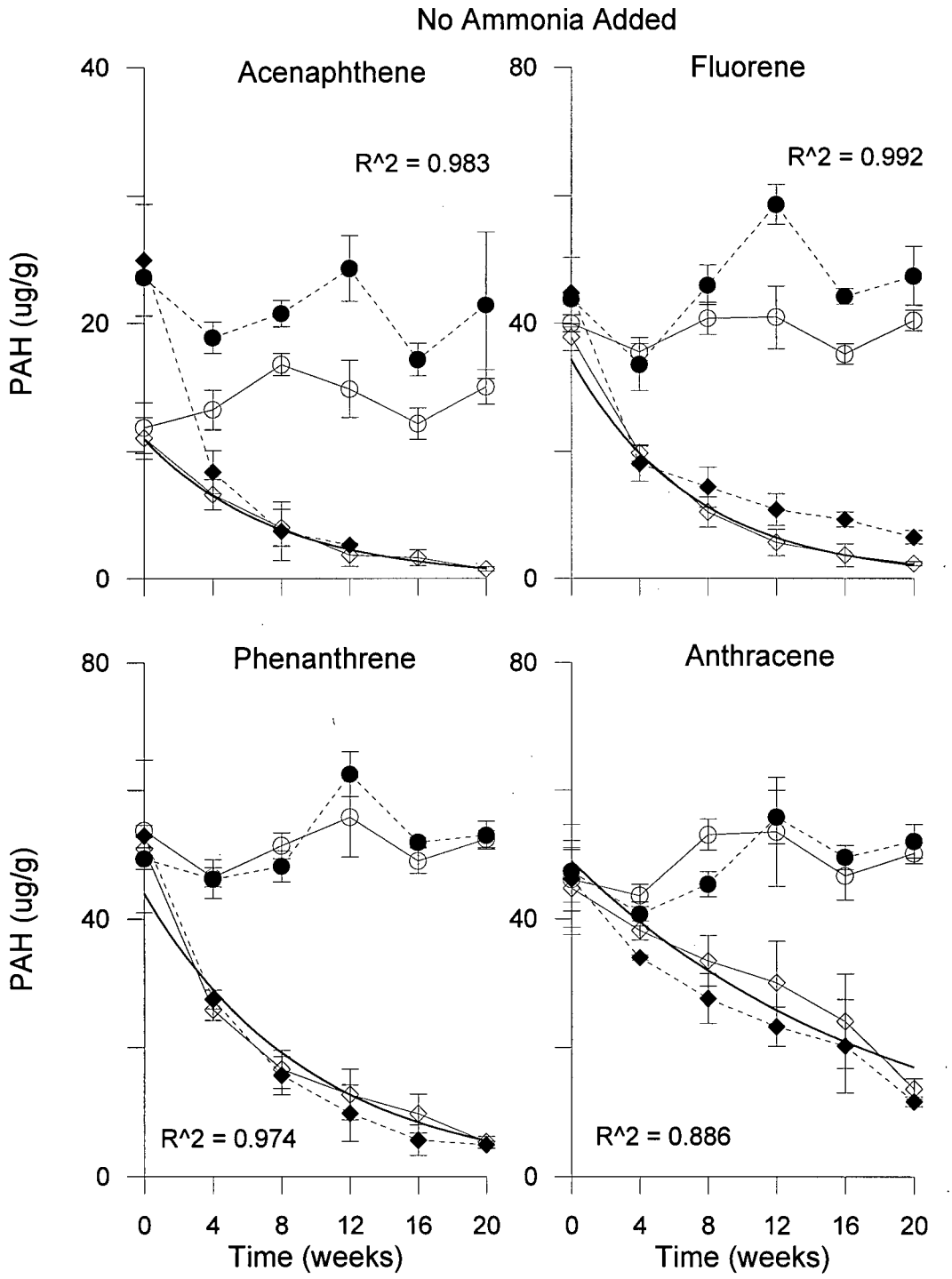


Figure 4.22 LMW PAH concentrations with time: No ammonia added
Concentration (µg/g dry weight) vs. Time (Weeks) plots for acenaphthene, fluorene, phenanthrene and anthracene without added ammonia (n=6). Symbols: nitrate-treated sediment (◊), control sediment (O), nitrate-treated rSPMD (◆), control rSPMD (●) sample extracts. Also included are the best fit first order degradation curves (thick lines) and correlation coefficients (r^2) for nitrate-treated sediments.

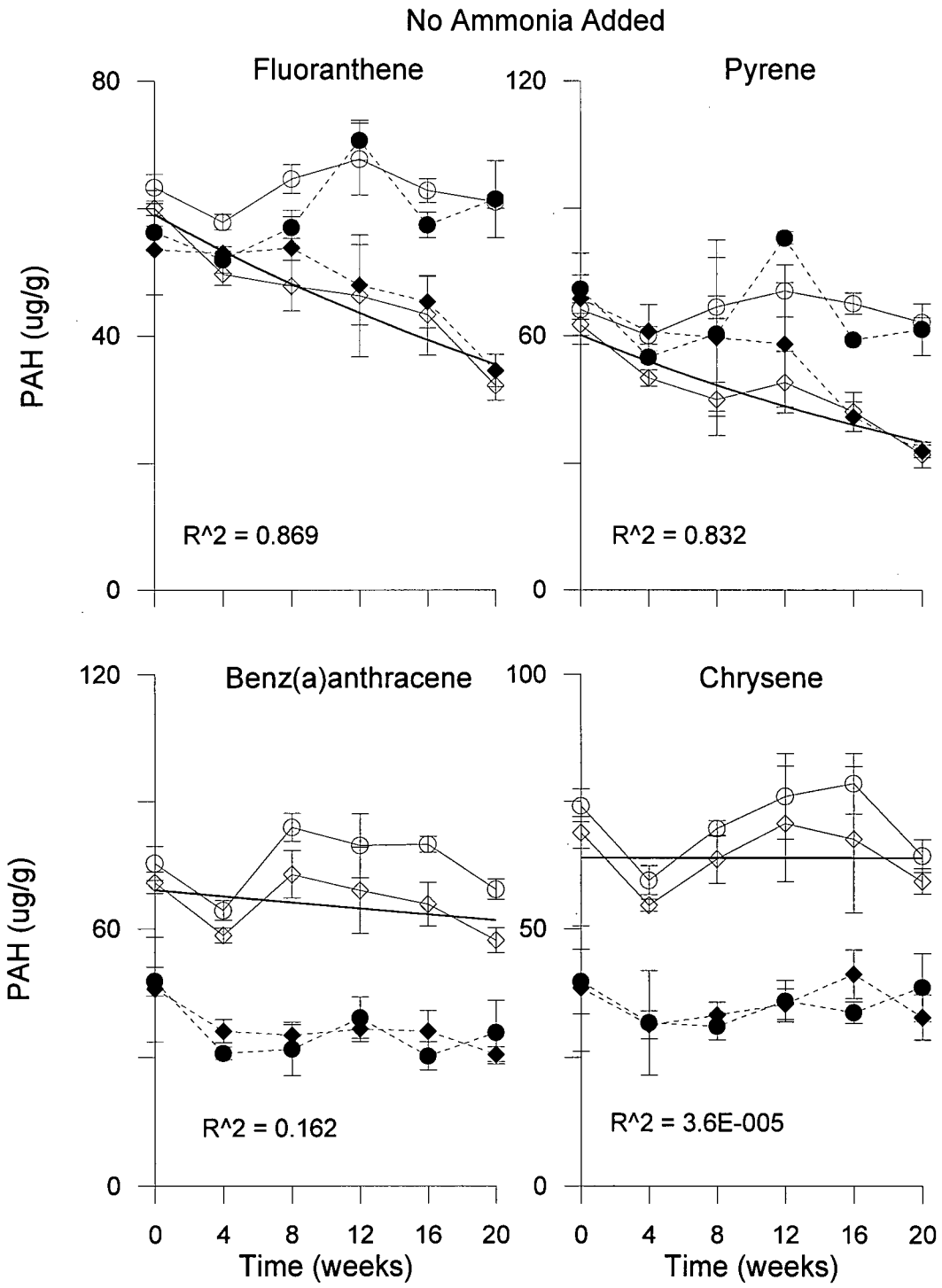


Figure 4.23 HMW PAH concentrations with time: No ammonia added
Concentration (μg/g dry weight) vs. Time (Weeks) plots for fluoranthene, pyrene, benz(a)anthracene and chrysene without added ammonia (n=6). Symbols: nitrate-treated sediment (◊), control sediment (O), nitrate-treated rSPMD (◆), control rSPMD (●) sample extracts. Also included are the best fit first order degradation curves (thick lines) and correlation coefficients (r^2) for nitrate-treated sediments.

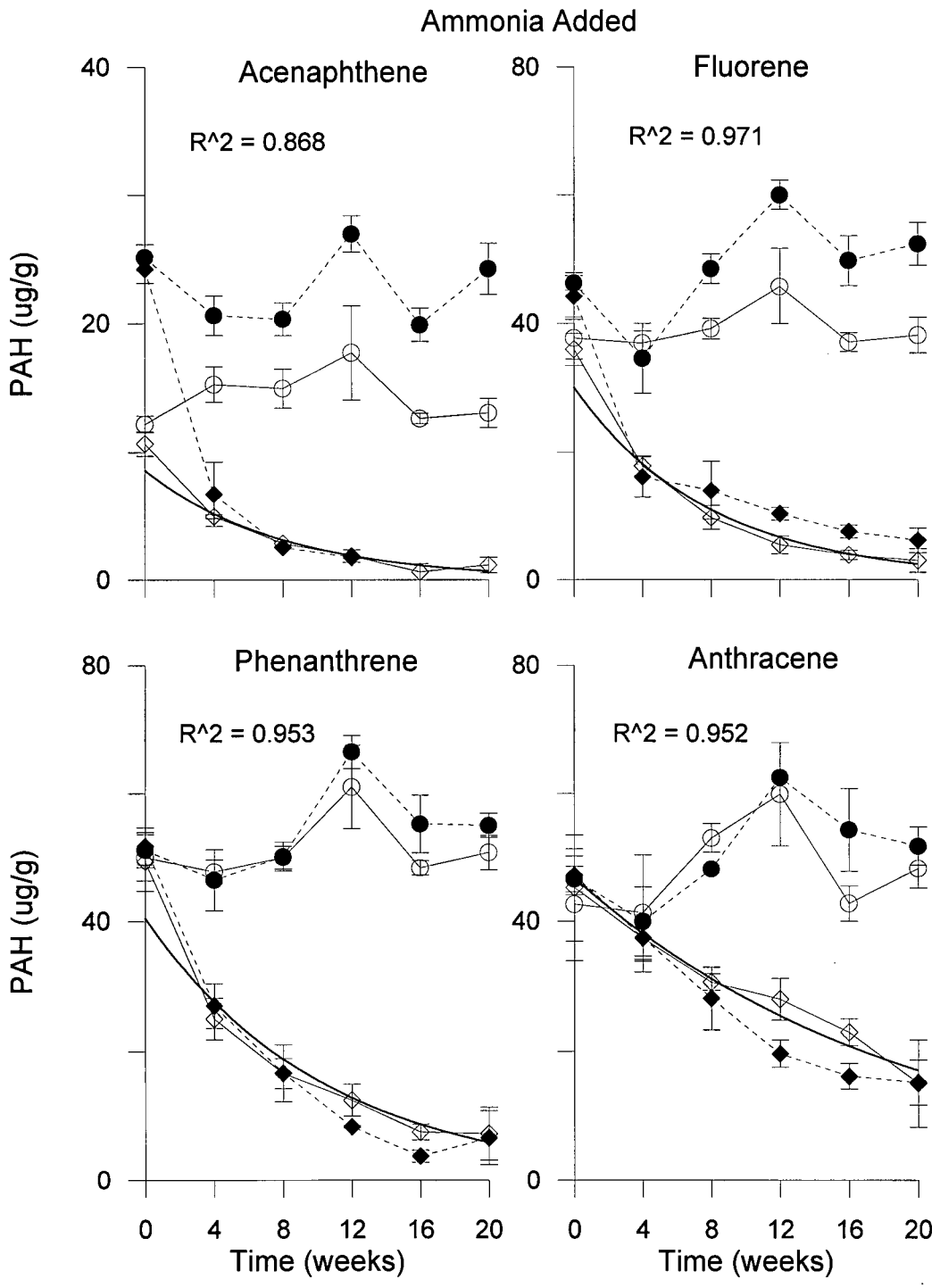


Figure 4.24 LMW PAH concentrations with time: Ammonia added
Concentration (μg/g dry weight) vs. Time (Weeks) plots for acenaphthene, fluorene, phenanthrene and anthracene with ammonia added (n=6). Symbols: nitrate-treated sediment (◊), control sediment (O), nitrate-treated rSPMD (◆), control rSPMD (●) sample extracts. Also included are the best fit first order degradation curves (thick lines) and correlation coefficients (r^2) for nitrate-treated sediments.

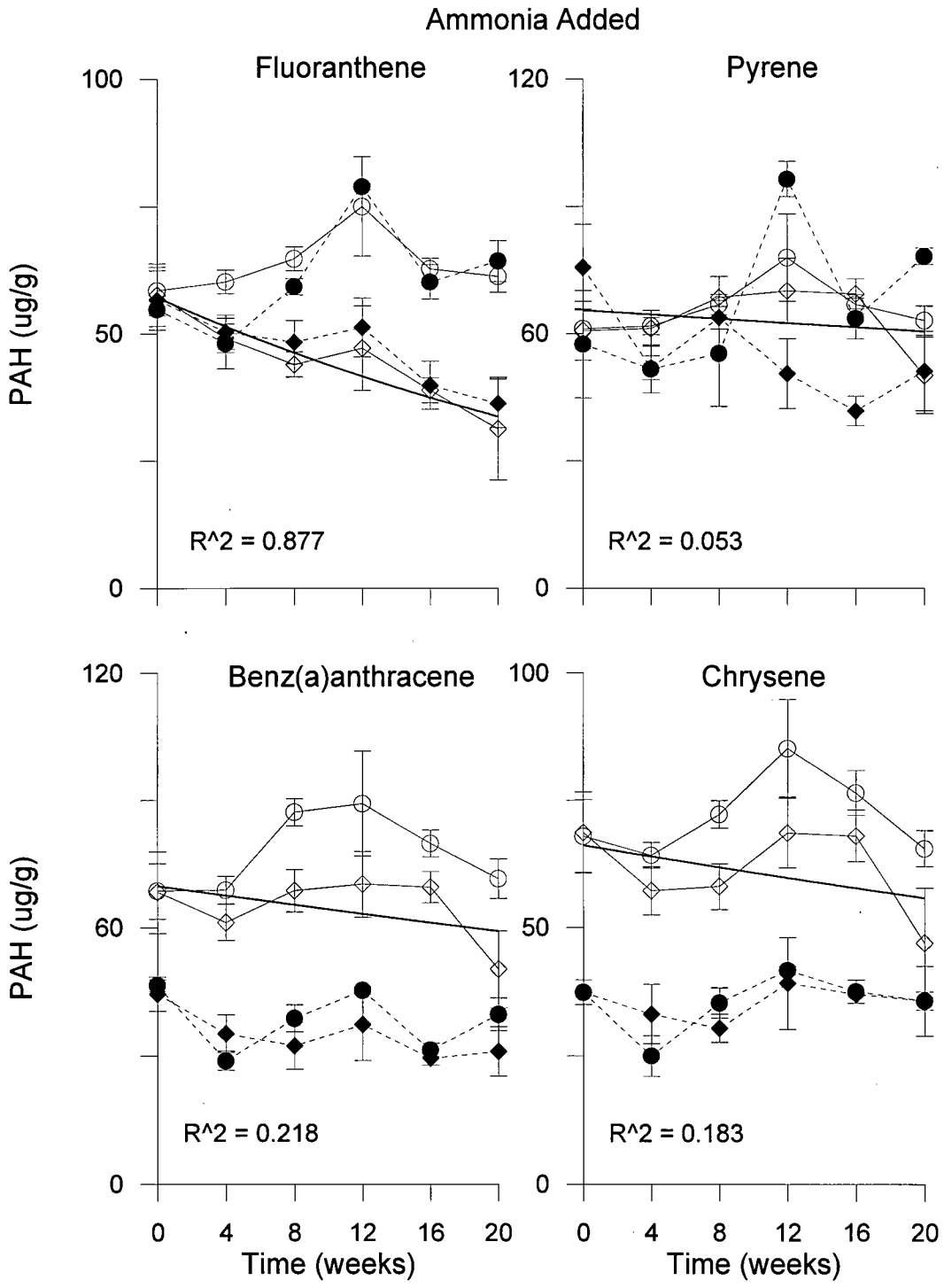


Figure 4.25 HMW PAH concentrations with time: Ammonia added
Concentration (μg/g dry weight) vs. Time (Weeks) plots for fluoranthene, pyrene, benz(a)anthracene and chrysene with ammonia added (n=6). Symbols: nitrate-treated sediment (◊), control sediment (O), nitrate-treated rSPMD (◆), control rSPMD (●) sample extracts. Also included are the best fit first order degradation curves (thick lines) and correlation coefficients (r^2) for nitrate-treated sediments.

The half lives of the PAH were calculated as in the High/Low Spike experiment (section 4.3.2) by plotting the natural log of the PAH concentration in nitrate-treated sediments versus time. The slope represents the decay coefficient and the half life is calculated as ln(0.5)/decay coefficient (Table 4.10). The PAH degradation rates with and without ammonia added are not significantly different.

Table 4.10 PAH degradation rates: Ammonia and Availability experiment
Decay rates (ln[μg PAH/g sediment dry wt]/week), half lives (weeks) and correlation coefficients (r^2) of PAH in sediments treated with nitrate, with or without ammonia addition are given in weeks.

	no ammonia added			with ammonia addition		
PAH	decay	$T_{1/2}$	r^2	decay	$T_{1/2}$	r^2
Acenaphthene	-0.132	5.25	.89	-0.122	5.68	.865
Fluorene	-0.143	4.85	.905	-0.136	5.10	.899
Phenanthrene	-0.105	6.60	.904	-0.099	7.00	.87
Anthracene	-0.054	12.84	.761	-0.055	12.60	.769
Fluoranthene	-0.026	26.66	.654	-0.028	24.76	.584
Pyrene	-0.027	25.67	.705	-0.028	24.76	.586
Benz(a)anthracene	-0.005	138.63	.079	-0.010	69.31	.146
Chrysene				-0.011	63.01	.164

4.5.2 PAH degradation in unspiked False Creek sediment

Results of the availability experiments using spiked and unspiked False Creek sediment indicated that endogenous PAH are less available than PAH that are added to the sediment (see Table 4.9). The previous two degradation experiments demonstrated the potential of nitrate addition to stimulate the biodegradation of PAH in False Creek sediment by showing the degradation of added PAH with time. Unspiked sediment was used in the Kinetics experiment, but nitrate was added as calcium nitrate and phosphate may have been limiting. The degradability of endogenous PAH using sodium nitrate and with added phosphate was therefore examined.

The incubations were carried out in serum bottles with Teflon lined septa. They contained 10 g False Creek sediment (3.26 g dry weight, 15.2% loss on ignition), sea salts solution, trace elements, phosphate and sodium nitrate (or sodium chloride in the controls). Six replicates were made of each treatment, and two spiked samples were included as positive controls and for comparison with previous experiments. The incubation was for 12 weeks. More phosphate was added at 4 and 8 weeks, and nitrate was re-applied after 4 weeks. Sediment PAH, AVS and MICROTOX toxicity as well as supernatant pH, ammonia, NO_x and phosphate were measured at the start and finish of the experiment (Appendix H).

As in previous experiments, the sediments with added nitrate turned lighter in colour and produced gas. After several weeks, gas production was also observed in the control samples. This gas production in control sediments had not been observed in previous experiments.

Ammonia was formed over the course of the experiment, as was previously observed. The ammonia did not become limiting in the nitrate-treated samples by the end of this experiment, which was shorter than previous incubations.

Nitrate and phosphate were consumed in the nitrate-treated vessels, although both were present in the reaction vessels at the end of the experiment. Of a total of 100 mg N added per serum bottle, approximately 52 mg N was consumed, with or without added PAH. This corresponds to 16 mg N consumed per g dry weight of sediment or 208 mg N/g OC.

The acid volatile sulphide concentration increased slightly in the control sediments and decreased with nitrate treatment (Table 4.11). The toxicity of the treated sediments also decreased.

In the unspiked sediments, fluorene, phenanthrene, anthracene and pyrene were all significantly lower in the treated sediments than in untreated sediments after 12 weeks (Figure 4.26). This is consistent with observations from previous experiments in which only the smaller PAH were degraded. It also supports the conclusion that endogenous PAH degradation was inhibited in the Kinetics experiment due to a lack of phosphate.

A smaller percentage of the PAH was degraded in the unspiked samples than in spiked sediments (Figure 4.26, Table 4.12). This indicates that the availability of the PAH in the sediment limited the rate at which they were degraded. It is also interesting to note that the

percentage of PAH degraded in these spiked samples was less than in the Ammonia/Availability experiment after 12 weeks of incubation (see Table 4.12).

Table 4.11 AVS, toxicity, pH and nutrient levels in spiked and unspiked FCE reaction vessels
The means of six replicates for the unspiked sediments and duplicate samples of the spiked sediment are given. The MICROTOX EC_{50} of the bulk sediment was determined in triplicate at T=0. Nitrate was re-applied at 4 weeks, and phosphate at 4 and 8 weeks.

	No Spike				Spiked Samples			
	NO_3		Control		NO_3		Control	
Parameter	T=0	T=12	T=0	T=12	T=0	T=12	T=0	T=12
AVS (mmol/g)	1406	0.5	1581	1435	1314	0.6	1436	2159
EC_{50} (ppm)	108	37200	108	191	108	40000	108	190
NO_x (mg N/L)	400	376	0.3	1.03	378	378	0.3	0.6
phosphate (mg P/L)	8.6	8.1	10.8	35.7	7.7	8.4	10.4	37.5
Ammonia (mg N/L)	2.3	18.61	1.1	16.6	2.6	14.6	1.1	16.3
pH	8.33	7.81	8.14	7.34	8.27	7.94	8.17	7.61

Table 4.12 PAH loss (%) from FCE sediment at 12 weeks
Spiked and unspiked samples from this experiment are shown , and for comparison, the PAH losses at 12 weeks in the Ammonia and Availability experiment are included.

	This experiment		Ammonia/Availability	
PAH	spiked	no spike	spiked, $+NH_3$	spiked, $-NH_3$
acenaphthene	62	36	90	87
fluorene	55	48	88	86
phenanthrene	41	19	80	77
anthracene	23	15	53	44
fluoranthene	19	13	37	32
pyrene	19	13	40	30
benz(a)anthracene	9	1	21	13
chrysene	9	16	20	7
benzo(a)pyrene	5	-10	6	2

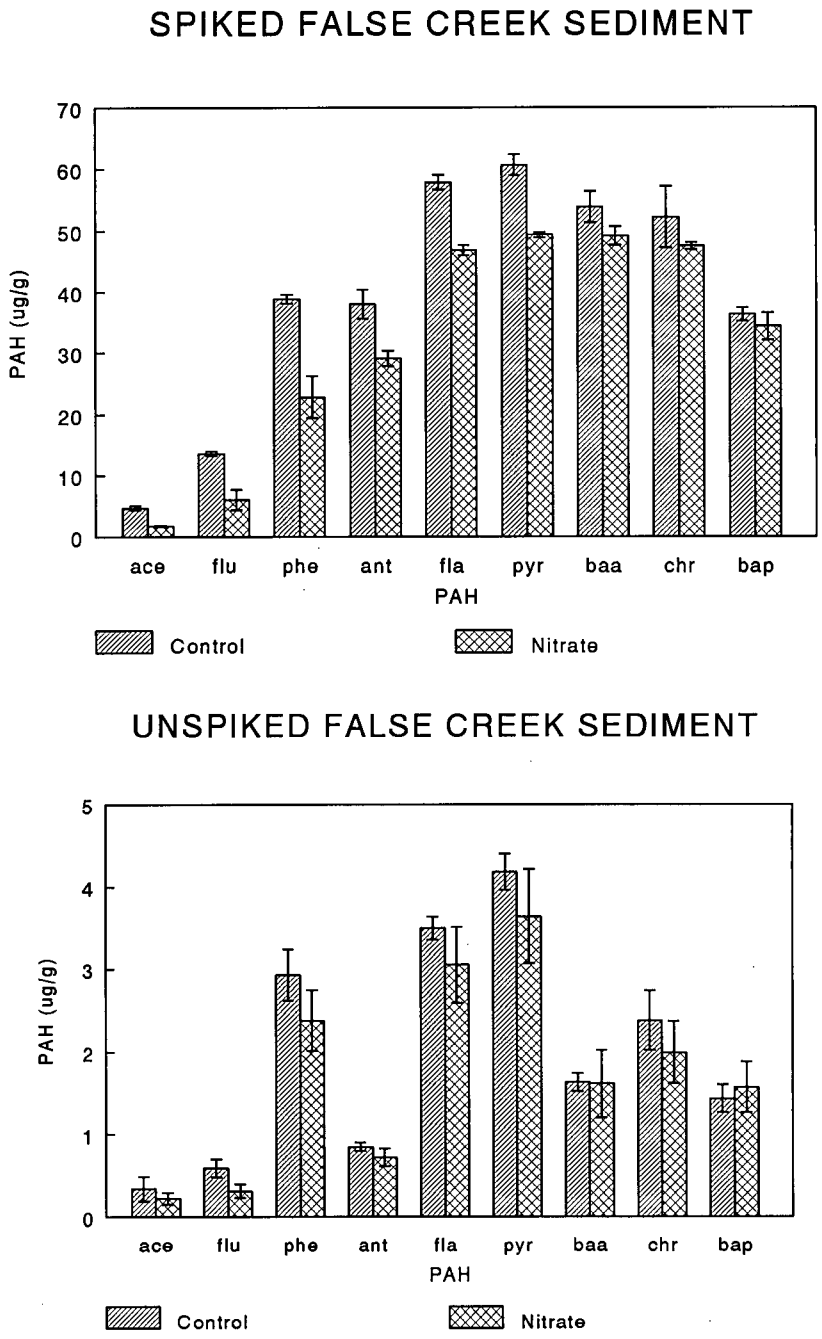


Figure 4.26 PAH concentrations in spiked and unspiked FCE sediment after 12 weeks
Spiked sample concentrations are in the top panel, and unspiked in the lower panel. The error bars indicate the standard deviation, n=6.

The results of the reverse SPMD extracts of the unspiked sediment in this experiment also produced values that were higher than the "total" sediment concentration, despite the lower degradation rates of the endogenous PAH. The errors associated with the unspiked sediment availability determinations were high, however, due to the low PAH levels in the samples relative to background noise.

4.5.3 Degradation of PAH in ESSO sediment

The source of PAH appears to influence their availability in the environment (Readman et al, 1984; McGroddy and Farrington, 1995; McGroddy et al., 1996). Consequently, PAH from the vicinity of the ESSO refinery might behave differently than those from the False Creek site. In an early experiment, the addition of calcium nitrate did not stimulate the biodegradation of PAH from the Port Moody site. To determine if the lack of PAH degradation in that experiment was due to phosphate limitation or other factors, the ESSO sediment was re-examined by treating it with sodium nitrate.

The following were added to serum bottles sealed with Teflon lined septa: 10 g ESSO sediment (2.88 g dry weight, 11.7 % loss on ignition), sea salts solution with trace elements, phosphate, and nitrate or sodium chloride. PAH-spiked bentonite was added to the spiked samples. More phosphate was added after three weeks and 1.8 mM ammonia was added at 3 weeks. Yeast extract (1 mL of a 10 mg/mL stock) was also added after 6 weeks of incubation because gas production was much less vigourous than in FCE sediment (Appendix H).

Sediment PAH, AVS and MICROTOX toxicity as well as water phase pH, ammonia, NO_x and phosphate were measured at the start of the experiment and after 12 weeks for both spiked and unspiked samples. Spiked samples were also evaluated at 6 weeks.

Sediment from the ESSO site was very different from the False Creek sediment. The initial AVS concentration and toxicity were much lower than at False Creek. Over the course of the experiment, the visible differences between the treated and control sediments were less pronounced.

Nitrate was consumed in the treated sediments, but more slowly than in False Creek sediment. Of the 50 mg N nitrate added to the bottles, only 11 mg N was consumed in the unspiked samples, and 14 mg N in the spiked samples. This corresponds to 3.8 and 4.9 mg N/g dry weight sediment, or 65 and 83 mg N/g OC for unspiked and spiked samples, respectively.

Neither ammonia or phosphate seemed to limit degradation, since both were present in reaction vessels at the end of the experiment. The toxicity of the treated sediment decreased 3 fold whereas the control sediment toxicity increased 3-5 fold during the 12 week incubation. Acid Volatile Sulfide concentrations also increased in the control sediments and decreased with treatment. These results are shown for unspiked ESSO sediment in Table 4.13 and in Table 4.14 for the spiked samples.

Table 4.13 AVS, toxicity, pH and nutrient levels in unspiked ESSO reaction vessels
Means of six replicates are shown. EC_{50} was determined by MICROTOX on bulk sediment at T=0 in triplicate. Note phosphate was reapplied at 3 weeks, and ammonia was also added at that time. N/d denotes none detected.

	NO_3		Control	
Parameter	T=0	T=12	T=0	T=12
AVS (mmol/g)	11.4	n/d	35.3	99
EC_{50} (ppm)	5100	19000	5100	1800
NO_x (mg N/L)	476	370	0.3	0.2
Phosphate (mg P/L)	10.7	15.1	13.2	34.2
Ammonia (mg N/L)	0.8	26.1	0.8	25.7
pH	8.08	7.15	8.08	7.06

Table 4.14 AVS, toxicity, pH and nutrient levels in spiked ESSO reaction vessels
Means of six replicates are shown. EC_{50} was determined by MICROTOX on bulk (unspiked) sediment at T=0 in triplicate. Note phosphate was reapplied at 3 weeks, and ammonia was also added at that time.

	NO_3			Control		
Parameter	T=0	T=6	T=12	T=0	T=6	T=12
AVS (mmol/g)	14.6	1.0	0.1	9.3	58	119
EC_{50} (ppm)	5100	16100	14100	5100	1460	1000
NO_x (mg N/L)	483	392	344	0.9	0.6	0.3
Phosphate (mg P/L)	8.8	14.6	14.7	9.3	26.9	32.4
Ammonia (mg N/L)	1.0	25.2	27.5	1.0	22.8	26.2
pH	7.96	7.3	7.4	7.89	7.00	7.21

In spiked samples, the concentrations of acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene and chrysene were all significantly lower in sediments treated with nitrate than in the controls (Figure 4.27). Acenaphthene, fluorene, phenanthrene and anthracene seem to have been degraded in the controls, although more slowly than with nitrate addition (Figure 4.28). The HMW PAH (fluoranthene, pyrene, benz(a)anthracene and chrysene) were not degraded in the control samples (Figure 4.29). Degradation of PAH in the controls could be due to oxygen originally in the supernatant and headspace, since this sediment contained a much lower sulfide concentration than the False Creek sediment. In the latter case, any oxygen present was quickly removed from the system, as seen by the colour change of resazurin, a redox indicator, added to samples in some experiments.

It also appears from the rSPMD extracts that the available fractions of benz(a)anthracene and chrysene were higher in the spiked ESSO sediment than in spiked False Creek sediments (compare Figure 4.29 to 4.23 and 4.25). In the ESSO sediment, the concentrations in the rSPMD extracts were similar to whole sediment extracts, but in the case of False Creek, the concentrations recovered from rSPMDs were only about half of the whole sediment values.

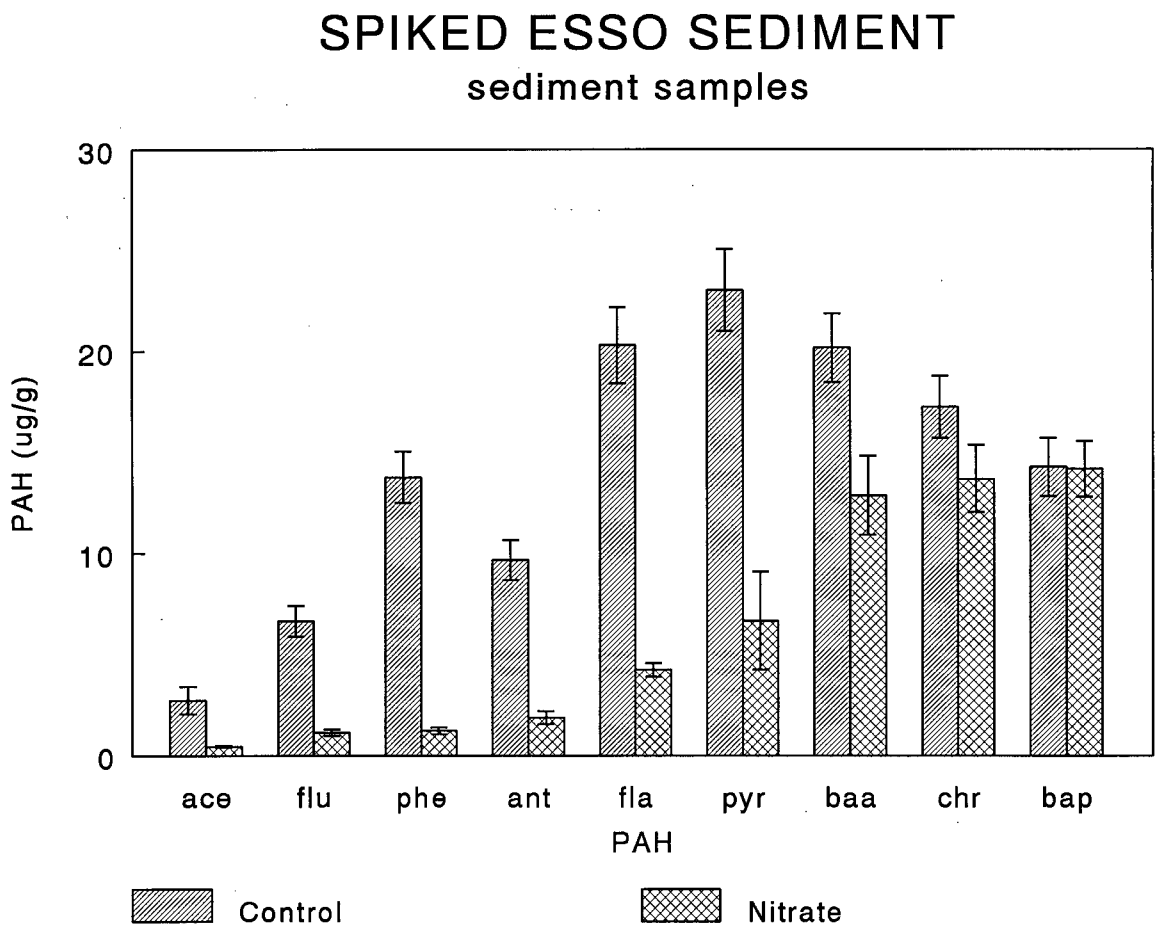


Figure 4.27 PAH concentrations in spiked ESSO sediments at 12 weeks
The difference between NO_3 treated and control sediment concentrations is significant at the 95% level for all PAH except benzo(a)pyrene (n=6).

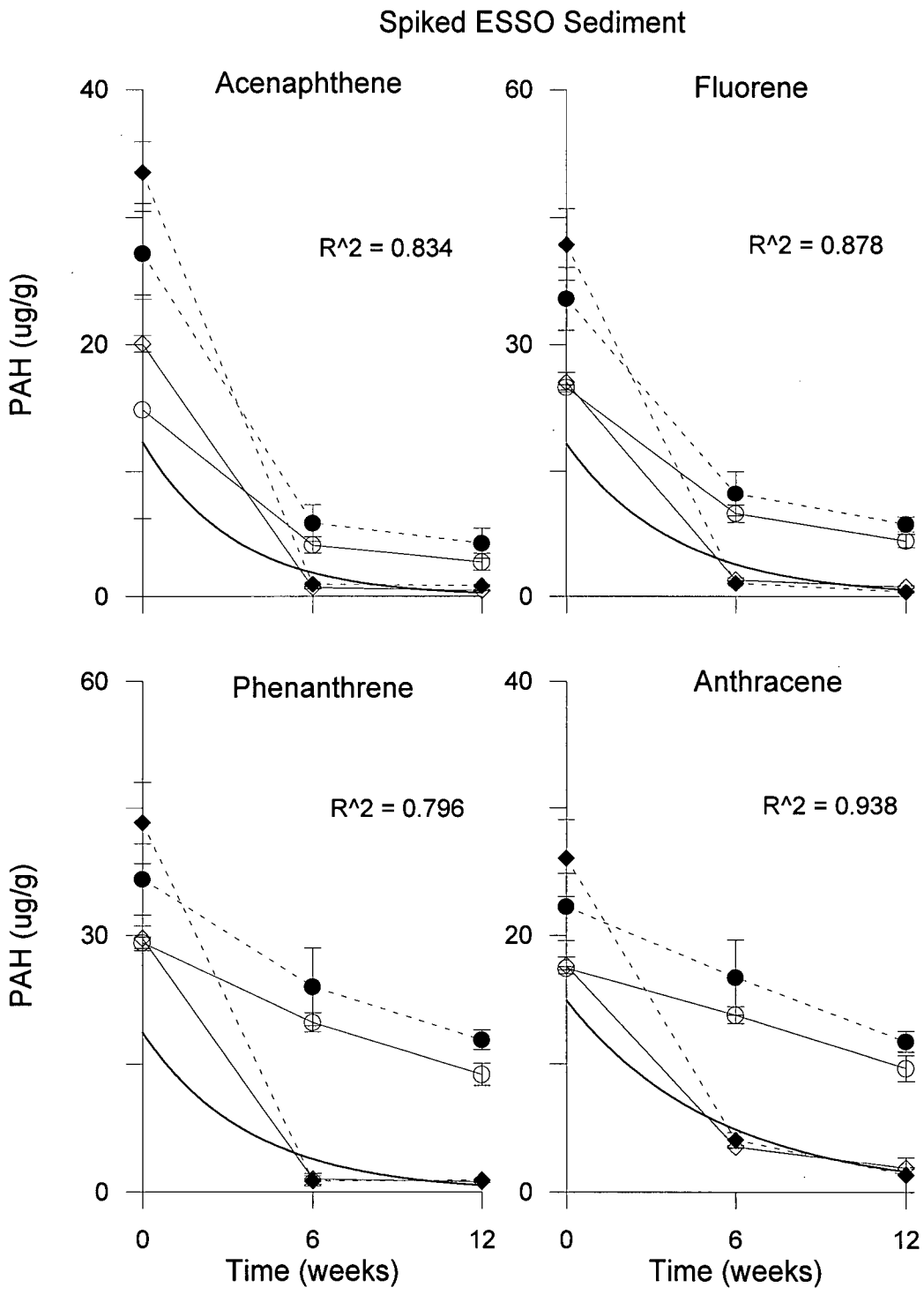


Figure 4.28 LMW PAH concentrations with time: Spiked ESSO sediments
Acenaphthene, fluorene, phenanthrene, and anthracene are shown: control sample sediment (O) and rSPMD (●); nitrate-treated sediment (◊) and rSPMD (◆) extract concentrations. Also included are the best fit first order degradation curves (thick lines) and correlation coefficients (r^2) for nitrate-treated sediments (n=6).

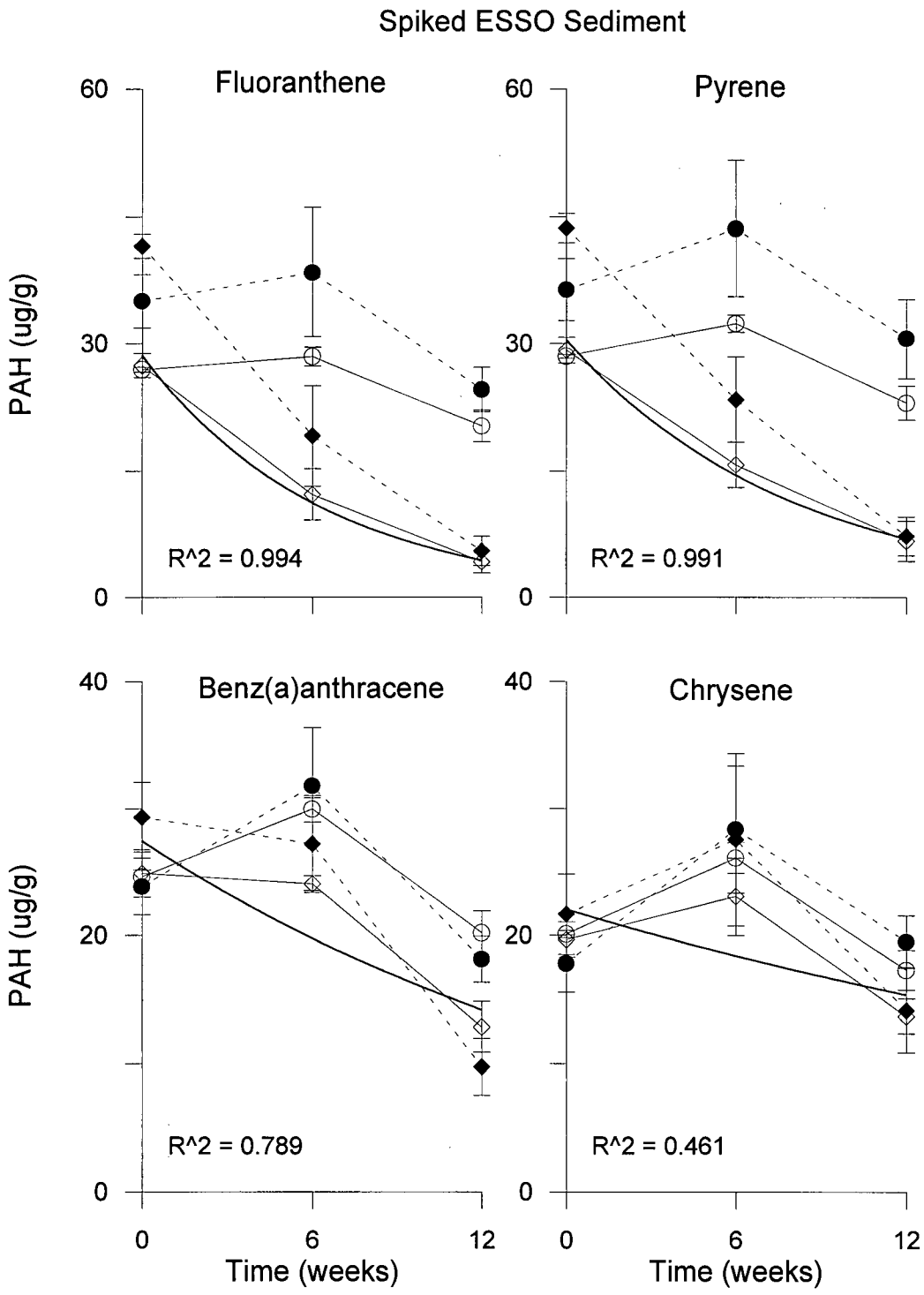


Figure 4.29 HMW PAH concentrations with time: Spiked ESSO sediments
Fluoranthene, pyrene, benz(a)anthracene and chrysene are shown: control sample sediment (O) and rSPMD (●); nitrate-treated sediment (◊) and rSPMD (◆) extract concentrations. Also included are the best fit first order degradation curves (thick lines) and correlation coefficients (r^2) for nitrate-treated sediments (n=6).

In unspiked sediments, the differences between the control and treated sediments were harder to assess. Although the average concentrations of all the PAH appeared lower with nitrate treatment than without, the differences are not statistically significant at the 95% level (Figure 4.30). However, the levels of PAH in the sediment were very low, which led to a higher variation in the measurements. The percent loss of PAH with treatment of unspiked ESSO sediment was less than in the spiked samples, which indicates that availability of the PAH may limit the degradation rate in this sediment as was concluded for the False Creek sediments (Table 4.15). The values in Table 4.15 were calculated by dividing the concentration in nitrate-treated samples by the control samples, rather than normalizing to the value at the beginning of the experiment so any degradation or losses that were not due to nitrate addition would not be included in the % loss. Contrary to the spiked control results, degradation of the smaller PAH in unspiked samples without nitrate addition was not observed.

Table 4.15 Percent loss of PAH in ESSO sediments with nitrate treatment
Percent loss here is expressed as 100*(1-concentration with nitrate/concentration in control), not change with time. The length of the incubation was 12 weeks.

	ESSO refinery sediment	
PAH	spiked	no spike
acenaphthene	83	
fluorene	83	31
phenanthrene	91	54
anthracene	80	71
fluoranthene	79	24
pyrene	71	27
benz(a)anthracene	36	24
chrysene	21	29
benzo(a)pyrene	1	15

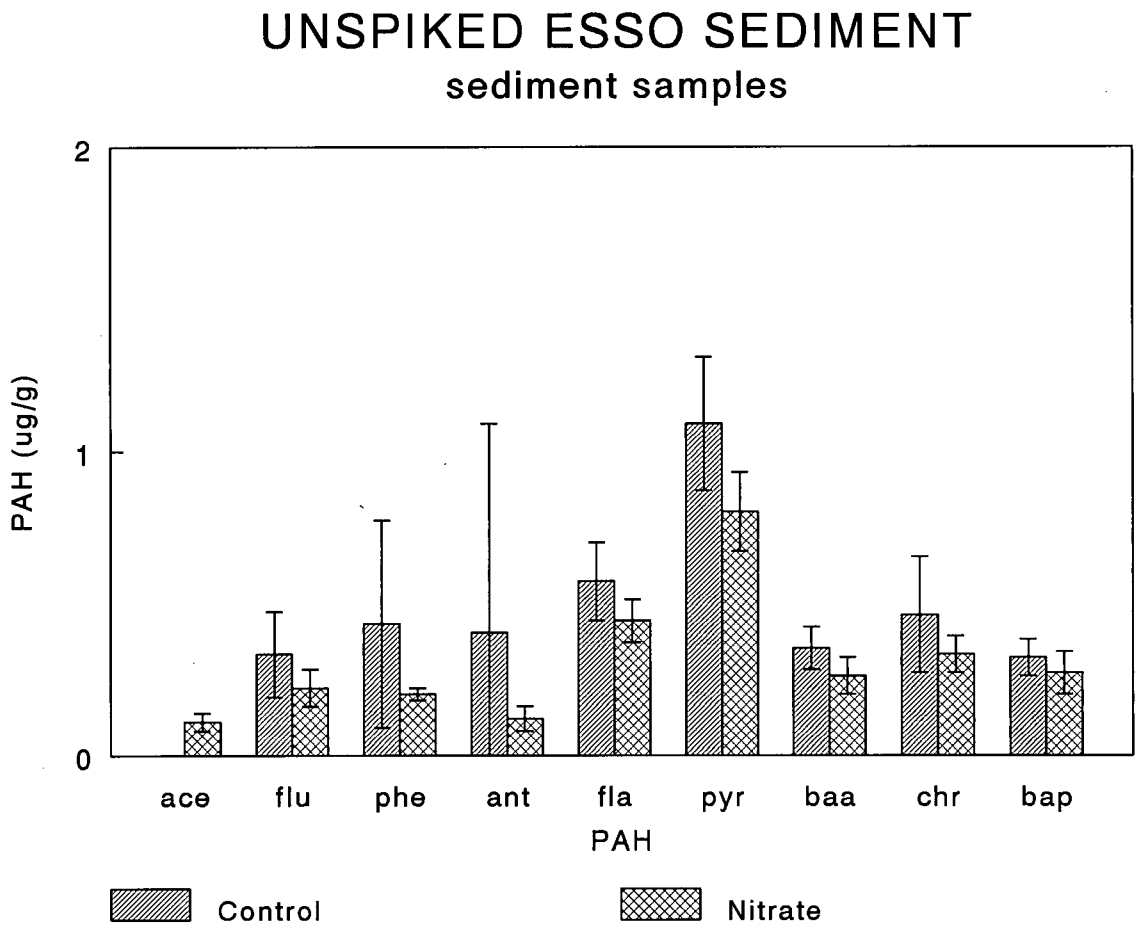


Figure 4.30 PAH concentrations in unspiked ESSO sediments at 12 weeks
The concentration differences between control and treated samples are not significant at the 95% level (n=6).

5. DISCUSSION

This research has established that the addition of nitrate with phosphate and trace elements stimulates the biodegradation of PAH in anoxic marine sediment from the east basin of False Creek and Port Moody near the ESSO site. The degradation of LMW PAH in the absence of oxygen has only recently been established (Mihelcic and Luthy, 1988a, b, 1990; Al-Bashir et al., 1990; Leduc et al., 1992, Murphy et al. 1995, Coates et al. 1996, Langenhoff et al., 1996). Biodegradation of PAH with more than three rings has only been reported in this research and that of Murphy et al. (1995).

5.1 Effects of nitrate addition on False Creek sediment

5.1.1 AVS and toxicity in treated sediments

The sediment from the east basin of False Creek is physicochemically reduced. Measured redox potentials were between -379 and -441 mV, and the sediment smelled strongly of sulphide. Several samples of False Creek sediment and one from the ESSO site were sent to Chemex Labs Ltd. for metals analysis (data in Appendix A). The concentration of silver in the False Creek sediments was quite high. Silver has been used as a marker of sewage contamination and the silver levels observed in the False Creek sediment (2.0-2.8 ppm) were similar to the highest value obtained near the Clark Drive Combined Sewer Overflow (2.73 ppm) (Seaconsult Marine Research and EVS Environmental Consultants, 1996). This indicates that the False Creek sediment receives sewage inputs, so a large oxygen demand and low redox potential would be expected. The ESSO sediment silver concentration was much lower (0.4 ppm), providing no evidence of sewage contamination.

Sulphide concentrations in False Creek sediments, as measured by AVS at the start of each experiment, ranged from approximately 150 to 1500 μmol/g dry weight sediment. Table 5.1 shows the AVS values at the start and finish of each experiment. The initial values could have been lower than *in situ* due to oxidation of sulphide in the sediments during sampling, sieving and addition to the reaction vessels, but the sediments were quite variable as seen by differences in the solids results as well as appearance and odour of the samples. In the control sediments which did not receive nitrate addition, AVS concentrations always remained high.

Given a sulphate concentration of 30 mM in full-strength seawater (Kinne, 1976), approximately 1300 μmol of sulphate was added to the reaction vessels. This amount of sulphate addition can account for the increase in AVS measured in the control sample sediments.

In all of the nitrate-treated samples, AVS dropped within the first four weeks of the experiment to levels close to or below the detection limit. This drop in AVS with nitrate treatment is important because hydrogen sulfide, which is included in the AVS measurement, is toxic to most organisms. The toxic effects of hydrogen sulphide in aquatic environments appear to be pH-dependent, but non-toxic levels for fish are in the parts per billion range, and for invertebrates, the concentration at which 50% of the test organisms are killed in 96 hours (96 hr LC_{50}s) ranges from 0.02-1.07 ppm (Environment Canada, 1984). In the case of False Creek, hydrogen sulphide could be a major cause of acute toxicity. The toxicity of the sediments as measured by MICROTOX decreased most sharply with the rapid decline in AVS values.

Table 5.1 AVS concentrations at the start and finish of all experiments
All values are given as μmol/g dry weight of sediment. Not detected recorded as n/d.

	NO_3 treated		Control	
Experiment	Start	Finish	Start	Finish
Kinetics	147	n/d	156	197
High Spike	279	n/d	284	678
Low Spike	363	n/d	431	523
No Ammonia	586	1	701	904
Ammonia added	739	2	624	2159
FCE unspiked	1406	0.5	1581	1435
FCE spiked	1314	0.6	1436	2159
ESSO unspiked	11	n/d	35	99
ESSO spiked	15	n/d	9	119

Aside from direct toxic effects, sulphide is important in the speciation of metals in marine systems. Sulphide-metal complexes are typically insoluble and non-toxic. When the sulphide concentration far outstrips the metals concentration (especially Fe II), sulphide toxicity

is observed. However, if the sulphide content in sediments is depleted by oxidation, metals which had previously been complexed with sulphide can be released into the water column (Morse et al., 1987, Calmano et al., 1994). Although only a small percentage of the total metal concentration is likely to be solubilized in this manner, there can be transiently high available metal concentrations. This is an important factor that would have to be addressed if *in situ* remediation or dredging of these marine sediments were to be undertaken.

Table 5.2 shows that some heavy metal concentrations in sediment samples from the east basin of False Creek were above the Benthic Apparent Effects Threshold (BAET) for Puget Sound, Washington (Barrick et al., 1988), the "effects range-median" values (ERM) from the US NOAA (Long et al., 1995) and the probable effects levels (PEL) from Environment Canada (Smith et al., 1995). The BAET is site-specific and depends on environmental conditions, but the conditions in Puget Sound should be similar to Burrard Inlet. Biological effects are probable at concentrations above the ERM and the PEL. Copper, zinc and lead values are above the ERM and PEL values in False Creek sediment, so this is of particular concern. ESSO sediment metal concentrations were below the effects levels in the analyzed sample except for copper, which was above the Environment Canada guideline.

Table 5.2 Metal concentrations (ppm) in FCE and ESSO sediments
Data for FCE and ESSO sediments are from CHEMEX Labs Ltd. analysis, FC 82* results were obtained in 1982 by Brothers and Sullivan (1984) and are included for comparison. The Benthic Apparent Effects Threshold (BAET) values are for Puget Sound (Barrick et al., 1988), the effects range-median values (ERM) were reported by Long et al. (1995), and the probable effects levels (PEL) are from Environment Canada (Smith et al.,1995).

Metal	BAET	ERM	PEL	FCE	ESSO	FC 82*
Cd	5.8	9.6	4.21	4.2	1.5	1.1-2.9
Cr	59	370	160	63	46	42-85
Cu	310	270	108	346	110	70-324
Hg	0.88	0.71	0.70	<1	<1	0.18-1.99
Ni	49	51.6	42.8	37	30	
Pb	300	218	112	313	52	31-985
Zn	260	410	271	705	140	72-1370

The MICROTOX assay uses inhibition of light production by bacteria to indicate the acute toxicity of water or sediment samples. In this research, the sediment (solid phase) test

was used and the concentration at which half the light production was inhibited (EC_{50}) was determined by exposing the bacteria to various dilutions of the material. A lower EC_{50} indicates that the sediment is more toxic. The toxicity as measured by this test followed a pattern similar to that of the AVS concentration (Table 5.3). The toxicity in the nitrate-treated samples decreased within the first time period, and in the control samples, the toxicity generally increased. The EC_{50} at the beginning of the ammonia and availability experiment (section 4.5.1) were very low for the control samples, but the 95% confidence interval for those samples was particularly broad (0.31-892 ppm for the sample without ammonia, and 0.33-970 ppm with ammonia). It is likely that the initial toxicity of the control samples was similar to that observed in the nitrate-treated samples.

Table 5.3 MICROTOX EC_{50} at the start and finish of all experiments
All values are given as EC_{50} (concentration at which half the light production is inhibited) in ppm for the solid phase MICROTOX test. Note that a low EC_{50} indicates high toxicity.

	NO_3 treated		Control	
Experiment	Start	Finish	Start	Finish
Kinetics	457	25495	488	227
High	588	35619	560	146
Low	526	47888	557	139
No Ammonia	309	88734	17	73
Ammonia added	328	80676	18	164
FCE unspiked	108	37159	108	191
FCE spiked	108	40014	108	190
ESSO unspiked	5100	18977	5100	1794
ESSO spiked	5100	14070	5100	1044

Both the AVS and the MICROTOX tests had fairly high analytical errors due to sediment heterogeneity and the small sample sizes involved, but the early rapid decrease in AVS and toxicity in nitrate-treated samples were observed in every experiment. Longer-term trends were more subtle , and therefore harder to resolve. The sulphide concentrations did not increase in the nitrate-treated samples because nitrate was added to the system in excess, and was re-applied as necessary. The initial increase in AVS in the control samples was probably

due to microbial reduction of sulphate in the flasks and was accompanied by an increase in toxicity. The control toxicity levels remained fairly constant throughout the remainder of the experiments. A general trend of slowly decreasing toxicity in the nitrate-treated sediments was observed following the rapid decline in toxicity which coincided with sulphide oxidation, but the possibility that this is an artifact within the analytical error cannot be ruled out. Such a decrease in toxicity would be expected, however, as toxic organic components of the sediment are degraded.

AVS concentrations did not change significantly with nitrate treatment in the bench scale experiments carried out by Murphy et al. (1993). These authors, however, observed a decrease in the concentration of hydrogen sulphide, which was measured independently. Toxicity was not measured in bench scale experiments in their laboratory, but at a field site near Sault Ste. Marie, Ontario, the ATP-TOX toxicity (similar to MICROTOX) was lower after treatment (Murphy et al., 1994).

5.1.2 Nutrient use in reaction vessels

Phosphate probably became limiting in the Kinetics experiment (section 4.3.1) due to precipitation with calcium. Calcium phosphate ($Ca_3(PO_4)_2$) has a solubility product of 2×10^{-29}, so it should have precipitated, given a PO_4^{3-} concentration of 9.9×10^{-9} to 1.41×10^{-7} M (between pH 7.5 and 8.5) and a calcium concentration of 0.018 M. The remaining soluble phosphate concentration and dissolution rate was probably insufficient to meet the demand of the PAH degraders. Certainly some microorganisms were able to continue to reduce nitrate since it was consumed in this experiment. To avoid precipitation of phosphate, nitrate was added as the sodium salt in subsequent experiments. In subsequent experiments in which sodium nitrate was used, phosphate levels remained fairly constant at the added levels in the control vessels, and dropped with time in the nitrate-treated samples.

The problem of phosphate availability with calcium nitrate addition was addressed by Murphy et al. (1995, 1994, 1993) by the addition of a proprietary organic amendment which also contained available phosphorus. The organic amendment might also have contained other growth factors that stimulated degradation. Calcium nitrate is used in their field-scale sediment bioremediation process (the NWRI/Golder system) since it is widely available and inexpensive. Gas production was used as a measure of biological activity in laboratory-scale research by

Murphy et al. (1994, 1993). When calcium nitrate alone was added to Red Rock and St Marys River sediments, gas production in the sediments was inhibited. Inhibition of gas production was not noted in samples from the Dofasco boatslip, which had a higher phosphorus concentration. The addition of inorganic phosphate did not improve gas production in the Red Rock and St Marys River sediments, although adding a phosphorus-containing organic amendment along with the calcium nitrate stimulated biodegradation 5-10 fold. These authors also observed an increase in the degradation rates of the target compounds, PAH and total petroleum hydrocarbons, when the organic amendment was included in the treatment (Murphy et al., 1995, 1994, 1993).

Nitrate concentrations were monitored in all of the degradation experiments described here. In the control samples, nitrate was not added, nor was it present in the samples initially. In nitrate-treated vessels, the nitrate consumption was most rapid at the beginning of the incubations and slowed with time. Much of the initial consumption of nitrate can probably be attributed to sulphide oxidation, and degradation of the easily degraded organic matter. Over time, the more easily degraded material would become depleted in the sediment, leaving behind a residue of more recalcitrant material. The residual organics would be degradable by less and less of the microbial population, so the rate of nitrate use would be expected to decline. A buildup of toxic products could also depress the nitrate utilization rate. In the latter case, the PAH degradation rates would also decline and the toxicity of the sediment would increase, unless the toxicity applied only to denitrifiers that could not use PAH. It is more likely that the readily available degradable carbon was being depleted.

The nitrate utilization rates per gram of sediment and per gram organic carbon were calculated to see if there was a consistent nitrate requirement for False Creek sediment treatment. The spread in the data is quite wide as shown in Table 5.4. The low nitrate use per g dry weight in the Kinetics (section 4.3.1) and Unspiked False Creek (section 4.5.2) experiments might be attributable to nutrient limitation or toxicity in those experiments. Both experiments also had slower spiked PAH degradation rates than were observed in other experiments. In the Kinetics experiment (and in the preliminary single time point experiments), the limitation was probably due to loss of phosphate by precipitation as the calcium salt. The limitation or toxicity in the Unspiked False Creek experiment might have been due to lower availability of organic matter in that sample. It should be noted that the nitrate utilization results shown in

Table 5.4 are not directly comparable because the incubation times were different, and the rate of nitrate use decreased with time. From the High/Low (section 4.3.2) and Ammonia/Availability (section 4.5.1) experimental data, it seems that the required nitrate dose is around 22-29 mg N/g dry weight of sediment in the absence of significant toxicity or limitation. For the ESSO sediment (section 4.5.3), the nitrate requirement was much lower, at approximately 5 mg N/g dry weight.

Table 5.4 Nitrate utilization in all experiments
The average and standard deviation values for nitrate utilization in False Creek sediment are shown at the bottom. Organic carbon (OC) was estimated as half the loss on ignition.

Experiment	Time (weeks)	mg N/g OC	**mg N/g (dry wt)**
Air Pretreatment	6	66	**2.5**
Nitrate Pretreatment	6	12	**0.5**
No Pretreatment	7	148	**7.1**
Kinetics	16	297	**13.3**
High Spike	16	420	**28.9**
Low Spike	16	420	**28.9**
No Ammonia	20	245	**21.7**
Ammonia added	20	273	**24.1**
FCE Unspiked	12	208	**16.0**
FCE Spiked	12	204	**15.6**
ESSO Unspiked	12	69	**4.0**
ESSO Spiked	12	85	**5.0**

In clean soil, 0.6-1.4% of the endogenous organic carbon is oxidizable under denitrifying conditions (Mihelcic and Luthy, 1988b). Given that the nitrate utilization rates were from 15 to 40% of the assumed organic carbon concentrations (without pretreatment), the lack of electron acceptors that can be used by bacteria in False Creek sediment might have prevented more oxidizable material from being degraded until nitrate was added. There would be abundant degradable organic material and nutrients in the sediment from sewage contamination as well as from other sources such as storm runoff, but some nitrate would also

be consumed in the oxidation of sulphide. Table 5.5 shows the assumed nitrate consumption from oxidation of AVS, sediment organic carbon and added PAH. Balanced chemical reactions were used to estimate nitrate requirements and complete oxidation was assumed, which might not be the case.

The assumed stoichiometry of nitrate consumption is as follows:

$$5CH_2O + 4NO_3^- + 4H^+ \Rightarrow 2N_2 + 5CO_2 + 7H_2O \qquad \text{OC}$$

$$5H_2S + 8NO_3^- \Rightarrow 4N_2 + 4H_2O + 5SO_4^{2-} + 2H^+ \qquad \text{AVS}$$

$$5C_{14}H_{10} + 66HNO_3 \Rightarrow 70CO_2 + 33N_2 + 58H_2O \qquad \text{Phenanthrene}$$

Table 5.5 Measured and predicted nitrate utilization
The measured nitrate use is labeled N Used, the predicted amounts of nitrate required to oxidize (a) oxidizable OC (1.4% of total), assuming 0.93 mg N used/mg OC (b) AVS, assuming 0.7 mg N/mg S, and (c) added PAH that were degraded, assuming 1.05 mg N used/mg PAH are shown as N_{oc}, N_{avs} and N_{pah} respectively. N_{tot} is the total predicted nitrate use. Pretx means pretreatment. AVS was not determined in preliminary experiments (-).

	Measured Values				Predicted Values			
Expt.	**N Used (mg)**	OC (mg)	AVS (mg)	PAH (mg)	Noc (mg)	Navs (mg)	Npah (mg)	**Ntot (mg)**
Air Pretx	**76**	16.10	-	0.73	14.97	-	0.77	**16**
NO_3 Pretx	**15**	18.20	-	0.13	16.93	-	0.13	**17**
No Pretx	**31**	2.94	-	0.22	2.73	-	0.24	**3**
Kinetics	**193**	9.10	68	0.21	8.46	48	0.22	**56**
High Spike	**105**	3.50	32	0.64	3.26	23	0.68	**27**
Low Spike	**105**	3.50	42	0.12	3.26	30	0.13	**33**
No Ammonia	**54**	3.08	47	0.57	2.86	33	0.60	**36**
Ammonia added	**60**	3.08	59	0.77	2.86	41	0.81	**45**
FCE Unspiked	**52**	3.50	147	0.01	3.26	103	0.01	**106**
FCE Spiked	**51**	3.50	137	0.29	3.26	96	0.31	**100**
ESSO Unspiked	**11**	2.36	1	0.01	2.19	1	0.02	**3**
ESSO Spiked	**14**	2.36	1	0.23	2.19	1	0.25	**3**

If all the reactions shown above accurately describe the processes in the flasks, most of the nitrate is used to oxidize AVS. Even if only 1.4% of the organic carbon in the sediment is

degradable under denitrifying conditions, the nitrate required to degrade the added PAH is small compared to the nitrate demand of the rest of the organic material.

In the False Creek Unspiked experiment (section 4.5.2), the lower than predicted nitrate use (in both spiked and unspiked samples) could be due to incomplete oxidation, especially of AVS. If so, the oxidation of sulphide might have led to production of elemental sulphur. Low nitrate use could also be due to erroneously high AVS results at the beginning of this experiment, since the value obtained at that time was much higher than from any other False Creek sample.

In all other cases, the nitrate consumption was higher than predicted. A higher than predicted consumption is likely to be due to a higher degradable organic carbon content than estimated. Since the estimate was based on the degradable portion of the organic content that can be degraded under denitrifying conditions in unpolluted soil rather than polluted sediment, this explanation seems likely. Another possibility is that nitrate reacted with, or was used to oxidize, other reduced compounds in the system that were not measured.

Ammonia levels were also monitored. In the control samples, the ammonia concentration rose slowly over the course of the experiment. In the nitrate-treated samples, ammonia levels rose slowly, as in the controls, and then dropped below the detection level. Since the level dropped at the end of the incubations, ammonia was added to one set of vessels in the Ammonia and Availability (section 4.5.1) experiment to prevent ammonia limitation. The ammonia level did not seem to become limiting until the last time point, so any difference between the samples receiving ammonia and those that didn't would only have become important at that time. If a difference existed, it was masked by the analytical variation (not statistically significant).

One of the main reasons for monitoring ammonia concentrations was to determine if nitrate reduction by bacteria in this system would result in the production of high levels of ammonia (dissimilatory nitrate reduction to ammonia). This did not occur. DNRA is most often observed in sediments where the nitrate concentration is low relative to the organic carbon concentration. In these experiments, nitrate was added in excess to favour denitrification. Some DNRA might have occurred in the reaction vessels, but if it did, the effect was offset by ammonia consumption by other microorganisms, because the ammonia levels did not rise above those observed in the control samples.

5.1.3 PAH biotransformation rates with nitrate addition

The addition of nitrate was shown to stimulate the biodegradation of PAH in False Creek sediment. It was realized in early experiments that phosphate precipitation could occur if calcium nitrate was added, leading to the possibility of phosphate limitation. Other factors that might have affected the degradation rates were the concentration of PAH and the organic content of the sediment at the time of sampling, which would affect the aqueous concentration of the PAH. The constituents of the sediment organic content and the microbial population could also influence PAH degradation. Differences in any of these factors should be reflected in the results of the degradation experiments to produce different degradation rates, if they were significant. Table 5.6 shows the half lives of PAH calculated assuming first order kinetics. Zero order degradation rates were also calculated for all compounds, since degradation of the HMW PAH (in particular) might have been limited by desorption rates. However, linear models were only better for anthracene and the HMW PAH in the Ammonia/Availability experiment (section 4.5.1). Included in Table 5.6 are half life values for the False Creek and ESSO (sections 4.5.2 and 4.5.3) sediments with and without spike. These values are putative since they assume similar kinetics to the High/Low and Ammonia and Availability experiments, and they are based on fewer data points. They are included merely for comparison, since the real nature of the degradation cannot be determined from concentrations at the beginning and end of the experiment, or from the three points generated for the spiked ESSO sediments.

The half life values from the High/Low and Ammonia/Availability experiments are in agreement with each other. Higher half lives were observed in the Kinetics experiment, except for Fluoranthene, which had wide 95% confidence limits, spanning 13.3-23.1 weeks. Benz(a)anthracene and chrysene degradation were not well described by first order kinetics. Typically, differences between the control and treated sediment concentrations for these compounds were not significant in the early part of the experiment. An apparent lag could be due to the effect of a small change in concentration coupled with the analytical variation, or degradation might have begun only after a real lag period. Degradation could have been zero order, due to desorption-limited use of these less soluble substrates, but linear models did not fit the data much better than first order models.

Table 5.6 Half life values (weeks) for PAH degradation
First order kinetics were assumed for all experiments. Numbers in bold face type represent values whose correlation coefficients (r^2) were greater than 0.5. The values are corrected for changes in the control concentrations, if control slopes were negative. The False Creek values for spiked and unspiked sediment (FCE + and -) and ESSO unspiked (ESSO-) values were calculated taking the final control value as time zero and the final treated concentration as time 12 weeks. Blanks (-) indicate that no half life was determined because the slope was zero or slightly negative, or the compound was not detected in the sample (ESSO-, Ace).

PAH	Kin	High	Low	$-NH_3$	$+NH_3$	FCE -	FCE +	ESSO -	ESSO +
Ace	**15.8**	**4.7**	**7.6**	**5.3**	**5.7**	21	8	-	4
Flu	**14.2**	**4.8**	**5.2**	**4.8**	**5.1**	13	10	27	5
Phe	21.7	**6.2**	**7.8**	**6.6**	**7.0**	43	15	14	4
Ant	69	**11.8**	**12.6**	**12.8**	**12.6**	46	31	18	5
Fla	16.9	**22.4**	**20.4**	**26.7**	**24.8**	58	39	33	5
Pyr	116	**19.3**	**28.9**	**25.7**	**24.8**	58	41	27	6
BaA	116	38.5	**38.5**	138	69	173	99	29	18
Chr	50	99	25.7	-	63	46	87	28	39
BaP	-	-	-	-	-	-	139	53	231

Lag periods have often been observed in pollutant degradation. Reported causes of lag periods include the length of time required for proliferation of a small population that can use the substrate, relief of toxicity with time, diauxie (when one substrate is used before degradation of a second occurs), predation by protozoa, the time required for the appearance of a new genotype by mutation, or enzyme induction (Alexander 1994). In the case of False Creek, the sediments have been exposed to PAH contamination for years, so a population capable of PAH degradation is likely to exist there. The degradation of the smaller compounds with no significant lag indicates that at least some microorganisms from False Creek can degrade PAH under denitrifying conditions. Enzyme induction occurs relatively quickly, and toxicity did not seem to be a problem, at least for LMW PAH degraders. Diauxie and proliferation of a small population can't be ruled out, since the lack of available HMW PAH could have limited the growth of microorganisms that could degrade them. Whether there was a lag period, or slow degradation of the HMW PAH was initially masked by the analytical variation, the degradation of benz(a)anthracene and chrysene was slow. The rate estimates

from this research are not very reliable because of the spread of the data coupled with the small changes observed.

A number of other studies have shown that degradation of sparingly soluble compounds is limited primarily by desorption or solubilization rather than by the maximal substrate utilization rate (e.g., Zhang et al., 1995). Such a dependence on desorption is reflected in the relationship between the degradation rates and the octanol-water coefficients (K_{ow}) and solubilities of the substrate. The half lives determined in the present research are related positively to the log (Kow) and inversely to solubility of the PAH (Figure 5.1). Similar relationships were observed for the half lives of added PAH in aerobic systems (dashed and dotted lines for sludge-amended and spiked soils, respectively; Wild and Jones, 1993). The coefficients of the relationships for the sludge amended soils used by Wild and Jones indicated that aerobic degradation of the LMW PAH was slower, and HMW PAH was faster than was reported here. These differences may be due to differences in the desorption rates of PAH from the sludge solids and False Creek sediments, substrate interactions, microbial populations and the data spreads. Higher degradation rates for all PAH were observed in the aerobic spiked soils (Figure 5.1, dotted lines) than for spiked sediments incubated anaerobically (the present research). The organic content of the spiked soil was much lower than that of the sludge-amended soil, and the PAH spike had not been in contact with the solids prior to the start of the degradation experiments, therefore the PAH would have been more easily desorbed. Faster substrate degradation under aerobic than anoxic conditions has often been observed (e.g., Mihelcic and Luthy, 1988a for PAH). This difference is probably due to the higher efficiency of substrate conversion to usable energy for growth and maintenance using aerobic metabolism

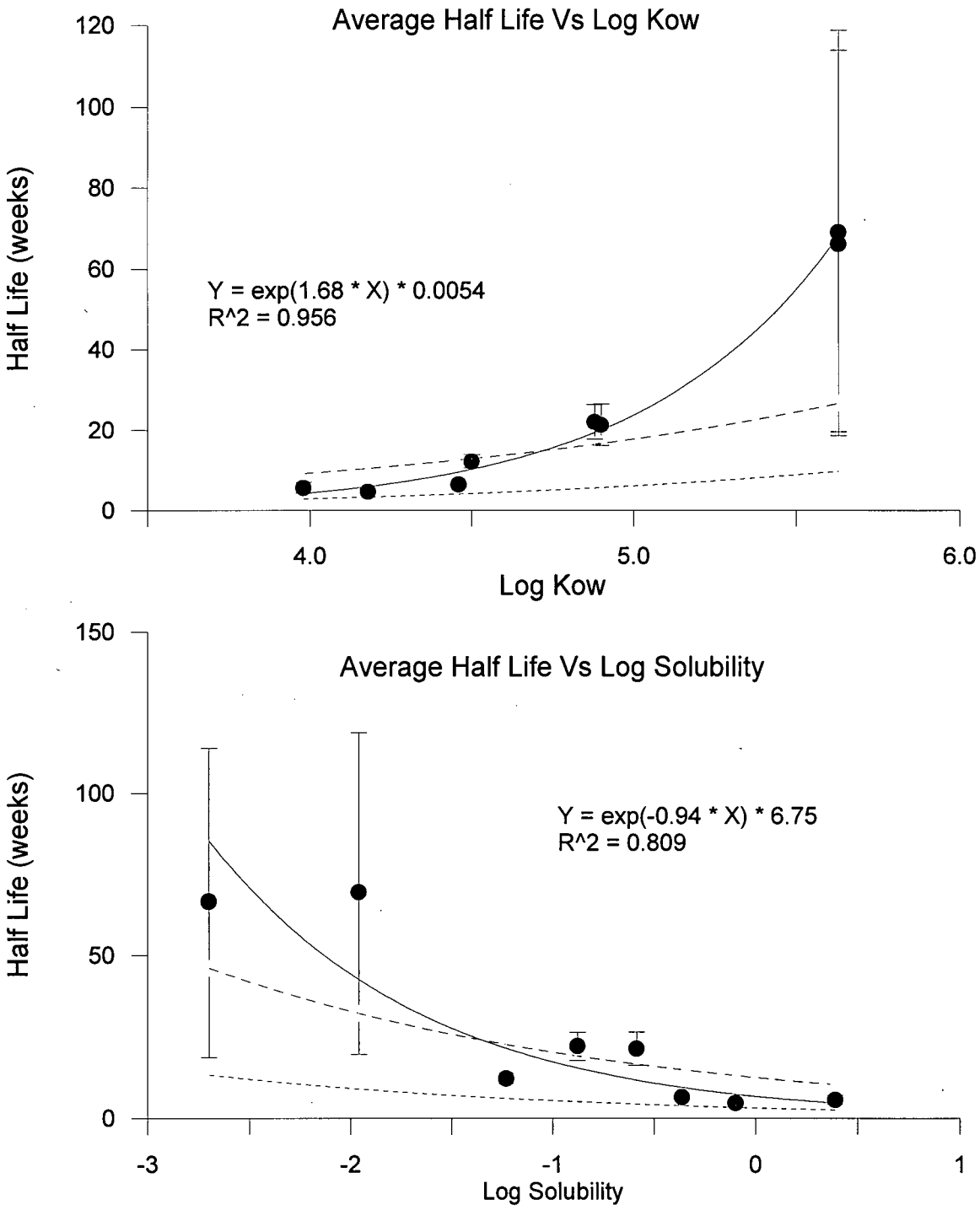


Figure 5.1 PAH half life vs. log Kow and log solubility plots
The top panel shows the relationships between the average PAH half life (High/Low and Availability/Ammonia experiments) vs. log Kow (Nagpal, 1993). Values for solubility in the bottom panel were also taken from Nagpal, 1993. The solid line shows the best fit for these data. The equation of the line and correlation coefficient (r^2) are also shown. The dashed lines represent the line of best fit taken from aerobic half lives for sludge amended soils and the dotted lines for PAH spiked soils reported by Wild and Jones, 1993.

Half life estimates from the single time point spiked and unspiked False Creek and ESSO sediment samples show that degradation of the spiked PAH proceeds faster than that of the endogenous material. Based on the availability results (section 4.4), this is not surprising. A higher percentage of spiked than endogenous PAH was extractable by reverse and conventional SPMDs, which required desorption from the solid phase. This difference could indicate that a portion of the endogenous PAH is occluded more or less permanently in the solid matrix, or that the PAH at the site have been weathered and are subject only to slow desorption. In the first case, the material may have come from the source in occluded form. This explanation has been put forward to account for results obtained by McGroddy et al. (1995, 1996). In the second instance, contaminants gradually migrate to microenvironments in which they form numerous interactions with the matrix. This material becomes available very slowly due to limited mass transfer according to slow desorption theory (Pignatello and Xing, 1996). Either or both of these possibilities could account for the lower degradation rates of endogenous PAH in FCE and ESSO sediments.

Although none of the nutrients that were measured (nitrate, ammonia, and phosphate) seemed to be limiting in the single time point experiment with spiked and unspiked False Creek sediment (section 4.5.2), the spiked PAH degradation rates were lower than in previous experiments using sodium nitrate. Some evidence suggests that the PAH were less available in this sample than in the Ammonia/Availability experiment (see section 5.5). This could indicate that the sediment organic content had a higher affinity for the PAH in this sample, which would have impeded degradation of endogenous PAH.

5.2 Effects of nitrate addition on ESSO sediment

The ESSO sediment was not as highly reduced as the False Creek sediment at the time of sampling for the comparison of spiked and unspiked sediments. The redox potential of the sediment was not measured at that time, but previous measurements had been between -310 and -372 mV. Since previous measurements of redox potential had been so low, it was assumed that the sediments would behave similarly to False Creek sediment. This did not occur. The AVS was much lower than in False Creek, being only between 10 and 35 μmol/g dry weight in the ESSO sample compared to 150 and 1500 μmol/g dry weight False Creek sediment (see Table 5.1, page 122). These low values might reflect the real sulphide concentration in ESSO

sediment, or it could have been due to losses during the manipulation of the samples. This difference between the sediments could account for losses of PAH in the ESSO control bottles: oxygen in the supernatant and headspace might have been used initially to degrade PAH in both control and nitrate-treated samples, then degradation could only continue in the nitrate-treated samples. In the False Creek sediments this was not observed because the oxidation of sulphides and easily degradable organics quickly consumed any free oxygen. Degradation of PAH in the controls could also have been due to degradation at the expense of sulphate, which was observed in two previous studies, albeit more slowly than was observed here (Coates et al., 1996; Langenhoff et al., 1996).

As in the False Creek experiments, the AVS concentrations rose in the ESSO control samples and dropped in the nitrate-treated sediments. Similarly the toxicity of ESSO control sediments increased with incubation, but the final toxicity was not as high as that observed in False Creek control sediments. The toxicity of the nitrate-treated sediment decreased with time, but this reduction was not as pronounced as in the False Creek sediments (see Table 5.3, page 124). The final toxicity of the treated ESSO sediment was greater than that of the False Creek sediments, however the length of the ESSO experiment was only 12 weeks, and the toxicity might have continued to decrease after that time as more of the organic matter was consumed.

The lower nitrate utilization rate in the ESSO sediment (compared to FCE sediment) corresponds to the lower initial sulphide concentration and could also indicate that less of the sediment organic material was oxidized (see Table 5.4, page 127). Low oxidation of sediment organic matter might be due to a higher proportion of that material that is not degradable under denitrifying conditions, or the breakdown of the organic content might have been inhibited by toxicity. Because the added PAH were degraded quite readily, it seems likely that the former was the case.

PAH contamination at the ESSO site is probably attributable to effluent from the refinery and later from the storage facility. There is probably aged oil in the sediment from which the easily degradable and more soluble material has been removed. With fewer substrates available in the system, there would be less competition (by microorganisms that do not degrade PAH) for other nutrients, and stronger selection for microbial populations that could use PAHs as carbon sources. In contrast, recent contamination in False Creek appears to

be largely from combined sewer overflows. This material would include domestic sewage as well as road runoff and rain water, and would probably have a higher degree of degradability as long as other limitations were satisfied. There is also historical contamination from industries that formerly lined False Creek. These varied sources would contribute different contaminants to the environment, leading to a more complex mixture of substrates and microorganisms, and probably greater competition for growth factors by non-PAH degraders.

Even after accounting for the losses of PAH in the control samples, the degradation rate of PAH in the spiked ESSO sediment was higher than in the spiked False Creek sediments for most of the PAH tested. The lower initial sulphide concentration and toxicity, lower concentrations of preferred (non-PAH) substrates, or greater bioavailability of the PAH could account for the observed degradation rates. Lower sulfide concentration and initial toxicity could result in less inhibition of the microorganisms in the sediment. Presumably, many of the organisms in the False Creek sediments are well adapted to strongly reducing conditions and a high hydrogen sulphide concentration, but those conditions might not be optimal for PAH degradation. Furthermore, the extreme change in environmental conditions with nitrate treatment to False Creek sediment might have required more significant adaptations or population shifts. Lower nitrate use in the ESSO sediment probably reflects a combination of lower demand from sulphide and organic substrate oxidation. With fewer alternative carbon sources, PAH degradation would be less likely to be inhibited due to non-PAH substrate preferences or competition from faster growing organisms that use different substrates. The bioavailability of PAH at the ESSO site will be discussed in section 5.5.

If the observed nitrate utilization rate was low in the ESSO flask test because of a limitation other than degradable carbon, that limiting factor could be added with the electron acceptor at the time of treatment, or might be addressed by the addition of an organic amendment. Some organisms require vitamins, amino acids or trace elements, which could have been or become unavailable in the laboratory test, and so, the degradation rates for PAH might increase with relief of the limitation.

5.3 Comparison of results to other PAH degradation studies

5.3.1 Comparison to anaerobic studies

Table 5.7 shows a comparison of the maximum degradation rates found in this work, using the High/Low and Ammonia/Availability experimental data (sections 4.3.2 and 4.5.1), and in other studies conducted on soil/water systems under denitrifying conditions. Relevant factors such as the organic carbon content, nitrate N concentration and spike levels are included. These results are expressed as maximal degradation rates in μg/g•day. For the results of this study, only the rate over the first 4 weeks is used, because degradation of the LMW PAH appeared to be first order, and the maximal degradation rate occurred at the beginning of the experiments. The apparent increase in linear degradation rates with molecular weight in the False Creek sediment is a result of the different starting concentrations of the PAH (listed as spike values in Table 5.7). In terms of percent loss, the trend was the opposite: acenaphthene 63-76%, acenaphthene 32-67%, fluorene 30-51%, phenanthrene 31-48%. Mihelcic and Luthy (1988b) measured PAH in the aqueous phase, and their rates were obtained from figures in their paper. The results of the present research are similar to those observed by the other researchers, despite quite different experimental conditions.

Table 5.7 Maximum PAH degradation rates under denitrifying conditions
Degradation rates are given in μg/g•day. Data from this research (False Creek) were taken from the first four weeks of the High/Low and Ammonia/Availability experiments, Mihelcic and Luthy (1988b) rates were estimated from their reported data. Blanks indicate no rate was determined for that compound.

PAH	Al-Bashir et al.	Mihelcic/Luthy	Leduc et al.	False Creek
Nap	1.3, 1.8, 1.8	0.15	-	-
Acy	-	-	0.35 +/- 0.03	0.12 - 0.17
Ace	-	0.013	0.32 +/- 0.03	0.12 - 0.31
Flu	-	-	0.29 +/- 0.03	0.30 - 0.65
Phe	-	-	0.29 +/- 0.03	0.50 - 0.90
OC (%)	2.1	2.9	2.1	7-9
NO_3^- as mg N/L	3300	181	800	500
spike (ppm)	50, 200, 500	4.5, 0.6	100	6, 11, 36, 50

Al-Bashir et al. (1990) monitored carbon dioxide release from labeled naphthalene added to soil-water slurries. The spike levels were well above the solubility of naphthalene in water. Most of their work was done on a soil with an organic content of 2.1%, although a contaminated soil with an organic content of 4.3% was also tested at the 200 ppm spike level. The method of organic content determination was not reported, therefore, the reported organic levels could be loss on ignition or organic carbon content, which would be about half of the former value. When the concentration was close to saturation for naphthalene, the degradation was zero order with respect to substrate concentration and was controlled by desorption. Given these findings, the degradation rate in False Creek and ESSO sediments would be expected to be lower because of the high organic contents and perhaps the lower spike levels and higher available (non-PAH) carbon from the sediment. Direct comparisons are not made here because of the analytical difficulties in quantifying naphthalene from False Creek and ESSO sediments.

Mihelcic and Luthy (1988b) measured naphthalene and acenaphthene in the water phase. The initial concentrations were lower than were used by Al Bashir et al. (1990) or in the present research. It is also unclear if loss of the compounds from the aqueous phase adequately describes the degradation of naphthalene and acenaphthene in their experiment, because as the PAH were degraded, some would have desorbed from the solids to replenish the aqueous concentration. These two factors probably account for the lower apparent degradation rates in Mihelcic and Luthy's research (1988b) than those observed by others. In the False Creek sediment there is more organic material, which may be used preferentially by heterotrophic nitrate respirers, than in the sediments used by the other researchers. The high organic content of FCE sediment could also decrease the aqueous concentration of PAH available for degradation. For these reasons, the rate of PAH degradation would be expected to be lower in the False Creek sediment-water system than in the system used by Mihelcic and Luthy.

Leduc et al., (1992) studied degradation of PAH in soil slurries spiked to a level of 100 ppm that were mixed continuously (in flasks) or periodically (in a bioreactor). Their experiments lasted for four months and degradation was roughly linear throughout. This is in contrast to the results of the present study where degradation was fastest in the initial stages of the experiments and decreased as the concentration of the PAH declined (first order kinetics). The maximum percent loss observed by Leduc et al. (1992) was 60% for acenaphthylene and acenaphthene, so the rate of degradation might not yet have measurably decreased, given the

variability of the data. The rates they calculated were similar to the range of rates determined for False Creek. The acenaphthylene degradation rate in False Creek sediment was lower than reported by Leduc and his colleagues. This could be due to the low concentration or recovery of acenaphthylene from the False Creek sediment matrix. Alternatively, this low rate could be a result of a lower available acenaphthene concentration due to the higher organic content of False Creek sediment. The higher degradation rates in False Creek sediment for fluorene and phenanthrene might be due to larger populations of organisms that can use these substrates, stimulatory effects of other matrix components or even a higher aqueous concentrations in the False Creek sediment porewater due to solubilization by colloidal material or biosurfactants.

Langenhoff et al. (1996) also reported degradation of naphthalene under denitrifying conditions. The organic content of the sludge/sediment/soil mixture they used was not given, nor was the dry weight of material in their sediment columns, but the removal rate of naphthalene through 60 mL reactor columns was approximately 120 μg/day after the system had been running for 300 days. This rate of removal was only observed when benzoate was also added in the feed. This effect might have been due to cooxidation of naphthalene when benzoate was used as the primary substrate, induction of the naphthalene degradation enzyme system by benzoate, or benzoate might have been a required electron donor for reduction of the aromatic ring. Whatever the mechanism, if the dry weight of the material in the columns used by Langenhoff et al. is assumed to be about 20 g, this rate (6 μg/g•day) is 3-40 times faster than was observed in the soil water systems of Mihelcic and Luthy and Al Bashir et al.

Degradation rates are not available for the work done by Murphy et al. (1995) on Hamilton Harbour sediment. The total PAH concentration decreased by 68% in 197 days in laboratory assays using sediment from the Dofasco site in Hamilton Harbour, with all PAH degrading roughly equally. The total PAH concentration in Hamilton Harbour sediment was initially >2000 ppm. Assuming linear degradation of PAH throughout the incubation period, the degradation rate would have been >6.9 μg total PAH/g•day. From a figure in their paper (Murphy et al., 1995) approximate degradation rates were calculated: naphthalene 2.2, acenaphthylene 0.6, acenaphthene 0.2, fluorene 0.2, phenanthrene 0.7 μg/g•day. All of these rates are based on the assumption that the degradation of all PAHs was equal and linear over the incubation period, which cannot be confirmed with the available data, but the rates are similar to those observed in the other studies (Table 5.7). In the field, the degradation rates

were slower: Total PAH decreased from 450 to 383 μg/g in two to three months, a total PAH loss of about 0.8 to 1.2 μg/g•day, and there was an increase in the naphthalene concentration over that time.

The most striking observation from the work carried out by Murphy et al. (1995) was that the degradation of the larger PAH in Hamilton Harbour sediment (with or without addition of the organic amendment) was similar to that observed for the smaller, more soluble compounds. The False Creek PAH degradation rates are more typical of what has been seen by other researchers: the degradation of the compounds decreases with decreasing solubility. The observed high degradation rate for HMW PAH in Hamilton Harbour sediment must mean that the organisms in that environment have some mechanism to solubilize PAH, conditions at the site are conducive to the solubilization of PAH or there is a population there that can use sorbed material. This observation is especially interesting because the low degradation rates of HMW PAH typically limit the success and speed of bioremediation attempts. Furthermore, the HMW compounds tend to be the carcinogenic and mutagenic ones, so it is of particular interest to ensure that they are degraded.

5.3.2 Comparison to aerobic studies

More research has been done on the degradation of PAH under aerobic conditions, so rates of the HMW PAH degradation can be included. Table 5.8 shows the range of half life values for PAH from a number of studies. The Sims and Overcash (1983) data were collated from a large number of degradation studies. The Wild et al. (1991) data (in years) are from a single sludge application to agricultural soil twenty years ago. Samples from the treated plot were compared to archived pre- and post- treatment samples. Park et al. (1990) studied spiked low organic content sandy and loamy soils. Wild and Jones (1993) investigated PAH degradation with spiked soils and soils to which PAH were added in a complex sludge mixture.

On the whole, the results show that degradation under denitrifying conditions is slower than in the presence of oxygen, although the difference is not consistent. The spread of the data is wide, reflecting different experimental conditions, soils, and spiking methods, but it does appear that the anoxic degradation of PAH requires more time. The differences are less pronounced for the HMW PAHs, for which the half life estimates are less accurate. The

Table 5.8 Half Lives of PAH under aerobic conditions
Half lives are expressed in days except for Wild et al. (1991) data which are in years. Data from this study were taken from the High/Low and Ammonia/Availability experiments. Values with asterisks are combined ACE/FLU or BAA/CHR data, and blanks indicate that the half lives were not determined.

PAH	Wild/ Jones (days)	Wild/ Jones (days)	Sims/ Overcash (days)	Park et al. (days)	**This Study (days)**	Wild et al. (years)
NAP	28	15	0.1-4.0	2.1-2.2	**-**	<2
ACE	-	-	0.3-4.0	-	**30-53**	-
FLU	65*	28*	2.0-39	-	**30-36**	<3.2*
PHE	124	14	2.5-26	16-35	**40-49**	<5.7
ANT	141	48	35-175	50-134	**69-95**	<7.9
FLA	137	16	44-182	268-377	**113-187**	7.8
PYR	25	51	3.0-35	199-260	**113-180**	8.5
BAA	-	-	4.0-6250	162-261	**180-970**	-
CHR	215*	84*	5.5-11	371-387	**143-809**	8.1*
BAP	211	112	2.0-694	229-309	**-**	8.2
OC %	6 to 58	6	various	0.5-1.1	**7-9**	-
R^2	.22-.98	.62-.996	-	.57-.93	**.01-.99**	-
Spike	sludge	yes	various	yes	**yes**	sludge

smaller differences are probably due to the fact that the degradation of higher molecular weight PAH is controlled to a greater extent by their desorption from the solid matrix.

The long time required for degradation of the PAH in the sludge-amended agricultural soils (Wild et al., 1991a, b) illustrates that the half lives of PAH can be much longer than predicted from laboratory assays, especially those using spiked samples. In similar research using a different sludge-amended soil, Wild et al. (1991) were unable to calculate half lives because the PAH concentrations had already dropped below one half of the original values by the first time point (four years) but had remained at elevated concentrations in subsequent samples. This could be indicative of limited availability of the PAH, nutrient limitation, toxicity, or a combination of all three.

The more rapid degradation of PAH added directly to soil as compared to PAH in sludge is also important to note. The rates of degradation reported in the present study are all for spiked samples. In the False Creek site, some of the contaminants present in sludge are certain to be found due to the CSO inputs. This is unlikely to be the case for the ESSO site, where the PAH would mainly reach the ESSO sediment as part of a complex mixture of primarily hydrocarbon contaminants. Sometimes the presence of other contaminants increases the rate of degradation, as in the case of benzoate requirement for naphthalene degradation under denitrifying conditions reported by Langenhoff et al. (1996) and others for which the initial transformation of the PAH is by cooxidation. It is possible that part of the reason for lower degradation rates of PAH with sludge application is the difference in partitioning and availability in the system, since the sludge amended soil would have a higher overall organic content to which PAH could partition or adsorb. Also, there could be heavy metal or organic components of the sludge that are toxic to the PAH degraders.

5.4 Measurement of PAH availability with SPMDs and rSPMDs

It is very difficult to assess the risk associated with pollutants in the environment because the same contaminant concentration in two different matrices can have very different toxicological effects. To exert its toxic effect, a contaminant must be taken up by the target organism or organ. Since testing biological tissues for trace contaminants is difficult and expensive, chemical testing of the matrices is more often carried out. In the case of sediments, the relationship between concentration and biological effects is not straightforward. Much of the material in the sediment is tightly bound to the particulate fraction, and the fraction of bound material depends on the characteristics of the solid phase, especially the organic content, the length of time the contaminant has been in the system, the nature of the contaminant and sometimes even the source of contamination. It is of interest to determine what fraction of the extractable contaminant is available to the organisms in the system, since that is the fraction which can harm them.

Two methods that are sometimes used to test available fractions are leaching and mild chemical extractions. The former is useful because it measures the desorption of contaminants in water or other solvents, but it doesn't differentiate between the truly dissolved and the colloidal or DOM-associated phases. Mild extractions do not address the biological availability

so much as the chemical extractability of contaminants. A study was carried out recently to try to find a chemical extraction method that would mimic bioavailability (Kelsey et al., 1997). Mild chemical extractions reflected the trend of lower bioavailability with increased sediment-contaminant contact time. The procedure that best described availability was dependent on the soil and the test organism used, however, so the best type of extraction will be specific to the test system (matrix, contaminant and test organism). Another recent measure of availability is the use of semipermeable membrane devices (SPMD). These are made from non-porous polyethylene tubing containing a thinly spread layer of triolein (Huckins et al., 1990, 1993).

The underlying assumption behind the use of SPMDs as measures of available contaminants is that only dissolved material is bioavailable. Any pollutants that are attached to particles or are associated with HMW colloidal material will be unable to pass through the polyethylene membrane, which is intended to mimic the function of biological membranes. Hydrophobic contaminants become concentrated in the lipid relative to the water phase according to their octanol-water partition coefficients, as they might in fish lipids or the lipids of other organisms (Huckins et al., 1993). This approach allows exposures to be determined without having to account for the variation in relevant biological factors between individuals or the metabolism or depuration rates in organisms. Furthermore, SPMDs can be deployed in the field at sites where animals might not survive.

Two methods were used to try to measure the availability of PAH from False Creek sediment: semipermeable membrane devices (SPMDs) and reverse SPMDs (rSPMDs). SPMDs have been used successfully to monitor PAH concentrations in the field. PAH with up to 5 rings were detected in samples using this approach (Lebo et al., 1992), so exclusion of PAH by the polyethylene membrane should not be a problem. Reverse SPMDs were developed to determine availability more quickly, inexpensively and using less sediment.

Several tests were carried out to determine if SPMDs could be used as a tool to measure PAH availability in FCE sediment. It was expected that the SPMD extracts would contain less of the HMW compounds than the LMW compounds because HMW compounds are less water-soluble. A sediment with a higher organic content would be expected to bind PAH more strongly than a low organic sediment. Spiked material was assumed be more easily desorbed and therefore more available than "endogenous" material that has "aged" *in situ*. In the present research, a higher proportion of PAH was extracted in SPMDs when they were exposed to

spiked clay alone than when spiked clay plus FCE sediment was used. This effect was especially pronounced for the HMW compounds, which was expected because the PAH could adsorb to the organic phase of the FCE sediment so less would have been in solution (Figure 4.14). The percentage of total PAH left in the sediment after exposure to SPMDs generally increased with increasing organic carbon content, indicating stronger binding of the PAH (Figure 4.15). The spiked PAH were more readily available than endogenous material, since a higher proportion of the added PAH was extracted in the SPMDs (Table 4.8). Therefore, it was concluded that SPMD extractions were good estimates of PAH availability.

SPMDs are expensive to make due to the cost of the triolein, and take a long time to process, so an alternative that would also use less sample was devised. Reverse SPMDs use the same membrane tubing but the sediment/water slurry is placed on the inside of the membrane and the contaminants have to diffuse out into a solvent (pentane) phase. Only the contaminants that are in solution can pass through the membrane, but the driving gradient is higher using the rSPMD because 150 mL of pentane is used compared to 1 mL of triolein in conventional SPMDs. Again, a higher proportion of spiked than endogenous PAH was extracted in the rSPMDs (Table 4.9). The data in Table 4.9 also show that the rSPMD method is a more stringent measure of availability than the conventional SPMD method, since a higher proportion of the PAH present is extracted by this method. According to Huckins et al. (1993), solvents like hexane, and presumably pentane, might be expected to dissolve in the polyethylene membrane and hamper the diffusion of pollutants as a result of the large flux of solvent going in the opposite direction. In the present research, solvent movement didn't seem to hamper pollutant extraction. Any solvent that did pass through the membrane might have served to lower the polarity of the water-sediment phase, allowing the sediment organic phase to swell. Less compact sediment organic matter would improve diffusion of contaminants within the particles and increase the rate of desorption. The method that most accurately reflects bioavailability will be specific to the organism of interest. Exposure of organisms depends on the lipid content, route of exposure, and sediment contact time. The rSPMD method is a fairly stringent assay of material that can desorb from the solid phase.

5.5 Implications of PAH availability on bioremediation

The major limitation of the application of *in situ* bioremediation to degradable hydrophobic organic compounds is probably the availability of the contaminants at the site. If physical methods are required to release contaminants from the matrix at a rate that sustains growth of microorganisms, *in situ* treatment is unlikely to be successful because these methods would be difficult to apply. In False Creek sediment, SPMDs and rSPMDs were used to examine the relative availability of added and endogenous PAH. As estimated by both methods, a higher percentage of the added material was available for partition into the SPMD lipid phase or the rSPMD solvent phase (see Table 4.9). In degradation studies using spiked and unspiked sediments, the PAH degradation rates were higher and half lives were lower in the spiked sediments. These results support the conclusion that the degradation of PAH in these systems is governed by their availability, as has been observed elsewhere (Zhang et al., 1996), rather than their maximum rate of utilization. Furthermore, after exposure to a series of SPMDs, the PAH content remaining in a spiked FCE sediment sample and an unspiked sample were compared. The PAH concentrations were very similar, indicating that all of the spiked material (not aged) was eventually extractable by SPMD, and that there was a fraction of the endogenous PAH content that was occluded (Figure 4.17). This material is probably recalcitrant to biological degradation.

One difficulty in interpreting the data from the rSPMD extracts of sediments in the degradation experiments is that the "available" fraction was higher than the "total" PAH measurement in some cases. This most likely reflects a technical problem that yields higher PAH recoveries from the rSPMD extracts than from the dried solids extracts. The rSPMD extracts would contain fewer high molecular weight compounds that could keep the PAH in the DMSO-water phase when it is extracted with pentane, and the cleanup of rSPMD extracts does not include the solvent exchange step required in the DMSO cleanup of dichloromethane extracts. Some other factors that contribute to variation in the data are the small sample size and low concentrations of PAH in the extracts. The observations regarding the "available" fractions are therefore limited to major differences.

The degradation of spiked PAH that exhibited lower availabilities by rSPMD, that is benz(a)anthracene, chrysene and especially benzo(a)pyrene, was very slow or did not occur in False Creek sediment. This could result from a lack of organisms in the system that could

degrade the larger PAH, or it could be due to the low rate of desorption of those compounds from the sediment. Degradation of the less soluble PAH has been observed by others, even under denitrifying conditions (Murphy et al., 1995), and so HMW PAH degradation was likely limited by their availability in FCE samples. Given the high organic carbon content of the False Creek sediment, the likelihood that a great deal of this organic content is oil and grease from runoff, and the high affinity of the larger PAH for the organic content of the solid phase, this is to be expected. Since the PAH were added to the sediment, they were not occluded in the sediment matrix, at least initially. This means that regardless of the source of the HMW PAH, they will become available for degradation only slowly, so several applications of nitrate might be required to treat this system.

Benz(a)anthracene and chrysene were degraded, although more slowly than the smaller compounds, in the spiked ESSO sediment with the addition of nitrate. This probably indicates that these PAH became available for degradation more quickly than the same compounds did from the False Creek sediment. The availability of these compounds as measured by reverse SPMD was also higher in the ESSO samples than was observed for False Creek samples (compare Figure 4.29 to Figure 4.23 and Figure 4.25; Table 5.9). This was most likely due to differences in the organic carbon contents of the sediments. Since unpolluted sediments have similar adsorptive properties (Kile et al., 1991), the discrepancy between the PAH availabilities in sediments from the two sites indicates that different pollutant compositions can give rise to sediments with different sorptive capacities or profiles. Unfortunately, the availability of the endogenous PAH was not accurately determined in ESSO sediment due to low concentrations, which also led to more variation in the sediment phase concentrations.

The relative availability of PAH in ESSO sediment (section 4.5.3) and False Creek sediment from the Ammonia and Availability experiment (section 4.5.1) and the last single time point experiment (section 4.5.2) are shown in Table 5.9. They are expressed as % PAH available, and numbers over 100% reflect the better recoveries obtained from SPMD extracts than whole sediment extracts. The availabilities were typically highest for ESSO sediment and lowest for the last single time point False Creek experiment. These observations might explain the lower degradation rates observed in the final experiment using False Creek sediment, even in spiked samples, and higher rates in ESSO sediment (see Table 5.6).

Table 5.9 Percent availability of PAH
The percent availabilities are expressed as 100*[rSPMD]/[sediment] at 12 weeks. NH_3 + and - values are from the Ammonia and Availability experiment, FCE+ is the spiked sample from the Unspiked False Creek experiment and ESSO+ is the spiked ESSO sediment data.

PAH	NH_3+	NH_3-	FCE+	ESSO+
Acenaphthene	185	143	97	153
Fluorene	137	117	97	162
Phenanthrene	108	101	98	128
Anthracene	107	104	74	121
Fluoranthene	105	101	85	120
Pyrene	124	102	83	132
Benz(a)anthracene	56	52	49	89
Chrysene	54	60	54	112

These results highlight one of the key difficulties in bioremediation. In False Creek sediments, added HMW PAH were not degraded in 20 weeks. This indicates that the rate of degradation of the larger compounds will be low, regardless of the form in which they reach the sediment. The acute and chronic toxicity of these compounds should also be low due to the reduced bioavailability of bound PAH, however there could be more risk associated with them if environmental conditions change their availability. The availability of the endogenous material was less than that of the added material A portion of the endogenous PAH could be permanently occluded or in a very slowly desorbing phase, however, it would be difficult to demonstrate that none of the material could become available given the right conditions. This will be a limitation to bioremediation in many sediments and soils that contain PAH.

Ex situ methods to increase the bioavailability of contaminants can be used to improve bioremediation in bioreactors, but most of these methods cannot easily be applied *in situ*. They include pH or temperature adjustments, solvent treatments to expand the organic component of the solid matrix and to speed up diffusion-limited mass transfer, and surfactant addition (Pignatello and Xing, 1996). In the case of PAH, there is even some debate as to whether PAH that are not readily exchangeable should be included in the concentration that can be regulated. Some PAH seem to be so strongly associated with the matrix that they remain biologically unavailable on a scale of years (McGroddy et al., 1996; Beck et al., 1995). It has been

demonstrated that elevated PAH levels in sediment do not necessarily give rise to toxic effects and deterioration of the environment (Paine et al., 1996). It could be that models of availability that incorporate the soot phase (Gustafsson et al., 1997) will better predict porewater PAH concentrations, bioavailability, and toxicity. Since these issues have not yet been resolved, PAH reductions in some solid phase systems will likely have to be reduced to regulated concentration limits that might not be attainable by bioremediation.

5.6 Potential for bioremediation at False Creek and ESSO

To ensure that all the alternatives are explored before embarking on a remediation project, a decision making tree was developed by the Contaminated Sediment Treatment Technology Programme (CoSTTeP) with funding from Environment Canada's Great Lakes Cleanup Fund. At the first level decisions are made about the viability of the most broad remediation options, namely: taking no action, capping, *in situ* treatment or removal. Subsequent levels represent refinements of each of the options. *In situ* treatment options includes fixation, biological or chemical treatment. Removal can be followed by treatment by fixation, incineration, alternate thermal treatment, biological or chemical treatment. Removed sediment can also be used as fill or confined in hazardous waste or other engineered landfills (Wardlaw et al., 1995). The options employed will depend on the degree of contamination, matrix factors, political and biological considerations as well as cost.

Nitrate could be used as a terminal electron acceptor for biodegradation of organic contaminants in both *in situ* and *ex situ* bioremediation projects. The *in situ* technique has the major advantage of avoiding the need to remove and handle hazardous material. In some cases, dredging does not remove all of the contaminated material and a highly contaminated sediment can be left behind. The *in situ* treatment is also relatively inexpensive, provided extensive treatments are not required (Murphy et al., 1994, 1993, 1995). In some cases, dredging may be necessary for navigation, when deeper treatment is required, when non-biodegradable contaminants such as metals are present, or for highly contaminated sediments where *ex situ* methods are more cost effective. *Ex situ* treatment allows more control over the process, results in the removal of the material from the site and can be optimized to speed up degradation. Conditions in slurry phase bioreactors can be controlled to improve the bioavailability of the contaminants.

In situ treatment of False Creek sediment using the NWRI/Golder *in situ* bioremediation method (Murphy et al., 1995) would result in the immediate benefits of lowered hydrogen sulphide concentrations and toxicity. If the strongly reducing conditions in the sediment were overcome, oxygen would likely penetrate more deeply into the sediment, and some of the benthic community might be able to recolonize the area. Bioturbation of sediments allows for the pumping of interstitial water out of the sediment, moving deeper sediments to the oxic surface and depositing fecal pellets with nutrients at the surface. This facilitated mixing and aeration, as well as potential changes to the compounds in the guts of the macrofauna, can improve the availability and degradability of contaminants in sediments (Baudo and Muntau, 1990; Bauer et al., 1988; Riser-Roberts, 1992). The efficacy of this method remains to be proven, especially if establishment of the benthic organisms is inhibited by heavy metals in the sediment. If, in the marine environment, the metal concentrations observed at the sites are in available form after treatment, it could be that the resolution of one problem results in the formation, or at least uncovering, of another.

In situ treatment will probably be limited to sites at which the metal concentrations do not exceed observed effects levels (e.g., PEL, ERM), or at least to sites where the toxic metal concentrations after treatment can be shown to be non-toxic. Since the sediment is not removed from the site and the metals are not destroyed by the *in situ* treatment, this could be a serious limitation to the application of the technique. In False Creek, some metal concentrations are quite close to or exceed the BAET levels determined for Puget Sound in Washington State (see Table 5.2), and some are in the probable effects ranges (above the ERM and PEL) determined by Long et al. (1995) and Environment Canada (1995).

The low availability of the HMW PAH at False Creek will prolong the duration of treatment required. That would lead to repeated nitrate applications and increased cost. Furthermore, the experience in solid phase bioremediation of PAH in soils has not been very successful, since the HMW compounds (in particular) persist (Wilson and Jones, 1993). A similar degradation pattern was observed in the present research. It could be argued that materials which are not available to microorganisms will not exert toxic effects. Conditions inside the guts of benthic organisms will not be the same as those in the surrounding environment, however, and the guideline PAH concentrations that must be met for treated

sediments to be used as fill or to be dumped are still based on the total concentrations of contaminants, not on their availability.

It will also be important to establish if treatment of the top 15 cm of sediment is sufficient to protect the biota in the system. If the contaminants are tightly bound and few organisms are exposed to the deeper material, the treatment of the top layer should be sufficient. This remains to be proven. The depth of the contamination problem will have to be addressed by taking core samples at any site for which the *in situ* method is considered until that question is addressed.

The lower overall nitrate use and higher degradation rates of PAH under the test conditions indicate that the ESSO site might be a better one for a pilot *in situ* remediation project, even though the degradation rates of endogenous PAH were lower than spiked ones. Less nitrate would have to be applied to achieve oxidation of the sediment at ESSO than at False Creek, and it is less likely that repeat applications would be required. The higher availability of the material also makes this a more attractive site. Another advantage is that most of the metal concentrations at the ESSO site do not exceed the PEL, ERM or BAET levels (see Table 5.2), so treatment of the organic contaminants could sufficiently improve conditions to alleviate many of the observed environmental effects. Furthermore, since the cessation of process effluent discharge to Port Moody Arm from the refinery in 1991, the input of contaminants is likely to be much lower than at the False Creek site which receives continued CSO inputs, so longer-term improvement of conditions at ESSO could be expected from the treatment.

Ex situ bioremediation can also be considered for these sediments. Slurry phase treatment has been more successful than solid phase remediation for the treatment of PAH contaminated soils since conditions can be controlled to improve the availability of organic compounds through temperature and pH control as well as by surfactant addition. In the case of False Creek slurry phase treatment might be effective since the rate of desorption seemed to limit the degradability of the HMW contaminants, however biological treatment of any kind would not address the problem of metals contamination. At the ESSO site, the lack of BaP degradation could also be addressed by treatment in a slurry phase bioreactor.

Slurry phase bioremediation can be carried out with air or with nitrate as the final electron acceptor. Nitrate treatment has recently received a lot of attention in petroleum

contaminated soil bioremediation both *in situ* and *ex situ* (Norris et al., 1995; Battermann and Meier-Lohr, 1995; Hutchins et al., 1995; Battermann et al., 1994). The major advantage of nitrate use is the lower cost, compared to aerobic treatment, which can be very important if remediation requires a long time. If the availability of the pollutants is not a problem it could be more economical to use an aerobic system with a lower retention time, since the degradation rate would be faster. If availability limits the degradation rate, using nitrate as the electron acceptor might be the more economical approach. The demonstration of PAH degradation in marine sediments under denitrifying conditions provides a valuable new bioremediation option.

There will likely be some cases in which desorption of PAH cannot reach a sufficient rate to maintain the cost advantage of bioremediation. In such cases, different techniques will be required to treat PAH-contaminated materials. This would likely occur in cases where the material had been aged over a long period of time, or when the PAH are tightly bound to particulate matter at the source. Two recent models have been proposed which are intended to predict whether removal of contaminants from soils and sediments is limited by the maximal degradation rate of the substrate by microorganisms, or the rate of mass transfer from the medium (Ghoshal and Luthy, 1996; Bosma et al., 1997). The specific limitation to the biodegradation rate should be assessed whenever bioremediation is being considered as a treatment option. Mass transfer and degradation rate constants should be determined under similar conditions as those of the proposed bioremediation mechanism(s).

5.7 Comparative costs of sediment bioremediation alternatives

The cost of bioremediation of sediment *in situ* using the Golder/NWRI nitrate injection process depends to some extent on the rate of nitrate and organic amendment application, but more on the number of treatments required. If a single treatment is required, the cost of remediation ranges from (Canadian) $25-35/m^3$. The maximum cost could go up to $75/m^3$ depending on the number of treatments (Jay Babin, Golder Associates, personal communication).

A cost comparison (in Canadian dollars) of various *ex situ* sediment treatments undertaken under the Contaminated Sediment Treatment Technology Programme (CoSTTeP) was carried out by Wardlaw (1994, 1996). Incineration was the most expensive option at approximately $350/tonne. Alternate thermal methods were slightly less expensive at $250-

300/tonne. Extraction methods and biological treatments overlapped in the $75-200/tonne range. In addition to the treatment costs, these *ex situ* methods would require sediment removal and transport to the treatment site, and final disposal of the treated material. Pretreatment of the sediment might also be required. Each of these actions would add to the overall cost of site remediation.

The final disposal options could influence the choice of sediment treatment. If the material could be used as residential or industrial fill, costs would be lower than if it required disposal in controlled landfill or hazardous waste sites. Although the PAH removals were very high (>90%) in some of the bioremediation and sediment extraction techniques, the final concentrations of some compounds, for example BaP, were still above those permitted in Ontario for use as industrial fill (Wardlaw, 1994, 1996). This means that disposal in controlled landfill would be required, and the cost would increase.

All of the bioremediation, extraction and alternate thermal techniques compared in the above papers were new and were carried out on sediment samples from single sites, so better removals could be possible given optimal conditions and the right sediments. More research in this area would certainly improve efficiencies, and would help clarify which conditions limit and enhance bioremediation. Both the unit costs of treatment and the removal efficiencies could be improved in time.

An analysis of sediment treatment and handling costs was done by Keillor (1993) to help decision makers choose options that can be carried out at a "reasonable" cost. The data reported were the lower limits of published remediation unit cost ranges from the literature. These cost estimates are therefore for remediation under the most favourable conditions (Table 5.10).

From Table 5.10, solidification or stabilization seems to be one of the least costly treatment options. Forstner (1989) cautions that these approaches should be limited to environments in which conditions remain fairly static. Changes in pH can cause changes in the solubility of stabilized materials. Some problems exist for sediment handling that are not observed in soils. For example, sulphide oxidation can cause the pH to drop, and degradation of organics can allow the release of previously bound metals (Forstner, 1989).

Table 5.10 Estimated costs of sediment treatment options
Estimates are taken from Keillor, 1993. Treatments marked with asterisks are low cost limits from the literature. All estimates are given in 1991 US dollars per cubic meter

Sediment Treatment Description	Cost Range (1991 US$/m^3)
Dredging and Disposal	4-28
Separation, Dewatering	5-44
Soil Washing	76-132
Inexpensive Treatment (Separation/Solidification/Stabilization)	14-69
Expensive Treatment (Chemical Extraction/Treatment and Incineration)	277-693
Extraction*	40-268
Biodegradation*	39-181
Chemical Destruction of Organics*	34-945
Thermal Processes*	70-257
Solidification/Stabilization*	33-158

Biodegradation is another relatively inexpensive option, although cost savings in soil treatment have only been observed in projects that are relatively short in duration. The ongoing monitoring required to maintain optimal conditions adds to the cost over time (Davis et al., 1995), so the cost advantage of bioremediation decreases with longer treatment times. The availability or desorption rate of the contaminants of interest will therefore be a controlling factor in the cost of bioremediation.

Keillor (1993) determined that the cost of sediment handling often increases substantially in cases where insufficient site investigation has been carried out. Detailed study of treatment sites must be undertaken to target the most contaminated zones. Areas of intermediate and low pollutant concentrations should be protected from further contamination during removal and treatment. Thus a more expensive monitoring programme at the beginning of a project could substantially lower the total cost by avoiding unnecessary removal and treatment of clean material, and by protecting areas of lower contamination.

Such an early site investigation should include extensive availability and degradability tests in cases where bioremediation is being considered. The limiting factor of bioremediation (the microbial degradation rate or the mass transfer rate: Ghoshal and Luthy, 1996; Bosma et al., 1997) and the fractions of "occluded" materials (by high temperature desorption studies and/or soot fraction determinations: Cornelissen et al., 1997; Gustafsson et al., 1997) should also be determined. In cases where the occluded fraction exceeds regulated levels, bioremediation should probably not be employed. When the mass transfer rate limits biodegradation, tests should be undertaken to determine if substrate availability can be improved, and low operating cost alternatives, such as operating bioreactors under denitrifying conditions, should be explored. *In situ* bioremediation can be applied when the microbial degradation rate limits the rate of contaminant degradation and can be increased by the addition of nitrate and organic amendments to the sediment.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This research consisted of experiments using marine sediment from False Creek and a single time point experiment using sediment from the ESSO site. Several conclusions can be drawn from the results:

(1) Nitrate addition to anoxic marine sediment can cause the rapid oxidation of acid volatile sulphides as well as a decrease in sediment toxicity evaluated by the MICROTOX bioassay.

(2) Low molecular weight PAH can be degraded relatively rapidly under denitrifying conditions in the absence of other limitations.

(3) High molecular weight PAH degradation was less successful. Fluoranthene and pyrene were degraded, but more slowly than the lower molecular weight PAH. Benz(a)anthracene and chrysene were sometimes degraded whereas benzo(a)pyrene biodegradation was never observed under the experimental conditions.

(4) PAH in sediments were extracted by SPMDs and reverse SPMDs. The more hydrophobic contaminants, and endogenous or "aged" PAH were not extracted as well as the added LMW PAH using these availability measures. A higher sediment organic content also led to lower PAH yields in SPMD extracts. Both methods should be useful in determining that fraction of the PAH that is desorbable from the solid phase, and hence potentially more bioavailable.

(5) Reverse SPMDs can be used as a less expensive and less time consuming alternative to conventional SPMD use, and they are also a more stringent measure of availability.

(6) Degradation of the HMW PAH seemed to depend on availability as measured by rSPMD. Compounds that were only sparingly extractable by this method were degraded very slowly, if at all.

(7) The addition of calcium nitrate caused phosphate limitation, inhibiting biodegradation. In this research, the problem was overcome by using sodium nitrate.

(8) Nitrate reduction was not accompanied by an increase in the ammonia concentration, indicating that the reduction pathway was denitrification, not dissimilatory nitrate reduction to ammonia (DNRA). The appropriate carbon to nitrate balance will have to be achieved when using nitrate as the electron acceptor for bioremediation to avoid potential ammonia toxicity caused by DNRA.

(9) The high metal concentrations, large nitrate demand, ongoing inputs and low degradation rates of the HMW PAH will probably limit the success of *in situ* bioremediation at the False Creek site. *Ex situ* bioremediation coupled to metals removal could be considered, using nitrate as the electron acceptor, especially if desorption limits the rate of degradation.

(10) *In situ* bioremediation at the ESSO site is more likely to be successful than at the False Creek site since more of the HMW PAH material was available to the microorganisms and the sediment demand for nitrate is much lower at the ESSO site. It might still be necessary to remove the sediment if benzo(a)pyrene removal is required.

6.2 Recommendations for further research

There are several interesting questions that have been raised by this work, and there is a great deal of room for further research in the areas of the microbiology of anoxic PAH degradation, bioavailability of sediment associated PAH, and in finding the right method(s) of site remediation. The latter question will necessarily be more site specific than the others, since bioremediation options are very site sensitive.

To begin with the microbiological aspects, it would be useful to isolate the organisms responsible for PAH degradation to better understand their nutritional requirements, optimal growth conditions, minimal substrate concentration requirements and other factors that would affect the success of bioremediation using this method. The degradative pathways of these microorganisms should be studied to determine the possibility of toxic intermediate formation and to identify trace elements, cofactors, inducers and repressors required for PAH biodegradation. Degradation studies on mixed cultures could also be carried out using radio-labeled substrates to better understand the fate of PAH and their metabolites.

The question of bioavailability of contaminants is difficult. Adsorption to sediments and soils not only renders contaminants less toxic, but also less amenable to bioremediation, or

natural biodegradation. With PAH there is a great deal of difficulty in assessing the risk associated with contaminated soils and sediments, since the larger, carcinogenic compounds tend to be tightly associated with the solid phase. More research needs to be done on desorption of PAH from solids under conditions similar to those encountered in the digestive tracts of benthic organisms, bottom fish, and their predators. The influence of surfactant addition, temperature, and other variables that can alter PAH availability in slurry bioreactors should also be investigated.

It would also be useful to assess the biological relevance of SPMDs and rSPMDs by pairing "availability" determinations with experiments on uptake by organisms and toxicity. This research would lead to a better understanding of the biological relevance of these approaches to availability measurement, and would likely lead to improved methods. Additional information on the toxicity and carcinogenicity of individual PAH would also be useful in assessing the risks of exposure.

The *in situ* bioremediation method employed by NWRI/Golder is a new approach, so there are a number of issues that should be investigated. The range of organic matter that can be degraded under these conditions should be determined to establish the limits of the approach. In the research described in this thesis, only the PAH were monitored, although it appeared much more organic material in the sediments was degraded. The toxicity of sediments treated in this way should be determined to find out if the metals and residual organics are present in a form that causes harm to the organisms in the ecosystem. The adequacy of the depth of sediment treatment should also be assessed to determine if benthic organisms could be affected by contamination below the treated zone.

Research should also be carried out on the use of nitrate as an electron acceptor in slurry bioreactors. It would be helpful to determine if desorption-limited biodegradation could be carried out more economically under nitrate reducing conditions than aerobic conditions. This will likely depend on the difference between degradation rates in nitrate and oxygen treated systems and the length of time required to produce material that meets the required criteria. A rough guide to degradation rates vs. cost effectiveness using each method would be very useful to decision makers.

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Appendix A: Sampling Site Characteristics

False Creek Water Characteristics:

Date	Aug. 18/'92				Oct. 14/'92			
Depth (m)	Temp (^oC)	Cond. (uS/cm)	Salinity (ppt)	D.O. (mg/L)	Temp (^oC)	Cond. (uS/cm)	Salinity (ppt)	D.O. (mg/L)
1	21.3	28300	19.2	8.2	10.8	17000	13.6	8.0
2	19	29200	19.8	10.2	10.8	17700	14.5	7.9
3	19	28700	19.5	10.2	11.6	19800	16.4	7.5
4	17.3	29300	21.5	13	11.8	20800	17.3	6.9
5	15.8	29600	22.3	12	11.4	20200	17.6	6.5
6	14.8	29200	22.8	8.8	11.5	21800	18	4.3
7	13	29300	23.7	6.1	11.3	21000	16.5	3.8

False Creek Sediment Characteristics:

Sample	Redox (mV)	pH
Aug. 18/'92 #1	- 423	7.73
Aug. 18/'92 #2	- 411	7.61
Aug. 18/'92 #3	- 379	7.52
Aug. 18/'92 #4	- 423	7.41
Oct. 14/'92 #1	- 379	7.46
Oct. 14/'92 #2	- 385	7.64
Oct. 14/'92 #3	- 441	7.43
Oct. 14/'92 #4	- 439	7.49

False Creek Sediment Particle Size:

Size	3/93 I	3/93 II	3/93 III	3/93 IV	3/93	4/93	6/93	7/93
>2	9.9	4.6	11.7	8	3.5	11	2.6	4.1
>0.5	17.6	17.6	15.2	15.3	22.9	23	18	22.4
>0.25	8.9	9.4	10	9.7	21.1	18	41.5	37.2
>0.125	10.2	11.4	11.3	13	14.9	16	14.7	13.8
>0.063	14.9	14.3	17.7	14.8	13.6	16	7.7	7.8
<0.063	38.5	42.6	33.9	39.2	23.9	17	15.6	14.8

Note: The material was dried and disaggregated with a mortar and pestle prior to sieving. The spread in the data might have been caused by insufficient disaggregation since the consistency of the wet sediment was quite "soupy". Numbers in the table are % in each fraction. "ppt" is parts per thousand.

ESSO Water Characteristics:

Date	Aug. 18/'92				May 20/'93			
Depth (m)	Temp (°C)	Cond. (uS/cm)	Salinity (ppt)	D.O. (mg/L)	Temp (°C)	Cond. (uS/cm)	Salinity (ppt)	D.O. (mg/L)
0	21	25900	16.3	8.6	18	21700	15.3	10.8
1	20.9	26800	17.6					
2	20.2	27200	18.7	8.6	18.2	22500	15	10.6
3	19.8	27500	18.7					
4	18.5	26600	18.5	9.5	14.5	21600	15.2	10
5	15	25000	19					
6	14.2	24800	18.8	5.3	12	20200	16.8	9.3
7	14.8	24900	18.8					
8	13.3	25000	19.7	4.1	10.1	19000	17	7.8
9	13.2	25000	19.7					
10	13.2	25000	19.8	4.4	8.2	20200	18.3	7.2
11	13	25000	19.8					
12	13	25000	20.2	4.4	6.4	20200	18	6.5
13	12.9	25000	20.2					
14	12.9	23500	19	3.6	4.5	19000	18.8	4.6
15	13.2	23800	19.2					
16					3.5	18700	18.7	.4

ESSO Sediment Characteristics

Sample	Redox (mV)	pH
Aug. 18/'92 #1	- 327	7.84
Aug. 18/'92 #2	- 323	7.67
Aug. 18/'92 #3	- 372	7.31
Aug. 18/'92 #4	- 349	7.41
May 20/'93	- 310	7.28

ESSO Sediment Particle Size:

date\size	> 2	> 0.5	> 0.25	> 0.125	> 0.063	< 0.063
5/93	16.2	34	12.9	8.6	5.7	22.6

Numbers are % in each fraction. The wet sediments appeared to be made up of fine material. Some of the material in the larger size fractions might not have been sufficiently disaggregated.

Metals Concentrations: Data from CHEMEX Labs Ltd.

Metal	units	9/94	9/94	3/94	3/94	5/93	**avg FCE**	ESSO
Ag	ppm	2.8	2	2.8	2.4	2	**2.4**	0.4
Al	ppm	1.92	1.75	1.82	1.7	1.53	**1.744**	2.22
As	ppm	20	18	20	20	20	**19.6**	6
Ba	ppm	80	60	100	100	100	**88**	60
Be	ppm	<0.5	<0.5	<0.5	<0.5	<0.5	**<0.5**	<0.5
Bi	ppm	<2	<2	4	<2	<2	**<2.4**	2
Ca	%	1.36	1.38	1.45	1.32	1.39	**1.38**	0.79
Cd	ppm	4.5	4.5	4	4	4	**4.2**	1.5
Co	ppm	10	10	9	10	10	**9.8**	9
Cr	ppm	64	62	63	65	61	**63**	46
Cu	ppm	345	366	366	325	330	**346.4**	110
Fe	%	4.46	4.56	4.55	4.14	4.74	**4.49**	3.25
Ga	ppm	<10	<10	<10	<10	<10	**<10**	<10
Hg	ppm	1	<1	1	<1	<1	**1**	<1
K	%	0.28	0.29	0.26	0.23	0.24	**0.26**	0.39
La	ppm	<10	<10	<10	<10	<10	**<10**	<10
Mg	ppm	1.03	1.08	1.01	0.9	0.92	**0.988**	1.22
Mn	ppm	300	310	320	285	300	**303**	310
Mo	ppm	6	6	7	7	6	**6.4**	3
Na	%	1.86	1.86	1.22	1.28	1.63	**1.57**	1.84
Ni	ppm	38	37	38	38	34	**37**	30
P	ppm	1280	1140	1320	1230	1510	**1296**	900
Pb	ppm	304	304	322	318	318	**313.2**	52
Sb	ppm	<2	<2	<2	<2	2	**2**	<2
Sc	ppm	4	4	4	3	3	**3.6**	5
Sr	ppm	95	99	105	91	100	**98**	74
Ti	%	0.1	0.1	0.1	0.09	0.08	**0.094**	0.09
Tl	ppm	<10	<10	<10	<10	<10	**<10**	<10
U	ppm	<10	<10	<10	<10	<10	**<10**	<10
V	ppm	79	72	74	71	65	**72.2**	62
W	ppm	<10	<10	<10	<10	<10	**<10**	<10
Zn	ppm	748	714	706	686	670	**704.8**	140

note the Aqua Regia digest may be incomplete for: Al, Ba, Be, Ca, Cr, Ga, K, La, Mg, Na, Sr, Ti, Tl, W

Appendix B: Air and Nitrate Pretreatment Data

Solids, supernatants and toxicity data

Solids:

	% Dry weight	St Dev	% Loss on ignit.	St Dev	n
Start	50.4	0.05	7.1	0.03	3
Air: after pretreatment	57.31	0.96	7.39	0.20	2
NO3: after pretreatment	52.65	0.33	9.17	0.01	2

Supernatant nitrate, phosphate, ammonia and pH:

Sample	NOx (mgN/L)	Phosphate (mgP/L)	Ammonia (mgN/L)	pH
AIR Start	0.369	35.92	2.971	
Air: after pretreatment	2.2	0.5	5.3	5.93
AIR/control, end	0.271	0.063	3.2	6.36
AIR/NO3, end	0.271	0.132	2.8	6.88
NO3 Start	501.8	5.523	0.7	
NO3: after pretreatment	84 (2.4)	0.2	37.5	8.06
NO3/control, end	0.312	1.132	3.9	7.63
NO3/NO3, end	463	0.266	2.9	7.17

MICROTOX toxicity (ppm):

SAMPLE	EC50	95% Confidence Range
Start	325	269-392
Air: after pretreatment	348	273-444
NO3: after pretreatment	3622	3120-4205
AIR/control, end	3379	3163-3610
AIR/NO3, end	9853	9187-10567
NO3/control, end	2696	599-12143
NO3/NO3, end	5988	2873-12482

PAH data

Air pretreatment/control (μg/g dry weight):

PAH	1	2	3	4	5	Average	St Dev
NAP	0.17	0.17	0.12	0.10	0.21	0.15	0.04
ACY					0.22		
ACE	1.21	1.25	1.09	1.10	1.22	1.17	0.07
FLU	2.45	2.57	2.42	2.43	2.56	2.49	0.07
PHE	4.13	4.90	4.24	5.02	8.23	5.30	1.68
ANT	1.36	1.39	1.45	1.27	1.37	1.37	0.07
FLA	6.85	7.79	11.75	8.24	8.75	8.67	1.85
PYR	5.82	6.64	10.65	7.08	7.83	7.60	1.85
BaA	5.07	5.60	6.99	5.80	6.11	5.91	0.71
CHR	6.26	6.83	9.71	7.56	9.24	7.92	1.50
BaP	2.56	2.82	2.83	3.74	3.12	3.01	0.45
TOTAL	35.89	39.95	51.25	42.34	48.85	43.66	6.33

Air pretreatment/NO3(μg/g dry weight):

PAH	1	2	3	4	5	Average	St Dev
NAP	0.13	0.17	0.15			0.15	0.02
ACY					0.30		
ACE	0.27	0.46	0.30	0.11	0.36	0.30	0.13
FLU	0.37	0.58	0.37	0.34	0.42	0.42	0.10
PHE	1.09	3.16	1.23	1.08	1.73	1.66	0.88
ANT	0.61	0.96	0.66	0.58	0.72	0.71	0.15
FLA	3.15	4.35	3.00	2.82	3.14	3.29	0.61
PYR	5.20	6.39	5.16	4.88	5.65	5.45	0.59
BaA	3.33	4.10	3.18	3.03	3.30	3.39	0.42
CHR	4.64	5.96	4.34	4.10	4.95	4.80	0.72
BaP	3.14	3.47	3.02	3.03	3.13	3.16	0.18
TOTAL	21.93	29.61	21.40	19.96	23.70	23.32	3.76

All data are reported as μg/g dry weight and were generated using the DB-5 column. Blanks were below the detection limit.

NO3 pretreatment/control (μg/g dry weight):

PAH	1	2	3	4	5	Average	StDev
NAP	0.21	0.13	0.13	0.11	0.09	0.13	0.04
ACY	0.08						
ACE	0.95	0.81	0.78	0.81	0.67	0.80	0.10
FLU	0.91	0.77	0.73	0.78	0.65	0.77	0.10
PHE	1.87	1.66	1.64	1.65	1.37	1.64	0.18
ANT	1.18	1.03	1.03	1.07	0.89	1.04	0.10
FLA	8.32	7.25	7.01	6.88	5.73	7.04	0.93
PYR	6.92	6.12	6.08	6.09	5.07	6.06	0.66
BaA	6.37	6.13	5.61	5.78	4.81	5.74	0.60
CHR	7.73	7.53	6.88	7.41	6.17	7.14	0.63
BaP	3.38	2.98	2.92	2.93	2.44	2.93	0.33
TOTAL	37.92	34.41	32.81	33.50	27.89	33.31	3.61

NO3 pretreatment/NO3 (μg/g dry weight):

PAH	1	2	3	4	5	Average	StDev
NAP	0.19	0.14	0.14	0.14	0.15	0.15	0.02
ACY							
ACE	0.31	0.30	0.28	0.27	0.28	0.29	0.02
FLU	0.46	0.50	0.46	0.45	0.47	0.47	0.02
PHE	1.34	1.29	1.29	1.20	1.28	1.28	0.05
ANT	0.92	0.87	0.88	0.82	0.88	0.87	0.04
FLA	4.94	5.04	5.26	4.99	4.77	5.00	0.18
PYR	6.82	7.05	6.87	6.49	6.59	6.77	0.22
BaA	5.47	4.99	4.95	4.70	5.05	5.03	0.28
CHR	6.67	6.40	5.95	5.68	6.21	6.18	0.38
BaP	3.64	3.60	3.57	3.33	3.49	3.52	0.12
TOTAL	30.75	30.19	29.63	28.07	29.17	29.56	1.02

All data are reported as μg/g dry weight and were generated using the DB-5 column. Blanks were below the detection limit.

Appendix C: No Pretreatment Data

Solids, supernatants and toxicity data

Solids: % dry weight = 43.71% St Dev = 0.27 (n=6)

% loss on ignition = 9.56% St Dev = 0.14 (n=6)

MICROTOX toxicity, and supernatant pH, ammonia, nitrate and phosphate:

	EC50 (ppm)	95% range (ppm)	pH	Ammonia (mg N/L)	NOx (mg N/L)	Phosphate (mg P/L)
Control	2260	1822-2803	7.17	6.85	0.784	0.897
NO3	22578	17618-28934	7.27	9.65	173.46	0.07

PAH data

Control:

PAH	1	2	3	4	5	Average	St Dev
NAP	0.13	0.21	0.17	0.24	0.11	0.17	0.05
ACY	0.22	0.29	0.25	0.21	0.21	0.24	0.03
ACE	1.80	2.17	1.89	2.12	1.65	1.92	0.22
FLU	4.88	5.36	4.52	4.90	4.15	4.76	0.45
PHE	5.13	7.18	6.30	10.64	5.00	6.85	2.30
ANT	3.92	4.53	3.74	4.56	3.50	4.05	0.48
FLA	16.99	18.06	14.59	17.71	14.42	16.35	1.73
PYR	12.94	14.17	11.36	14.22	11.04	12.75	1.51
BaA	14.05	15.16	12.01	14.74	12.41	13.68	1.40
CHR	16.68	18.24	14.53	18.51	15.30	16.65	1.76
BaP	6.36	7.11	5.73	7.09	5.35	6.33	0.79
TOTAL	83.10	92.48	75.11	94.94	73.13	83.75	9.87

Nitrate-treated:

PAH	1	2	3	4	5	Average	St Dev
NAP	0.10	0.15	0.25	0.09	0.13	0.14	0.06
ACY	0.52	0.15	0.15	0.17	0.16	0.23	0.16
ACE	0.53	0.36	0.52	0.28	0.27	0.39	0.13
FLU	1.23	0.61	0.82	0.47	0.49	0.72	0.31
PHE	2.02	1.96	3.36	1.14	1.13	1.92	0.91
ANT	2.06	1.24	1.53	1.08	0.94	1.37	0.44
FLA	13.04	5.80	8.14	4.32	5.51	7.36	3.46
PYR	7.81	5.05	6.32	3.69	4.10	5.40	1.69
BaA	11.97	7.52	9.03	5.69	6.21	8.09	2.53
CHR	13.98	10.25	11.80	7.34	8.56	10.39	2.62
BaP	6.07	6.27	6.28	5.20	5.42	5.85	0.50
TOTAL	59.34	39.34	48.22	29.48	32.93	41.86	12.10

Data are expressed as μg/g dry weight, and were obtained using the DB-5 column. Blanks were below the detection limit. All data are for the end of the incubation period (6 weeks). 2.0 g samples were made up to 1 mL with solvent and internal standard was added to 20 ppm.

Appendix D: Kinetics Data

Solids:

% dry weight = 48.28% St Dev = 0.32 n=5

% Loss on ignition = 8.99% St Dev = 0.14 n=5

Acid Volatile Sulfide and toxicity data

	AVS (μmol/g)						
Sample	T=0	T=1	T=2	T=4	T=8	T=12	T=16
NO3 - 1	159.5	0.49	0.06	0.04	0.04	0.23	0.02
NO3 - 2	134.4	0.49	0	0.18	0.04	0.19	0.03
Control - 1	150.6	215	271	281	201	508	225
Control - 2	161.9	248	261	216	201	637	168
	MICROTOX EC_{50} (ppm)						
Sample	T=0	T=1	T=2	T=4	T=8	T=12	T=16
NO3 - 1	414	8728	10131	13922	13723	16763	30235
Range	(379 -452)	(8505 -8957)	(8633 -11888)	(13055 -14846)	(11786 -15978)	(12502 -22476)	(17911 -51039)
NO3 - 2	501	8736	11363	18663	25824	18077	20755
Range	(482-520)	(7020-10869)	(8095-15948)	(9616-36223)	(14849-44910)	(13812-23660)	(12339-34911)
Control - 1	534	211	296	260	304	326	291
Range	(378 -757)	(46 -962)	(253 -345)	(234 -289)	(247 -373)	(209 -508)	(254 -333)
Control - 2	442	188	206	220	237	270	264
Range	(394 -497)	(45 -784)	(159 -267)	(202 -241)	(199 -282)	(228 -320)	(205 -339)

"Range" is the 95% confidence range of the MICROTOX determinations. Time (T=#) is in weeks. The AVS values are given on a dry weight basis.

Supernatant chemistry data:

		Spiked Samples						
Test	Sample	T=0	T=1	T=2	T=4	T=8	T=12	T=16
pH	NO3 - 1	7.88	7.18	7.37	7.36	7.56	7.59	7.24
	NO3 - 2	7.9	7.19	7.39	7.36	7.56	7.6	7.22
	Control - 1	8.24	7.36	7.43	7.09	7.26	7.32	7.02
	Control - 2	8.3	7.48	7.39	7.1	7.24	7.28	6.99
	Blank	9.57	9.37	9.28	9.03	9.04	9.09	8.97
	Killed	8.15	7.99	7.68	7.59	7.84	8.09	8.03
Ammonia	NO3 - 1	2.319	0.212	2.944	9.095	14.683	14.402	0.091
(mg N/L)	NO3 - 2	2.372	0.145	2.528	9.145	12.218	13.5	0.056
	Control - 1	2.028	4.022	6.853	9.309	12.772	13.217	12.691
	Control - 2	1.986	4.005	6.671	9.604	12.654	13.117	13.696
	Blank	0.268	0.203	0.293	0.247	0.244	0.252	0.218
	Killed	3.109	3.111	8.934	9.976	7.077	9.648	9.16
Nitrate	NO3 - 1	459.8	262.4	85.1	120	191	154	261.5
(mg N/L)	NO3 - 2	446.7	268.1	101.7	110	191	148	252.6
	Control - 1	1.475	0.373	0.116	0.232	0.361	0.512	0.834
	Control - 2	0.269	0.268	0.076	0.265	0.409	0.555	0.862
	Blank	0.266	0.092	0.084	0.244	0.293	0.364	0.733
	Killed	231.6	267.3	261.4	215	224	214	229.5
Phosphate	NO3 - 1	5.722	0.009	0.09	0.08	0.064	0.083	0.155
(mg P/L)	NO3 - 2	6.208	0.08	0.148	0.102	0.083	0.103	0.099
	Control - 1	17.676	26.16	24.855	23.85	22.525	16.13	19.92
	Control - 2	18.612	25.075	25.52	24.65	22.985	20.26	19.17
	Blank	31.14	32.5	32.96	32.58	34.41	33.485	31.865
	Killed	9.653	1.04	1.311	1.062	0.898	0.829	0.715
		No Spike						
pH	NO3 - 1	8.05				7.5		7.27
	NO3 - 2	8.06				7.46		7.28
	Control - 1	8.52				7.37		7.05
	Control - 2	8.5				7.38		7.06
Ammonia	NO3 - 1	2.348				13.947		0.063
(mg N/L)	NO3 - 2	2.08				12.093		0.053
	Control - 1	1.827				13.279		14.184
	Control - 2	1.713				13.158		13.82
Nitrate	NO3 - 1	450.8				253		331.2
(mg N/L)	NO3 - 2	445.7				262		308.6
	Control - 1	0.331				0.363		0.828
	Control - 2	0.287				0.381		0.804
Phosphate	NO3 - 1	6.533				0.06		0.10
(mg P/L)	NO3 - 2	6.739				0.05		0.06
	Control - 1	19.986				25.02		23.01
	Control - 2	20.394				26.28		22.49

PAH data:

Blank Samples (spiked clay only):

PAH	T=0	T=1	T=2	T=4	T=8	T=12	T=16
NAP	0.68	0.45	0.54	0.66	0.48		
ACY	1.60	1.09	1.39	3.11	3.76	3.72	2.55
ACE	5.17	3.64	3.84	4.28	3.77	4.01	3.39
FLU	28.66	16.10	15.43	16.53	14.79	15.86	4.07
PHE	65.28	51.88	54.11	54.41	48.68	52.54	3.93
ANT	40.98	25.07	40.12	27.36	32.66	34.79	19.75
FLA	64.84	62.82	67.05	67.45	60.82	63.85	11.81
PYR	68.81	68.83	76.32	77.00	69.10	69.18	29.22
BaA	100.64	92.78	100.99	101.20	94.64	95.82	92.89
CHR	122.54	122.83	118.90	128.75	109.00	115.03	106.14
BaP	90.70	14.36	21.65	18.39	16.92	14.10	16.71
Total	589.89	459.85	500.33	499.15	454.62	468.90	290.46

Killed Samples (n=2):

	T=0		T=1		T=2		T=4	
PAH	killed	St Dev	killed	st dev	killed	st dev	killed	st dev
NAP	0.20	0.02	0.38	0.06	0.36	0.02	0.39	0.04
ACY	0.46	0.16	1.09	0.17	1.14	0.05	1.52	0.21
ACE	1.76	0.32	2.22	0.36	2.28	0.10	2.74	0.29
FLU	3.35	0.56	3.77	0.60	3.69	0.15	4.13	0.52
PHE	6.71	0.54	6.89	1.10	6.76	0.03	7.34	0.60
ANT	2.12	0.30	2.60	0.42	2.69	0.08	3.36	0.35
FLA	6.94	0.35	7.08	1.13	6.67	0.14	6.94	0.53
PYR	6.99	0.27	7.19	1.15	7.09	0.27	7.47	0.38
BaA	7.12	0.17	7.78	1.24	7.14	0.16	7.43	0.68
CHR	9.39	0.13	10.59	1.69	9.23	0.21	8.75	0.60
BaP	10.30	0.07	2.96	0.47	2.54	0.23	2.77	0.17
TOTAL	55.32	2.76	52.53	8.40	49.59	1.40	52.84	4.37

	T=8		T=12		T=16	
PAH	killed	st dev	killed	st dev	killed	st dev
NAP	0.61	0.00	0.65	0.11	0.45	0.08
ACY	0.81	0.01	1.67	0.20	0.85	0.29
ACE	2.46	0.02	3.09	0.36	2.34	0.01
FLU	3.88	0.03	4.43	0.50	3.81	0.28
PHE	7.40	0.06	8.41	0.98	9.96	3.59
ANT	2.63	0.02	4.08	0.24	3.41	0.36
FLA	7.13	0.06	8.57	0.53	9.70	2.62
PYR	7.83	0.06	8.93	0.92	10.25	2.84
BaA	7.75	0.06	9.34	0.93	10.30	2.71
CHR	10.40	0.08	12.30	1.42	12.67	4.12
BaP	2.83	0.02	4.13	0.29	4.66	1.59
TOTAL	53.72	0.42	65.60	6.51	68.40	17.93

Control and nitrate treatment samples (n=6):

	Time = 0 weeks				Time = 1 week			
PAH	NO3	St Dev	Control	St Dev	NO3	St Dev	Control	St Dev
NAP	0.48	0.26	0.55	0.40	0.49	0.09	0.62	0.50
ACY	0.66	0.24	0.63	0.23	0.82	0.23	0.70	0.32
ACE	1.98	0.39	2.03	0.50	2.20	0.23	2.19	0.92
FLU	3.35	0.44	3.35	0.73	3.60	0.33	3.40	1.01
PHE	9.46	3.47	8.44	3.63	8.26	2.35	9.33	6.78
ANT	2.37	0.51	2.47	0.70	2.65	0.41	2.51	1.36
FLA	9.20	2.58	7.71	1.97	7.90	1.55	8.35	4.04
PYR	9.20	2.05	8.32	2.10	8.12	1.51	8.73	4.27
BaA	8.31	1.10	8.21	1.80	8.02	1.44	8.62	3.95
CHR	12.23	1.94	11.96	2.77	10.96	2.21	11.96	5.92
BaP	13.76	3.14	12.47	4.00	3.37	0.72	3.53	1.96
TOTAL	70.98	14.58	66.14	18.46	56.39	10.40	59.93	30.79
	Time = 2 weeks				Time = 4 weeks			
PAH	NO3	St Dev	Control	St Dev	NO3	St Dev	Control	St Dev
NAP	0.55	0.14	0.51	0.12	1.14	1.50	0.53	0.07
ACY	0.79	0.37	0.67	0.35	1.19	0.27	0.81	0.32
ACE	2.34	0.37	2.27	0.43	2.90	0.73	2.25	0.36
FLU	3.51	0.47	3.52	0.56	4.57	1.25	3.72	0.48
PHE	7.30	1.69	8.37	2.61	12.32	8.91	7.37	0.50
ANT	2.57	0.46	2.46	0.78	3.80	1.78	2.52	0.79
FLA	7.30	0.91	8.04	1.78	10.81	5.93	7.51	0.39
PYR	7.71	1.05	8.38	1.86	11.12	5.84	7.95	0.36
BaA	7.50	0.73	8.27	1.71	10.54	4.79	7.42	0.33
CHR	10.20	1.18	11.45	2.53	13.93	7.16	10.11	0.69
BaP	2.79	0.42	3.22	1.26	4.69	2.76	2.76	0.28
TOTAL	52.55	6.82	57.17	13.49	77.00	40.71	52.92	3.16
	Time = 8 weeks				Time = 12 weeks			
PAH	NO3	St Dev	Control	St Dev	NO3	St Dev	Control	St Dev
NAP	0.36	0.05	0.90	0.44	0.42	0.11	0.88	0.33
ACY	0.73	0.09	0.75	0.20	0.60	0.11	0.88	0.25
ACE	1.82	0.13	2.56	0.69	1.49	0.27	2.66	0.27
FLU	2.80	0.29	3.92	0.97	2.36	0.35	3.86	0.22
PHE	5.46	0.44	9.28	4.69	6.06	1.76	7.90	0.61
ANT	2.27	0.36	2.75	1.20	2.61	0.40	2.81	0.37
FLA	6.58	0.40	8.65	2.93	7.71	0.89	8.45	0.90
PYR	6.99	0.41	9.12	3.17	8.38	1.03	8.49	0.26
BaA	7.00	0.38	8.81	2.64	8.14	0.92	8.46	0.84
CHR	9.01	0.49	12.72	3.92	10.80	1.28	11.13	0.64
BaP	2.73	0.17	3.57	1.35	3.74	0.59	3.66	0.37
TOTAL	45.75	2.89	63.01	21.90	52.30	6.93	59.17	2.23

Spiked samples (continued)

	Time = 16 weeks			
PAH	NO3	St Dev	Control	St Dev
NAP	0.45	0.23	0.74	0.25
ACY	0.36	0.07	0.63	0.40
ACE	1.09	0.33	2.48	0.53
FLU	1.61	0.43	3.94	0.64
PHE	5.71	2.17	9.18	2.53
ANT	2.18	0.62	2.76	0.69
FLA	6.98	1.56	8.88	1.31
PYR	7.93	1.67	9.16	1.37
BaA	7.48	1.38	8.31	1.24
CHR	8.86	1.68	9.92	1.71
BaP	3.69	0.88	3.83	0.66
TOTAL	46.15	10.74	59.83	10.02

Unspiked samples:

	Time = 0 weeks				Time = 8 weeks			
PAH	NO3	St Dev	Control	St Dev	NO3	St Dev	Control	St Dev
NAP	0.19	0.06	0.15	0.03	0.32	0.30	0.22	0.16
ACE	0.24	0.07	0.17	0.05	0.32	0.22	0.30	0.18
FLU	0.32	0.12	0.34	0.21	0.38	0.25	0.35	0.20
PHE	1.90	0.89	1.61	0.28	2.41	1.65	2.33	1.52
ANT	0.56	0.16	1.16	1.50	0.68	0.27	0.70	0.27
FLA	3.00	0.39	2.94	0.39	3.51	1.20	3.66	1.05
PYR	3.09	0.39	3.01	0.62	3.48	1.19	3.56	1.14
BaA	2.19	0.29	2.21	0.39	2.54	0.93	2.54	0.89
CHR	3.95	0.49	4.19	0.91	4.34	1.58	4.43	1.41
BaP	5.10	0.64	5.16	1.51	1.57	0.52	1.47	0.42
TOTAL	20.45	3.42	20.91	5.06	19.31	8.08	19.49	7.20

	Time = 16 weeks			
PAH	NO3	St Dev	Control	St Dev
NAP	0.21		0.41	0.24
ACE	0.25		0.55	0.14
FLU	0.29		0.62	0.22
PHE	1.55	0.49	3.43	1.96
ANT	0.43	0.10	0.75	0.34
FLA	2.85	0.73	4.23	1.28
PYR	3.08	0.62	4.21	1.26
BaA	1.99	0.31	3.15	0.89
CHR	3.40	0.71	5.42	1.58
BaP	1.65	0.23	2.33	0.62
TOTAL	15.08	3.04	24.64	8.62

All PAH data are expressed as μg/g dry weight and were generated using the DB-5 column. Blanks indicate the concentration was below the detection limit. A column of blanks indicates that a sample was lost. 2.0 g samples were made up to 2 mL in solvent with 20 ppm internal standard.

Appendix E: High/Low Spike Data

Solids, supernatants and toxicity data

Solids:

% dry weight = 36.38 st dev = 0.38 n = 5
% loss on ignition = 14.26 st dev = 0.22 n = 5

AVS and Toxicity (single determinations):

	AVS (μmol/g dry wt.)					MTX EC50 (ppm)				
weeks	0	4	8	12	16	0	4	8	12	16
Low/NO3	363	0.27	0.14	0.27	0.03	526	20197	23017	27702	47888
High/NO3	279	0.16	0	0.27	0.03	588	19655	29161	37850	35619
Low/Con	431	832	675	746	523	557	151	118	160	139
High/Con	284	679	682	1035	678	560	142	117	133	146

Supernatant nitrate, phosphate, ammonia and pH (duplicate values given):

	pH				
weeks	0	4	8	12	16
Low/NO3	8.35/8.34	7.73/7.75	7.65/7.67	7.75/7.75	7.92/7.96
High/NO3	8.34/8.41	7.72/7.74	7.64/7.74	7.77/7.77	7.83/7.93
Low/Con	8.24/8.27	7.22/7.28	7.24/7.25	7.27/7.26	7.36/7.42
High/Con	8.22/8.28	7.28/7.24	7.22/7.21	7.26/7.27	7.38/7.39
	Phosphate (mg P/L)				
weeks	0	4	8	12	16
Low/NO3	12.8/14.5	0.16/0.15	0.26/0.22	6.3/3.5	3.14/3.07
High/NO3	12.5/14.2	0.01/0.12	0.20/0.21	3.3/3.1	2.68/2.86
Low/Con	14.9/15.1	16.6/16.4	16.1/16.3	28.2/29.4	28.1/28.9
High/Con	13.9/14.8	16.4/16.3	16.1/16.1	29.4/28.1	28.9/29.0
	Ammonia (mg N/L)				
weeks	0	4	8	12	16
Low/NO3	2.45/2.51	11.2/10.9	8.69/8.83	0/0	0.06/0.04
High/NO3	2.62/2.80	10.6/11.1	11.0/11.0	0/0	0.35/0.04
Low/Con	2.13/2.04	8.14/8.16	9.19/12.7	11.5/10.8	13.9/13.7
High/Con	2.18/2.12	8.11/8.06	11.1/11.3	11.5/11.6	13.8/13.9
	Nitrate (mg N/L)				
weeks	0	4	8	12	16
Low/NO3	461/456	79.1/74.3	236/242	390/404	412/421
High/NO3	436/426	84.1/76.4	253/250	422/423	423/401
Low/Con	0.53/0.56	1.13/1.18	1.43/1.16	0.30/0.32	0.40/0.40
High/Con	0.54/0.59	1.17/1.08	1.44/1.43	0.28/0.34	0.40/0.45

PAH data

High Spike

	Time = 0				Time = 4			
PAH	NO3	st dev	control	st dev	NO3	st dev	control	st dev
ACY	0.99	0.11	1.14	0.10	1.26	0.08	1.32	0.08
ACE	7.34	1.18	8.14	0.55	3.99	0.40	5.90	0.87
FLU	21.59	2.35	22.62	1.18	12.94	0.97	18.54	1.98
PHE	40.63	4.53	43.92	4.97	26.26	1.49	37.99	2.70
ANT	17.92	4.15	18.53	3.40	16.77	2.42	16.35	4.29
FLA	46.62	5.63	50.34	4.56	42.10	2.17	44.71	3.48
PYR	50.58	6.89	54.60	4.65	43.81	2.22	48.86	3.96
BaA	65.30	8.25	71.23	5.43	63.06	4.00	62.69	5.30
CHR	82.91	7.55	100.39	8.01	75.69	6.83	85.67	6.81
BaP	22.93	4.37	25.02	3.16	25.85	2.24	25.09	3.01
TOTAL	356.87	43.89	396.05	26.38	311.71	22.12	347.12	25.11
	Time = 8				Time = 12			
PAH	NO3	st dev	control	st dev	NO3	st dev	control	st dev
ACY	1.15	0.14	1.51	0.31	1.22	0.05	1.38	0.10
ACE	1.68	0.17	6.85	2.31	0.87	0.08	5.46	0.76
FLU	5.57	0.58	20.45	5.68	2.95	0.18	16.29	1.41
PHE	14.09	1.44	39.85	10.14	8.94	0.45	34.15	2.03
ANT	11.26	1.61	17.43	7.80	8.49	0.91	15.32	3.52
FLA	41.08	4.17	48.17	10.90	30.53	1.41	42.10	2.01
PYR	40.07	4.09	57.17	14.31	30.07	1.31	45.96	2.76
BaA	82.58	9.56	99.81	24.77	60.29	3.46	72.89	6.11
CHR	101.33	14.86	133.53	26.42	78.60	4.47	93.51	9.71
BaP	40.03	5.41	42.27	11.83	28.30	2.04	28.37	3.59
TOTAL	338.99	37.20	467.22	104.57	250.34	12.50	355.62	31.41
	Time = 16							
PAH	NO3	st dev	control	st dev				
ACY	1.19	0.15	1.43	0.10				
ACE	0.59	0.08	6.00	0.92				
FLU	1.73	0.19	16.99	1.99				
PHE	6.08	1.08	37.45	3.67				
ANT	6.08	0.83	15.45	3.68				
FLA	25.15	2.06	44.43	3.39				
PYR	25.68	1.89	49.15	3.93				
BaA	47.84	3.55	69.53	7.19				
CHR	72.73	11.19	98.49	12.67				
BaP	25.72	2.49	30.58	3.01				
TOTAL	212.93	22.11	369.83	39.11				

Low Spike:

	Time = 0				Time = 4			
PAH	NO3	st dev	control	st dev	NO3	st dev	control	st dev
ACY	0.30	0.01	0.35	0.02	0.35	0.11	0.37	0.06
ACE	0.73	0.18	0.79	0.09	0.70	0.10	1.33	0.69
FLU	4.17	0.45	4.11	0.22	2.39	0.26	4.01	0.57
PHE	9.94	0.88	10.07	0.92	6.95	1.06	10.21	1.35
ANT	2.30	0.35	2.17	0.31	2.67	0.27	2.94	0.64
FLA	13.68	1.15	13.83	0.89	13.76	1.71	14.03	1.49
PYR	13.27	3.07	12.50	1.00	12.80	1.67	13.01	1.26
BaA	15.25	1.15	17.99	2.24	15.29	1.49	15.23	1.85
CHR	21.00	1.87	22.08	2.50	28.68	14.29	22.83	6.90
BaP	2.69	1.05	2.41	0.39	2.82	0.62	3.03	0.30
TOTAL	83.41	7.56	86.39	7.43	86.24	16.22	87.01	10.91
	Time = 8				Time = 12			
PAH	NO3	st dev	control	st dev	NO3	st dev	control	st dev
ACY	0.36	0.07	0.34	0.04	0.29	0.04	0.31	0.03
ACE	0.45	0.06	1.06	0.30	0.29	0.06	1.00	0.17
FLU	1.19	0.12	3.77	0.81	0.56	0.05	3.33	0.37
PHE	4.20	0.61	9.16	1.87	2.51	0.56	9.11	1.53
ANT	1.85	0.23	2.90	1.19	1.51	0.14	2.82	0.57
FLA	12.59	2.26	14.82	2.23	8.42	0.45	13.05	1.85
PYR	12.56	2.43	13.84	2.25	8.67	0.47	12.04	1.93
BaA	16.00	1.83	19.78	4.62	13.44	0.84	17.22	2.09
CHR	18.51	3.00	22.04	3.28	16.47	1.85	22.86	6.81
BaP	3.86	0.55	4.33	1.67	3.65	0.44	3.74	1.33
TOTAL	71.66	10.29	92.25	16.75	55.95	3.80	85.79	9.42
	Time = 16							
PAH	NO3	st dev	control	st dev				
ACY	0.32	0.07	0.31	0.04				
ACE	0.17	0.14	0.94	0.21				
FLU	0.42	0.06	3.23	0.53				
PHE	2.14	0.32	8.03	1.06				
ANT	1.11	0.16	2.42	0.56				
FLA	7.37	0.68	12.01	1.37				
PYR	8.40	0.71	11.22	1.16				
BaA	9.43	0.94	14.13	2.72				
CHR	13.40	1.73	19.71	5.52				
BaP	2.72	0.51	2.68	0.34				
TOTAL	45.58	4.81	74.87	12.70				

Data are given as μg/g dry weight. One gram samples were extracted, cleaned up by the DMSO method and made up to 1 mL and 20 ppm internal standard was added (n=6).

Appendix F: Availability Experimental Data

SPMD extracts of spiked clay with and without sediment added:

Extract concentrations (expressed as μg/g spiked clay)

	Spiked clay		Spiked clay plus sediment	
PAH	SPMD	sediment	SPMD	sediment
NAP				2.14
ACY	1.80			2.21
ACE	9.32		7.64	2.45
FLU	41.93		33.50	5.65
PHE	70.14		55.03	20.82
ANT	25.37	1.66	18.11	9.45
FLA	66.23		53.65	50.43
PYR	60.64	1.26	43.12	51.51
BaA	78.20	12.50	20.36	87.31
CHR	89.21	21.56	27.92	250.42
BaP				18.20
TOTAL	442.84	36.99	259.33	500.58

These data are from single determinations. The SPMD and sediment extracts were made up in 2 mL solvent with 20 ppm internal standard. Since 0.5 g spiked clay was used, the extract concentrations were multiplied by 2 mL/0.5 g to give results as μg/g spiked clay. All of the clay or sediment plus clay was extracted after exposure to the SPMD.

Effect of sediment organic content on availability

PAH	Average							Standard Deviation						
	1	2	3	4	5	6	sed	1	2	3	4	5	6	sed
	0 % OC													
NAP	1.56	1.08					3.13	0.12	0.56					0.23
ACY	0.34							0.17						
ACE	0.90	0.50					1.25	0.52	0.42					
FLU	8.67	0.80					11.35	4.59	0.49					2.45
PHE	21.70	2.60					1.90	12.33	0.43					0.42
ANT	15.18	2.22					3.04	5.98	0.35					0.49
FLA	22.72	2.02					1.43	11.79	0.52					0.17
PYR	28.37	2.31					4.67	12.80	0.61					1.16
BAA	24.10	2.02					4.32	2.30	0.26					0.67
CHR	18.81	3.21					16.44	0.64	0.08					2.15
BAP	13.24	1.88					4.89	1.55	0.05					0.92
DBA	4.01	3.97					25.61	0.28	0.22					4.70
BGHIP	3.03	2.09					10.53	0.50	0.21					1.84
	1.71 % OC													
NAP	1.06	1.44	0.50				4.09	0.49	0.13	0.14				0.47
ACY	0.17							0.05						
ACE	0.84	0.14	0.20				1.21	0.14	0.03					
FLU	8.08	0.90	0.89				9.90	0.96	0.10	0.78				1.26
PHE	23.87	2.10	1.17				4.37	2.62	0.24	0.45				1.17
ANT	15.04	1.78	1.22				4.85	1.55	0.21	0.55				1.29
FLA	26.86	3.54	4.19				19.89	1.07	0.31	3.10				2.48
PYR	31.78	4.34	1.75				7.83	1.43	0.51	0.55				1.75
BAA	13.45	5.23	2.76				14.40	0.11	0.22	0.45				1.95
CHR	10.92	4.88	3.12				34.61	0.05	0.19	0.68				6.71
BAP	4.06	2.97	2.28				20.33	0.09	0.16	0.54				1.90
DBA	1.30	1.00	1.24				45.38	0.04	0.04	0.53				5.21
BGHIP	0.58	0.47	0.55				20.73	0.03	0.03	0.24				2.01
	3.43 % OC													
NAP	1.00	1.25	0.55	0.77			2.68	0.51		0.10	0.01			0.37
ACY	0.14						0.80	0.00						0.16
ACE	0.90	0.18	0.27	0.26			1.85	0.04		0.03	0.04			0.71
FLU	8.82	1.18	1.47	1.25			10.84	0.57		0.03	0.13			1.43
PHE	25.41	3.07	0.90	0.54			8.23	2.36		0.05	0.04			2.75
ANT	15.71	2.31	1.02	0.66			5.37	1.38		0.05	0.14			0.70
FLA	29.28	6.04	5.53	5.35			17.59	3.39		4.16	2.68			9.40
PYR	32.78	7.37	2.22	1.18			11.87	3.60		0.22	0.20			1.92
BAA	10.63	6.17	3.33	1.97			20.18	1.03		0.23	0.27			3.89
CHR	8.90	5.88	3.02	2.29			42.14	0.95		0.19	0.20			6.62
BAP	2.91	3.01	1.34	0.91			27.63	0.18		0.05	0.15			3.37
DBA	0.84	0.78	0.64	0.54			58.92	0.07						9.19
BGHIP	0.40	0.44					25.86	0.04						3.79

	Average							Standard Deviation						
PAH	1	2	3	4	5	6	sed	1	2	3	4	5	6	sed
	5.14 % OC													
NAP	1.38	1.48	0.51	0.55	0.64		3.77	0.04	0.02	0.02	0.02	0.17		1.43
ACY	0.21						0.90	0.03						0.13
ACE	1.08	0.15	0.27	0.50	0.49		1.63	0.13	0.06	0.06	0.03	0.19		0.23
FLU	9.02	1.08	1.42	0.77	0.87		10.15	0.88	0.15	0.11	0.57	0.41		0.53
PHE	25.11	3.15	1.15	0.56	0.44		8.07	2.46	0.20	0.05	0.04	0.05		1.20
ANT	15.57	2.25	1.15	0.61	0.45		6.60	1.65	0.26	0.10	0.01	0.10		0.54
FLA	29.67	6.71	3.76	2.05	4.09		25.37	2.78	1.59	0.63	0.24	3.69		14.57
PYR	31.99	8.26	4.05	1.63	1.11		15.38	2.88	2.08	0.63	0.10	0.10		1.90
BAA	9.05	5.10	4.29	2.42	1.84		24.80	0.71	1.56	0.30	0.07	0.05		1.89
CHR	7.78	4.79	4.13	2.50	2.21		54.51	0.71	1.25	0.26	0.04	0.00		5.75
BAP	2.86	2.02	1.53	0.95	0.83		33.94	0.23	0.56	0.01	0.35	0.29		3.44
DBA	0.63	0.59					71.40	0.05	0.01					8.58
BGHIP	0.28	0.30					31.07	0.01	0.00					3.44
	6.85 % OC													
NAP	1.46	1.50	0.34	0.50	0.49	0.50	4.59	0.07	0.05	0.26	0.03	0.07	0.01	3.24
ACY							1.61							0.65
ACE	1.14	0.14	0.37	0.26	0.40	0.33	1.85	0.08	0.01	0.09	0.05	0.05	0.09	1.36
FLU	9.26	1.27	1.22	0.77	0.30	0.44	13.35	0.17	0.04	0.16	0.63	0.10	0.12	1.79
PHE	25.22	3.49	1.47	0.61	0.46	0.35	15.64	0.58	0.02	0.04	0.03	0.11	0.04	3.89
ANT	15.47	2.33	1.43	0.66	0.51	0.30	9.08	0.50	0.06	0.06	0.07	0.16	0.11	1.36
FLA	30.64	7.47	6.62	2.96	2.17	1.63	27.24	2.85	1.25	1.27	0.66	0.80	0.04	2.90
PYR	31.63	8.01	6.87	2.71	1.54	1.14	20.93	2.12	0.86	0.74	0.31	0.58	0.15	1.86
BAA	8.20	3.68	5.13	3.26	2.27	1.46	34.13	0.37	0.47	0.11	0.28	0.61	0.05	3.78
CHR	7.01	3.75	4.96	3.40	2.51	1.83	62.99	0.30	0.38	0.07	0.15	0.67	0.07	6.44
BAP	2.59	1.46	1.43	1.39	0.70	0.95	36.94	0.26	0.21	0.19	0.05	0.19		2.52
DBA	0.55						63.79	0.01						8.64
BGHIP	0.25	0.24				0.31	28.70	0.00						2.86

SPMD values were determined in duplicate, four sediment samples were used. All data are presented as μg/4.5 g dry sediment (all the material that was exposed). The organic carbon content was estimated as half the loss on ignition. Blanks indicate values were below detection or the sample was not extracted.

Availability of spiked and endogenous PAH

SPMD average data (n=2):

SPMD	1	2	3	4	5	6	7	8	9	10	11
PAH	Sediment										
ACY											
ACE	1.02										
FLU	1.37										
PHE	0.78								0.36		
ANT	1.45	0.49									
FLA	11.13	5.43	2.91	1.96	1.59	0.92	0.59	0.44	0.57		
PYR	9.67	5.21	2.50	3.84	4.47	4.06	2.65	2.27			
BAA	1.44	0.97	0.83	1.99	1.38	1.20	0.57				
CHR	1.77	1.35				0.64					
BAP											
total	28.64	13.44	6.24	7.79	7.44	6.82	3.81	2.71	0.93		
	Sediment plus spiked clay										
ACY	0.49										
ACE	3.15	0.52									
FLU	17.86	2.93	1.04								
PHE	27.80	4.84	1.17	0.47							
ANT	8.95	2.21	0.74	0.54	0.37						
FLA	31.49	10.66	5.47	2.98	2.13	1.00	0.69	0.58	0.56	0.59	0.46
PYR	24.87	9.64	5.65	4.83	4.53	3.03	2.48	2.46		0.80	0.79
BAA	9.19	2.17	3.77	4.75	4.51	2.14	1.54	1.21	0.47		
CHR	10.97	6.13	5.09	4.37	4.53	2.91	1.95	1.53	0.81	0.98	1.43
BAP											
total	134.8	39.11	22.93	17.94	16.07	9.07	6.66	5.78	1.83	2.37	2.68

SPMD standard deviations:

SPMD	1	2	3	4	5	6	7	8	9	10	11
PAH	Sediment										
ACY											
ACE											
FLU											
PHE									0.26		
ANT		0.03									
FLA		0.12	0.22	0.39	0.12	0.08	0.07	0.08	0.00		
PYR		0.10	0.33	1.09	0.50	0.29	0.14	0.39			
BAA		0.02	0.00	0.27	0.12	0.71	0.05				
CHR		0.05				0.45					
BAP											
Total		0.14	0.24	1.53	0.13	2.18	3.63	0.10	1.68		
PAH	Sediment plus spiked clay										
ACY											
ACE	0.11	0.04									
FLU	0.68	0.15	0.06								
PHE	0.45	0.05	0.00	0.04							
ANT	0.18	0.02	0.02	0.03	0.03						
FLA	0.10	0.65	0.15	0.34	0.03	0.19	0.03	0.00	0.01	0.04	0.16
PYR	0.39	0.69	0.18	0.33	0.52	0.45	0.08	0.42		0.57	0.25
BAA	0.42	2.33	0.14	0.58	0.42	0.15	0.34	0.25	0.33		
CHR	0.66	0.95	0.04	0.12	1.09	0.21	0.26	0.25	0.57	0.69	1.01
BAP											
Total	0.03	2.40	0.23	14.72	3.99	1.10	0.98	0.14	0.18	0.62	2.30

SPMD extracts were cleaned up by the DMSO method and made up to 1 mL in solvent with 20 ppm internal standard. Results are expressed here as μg/20 g wet weight sediment. The standard deviation of "Sediment" SPMD1 is missing because one sample was lost.

Sediment PAH concentrations after SPMD exposure and without exposure:

	Sediment				Sediment plus spiked clay			
PAH	End	st dev	No exp.	st dev	End	st dev	No exp.	st dev
NAP	4.14	0.87	3.84	0.15	5.89	0.17	3.93	0.67
ACY	4.75	0.19	2.31	0.55	3.88	1.58	4.70	1.08
ACE	2.24	1.57	0.40	0.22	2.05	0.83	3.22	0.36
FLU	0.23	0.10	0.22	0.00	0.23	0.04	1.68	0.04
PHE	29.53	13.08	14.71	0.84	29.06	5.83	49.92	4.50
ANT	8.90	3.39	7.19	0.33	7.80	0.70	18.69	4.00
FLA	23.58	11.52	33.95	0.26	25.62	5.57	67.99	4.92
PYR	26.88	11.52	33.63	0.34	28.25	5.48	63.43	4.94
BAA	33.67	8.28	29.13	2.40	29.64	1.95	74.41	10.02
CHR	27.19	8.27	22.05	1.74	25.10	1.91	54.73	6.62
BAP	28.64	4.00	16.17	2.07	39.25	23.37	23.13	2.09
TOTAL	189.75	44.28	163.59	2.32	196.77	30.96	365.82	23.52

Average and standard deviation (n=3) values are expressed as μg/20 g wet weight sediment (the total amount used in SPMD exposures). "End" samples are sediment concentrations after exposure to SPMDs, "No Exp." samples were not exposed to SPMDs. Samples (2 g dry wt.) were extracted and made up to 1 mL with solvent and 20 ppm internal standard was added.

Reverse SPMD extraction time:

Time	4		8		24		48	
PAH	Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev
nap	0.50	0.02	0.46	0.04	0.50	0.01	0.44	0.00
ace	0.27	0.03	0.25	0.04	0.24	0.09	0.24	0.01
flu	4.47	0.17	4.20	0.18	4.12	0.18	4.10	0.16
phe	6.51	0.16	6.70	0.41	6.98	0.33	7.32	0.35
ant	2.48	0.04	2.69	0.16	2.95	0.24	3.37	0.26
fla	5.77	0.19	6.50	0.54	7.18	0.24	7.58	0.72
pyr	6.67	0.17	7.55	0.71	8.48	0.90	8.57	0.60
baa	2.80	0.34	3.55	0.62	4.02	0.16	4.91	0.90
chr	1.84	0.17	2.45	0.36	3.21	0.26	3.96	0.74
bap	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
surr	21.14	0.60	21.21	0.20	19.83	0.47	20.13	0.39

Data are given as μg/mL extract using the DB-5 column. All rSPMDs contained 5 mL spiked sediment/water and the experiment was carried out in triplicate. Time is in hours.

Spiked and unspiked sediment extraction by reverse SPMD

	5 mL sample, no spike				10 mL sample. no spike			
PAH	SPMD	st dev	SED	st dev	SPMD	st dev	SED	st dev
NAP	2.30	0.36	1.61	0.33	1.08	0.08	0.97	0.34
ACY			0.03	0.06			0.16	0.03
ACE			0.09	0.18	0.13	0.09	0.23	0.01
FLU	1.10	0.25	0.51	0.13	0.63	0.06	0.10	0.21
PHE	0.94	0.67	0.75	0.65	0.89	0.29	0.87	0.12
ANT	0.59	0.10	0.02	0.04	0.55	0.07	0.34	0.07
FLA	6.62	2.78	4.35	0.35	2.47	1.11	1.91	0.38
PYR	2.87	0.43	1.93	0.14	2.59	0.43	2.10	0.52
BAA	1.17	0.13	0.76	0.61	1.05	0.35	1.11	0.25
CHR	1.51	0.24	1.21	0.26	1.43	0.19	1.38	0.25
BAP			0.76	0.63	0.65	0.79	0.93	0.44
surr			29.41	1.83			26.96	0.82
total	17.11	3.78	12.03	1.77	11.47	1.85	10.11	1.80
	5 mL sample, spiked				10 mL sample spiked			
PAH	SPMD	st dev	SED	st dev	SPMD	st dev	SED	st dev
NAP	2.90	0.89	3.10	1.02	1.52	0.20	1.79	0.16
ACY	2.46	0.42	1.94	0.05	1.81	0.37	2.53	0.20
ACE	5.12	0.28			5.21	0.32		
FLU	25.53	6.77	2.04	1.75	18.14	0.17	2.32	0.09
PHE	40.08	1.14	2.63	0.91	41.73	2.81	2.74	0.12
ANT	27.96	0.36	6.52	1.00	28.44	4.36	7.66	0.01
FLA	59.68	1.26	12.35	3.50	54.32	5.08	11.45	0.66
PYR	59.39	1.06	10.40	0.82	55.02	7.23	12.88	0.39
BAA	28.10	2.02	25.29	4.53	26.66	0.84	33.26	3.03
CHR	26.52	7.75	33.82	2.97	19.64	3.58	39.92	4.26
BAP	16.01	3.18	22.10	3.48	15.94	2.14	28.80	1.89
DBA	7.93	4.04	36.07	5.25	5.14	1.38	43.25	1.44
BGHIP	0.80	1.13	4.70	0.22	1.24	0.28	5.91	0.57
surr			17.16	0.42			17.62	0.11
total	302.47	16.01	160.96	21.98	274.81	23.52	192.51	9.89

Four replicates were analyzed both for rSPMD and sediment samples. These data were obtained using the DB-5 column and are given as μg/g dry weight. "Surr" values are the surrogate values. Surrogate was not added to rSPMD extracts.

Appendix G: Ammonia and Availability Data

Solids, AVS, toxicity and supernatant chemistry:

Solids:

Dry weight = 24.9 % St. dev. = 0.03 n=5

Loss on ignition = 17.79 % St. dev. = 0.13 n=5

AVS and MICROTOX toxicity (EC50) data:

sample\time	0	4	8	12	16	20
	AVS (µmol/g dry weight)					
NO3	586	n/d	n/d	n/d	1	1
control	701	526	859	1177	1435	904
NO3+NH3	739	n/d	n/d	n/d	2	2
control+NH3	624	557	941	1134	2159	leak
	MICROTOX (EC50 in ppm)					
NO3	308.87	71470	46575	56771	72655	88734
control	17	112	73	86	93	73
NO3+NH3	328	69301	62059	74414	67006	80676
control+NH3	18	74	62	42	139	165

Supernatant chemistry:

sample\time	0	4	8	12	16	20
	pH					
NO3 avg	8.48	7.59	7.64	7.69	7.73	7.77
NO3 sd	(0.04)	(0.08)	(0.02)	(0.02)	(0.02)	(0.02)
NO3 k	7.20	7.88	8.04	8.04	8.04	8.06
control avg	8.48	7.58	7.52	7.55	7.51	7.48
control sd	(0.07)	(0.09)	(0.02)	(0.05)	(0.02)	(0.04)
control k	7.23	7.9	7.99	7.93	8.05	8.04
NO3+NH3 avg	8.38	7.57	7.59	7.65	7.65	7.60
NO3+NH3 sd	(0.04)	(0.11)	(0.02)	(0.07)	(0.10)	(0.05)
NO3+NH3 k	7.23	7.77	8.24	7.38	8.23	7.84
control+NH3 avg	8.37	7.53	7.56	7.54	7.47	7.45
control+NH3 sd	(0.02)	(0.16)	(0.03)	(0.10)	(0.03)	(0.04)
control+NH3 k	7.02	7.85	6.86	6.83	6.88	6.78
	Ammonia (mg N/L)					
NO3 avg	7.71	8.60	11.13	2.73	0.03	0.03
NO3 sd	(0.34)	(0.05)	(0.41)	(2.25)	(0.01)	(0.01)
NO3 k	7.76	7.116	7.182	8.135	8.091	8.014
control avg	7.44	9.44	12.04	15.02	17.95	19.22
control sd	(0.21)	(0.10)	(0.29)	(0.22)	(0.15)	(0.14)
control k	7.76	6.829	7.367	7.917	8.019	7.398
NO3+NH3 avg	31.86	31.18	37.81	12.60	5.09	5.25
NO3+NH3 sd	(0.73)	(3.23)	(3.28)	(3.97)	(7.77)	(3.77)
NO3+NH3 k	28.65	29.02	33.1	28.38	29.57	53.13
control+NH3 avg	30.52	32.83	39.04	34.44	38.12	55.99
control+NH3 sd	(1.46)	(0.57)	(0.01)	(0.43)	(0.30)	(13.55)
control+NH3 k	33.57	27.01	34.12	30.1	41.56	63.82

sample\time	0	4	8	12	16	20
	Phosphate (mg P/L)					
NO3 avg	9.94	0.14	1.65	1.76	1.69	7.79
NO3 sd	(0.16)	(0.10)	(0.22)	(0.38)	(0.24)	(0.71)
NO3 k	15.49	0.81	1.92	1.653	2.13	5.869
control avg	10.35	19.39	34.46	32.56	33.58	49.32
control sd	(2.26)	(0.28)	(0.69)	(0.28)	(0.36)	(1.01)
control k	17.64	1.105	3.108	1.47	0.287	7.11
NO3+NH3 avg	9.75	0.08	1.56	1.38	1.59	6.39
NO3+NH3 sd	(2.06)	(0.01)	(0.32)	(0.19)	(0.27)	(0.69)
NO3+NH3 k	17.46	0.6	3.511	0.471	0.597	4.83
control+NH3 avg	9.96	19.11	34.36	32.54	34.74	50.76
control+NH3 sd	(1.09)	(0.28)	(0.85)	(0.58)	(0.68)	(2.38)
control+NH3 k	20.76	1.081	31.666	32.83	31.54	46.2
	Nitrate (mg N/L)					
NO3 avg	457.83	110.83	331.73	483.60	418.33	415.40
NO3 sd	(3.09)	(12.42)	(41.89)	(34.49)	(32.81)	(23.83)
NO3 k	420.40	322.4	594	879.3	860.4	886.4
control avg	0.46	0.82	0.89	0.36	0.53	0.40
control sd	(0.05)	(0.05)	(0.10)	(0.02)	(0.22)	(0.10)
control k	0.39	0.476	0.183	0.245	0.241	0.334
NO3+NH3 avg	421.20	108.40	365.40	426.40	371.30	360.13
NO3+NH3 sd	(11.68)	(22.34)	(30.48)	(9.94)	(20.45)	(6.43)
NO3+NH3 k	434.90	401.5	566.3	593	739	693.8
control+NH3 avg	0.35	0.74	0.75	0.42	0.56	0.44
control+NH3 sd	(0.08)	(0.03)	(0.09)	(0.09)	(0.03)	(0.03)
control+NH3 k	0.26	0.419	0.042	0.256	0.647	0.52

"NO3" means nitrate-treated, "+NH3" indicates ammonia was added to samples, "avg" means average of triplicate determinations and "sd" is the standard deviation, "k" denotes killed samples (autoclaved 3 times on successive days, unique samples). Time is in weeks.

PAH data:

No Ammonia added (μg/g dry weight):

	Nitrate-treated											
time	0		4		8		12		16		20	
PAH	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd
Nap	1.14	0.06	0.33	0.05	0.21	0.03	0.20	0.15	0.19	0.15	0.54	0.06
Acy	5.76	2.03	2.21	0.34	1.85	0.44	2.51	0.68	1.87	0.35	1.57	0.13
Ace	11.05	1.61	6.63	1.18	4.03	1.45	1.88	0.91	1.65	0.65	0.77	0.18
Flu	37.82	2.09	19.76	1.25	10.50	2.37	5.71	2.07	3.70	1.79	2.37	0.32
Phe	50.88	1.71	25.92	1.63	16.61	2.94	12.67	3.90	9.73	3.02	5.31	0.90
Ant	44.71	5.99	38.22	1.47	33.47	3.97	30.05	6.49	24.09	7.35	13.58	1.54
Fla	60.05	2.78	49.76	1.77	47.87	4.03	46.30	9.59	43.27	6.30	32.28	2.33
Pyr	62.61	2.55	50.08	1.88	45.05	4.01	49.07	7.23	42.18	4.63	31.96	3.04
BaA	70.78	2.49	58.47	1.72	72.72	5.51	68.94	10.07	65.76	5.07	57.35	2.97
Chr	68.74	3.15	54.57	1.17	63.53	4.67	70.48	11.34	67.39	14.34	59.19	2.45
BaP	63.83	4.70	55.56	1.75	72.62	4.75	70.06	11.08	75.88	3.52	72.32	3.33
DBA	150.68	15.55	83.05	1.94	141.05	10.71	85.91	14.52	113.29	17.77	92.24	6.10
BghiP	20.00	5.38	10.16	0.59	13.48	0.97	19.89	3.24	11.83	0.69	12.54	0.49
Surr			15.70	0.64	17.86	3.58	18.06	2.16	16.93	1.03	16.19	1.00

	Control samples											
time	0		4		8		12		16		20	
PAH	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd
Nap	1.18	0.09	0.29	0.05	0.26	0.02	0.50	0.29	0.28	0.10	0.64	0.04
Acy	5.76	1.73	5.96	0.56	7.90	0.65	7.48	1.36	5.28	1.01	6.56	0.92
Ace	11.85	1.96	13.25	1.56	16.82	0.87	14.89	2.26	12.15	1.23	15.04	1.36
Flu	39.97	1.34	35.53	1.29	40.78	2.53	40.98	4.94	35.21	1.55	40.43	1.63
Phe	53.69	0.84	46.42	1.49	51.33	1.99	55.74	6.16	48.98	1.99	52.35	1.33
Ant	46.10	4.90	43.56	1.71	53.01	2.38	53.45	8.48	46.61	3.74	50.15	1.63
Fla	63.29	2.09	57.88	1.22	64.60	2.26	67.73	5.63	62.80	1.86	61.02	1.04
Pyr	66.14	2.31	60.05	2.08	66.77	2.71	70.60	6.21	67.55	2.58	63.04	1.18
BaA	75.31	3.95	64.29	2.32	83.74	3.35	79.44	7.52	79.71	1.89	69.24	2.33
Chr	74.07	3.26	59.41	2.80	69.52	1.52	75.90	8.48	78.39	5.96	64.12	3.17
BaP	68.47	6.51	59.29	2.45	75.24	4.24	71.65	7.43	80.88	3.08	59.93	2.22
DBA	156.87	24.37	94.63	5.50	145.58	8.83	89.64	9.78	129.77	8.01	78.16	5.10
BghiP	19.77	8.46	12.15	0.59	14.05	1.12	21.09	1.49	13.82	0.52	11.80	0.84
Surr			18.11	0.27	21.78	0.74	20.22	1.36	17.76	0.33	18.18	0.43

Ammonia added (μg/g dry weight):

	Nitrate-treated samples											
time	0		4		8		12		16		20	
PAH	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd
Nap	1.13	0.08	0.33	0.03	0.19	0.01	0.36	0.28	0.00	0.09	0.58	0.07
Acy	5.26	1.95	1.65	0.08	1.41	0.08	2.43	0.17	1.73	0.19	1.62	0.22
Ace	10.68	0.95	4.98	0.16	2.84	0.17	1.84	0.49	0.62	0.28	1.15	0.60
Flu	35.98	2.47	17.92	1.52	9.80	1.90	5.45	1.40	3.87	0.73	3.00	1.83
Phe	49.28	4.64	24.97	3.19	16.52	2.35	12.40	2.44	7.52	1.18	7.25	4.10
Ant	45.16	8.26	37.41	3.58	30.59	1.29	27.99	3.20	22.90	2.07	14.97	6.74
Fla	57.64	6.16	49.13	2.80	43.99	2.44	47.16	8.29	38.94	2.45	31.32	10.08
Pyr	60.70	6.90	50.45	2.84	43.07	1.67	46.44	8.30	43.73	2.08	31.26	9.00
BaA	68.22	9.59	61.19	4.22	68.53	4.96	70.08	7.74	69.36	3.67	50.21	10.64
Chr	68.63	8.06	57.10	4.69	57.88	4.46	68.49	6.92	67.92	5.14	46.94	8.93
BaP	59.50	10.76	59.34	3.83	67.32	6.18	74.59	6.10	80.76	26.41	55.06	7.38
DBA	124.19	14.64	87.98	6.65	120.00	11.94	88.92	8.12	115.38	8.52	67.03	8.91
BghiP	11.76	1.91	10.51	0.61	10.89	0.91	21.13	1.94	11.63	0.62	8.64	1.01
Surr			15.67	0.76	17.63	1.32	19.07	1.7	16.08	0.70	16.20	1.57
	Control samples											
PAH	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd
Nap	1.11	0.08	0.38	0.04	0.23	0.02	0.48	0.33	0.30	0.09	0.66	0.05
Acy	5.74	2.33	6.89	1.12	7.00	0.53	8.70	2.08	5.44	0.42	6.06	0.44
Ace	12.20	0.65	15.31	1.38	14.99	1.52	17.74	3.64	12.65	0.40	13.09	1.14
Flu	37.75	3.25	36.94	1.92	39.20	1.61	45.81	5.84	37.11	1.47	38.17	2.74
Phe	49.94	3.66	47.67	1.90	49.91	1.79	61.01	6.53	48.27	1.16	50.70	2.70
Ant	42.58	8.65	41.23	9.09	52.96	2.26	59.83	8.13	42.68	2.72	48.16	3.08
Fla	58.54	4.60	60.27	2.35	64.75	2.33	75.01	9.74	62.74	2.07	61.31	3.13
Pyr	61.09	4.72	61.70	1.98	66.82	2.43	77.90	10.27	66.84	2.33	63.04	3.40
BaA	68.49	6.45	68.68	3.22	87.17	3.22	89.17	12.43	79.74	3.19	71.34	4.65
Chr	67.89	7.22	64.07	2.54	72.20	2.73	85.22	9.60	76.40	4.37	65.43	3.58
BaP	58.26	8.51	63.51	3.12	76.61	3.95	79.24	12.30	79.34	4.49	62.81	4.75
DBA	123.79	16.51	99.25	2.63	141.76	9.71	101.25	12.30	126.56	6.54	83.83	6.89
BghiP	12.31	1.94	12.12	0.20	13.10	1.02	24.25	3.67	13.34	0.55	12.31	1.47
Surr			17.83	0.11	21.56	0.93	21.54	1.55	18.39	0.27	17.85	0.52

No ammonia added, rSPMD samples (μg/g dry weight):

	Nitrate-treated samples											
time	0		4		8		12		16		20	
PAH	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd
Nap	3.71	3.31	0.90	0.05			3.04	0.10			4.01	0.24
Acy	17.69	2.15	2.15	0.63								
Ace	24.97	4.38	8.39	1.69	3.74	2.31	2.64					
Flu	44.87	5.47	18.08	2.77	14.40	3.19	10.86	2.48	9.33	1.18	6.53	1.02
Phe	52.87	11.89	27.51	1.44	15.63	2.94	9.81	4.39	5.62	2.36	4.90	0.58
Ant	46.13	8.51	33.99	0.40	27.61	3.84	23.26	3.08	20.22	7.23	11.61	0.74
Fla	53.62	7.16	53.01	0.35	53.85	5.88	48.01	6.32	45.32	4.11	34.58	2.52
Pyr	68.73	10.78	60.98	6.35	59.55	23.01	57.96	14.64	40.95	3.56	32.80	1.52
BaA	45.89	10.87	36.09	0.69	35.16	1.73	36.59	3.03	36.05	2.98	30.70	1.75
Chr	38.40	12.20	31.27	2.68	33.13	2.48	35.24	2.94	41.10	4.80	32.69	4.37
BaP	24.93	16.77	23.43	1.30	21.64	0.79	27.81	2.07	28.80	1.60	26.71	2.12
DBA	50.31	18.41	9.11	2.04	6.35		11.68	1.03	22.40	4.54	14.01	4.28
BghiP	10.06	3.68	2.48	0.19			6.64	0.37	4.24	2.86	0.95	0.74
Surr			17.92	0.77	20.33	0.79	18.85	0.73	17.94	0.48	18.20	0.51
	Control samples											
PAH	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd
Nap	4.14	2.38	1.00	0.05			3.14	0.09			2.71	0.90
Acy	18.33	0.44	10.73	0.66	11.60	1.34	11.16	1.65	8.32	0.77	9.42	2.28
Ace	23.64	0.48	18.89	1.21	20.77	1.06	24.31	2.57	17.18	1.25	21.45	5.74
Flu	43.82	0.83	33.64	4.13	46.05	3.10	58.68	3.12	44.29	1.26	47.48	4.63
Phe	49.36	1.70	46.13	3.00	48.11	2.44	62.50	3.52	51.86	0.71	52.96	2.16
Ant	47.33	4.80	40.72	1.86	45.34	1.96	55.76	4.17	49.50	1.88	52.02	2.58
Fla	56.31	2.64	52.01	2.08	57.03	1.68	70.68	3.12	57.39	1.97	61.44	6.02
Pyr	71.09	3.37	55.00	1.42	60.41	18.14	83.07	1.41	58.97	0.90	64.19	5.01
BaA	47.65	3.38	30.94	1.45	31.95	6.26	39.15	4.78	30.29	3.28	35.79	7.40
Chr	39.66	6.31	31.66	10.14	30.98	2.56	35.85	4.03	33.62	2.08	38.53	6.62
BaP	51.57	14.35	24.01	6.13	57.07	5.70	158.10	34.36	21.58	3.26	22.99	4.15
DBA	42.48	12.38	12.54	9.15			10.75	2.11	9.01	1.50	13.35	5.87
BghiP	6.70	3.28	1.61	0.05			4.34	0.18	1.01		1.37	0.11
Surr			17.65	0.49	20.26	1.12	21.37	0.32	17.59	0.22	18.19	0.47

Ammonia added, rSPMD samples (μg/g dry weight):

	Nitrate-treated samples											
time	0		4		8		12		16		20	
PAH	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd
Nap	5.83	3.62	1.48	0.69			2.99				4.16	0.64
Acy	18.99	0.56	1.53	0.15								
Ace	24.24	1.06	6.73	0.63	2.53		1.74	0.09			1.98	
Flu	44.28	3.66	16.17	3.14	14.07	4.57	10.38	0.99	7.56	1.03	6.16	1.99
Phe	51.71	2.95	27.00	3.41	16.59	4.42	8.34	0.16	3.77	0.97	6.62	4.19
Ant	47.10	3.05	37.45	3.33	28.09	4.81	19.60	2.13	16.08	1.99	15.12	3.38
Fla	56.64	5.88	50.30	3.37	48.35	4.28	51.25	5.76	39.89	4.70	36.32	4.76
Pyr	75.64	10.13	51.90	2.79	63.70	2.63	50.56	8.23	41.81	3.49	51.22	9.37
BaA	44.43	3.98	35.26	4.41	32.43	5.58	37.38	8.44	29.46	1.59	31.07	5.98
Chr	37.28	0.52	33.12	5.70	30.40	2.73	39.07	8.92	36.93	0.32	35.67	6.76
BaP	41.73	14.34	23.20	4.10	16.34	7.01	33.42	8.07	25.20	0.78	24.30	4.36
DBA	37.72	0.52	12.42	5.06			14.48	3.72	14.72	1.61	13.48	4.91
BghiP	6.92	0.67	2.72	0.38			4.66	1.04	1.96		0.90	0.94
Surr			17.68	0.59	20.00	0.75	20.55	1.28	17.55	0.52	19.51	1.66
	Control samples											
PAH	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd
Nap	3.25	3.40	1.05	0.02			3.08	0.56			4.17	0.94
Acy	19.42	1.62	11.15	1.25	9.67	1.25	13.45	1.46	10.54	0.82	10.75	1.05
Ace	25.13	1.01	20.63	1.55	20.33	1.26	26.95	1.42	19.79	1.29	24.26	1.97
Flu	46.33	1.14	34.63	5.36	48.51	2.27	60.02	2.28	49.76	3.82	52.36	3.34
Phe	51.12	2.73	46.40	4.79	50.08	2.31	66.49	2.61	55.17	4.55	54.96	1.88
Ant	46.51	1.98	39.93	5.31	48.08	0.60	62.45	0.28	54.22	6.50	51.68	2.99
Fla	54.74	1.17	48.11	4.93	59.31	1.64	78.89	0.61	60.23	3.40	64.31	4.02
Pyr	57.54	12.65	51.67	5.63	55.31	12.47	96.41	4.17	63.50	4.82	78.26	1.96
BaA	46.39	1.36	28.92	2.27	38.84	3.09	45.25	0.81	31.38	1.59	39.76	3.75
Chr	37.36	2.39	25.02	4.00	35.28	2.89	41.57	0.84	37.44	2.21	35.64	1.75
BaP	40.33	9.05	26.12	4.44	45.36	6.64	94.99	75.52	20.96	2.31	23.05	2.80
DBA	40.59	7.45	6.74	0.43			9.88	2.17	12.92	1.89	9.88	0.88
BghiP	7.43	2.01	1.50	0.46			3.34	0.07			1.39	
Surr			17.95	0.22	20.10	1.55	19.92	0.58	18.39	0.58	18.49	0.55

In the PAH data section, blanks indicate values below the detection level. Six replicates for the sediment extracts and three for the rSPMDs: "avg" means average and "sd" is the standard deviation of the determinations. Time is in weeks. 1.0 g sediment samples were extracted, cleaned up and made up to 1 mL. Five mL sediment/water suspensions (0.112 g dry wt) were added to the rSPMDs and dialyzed against pentane. These were cleaned up and made up to 1 mL with toluene for gc analysis. All of the data shown were from the DB-5 column. Peaks were confirmed using the DB-1 column.

Killed samples:

	No Ammonia Added											
	Nitrate-treated						Control					
time	0	4	8	12	16	20	0	4	8	12	16	20
Nap	1.08	0.53	0.23	0.22	0.16	0.65	1.20	0.53	0.25	0.31	0.21	0.49
Acy	8.81	9.03	6.65	8.19	6.75	7.40	10.30	9.64	7.62	7.96	6.44	7.33
Ace	15.26	20.45	13.36	14.98	12.30	15.12	18.63	20.78	14.77	15.47	13.60	14.91
Flu	41.11	46.39	36.92	48.20	46.60	41.06	44.59	44.90	40.81	45.93	41.15	39.85
Phe	53.13	56.90	47.80	63.25	68.64	55.56	55.14	53.86	51.30	59.83	52.19	54.59
Ant	46.17	48.58	44.14	56.87	56.41	51.10	50.04	44.22	47.38	51.55	45.03	47.73
Fla	60.94	65.59	57.18	73.29	87.27	60.97	63.32	61.92	60.92	68.55	60.55	60.27
Pyr	63.59	66.65	59.37	76.51	91.76	62.57	65.96	62.46	63.05	71.52	62.40	61.45
BaA	71.88	75.48	75.40	85.67	115.0	69.13	77.63	76.96	80.54	80.11	76.82	69.13
Chr	68.44	73.73	60.67	79.80	79.28	64.56	74.20	65.84	64.18	70.68	73.90	64.83
BaP	61.74	69.35	65.81	73.96	120.3	58.51	66.05	85.05	69.81	70.32	73.83	59.07
DBA	116.4	108.9	114.4	88.46	128.3	78.50	122.7	127.1	121.5	82.55	113.6	80.07
BghiP	12.05	13.86	11.60	22.09	17.60	11.58	12.22	11.95	12.18	21.58	12.12	11.28
Surr		18.53	19.41	21.20	16.54	17.21		18.10	20.64	19.71	16.55	16.71
	Ammonia Added											
	Nitrate-treated						Control					
time	0	4	8	12	16	20	0	4	8	12	16	20
Nap	1.04	0.48	0.13	0.67	0.26	0.63	1.05	0.54	0.27	0.59	0.21	0.65
Acy	5.75	7.72	5.17	7.80	6.11	4.84	4.77	7.88	6.80	7.00	4.17	6.34
Ace	16.28	18.24	9.97	16.81	13.20	9.70	13.69	18.50	14.00	12.96	9.35	13.47
Flu	41.43	39.87	29.97	45.52	38.19	29.47	37.09	39.74	37.84	41.92	35.16	36.51
Phe	51.69	49.87	38.20	57.44	51.50	39.99	47.40	49.51	49.26	56.12	48.95	51.01
Ant	36.90	42.05	38.21	58.99	48.30	35.95	34.36	40.08	41.05	43.56	34.43	39.81
Fla	59.36	55.62	44.41	64.68	57.55	44.65	54.85	54.90	58.71	61.98	59.51	56.98
Pyr	62.04	56.10	45.17	67.96	60.00	46.13	57.43	56.40	60.47	64.14	62.34	57.95
BaA	65.18	64.15	58.51	73.99	74.10	50.63	57.17	65.01	79.86	72.98	76.59	68.02
Chr	63.13	61.47	51.01	70.15	65.59	45.13	62.21	60.16	65.73	70.26	70.92	62.70
BaP	52.27	58.47	49.20	68.39	70.88	42.47	50.38	59.03	70.69	64.57	75.13	58.11
DBA	119.3	89.93	85.03	79.85	99.58	50.93	100.6	88.05	129.6	82.36	112.1	79.30
BghiP	16.38	11.31	7.49	18.40	10.46	7.37	9.97	11.07	13.12	20.59	12.81	12.19
Surr		17.48	15.01	18.31	15.54	13.24		17.62	19.95	20.32	17.29	17.61

Time is in weeks, and all data are for single determinations. 1.0 g sediment samples were extracted, cleaned up and made up to 1 mL. All of the data shown were from the DB-5 column. Peaks were confirmed using the DB-1 column.

Appendix H: FCE and ESSO spiked and unspiked

FCE data:

Solids:

Dry weight = 32.8 % St. dev. = 0.88 n=5

Loss on ignition = 15.08 % St. dev. = 0.77 n=5

No spike:

	Sediment samples							
	Time = 0				Time = 12			
	Nitrate		Control		Nitrate		Control	
PAH	avg	stdev	avg	stdev	avg	st dev	avg	st dev
NAP	0.49	0.29	0.44	0.10	0.32	0.03	0.39	0.06
ACY	0.26	0.07	0.22	0.12	0.23	0.04	0.40	0.11
ACE	0.45	0.05	0.31	0.04	0.22	0.07	0.34	0.15
FLU	0.46	0.05	0.67	0.11	0.31	0.08	0.59	0.11
PHE	4.51	0.87	3.37	0.16	2.38	0.37	2.93	0.31
ANT	1.02	0.21	1.00	0.27	0.72	0.11	0.85	0.05
FLA	5.93	0.68	4.12	0.22	3.05	0.46	3.50	0.14
PYR	6.76	0.92	4.79	0.15	3.64	0.57	4.18	0.22
BAA	3.13	0.54	2.15	0.23	1.61	0.41	1.63	0.11
CHR	3.78	0.65	2.68	0.36	1.99	0.38	2.38	0.36
BAP	3.31	0.72	2.01	0.65	1.57	0.31	1.43	0.17
DBA	6.49	3.38	6.91	2.98	0.24	0.06	0.24	0.18
BGHIP	2.83	1.17	3.70	2.63	1.35	0.13	1.22	0.31
surr	21.93	0.90	18.38	0.55	8.80	1.09	9.78	5.12
	rSPMD extracts							
PAH	avg	stdev	avg	stdev	avg	st dev	avg	st dev
NAP	1.51	0.12	1.78	0.14	9.92	3.86	10.34	6.62
ACY					0.68	0.77	0.24	0.10
ACE	0.38	0.06	0.38	0.04	0.76	0.15	1.03	0.27
FLU	0.71	0.09	0.68	0.09	0.72	0.14	0.91	0.20
PHE	3.67	0.38	3.61	0.50	4.56	0.41	5.24	0.60
ANT	1.06	0.18	1.31	0.61	0.89	0.56	0.74	0.12
FLA	5.20	0.94	4.72	1.12	5.82	0.86	5.97	0.67
PYR	5.47	0.94	3.69	0.76	4.63	0.57	4.07	0.47
BAA	1.67	0.44	1.74	0.51	1.63	0.21	1.35	0.31
CHR	1.76	0.19	2.02	0.29	1.86	0.29	1.70	0.37
BAP	0.86	0.26	0.73	0.21	1.18	0.21	1.43	0.90
DBA			0.09	0.18			0.63	0.15
BGHIP	0.48	0.20	0.36	0.11	0.73	0.15	0.55	0.27
surr	31.00	1.31	28.80	1.33	19.37	1.58	22.01	3.97

Samples (3 g) of sediment were made up to 1 mL with toluene for gc analysis at 0 weeks (n=4) and 0.5 mL at 12 weeks (n=6). The rSPMD samples (0.3 g dry weight) were concentrated to approximately 0.2 mL to increase the sensitivity of the analysis. "Avg" is the average of the replicates and "sd" is the standard deviation. Results in μg/g dry wt.

Spiked samples (n=2):

	Sediment samples							
	Time = 0				Time = 12			
	Nitrate		Control		Nitrate		Control	
PAH	avg	stdev	avg	stdev	avg	st dev	avg	st dev
NAP	0.65	0.24	0.51	0.00	0.31	0.01	0.49	0.01
ACY	4.47	0.04	3.47	0.11	12.33	0.76	13.95	0.42
ACE	5.07	0.16	4.58	0.14	1.82	0.77	4.73	0.40
FLU	14.22	0.20	13.26	0.13	6.08	1.64	13.65	0.34
PHE	40.24	0.18	37.19	0.08	22.83	3.38	38.85	0.77
ANT	28.27	0.82	25.61	0.74	29.08	1.27	37.99	2.40
FLA	55.05	0.98	52.10	0.03	46.88	0.84	57.89	1.21
PYR	55.97	0.02	55.51	0.19	49.38	0.42	60.72	1.72
BAA	52.04	0.35	49.59	0.11	49.30	1.52	53.89	2.49
CHR	41.73	0.41	39.40	0.55	47.59	0.56	52.24	4.95
BAP	46.34	0.17	43.96	0.04	34.34	2.26	36.33	1.12
DBA	120.81	1.43	134.17	17.89	30.16	1.03	36.12	2.11
BGHIP	16.86	0.61	16.15	1.87	10.23	0.37	11.71	0.37
surr	46.47	0.28	46.39	0.37	14.20	1.48	15.43	0.05
	rSPMD extracts							
PAH	avg	stdev	avg	stdev	avg	st dev	avg	st dev
NAP	1.23	0.01	0.94	0.07			1.87	
ACY	1.92	0.05	1.48	0.02	5.12	0.08	5.65	1.66
ACE	4.11	0.32	4.42	0.39	2.70		4.58	0.05
FLU	10.52	3.34	11.29	0.97	6.49	2.51	13.21	0.51
PHE	28.91	0.92	32.03	0.07	21.76	7.45	37.97	1.73
ANT	15.73	0.19	16.22	1.26	23.18	9.46	28.05	5.57
FLA	37.09	0.21	36.88	1.86	39.35	4.61	49.35	3.99
PYR	39.86	0.81	39.04	2.15	41.81	5.60	50.57	4.48
BAA	20.33	0.40	17.77	2.10	24.66	2.38	26.53	6.09
CHR	14.95	0.23	14.13	0.72	30.43	11.97	28.13	11.07
BAP	10.48	1.27	10.50	1.33	11.15	0.40	10.33	2.97
DBA	14.76	0.18	15.13	0.92	6.93	2.41	6.35	4.39
BGHIP	2.69	0.06	2.39	0.34				
surr	18.12	0.12	17.98	0.12	17.13	0.70	16.79	0.47

Three gram samples of sediment were used, and they were made up to 2 mL with toluene for gc analysis after cleanup. rSPMDs contained 0.3 g dry weight sediment and the extracts were made up to 1 mL. Duplicate samples were used for the analyses. . Results expressed as μg/g dry wt.

ESSO data:

Solids:

Dry weight = 28.79 % St. dev. = 0.6 n=5
Loss on ignition = 11.7 % St. dev. = 0.4 n=5

No spike:

	Sediment samples							
	Time = 0				Time = 12			
	Nitrate		Control		Nitrate		Control	
PAH	avg	st dev	avg	st dev	avg	st dev	avg	st dev
NAP	0.45	0.03	0.38	0.21	0.22	0.03	0.28	0.06
ACY	0.06	0.01	0.07	0.01	0.03	0.01	0.05	0.01
ACE					0.11	0.03		
FLU	0.25	0.05	0.27	0.03	0.22	0.06	0.33	0.14
PHE	0.55	0.08	0.57	0.14	0.20	0.02	0.43	0.34
ANT	0.28	0.02	0.26	0.03	0.12	0.04	0.40	0.69
FLA	0.69	0.07	0.79	0.18	0.44	0.07	0.57	0.13
PYR	1.31	0.10	1.43	0.16	0.79	0.13	1.09	0.22
BAA	0.45	0.08	0.61	0.08	0.26	0.06	0.35	0.07
CHR	0.44	0.08	0.48	0.06	0.33	0.06	0.46	0.19
BAP	0.41	0.03	0.45	0.05	0.27	0.07	0.32	0.06
DBA			0.50		0.30	0.19	0.31	0.12
BGHIP	0.80	0.13	1.04	0.06	0.47	0.12	0.50	0.12
surr	11.12	0.96	11.03	0.28	15.54	1.22	14.57	1.70
	rSPMD extracts							
PAH	avg	st dev	avg	st dev	avg	st dev	avg	st dev
NAP	0.81	0.17	0.68	0.06	2.39	0.90	2.60	1.02
ACE					0.63	0.30	0.83	0.43
FLU	1.13	0.50	0.79	0.37	3.37	0.74	2.82	0.82
PHE	0.38	0.07	0.40	0.03	0.94	0.13	0.94	0.27
ANT					1.63	0.37	2.02	0.55
FLA	0.95	0.60	0.67	0.15	1.84	0.61	1.81	0.68
PYR	1.60	0.47	1.20	0.07	1.93	0.27	3.90	1.18
BAA	0.56	0.41	0.35		0.84			
CHR	0.70	0.30	0.45		0.94	0.53	1.00	0.29
BAP							0.48	
surr	106.94	9.24	97.61	3.11	26.13	4.07	26.10	6.84

Four replicates were analyzed at the start of the experiment, and six replicates at 6 and 12 weeks. The sediment extracts (2.6 g) were made up to 0.5 mL and the rSPMD (0.26 g) were made up to 0.2 mL. "Avg" is the average of the replicates and "sd" the standard deviation. Results expressed as μg/g dry wt.

Spiked samples, sediment extracts (at time = 0, n=4; at time = 6, 12, n=6):

	Nitrate-treated samples					
Time	0		6		12	
PAH	avg	st dev	avg	st dev	avg	st dev
NAP	2.26	0.19	0.68	0.14	0.54	0.10
ACY	2.61	0.27	0.26	0.02	0.28	0.05
ACE	20.06	0.65	0.72	0.11	0.47	0.05
FLU	25.59	1.15	1.98	0.22	1.14	0.16
PHE	29.64	1.42	1.53	0.65	1.26	0.15
ANT	17.65	1.06	3.51	1.05	1.89	0.32
FLA	27.45	1.37	12.25	3.05	4.24	1.34
PYR	29.23	1.49	15.75	2.66	6.67	2.41
BAA	24.86	1.87	24.02	0.66	12.87	1.96
CHR	19.66	1.74	23.03	1.27	13.69	1.66
BAP	19.03	2.53	21.78	1.67	14.17	1.38
DBA	30.23	4.24	36.06	3.32	23.97	3.57
BGHIP	23.16	2.81	30.67	3.73	21.75	3.07
surr	16.50	0.37	20.55	0.95	15.11	0.88
	Control samples					
PAH	avg	st dev	avg	st dev	avg	st dev
NAP	2.07	0.18	0.82	0.08	0.58	0.06
ACY	2.50	0.11	0.38	0.05	0.34	0.05
ACE	14.88	8.67	4.10	0.65	2.75	0.67
FLU	24.99	0.30	9.90	1.05	6.65	0.77
PHE	29.14	0.66	19.84	1.11	13.78	1.27
ANT	17.44	0.12	13.79	0.65	9.66	0.99
FLA	26.99	0.29	28.48	1.10	20.32	1.90
PYR	28.52	0.23	32.39	1.05	23.06	2.03
BAA	24.61	0.52	29.97	1.07	20.20	1.70
CHR	20.10	0.28	26.08	1.22	17.26	1.56
BAP	18.03	0.59	23.24	0.73	14.27	1.43
DBA	32.26	0.52	37.69	2.63	22.72	3.06
BGHIP	24.55	1.47	33.18	1.66	20.78	1.98
surr	16.06	0.81	19.93	0.59	14.57	1.43

Four replicates were analyzed at the start of the experiment, and six replicates at 6 and 12 weeks. The sediment extracts (2.6 g) were made up to 2 mL and the rSPMD (0.26 g) were made up to 0.2 mL. "Avg" is the average of the replicates and "sd" the standard deviation. Results expressed as μg/g dry wt.

Spiked samples rSPMD extracts (at time = 0, n=4; at time = 6, 12, n=6):

	Nitrate-treated samples					
Time	0		6		12	
PAH	avg	st dev	avg	st dev	avg	st dev
NAP	7.99	0.32	1.05	0.35	1.58	0.10
ACY	5.71	0.35	0.03	0.07		
ACE	33.51	2.42	0.98	0.17	0.86	0.09
FLU	41.82	4.17	1.59	0.30	4.40	0.49
PHE	43.25	4.84	1.31	0.59	1.43	0.04
ANT	26.03	3.03	4.06	0.59	1.36	0.33
FLA	41.60	1.40	19.17	5.94	5.51	1.72
PYR	43.70	1.74	23.45	5.00	7.25	2.31
BAA	29.32	2.77	27.18	3.68	9.76	2.22
CHR	21.65	3.16	27.54	6.81	14.14	3.28
BAP	10.85	1.28	13.48	1.96	5.76	1.35
DBA	18.57	5.57	25.55	10.64	9.62	2.82
BGHIP	8.65	2.03	13.80	3.70	4.41	2.70
surr	121.80	3.49	27.88	3.40	19.74	1.76
	Control samples					
PAH	avg	st dev	avg	st dev	avg	st dev
NAP	6.60	1.38	1.45	0.34	1.62	0.14
ACY	4.32	0.49	0.26	0.13	0.23	0.03
ACE	27.19	3.27	5.84	1.46	4.25	1.19
FLU	35.40	3.73	12.31	2.67	10.88	0.80
PHE	36.55	4.22	23.99	4.51	17.81	1.14
ANT	22.22	2.63	16.73	2.92	11.74	0.81
FLA	35.08	3.19	38.48	7.64	24.64	2.60
PYR	36.44	3.70	43.63	8.03	30.60	4.69
BAA	23.83	2.23	31.82	4.56	18.13	1.81
CHR	17.79	2.23	28.34	5.02	19.49	2.01
BAP	8.74	1.52	14.66	2.28	8.09	1.06
DBA	14.77	3.47	23.07	5.80	10.91	1.70
BGHIP	8.35	3.16	13.18	4.46	4.42	1.90
surr	99.33	13.07	28.18	5.07	18.75	1.30

At time = 0 there were 4 replicates of both sediment and rSPMD data. At times 6 and 12 weeks, 6 replicates were analyzed. rSPMDs were made with 10 mL sediment/water. Results expressed in μg/g dry weight.