

## A NOVEL METHOD FOR EVALUATING BIOAVAILABILITY OF POLYCYCLIC AROMATIC HYDROCARBONS IN SEDIMENTS OF AN URBAN STREAM

Fumiyuki Nakajima\*, Anders Baun\*\*, Anna Ledin\*\*, Peter Steen Mikkelsen\*\*

- Department of Urban Engineering, the University of Tokyo, 7-3-1 Hongo, Bunkyo, Tokyo 113-8656, Japan
- (E-mail: nakajima@env.t.u-tokyo.ac.jp)

\*\* Environment & Resources DTU, Technical University of Denmark, Bygningstorvet, Building 115, DK-2800 Kgs. Lyngby, Denmark

### ABSTRACT

Hydrophobic organic pollutants in urban wet weather discharges have a potential to accumulate in the sediments of receiving water and may have adverse effects on the ecological system, especially on benthic organisms. In the present study, a novel method is developed for evaluating the bioavailability of such hydrophobic organic pollutants with a consideration for digestive guts in deposit-feeding polychaetes. We quantitatively compared the amount of polycyclic aromatic hydrocarbons (PAHs) extracted by an organic solvent and by sodium dodecyl sulfate (SDS) solution (as a hypothetical digestive gut fluid of polychaetes) and interpreted the ratio of the two values as bioavailability. The sediment extracts were applied to bacterial acute toxicity tests and algal growth inhibition tests. Sediment samples were collected from an urban stream system (Store Vejleå, Denmark) receiving wet weather discharges. The bioavailability of the total amount of 12 PAHs in the sediments was evaluated to be in the range 14-38 % based on the results from the GC/MS determination of the two different extracts. Lower molecular PAHs showed higher bioavailability compared to the higher molecular ones. The sediment extracts were shown to be toxic towards both algae and bacteria. The SDS extracts showed similar or higher toxicity in the two biotests compared to the organic solvent extracts in spite of their lower PAHs content.

**KEYWORDS:** bioavailability, digestive gut fluids, polycyclic aromatic hydrocarbons, sediment, toxicity, urban runoff

### INTRODUCTION

Hydrophobic organic pollutants in urban wet weather discharges have a potential to accumulate in sediment of receiving water and may have adverse effects on the ecological system, especially on benthic organisms (Maltby et al., 1995a,b; Boxall and Maltby, 1997). To assess the toxicity of such contaminated sediments, biotests became powerful tools (Marsalek et al., 1999). Chemical analysis, usually including a process of organic solvent extraction, provides basic and quantitative information of selected compounds, however the procedure does not reflect the biological process of ingestion, or bioavailability of the compounds.

Polychaetes may be an important link in the food chain from sediments to fish, birds and, possibly, to humans. According to Ahrens et al. (2001), absorption of hydrophobic contaminants by deposit-feeding polychaetes was well predicted by the degree of desorption of the contaminants by their digestive gut fluids, and the desorptive property of real gut fluids were similarly given by a solution of a synthetic surfactant, sodium dodecyl sulfate (SDS). Mayer et al. (2001) have conducted a great number of extraction experiments with real gut fluids of various species and showed a correlation between benzo[a]pyrene solubilization and the digestive biochemical properties. The mechanism of solubilization was still unclear but contact angle of the digestive fluid (i.e. surfactancy) was one of the strongly positively correlated factors.

In this study, we quantitatively compared the amount of polycyclic aromatic hydrocarbons (PAHs) extracted by an organic solvent and by an SDS solution (as a hypothetical digestive gut fluid of polychaetes) from sediments collected from an urban stream system (Store Vejleå, Denmark) receiving wet weather discharges and interpreted the ratio of the two values as bioavailability. The extracts from the sediments by an organic solvent (n-pentane) and by the SDS solution were applied to standardized bacterial acute toxicity tests (*Vibrio fischeri*) and algal growth inhibition tests using *Pseudokirchneriella subcapitata*.

### MATERIALS AND METHODS

#### *Sediment Samples*

Sediment samples were collected from an urban stream system (Store Vejleå, Denmark) receiving wet weather discharges (Figure 1, locations D, E, G and H) as well as from a small pond receiving highway runoff (Figure 1, locations A, A1, A2 and A3) (Baun et al., 2003). Sediments A, D, E, G and H were collected in July, 2001 and A1, A2 and A3 were collected in September, 2001. Coarse and fibrous particles were removed using a stainless steel sieve (approx. 1.5 mm) before further processing.

#### *Extraction with an organic solvent and hypothetical digestive gut fluids of polychaetes*

Two different solvents were used for evaluating bioavailability of PAHs in sediments: one is n-pentane and another is 1% (w/v) SDS solution in artificial seawater, which is considered as a hypothetical digestive gut fluid of polychaetes.

N-pentane extract (referred to as "P extract"):

A known amount (about 5 g) of wet sediment was well mixed with sodium sulfate (anhydrous). N-pentane (40 mL) was added to the dried sediment in an amber glass bottle. The bottle was shaken for 1 hour (100 rpm) in the dark at 20 °C. The pentane was recovered by centrifugation and the extraction procedure was repeated 3-6 times with new solvent until the recovered pentane was clear. The recovered pentane was concentrated by flushing with air.

1% SDS extract ("S extract"):

The wet sediment was centrifuged (4000 rpm, 3 minutes). The settled sediment was placed into a mortar and the supernatant was centrifuged again (4000 rpm, 3 minutes), followed by recovery of the settled sediment and adding it to the mortar. The recovered sediment was mixed gently with a stainless steel spoon to be homogenized but not to be ground. A known amount of the homogenized sediment was put into an amber glass bottle, adding 40 mL of 1.0 % SDS solution. The bottle was shaken in the same way as for the n-pentane extraction. The supernatant was collected and distributed into several centrifuge tubes. The division into several tubes for further extraction before dilution was thought to be very important because the dilution might cause destruction of micelles and release of the hydrophobic contaminants. After the release, it may be difficult to keep the contaminants homogenized in the solution. The divided solutions in tubes were diluted 20 times by artificial seawater into 0.05 % SDS, which is lower than the critical micelle dilution of SDS in seawater (reported as 0.1 % according to Ahrens et al. (2001)). N-pentane was added to the diluted SDS solution in the ratio of 1:10 (pentane : 0.05 % SDS) in volume. The tubes were vigorously mixed for 15 minutes and the n-pentane layers were recovered and were unified into a tube. The recovered pentane was concentrated by flushing with air. The aim of the liquid-liquid extraction between n-pentane and 0.05 % SDS solution was to focus on hydrophobic contaminants although SDS solution may solubilize both hydrophilic and hydrophobic fractions in sediment.

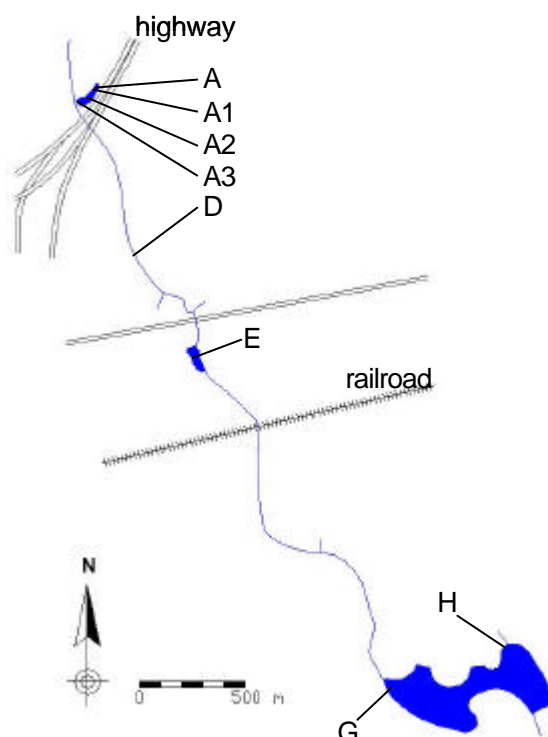


Figure 1 Sampling locations  
(Store Vejleå, Denmark)

***Determination of polycyclic aromatic hydrocarbons and definition of bioavailability***

Sixteen polycyclic aromatic hydrocarbons in the extracts were quantified with GC-MS (Hewlett Packard HP6890+MS5973) in the SIM mode. A commercially available deuteriated PAHs mixture (SUPELCO 4-8902) was used as internal standard. Bioavailability was defined as ratio of PAH in S extract to that in P extract.

**Table 1 Content of the PAHs (ng/g dry wt) in sediment collected from Store Vejle å Denmark**

sediment extract	<i>July, 2001</i>										<i>September, 2001</i>					
	A		D		E		G		H		A1		A2		A3	
	P	S	P	S	P	S	P	S	P	S	P	S	P	S	P	S
Naphthalene	150	240	234	280	2	48	14	145	5	35	40	761	15	121	51	168
acenaphthylene	7	3	4	2	0	0	1	2	0	0	11	8	36	3	24	3
acenaphthene	5	3	4	1	1	1	2	2	4	1	13	42	7	4	25	6
fluorene	14	12	12	6	0	2	4	6	5	1	19	36	22	9	27	17
anthracene	13	3	10	2	1	1	11	4	41	1	53	21	34	6	38	9
phenanthrene	85	28	72	30	4	9	65	33	58	8	248	185	158	41	219	83
fluoranthene	291	66	101	39	4	6	114	31	106	9	561	185	626	81	525	186
pyrene	157	60	276	51	16	13	244	95	195	33	368	152	281	236	436	167
benzo[a]anthracene	99	18	110	16	6	2	75	14	82	4	296	52	264	26	306	58
chrysene	232	67	177	58	9	7	169	68	101	18	596	230	544	181	685	252
benzo[b]fluoranthene +benzo[k]fluoranthene	211	50	207	58	12	13	176	87	111	22	550	162	666	118	716	219
benzo[a]pyrene	155	31	189	37	13	4	135	62	82	12	417	92	446	59	508	90
indeno[123-cd]pyrene	98	11	85	22	4	3	53	13	10	3	178	52	272	26	260	65
dibenz[a,h]anthracene	49	9	87	9	2	2	28	10	9	3	81	17	95	14	109	22
benzo[ghi]perylene	214	30	117	36	4	4	86	21	20	5	357	100	425	38	432	95
Total PAHs	1,779	632	1,683	648	79	113	1,177	592	831	156	3,789	2,094	3,890	962	4,362	1,441
Total 12PAHs*	1,603	373	1,429	359	76	63	1,156	437	816	118	3,706	1,248	3,812	825	4,235	1,248

\*Excluding naphthalene, acenaphthylene, acenaphthene and fluorene (the four PAHs above the dotted line).

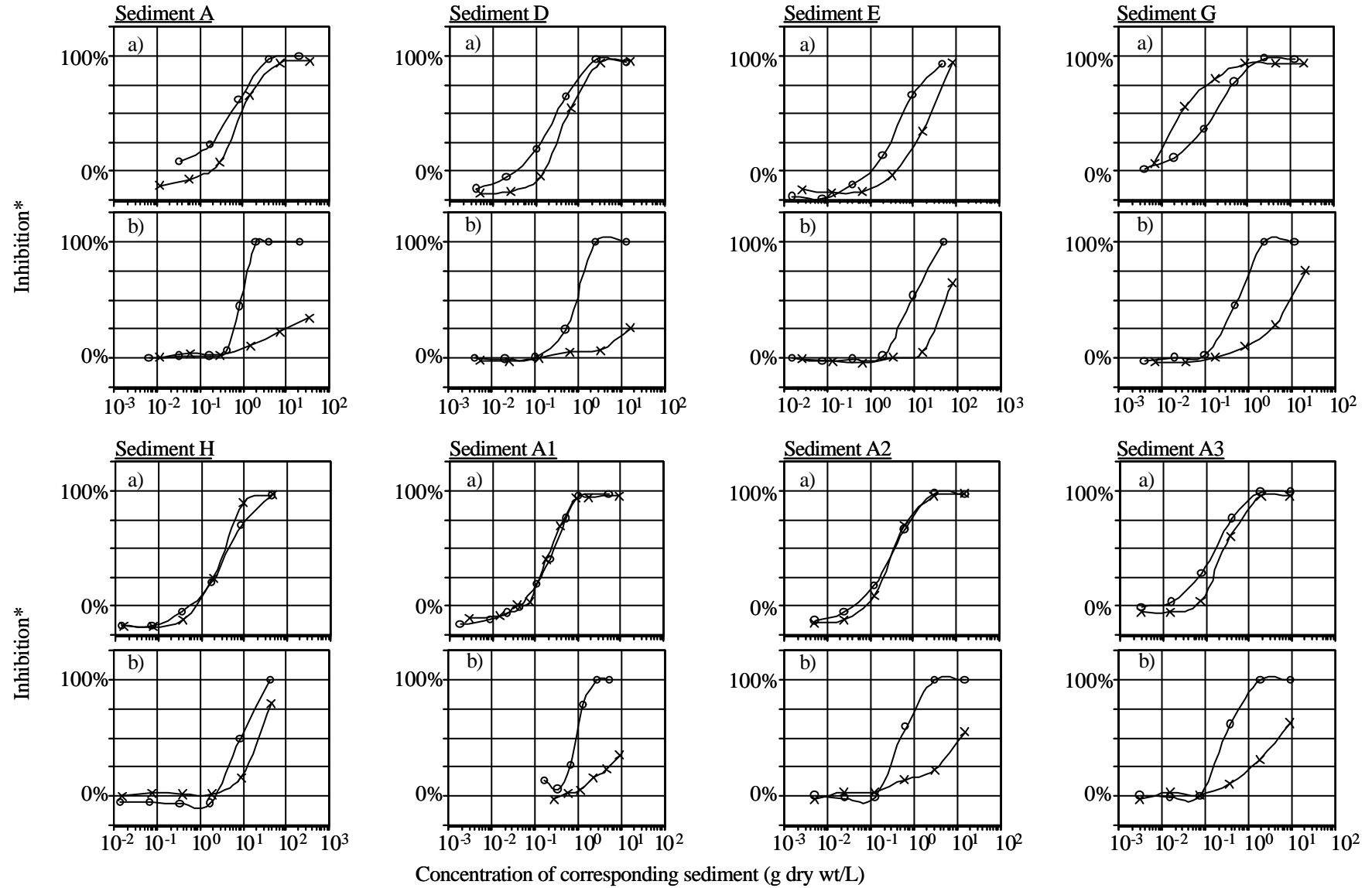


Figure 2. Toxicity of n-pentane extract (X) and of SDS extract (O) of each sediment in BioTox assay (a) and in algal toxicity test (b)

**Toxicity tests**

The extracts in n-pentane were transferred into DMSO (dimethyl sulfoxide) for two toxicity tests: bacterial acute toxicity test (*Vibrio fischeri*) and algal growth inhibition test using *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*). The concentrations of DMSO in all the test solutions were kept at 1.0 % (v/v) with appropriate addition of DMSO (a preliminary test showed that 1.0 % DMSO did not give a significant inhibition). Residual SDS in the S extracts was not detected by a simple test kit for anionic surfactants (WA-DET-II, Kyoritsu Chemical-Check Lab. Corp., Japan), whose detection limit was 0.5 mg/L (a preliminary test showed that 0.5 mg/L SDS with 1.0 % DMSO did not give a significant inhibition). The bacterial test was conducted according to the instruction manual of BioTox<sup>TM</sup> Kit (BioOrbit). Decrease of the bacterial luminescence after 30 minutes was used as an index of the toxicity of the extract. The algal test was conducted according to the modified ISO-method described by Arensberg et al. (1995). The test vials were incubated on a shaker (100 rpm) in continuous white fluorescent light (80-100  $\mu\text{E}/\text{m}^2/\text{s}$ ) at  $20 \pm 2$  °C for 72 hours. The tests were conducted at pH  $8.1 \pm 0.2$  with typical control growth rates of  $1.7-1.9 \text{ d}^{-1}$ . Algal growth rates were determined from acetone extractions as described by Mayer et al. (1997). The inhibition was evaluated by decrease of the algal growth rate.

**RESULTS AND DISCUSSION**

Table 1 gives the content of the PAHs in the sediments, extracted with n-pentane (P) and with SDS solution (S), respectively. The degree of sediment contamination by PAHs was moderate in comparison with literature values (Kim et al., 1999; Mackay and Hickie, 2000; Foster et al., 2000; Zakaria et al., 2002), except for sediment E, which had rather low PAHs content. The sediments A, A1, A2 and A3 contained more PAHs compared to others sediments, probably due to the influence of highway runoff.

Table 2 shows the ratio of extracted PAHs with the two solvents (P and S), defined as bioavailability (sediment E was omitted in Table 2 because of the low PAHs content that made the S/P ratio highly uncertain). The calculated bioavailability of the total amount of 12 PAHs in the sediments was evaluated to be in the range 14-38 %. Lower molecular PAHs such as naphthalene, acenaphthylene, acenaphthene and fluorene, having higher water solubility and higher vapor pressure, showed higher bioavailability compared to the higher molecular ones. Especially, the extracted amount of naphthalene was greater in the SDS solution compared to the n-pentane fraction for all the sediments. This indicates that n-pentane extraction method could not properly measure the content of the selected PAHs. Sodium dodecyl sulfate is a detergent, and the inherent properties of such a molecule (a hydrophilic and a hydrophobic part) could be the explanation for the higher extraction of naphthalene observed for SDS. The PAHs are adsorbed or physically incorporated in organic matter in the sediments. The addition of SDS will increase the solubility of organic matter in general and thereby also the PAHs.

**Table 2 Ratio of extracted amount (S) of PAHs in percent of total amount (P).**

sediment	A	D	G	H	A1	A2	A3
naphthalene	160	120	1062	677	1922	835	330
acenaphthylene	48	47	129	47	69	7	14
acenaphthene	62	23	115	35	329	57	22
fluorene	85	51	163	26	190	43	62
anthracene	25	18	35	2	40	16	24
phenanthrene	33	42	50	13	74	26	38
fluoranthene	23	38	27	9	33	13	35
pyrene	38	18	39	17	41	84	38
benzo[a]anthracene	18	14	19	5	17	10	19
chrysene	29	33	40	18	39	33	37
benzo[b]fluoranthene +benzo[k]fluoranthene	24	28	50	20	29	18	31
benzo[a]pyrene	20	20	46	14	22	13	18
indeno[123cd]pyrene	11	26	24	32	29	9	25
dibenz[a,h]anthracene	19	11	34	33	21	15	21
benzo[ghi]perylene	14	31	24	24	28	9	22
Total PAHs	36	39	50	19	55	25	33
Total 12PAHs*	23	25	38	14	34	22	29

*Excluding naphthalene, acenaphthylene, acenaphthene and fluorene (the four PAHs above the dotted line).*

Figure 2 shows for selected sediment samples the inhibitory profiles of the P and S extracts for the two biotests. Although the n-pentane extract was considered to embrace the contaminants in the SDS extract, the toxicity of SDS extract was higher than that of the n-pentane extract in most cases, especially in algal toxicity tests. This indicates that the higher molecular PAHs were not the major compounds responsible for the toxicity observed in the algal tests. Sediments E and H were less toxic compared to the sediments G and A3 that showed higher toxicity. A qualitative comparison of the sediment toxicity showed that the two different biotest generally agreed with each other, however with a tendency for that the algae

test was less sensitive than the biotox test. It also shows that the toxicity results didn't always comply with the measured content of PAHs (Table 1). Sediment G had higher toxicity but moderate content of PAHs compared with other sediments.

In this paper, a novel method for evaluating the bioavailability of PAHs is introduced. But the method is based on limited numbers of previous studies on desorption mechanism of spiked PAHs in sediments by digestive gut fluids of marine invertebrates (Weston and Mayer, 1998a,b; Mayer et al., 2001; Ahrens et al., 2001). Information on digestive gut fluids in benthic organisms especially in freshwater environment is very limited and the validity of the SDS solution, the hypothetical digestive gut fluid in this study, should therefore be investigated in further studies. The desorption mechanism may be governed by the strength of PAHs binding on the sediment particles as well as by the surfactancy of the fluid. Chemical characterization of the particles in urban runoff and its biological transformation will also be one of the important aspects on this matter.

## CONCLUSION

A novel method for evaluating the bioavailability of polycyclic aromatic hydrocarbons (PAHs) was developed and applied to sediments collected in an urban stream system (Store Ve jleå, Denmark) receiving wet weather discharges from urban areas. The method is aimed at simulating the digestive guts in deposit-feeding polychaetes. The study showed, that:

- The bioavailability of the total amount of 12 PAHs in the sediments was evaluated to be in the range 14-38 %. Lower molecular PAHs showed higher bioavailability compared to the higher molecular ones. Especially, the extracted amount of naphthalene was greater in the SDS solution (the hypothetical digestive gut fluid) compared to the n-pentane fraction.

- A qualitative comparison of the sediments showed that the results of two different biotests agreed with each other, the biotox test showing slightly higher sensitivity than the algae test, but not with the measured content of PAHs. The SDS extracts showed similar or higher degree of toxicity in the biotests than the organic solvent extracts in spite of lower PAHs content.

In further studies, the validity of SDS solution, the hypothetical digestive gut fluid in this study, should be investigated with a consideration for the mechanism of PAHs solubilization in benthic organisms.

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