The role of sediment composition on the bioavailability of laboratory-dosed sediment-associated organic contaminants to the amphipod, *Diporeia* (spp.)

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Abstract

Bioavailability of sediment-associated contaminants depends on contaminant and sediment characteristics and organism behavior and physiology. Bioavailability of a sediment-associated contaminant can be defined by the uptake clearance. Uptake clearance is the volume or mass of the source compartment scavenged of contaminant per mass of organism per time. For non-polar organic contaminants, carbon normalization has been suggested as a means of normalizing the relative bioavailability among sediments of differing compositions. To examine this, amphipods, *Diporeia* spp., were exposed to five Lake Michigan sediments and one soil from Florissant, MO, each spiked with selected polycyclic aromatic hydrocarbon (PAH) and polychlorinated biphenyl (PCB) congeners. The uptake clearances (k_s) were then determined. The PAH congeners were generally less bioavailable, based on k_s, than the PCB congeners after accounting for contaminant hydrophobicity. The k_s values generally declined as the fraction of fine particles (<63 μm) and organic carbon increased among the sediments. Organic carbon normalization reduced the variability among the k_s values to approximately a factor of two among the Lake Michigan sediments, but the Florissant soil was an outlier. The compounds were more bioavailable from the Florissant soil than from the Lake Michigan sediments, and the relative availability increased with increasing contaminant hydrophobicity up to a factor of eight for hexachlorobiphenyl.

Keywords: Polychlorinated biphenyls, polycyclic aromatic hydrocarbons, amphipods, bioavailability, sediment.

Introduction

Determining the bioavailability of sediment-associated contaminants among sediments of differing compositions is a major problem that must be solved before consistent exposures can be predicted and sediment quality criteria established. For non-polar organic contaminants, normalization to organic carbon has been suggested as a useful approach for establishing the differences in exposure because organic carbon is important in establishing the equilibrium among the solid and liquid phases in sediment (Di Toro *et al.*, 1991). A significant body of work exists that demonstrates that variability in biological exposure is reduced when carbon normalization is applied.

Reduction in variation with carbon normalization does not necessarily define the current range of variation remaining after normalization nor the factors that influence that variation. Variation among accumulation factors (lipid normalized concentration in organisms divided by carbon normalized concentration in sediment) for non-polar compounds can range over a factor of 100 among studies (Lake *et al.*, 1990, Lee, 1992). While the variation has been attributed, in part, to variation in the composition of the organic carbon (Meyer *et al.*, 1993, Versteeg and Shorter, 1992, Ortego and Benson, 1992), one study suggests that the composition is unimportant (DeWitt *et al.*, 1992). In the above cases, variation due to changes in composition was measured as changes in toxicity not compound accumulation. Thus, the source and magnitude of the variation after carbon normalization remains to be defined. The mechanisms for reduction in net accumulation in the presence of organic matter that have been investigated have suggested both reductions in accumulation (Versteeg and Shorter, 1992, Landrum and Faust, 1991) and enhanced elimination (Lydy *et al.*, 1992).

Our objective was to examine the differences in accumulation of selected polycyclic aromatic hydrocarbon (PAH) and polychlorinated biphenyl (PCB) congeners dosed to sediment with differing characteristics. The relative bioavailability was examined by measuring the uptake clearances. The uptake clearance is defined as the volume or mass of the source compartment scavenged of contaminant per mass of organism per time. The influence of organic matter was determined by

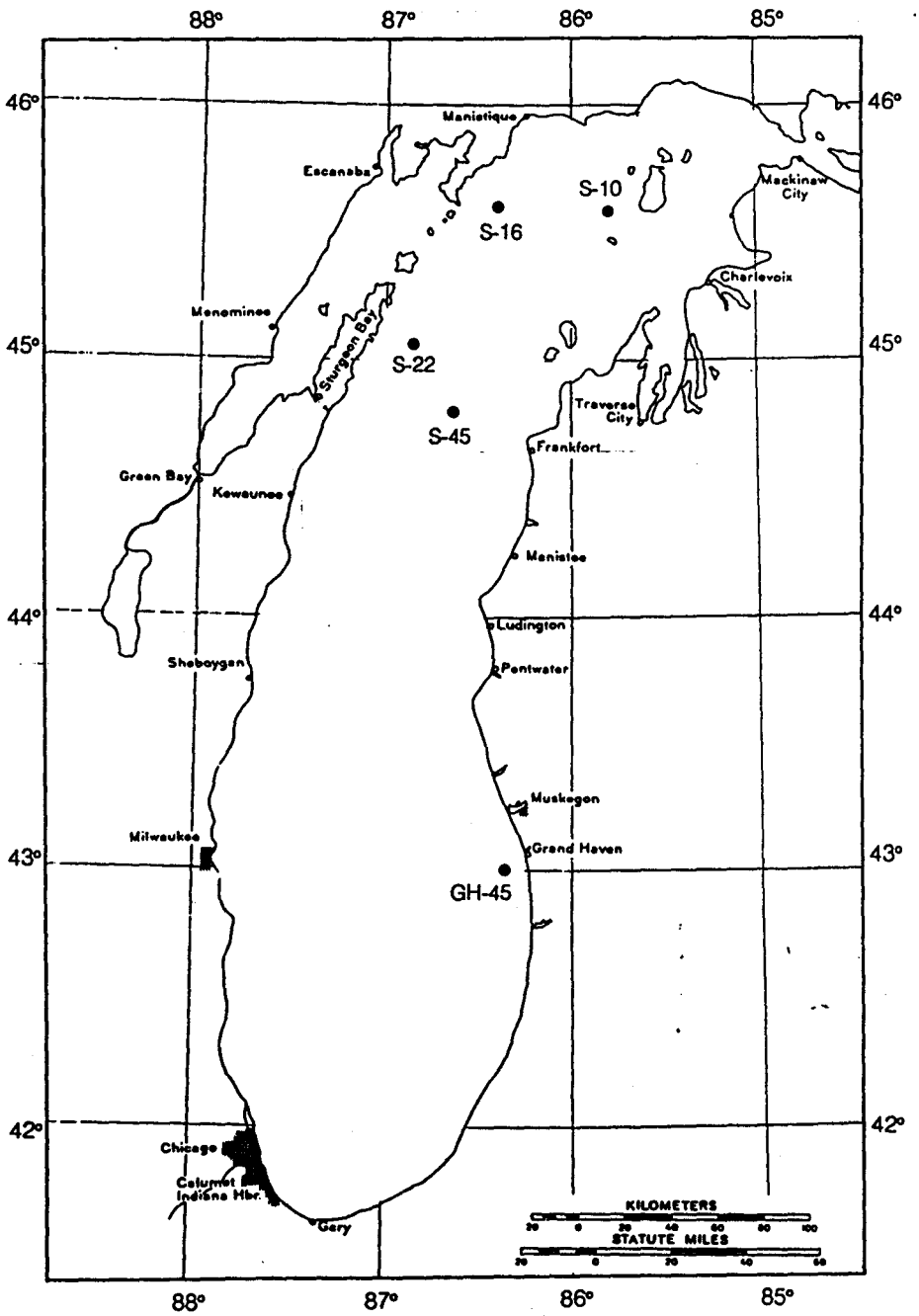

Figure 1 *Station locations for the five Lake Michigan sediment collections.*

normalizing the uptake coefficients to sediment organic carbon (SOC) content. Thus, we hypothesized that variation in the uptake clearances among sediment types could be eliminated by organic carbon normalization. In addition to measuring organic carbon, the percent of fine particles was also measured as a potential variable for reducing variance among sediment types.

Materials and Methods

Experimental design

The accumulation of selected xenobiotics by *Diporeia* spp. from five Lake Michigan sediments and one soil was followed over a 28–30 day period at 4°C. Radioactive tracers were added to the six different substrates; each differed in organic carbon content and fraction of fine-grained material (Table 1). The purpose of the experiment was to examine what effect the amount of fine-grained material and organic carbon had on the contaminant uptake clearance by *Diporeia*.

Groups of 20 *Diporeia* were exposed in 400-mL beakers each containing 40 g sediment (wet weight) and 250 mL lake water. The sediment was spiked with two radiotracers, ^{3}H-pyrene (PY, specific activity 34 Ci mmol^{-1}, Chemsyn) and ^{14}C-2,5,2′,5′- tetrachlorobiphenyl (TCBP, specific activity 12.6 mCi mmol^{-1}, Sigma Chemical Co.). Three such beakers of animals were sampled at each time point (6, 10, 15, 21, and 28 days) for each of the six substrate types. A second uptake experiment was conducted with ^{3}H-benzo(a)pyrene (BaP, specific activity 33.1 Ci mmol^{-1}, Amersham) and ^{14}C-2,4,5,2′,4′,5′-hexachlorobiphenyl (HCBP, specific activity 17.6 mCi mmol^{-1}, Sigma Chemical Co.) except that final samples were taken after 30 days.

Environmental media

The amphipods, *Diporeia* spp. (formerly *Pontoporeia hoyi*, Bousfield, 1989), were collected from Lake Michigan approximately 5 km south-west of Grand Haven, MI (43°01.2′N, 86°17.6′W) at a 29 m depth using a PONAR grab sampler. The grab samples were mixed with lake water and the *Diporeia* gently removed with a screen. *Diporeia* were placed in polyethylene bags of clean lake water and were kept cold with ice while transported to the laboratory. The *Diporeia* were held in aquaria containing about 3 cm sediment and 10 cm lake water at 4°C. The water used throughout the work was Lake Michigan surface water (collected about 1 m below the surface) and stored at 4°C.

The Lake Michigan sediments were obtained by PONAR grab at five different stations (Figure 1). The stations were chosen to provide substrates with differing amounts of organic carbon and fine-grained material. The soil sample from Florissant, MO was provided by the National Fisheries Contaminants Research Laboratory, National Biological Survey, Columbia, MO, and has been used as a control substrate in sediment toxicity tests by several laboratories. The substrates were sieved through a 1 mm screen to remove debris and indigenous animals and kept at 4°C. The sediments were expected to contain sufficient organic matter to serve as food for the animals as well as some anthropogenic contaminants; the soil sample is known to be low in contaminants.

Sediment characterization

The fraction of combustible solids (CS) and dry-to-wet weight ratios for sediments were determined by weighing a 1–2 g sample into a preweighed glass petri dish. The sediment was dried to a constant weight at 60°C. The dry sediment was combusted at 500°C for 5 h. The samples were cooled in a desiccator and the ash weight determined. CS is the percent ash-free weight of the dry sediment.

The sediment organic carbon (SOC) content was determined by treating 50–100 mg dry sediment with 1 mL of 1 N HCl and agitating the samples on a shaker overnight to remove the carbonates. The samples were dried at 80°C, and the SOC content determined with a Perkin-Elmer 2400 CHN Elemental Analyzer.

The percent of fine material in the sediments (particles <63 μm) was determined by wet sieving approximately 1 g of sediment. After the addition of 5 mL distilled water, the mixture was agitated in a sonic bath to disaggregate the particles. The material was then wet sieved through a 63 μm stainless steel sieve, and each fraction washed into a beaker. After drying to constant weight at 60°C, the fine and coarse fractions were weighed and the proportion of fine material determined.

Sediment preparation

An aliquot of each sediment was mixed until it appeared to be homogeneous. An amount of wet sediment corresponding to 500 g dry weight was placed into a 4-L beaker, and lake water was added to produce a total volume of 3 L. The sediment from Station S-45 required additional water in order to achieve a mixture which could be stirred (4 L total volume). The sediment was stirred vigorously at room temperature while a solution of one pair of compounds in 1.0 mL acetone was slowly added in a dropwise manner. The mixture was stirred at room temperature for an additional 4 h and kept at 4°C thereafter.

Experimental procedure

Two days after dosing each sediment, the overlying water was decanted to remove most of the acetone carrier. The sediment was stirred until homogeneous, and an amount of wet sediment equivalent to 25 g dry weight was added to each of fifteen 400-mL beakers. Samples were taken from each sediment at the start, middle, and end of the distribution to the beakers: 2 g for wet to dry weight determination and 0.2 g to be weighed into a

Table 1 *Location and characteristics of the sediments.*

Station	Latitude	Longitude	SOC[a]	CS[b]	FF[c]
S-45	44.88°N	86.56°W	3.13	12.6	0.99
			(0.17)	(0.6)	(0.01)
Florissant	NA	NA	1.25	4.4	0.975
			(0.12)	(0.3)	(0.004)
S-16	45.59°N	86.32°W	1.11	8.6	0.893
			(0.07)	(0.35)	(0.015)
GH-45	43.03°N	86.37°W	0.48	3.35	0.444
			(0.043)	(0.32)	(0.012)
S-22	45.09°N	86.76°W	0.47	8.08	0.588
			(0.05)	(0.67)	(0.03)
S-10	45.59°N	85.69°W	0.23	2.96	0.194
			(0.07)	(0.69)	(0.01)

[a] Percent sediment organic carbon, mean ± sd, n = 12.
[b] Percent combustible solids, mean ± sd, n = 15.
[c] Fraction of fines, mean ± sd, n = 6.

scintillation vial, dispersed into 12-mL scintillation cocktail sonicated for 90 s, and counted to determine radioisotope activity. Lake water (250 mL) was carefully added to each beaker to minimize disturbance of the sediment. On the following day (3 days after dosing of the sediments), a water sample (2 mL) was taken to determine the levels of radioisotopes in the aqueous phase, and 20 test *Diporeia* were added to each beaker.

Three beakers of animals exposed to each sediment type were sampled at each time point (6, 10, 15, 21, and 28 days), and the following samples were taken from each beaker:

1. Overlying water (2 mL) was counted to determine radioisotope activity in the aqueous phase.

2. The number of *Diporeia* above the sediment was recorded to check for sediment avoidance behavior; the number surviving was determined and the wet weight of several groups of three individuals each was recorded. Typically, three replicates of three *Diporeia* were dispersed into 12 mL of scintillation cocktail by sonicating for 60 s to determine the radioisotope concentration in the animals. Three individuals were placed in preweighed tubes, dried, and their lipid content determined by microgravimetric analysis (Gardner *et al.*, 1985).

3. A weighed sample of wet sediment was dried to constant weight at 60°C to determine the wet to dry weight ratio. A small sample of wet sediment (approximately 200 mg) was weighed out into a scintillation vial and dispersed into 12 mL of scintillation cocktail by pulsed sonication for 90 s to determine the radioisotope activity of the sediment.

4. On days 6, 15, and 28, a wet sediment sample was centrifuged, and the total, free, and bound concentration of the two radioisotopes were determined in the porewater similar to Eadie *et al.* (1990). Porewater (2 mL) was counted for total

Table 2 *Uptake coefficients (mg sediment g organism^{-1} h^{-1}, mean ± sd) for* Diporeia *exposed in different sediments.*

Station	PY	BaP	TCBP	HCBP
S-45	2.93	0.10	3.96	1.38
	(0.39)	(0.03)	(0.86)	(0.04)
Florissant	18.3	0.79	58.6	19.2
	(0.8)	(0.05)	(7.1)	(0.9)
S-16	14.3	0.36	24.9	3.87
	(0.9)	(0.04)	(2.4)	(0.6)
GH-45	15.5	0.94	22.3	6.6
	(0.9)	(0.07)	(0.8)	(0.2)
S-22	30.6	1.23	51.6	11.1
	(2.4)	(0.14)	(3.1)	(1.1)
S-10	50.5	1.9	92.9	19.5
	(6.3)	(0.2)	(4.7)	(0.8)

activity. The remainder was filtered (with a back-up filter to correct for sorption), and a sample of filtrate (2 mL) was counted. The filtrate was passed through a C-18 Sep Pak® (to absorb freely dissolved material), and the isotope contained in the elutriate (2 mL) was counted and presumed to be bound to organic matter. The isotope sorbed to the Sep Pak (determined by difference) plus two times the amount sorbed to the backup filter was presumed to be freely dissolved.

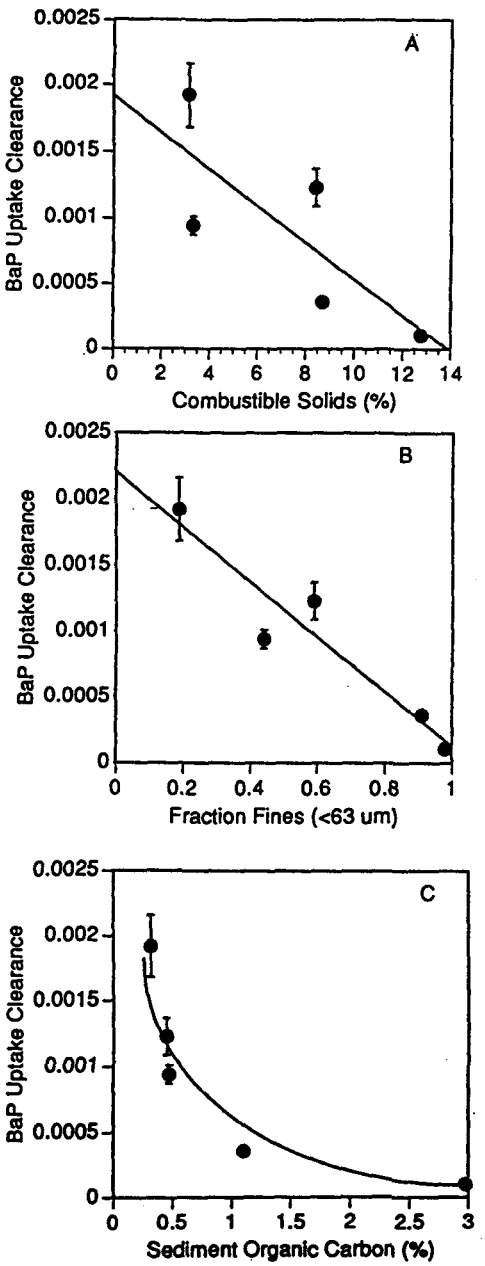

Figure 2 *Dependence of the Diporeia uptake clearance for benzo(a)pyrene on the amount of combustible solids (A), fraction of fines <63 μm (B) and amount of organic carbon (C), in Lake Michigan sediments.*

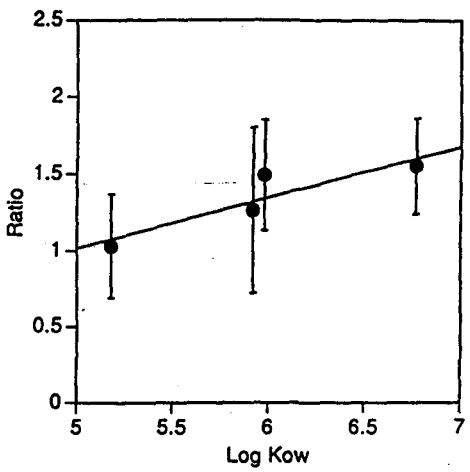

Figure 3 *Ratio of the carbon normalized uptake clearance ($k_{s\text{-}oc}$) for station S-10 divided by those of the other Lake Michigan stations with the change in compound hydrophobicity as represented by the log of the octanol:water partition coefficient (log K_{ow}).*

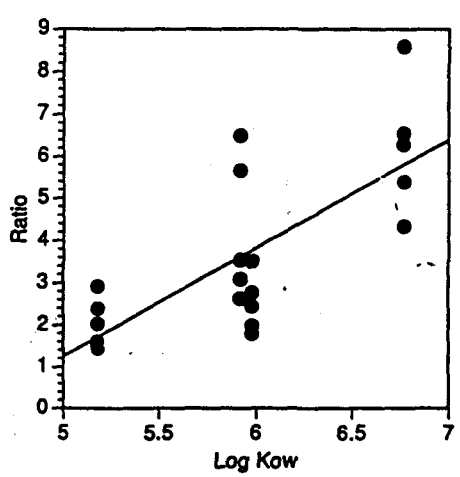

Figure 4 *Ratio of the carbon normalized uptake clearance ($k_{s\text{-}oc}$) for Florissant soil divided by those of the Lake Michigan Stations with the change in compound hydrophobicity as represented by the log of the octanol:water partition coefficient (log K_{ow}).*

Table 3 *Mean organic carbon normalized uptake clearance (mg organic carbon g organism^{-1} h^{-1}) for* Diporeia *exposed in different sediments.*

Station	PY	BaP	TCBP	HCBP
S-45	0.092	0.003	0.125	0.043
Florissant	0.229	0.010	0.733	0.240
S-16	0.157	0.004	0.274	0.043
GH-45	0.074	0.005	0.107	0.032
S-22	0.144	0.006	0.243	0.052
S-10	0.116	0.004	0.214	0.045

Radioactivity determination

All scintillation counting was performed on an LKB 1217 RACKBETA liquid scintillation counter using RPI scintillation cocktail 3a70B. Quench corrections were made via the external standards ratio method after subtracting background.

Calculations

The accumulation data were fit to a first order accumulation model to estimate the uptake clearance, k_s (g sediment g^{-1} organism h^{-1}), as a measure of bioavailability (Lee, 1991). PY and TCBP were fit to a two compartment model, while BaP and HCBP were fit to a one compartment model assuming initial rates conditions (Landrum, 1989). The data were fit by nonlinear curve fitting NLIN for the two compartment data and with the general linear method (GLM) for linear regression for the one compartment simplification (SAS Institute, 1985).

Comparisons of the k_s values to sediment characteristics were performed using the multivariate general linear hypothesis package in Systat™ (Wilkinson, 1984). Means and slopes were compared using Student's *t*-test. Relationships were considered statistically significant if $p < 0.05$.

Organic carbon normalized partition coefficients (K_{oc}) were calculated as the ratio of the concentration of contaminant on the sediment particles normalized for the organic carbon content divided by the freely dissolved concentration in the porewater.

Results

Sediment characteristics and partition coefficients

The sediment and soil samples covered a range of organic carbon of more than a factor of ten and a range for the fraction of fines of approximately four (Table 1). The combustible solids content did not correlate with the amount of organic carbon even among the Lake Michigan sediments (Table 1). The fraction of fines did correlate with the inverse of the organic carbon content among the Lake Michigan sediments, but the correlation was weakened when the Florissant soil was included in the regression. The Florissant soil is a material from a totally different environment, and so it is not surprising that the relations found among the Lake Michigan sediments did not predict the characteristics for a material of differing origin.

There was considerable variability in the partition coefficient data both within and among the sediments. However, the only apparent differences in the organic carbon normalized partition coefficients were for sediments from stations S-45 and S-10. Station S-45 had the highest organic carbon content and for which the amount of activity in the porewater was extremely low. Station S-10 had the lowest organic carbon content. The log K_{oc} values excluding these stations were PY 5.3, BaP 5.4, TCBP 5.6 and HCBP 5.5. The coefficients of variation for these partition coefficients were 40–67%, so the values can only be considered crude approximations. The values for station S-45 were similar to those above but were somewhat lower than the mean values. The values for station S-10 were approximately two to three times greater, suggesting that the small amount of organic carbon at station S-10 may partition these non-polar organic compounds more strongly than the organic matter from the other stations.

Kinetics

The uptake clearance coefficient, k_s (mg sediment g organism^{-1} h^{-1}) for each sediment suggested that the PCB congeners were generally more bioavailable than the PAH congeners after accounting for their hydrophobicity, as measured by the octanol : water partition coefficient (K_{ow}) (Table 2). Across sediments, the k_s values for each compound varied for each sediment such that the sediments with the greatest amounts of organic carbon, percent fines, and combustible solids yielded the lowest k_s values. The k_s values for each compound declined linearly with respect to the fraction of combustible solids and fines as shown for BaP (Figure 2a and b) among the Lake Michigan sediments. The k_s values for each compound exhibited a curvilinear relationship with the percent organic carbon as shown for BaP (Figure 2c) among the Lake Michigan sediments. The Florissant soil was an outlier in each case.

The variation in the k_s values was not attributable to changes in organism health based on lipid content measured at the beginning and end of the experiment 33.3 ± 4.64% (mean ± SD, n = 15) and 32.4 ±6.9% (n = 44) for the PY/TCBP

experiment and 30.1 ± 3.18% (n = 9) and 31.8 ± 5.5% (n = 45) for the BaP/HCBP experiment. Thus, there were no detectable differences in organism health among sediments or between experiments.

Because organic carbon is recognized as the component of the sediment that is most important for the partitioning of organic contaminants and, subsequently, affects the bioavailability, the uptake clearances were normalized to the sediment organic carbon content. If organic carbon controls the bioavailability, then the carbon normalized uptake clearance, $k_{s\text{-}oc}$, for a particular compound should be constant. Therefore, the $k_{s\text{-}oc}$ for each compound from each of the sediments was divided into the $k_{s\text{-}oc}$ value found in S-10 for the respective compound. If carbon normalization adjusts for the variability among sediments, then the resultant ratio should be 1. Among the Lake Michigan sediments, the ratio of the $k_{s\text{-}oc}$ values vary by approximately a factor of two, and the mean ratio of the $k_{s\text{-}oc}$ values range from approximately 1 to 1.5 across all the compounds studied (Figure 3). When the Florissant $k_{s\text{-}oc}$ value was divided by carbon normalized k_s for station S-16, which had a similar organic carbon content, the ratio ranged from 1.44 for PY to 6.3 for HCBP. When the Florissant $k_{s\text{-}oc}$ values were divided by the Lake Michigan station $k_{s\text{-}oc}$ values, the variation in the ratio for any one compound was approximately a factor of 2 to 3 (Figure 4). Pyrene came the closest to the theoretical value of 1 with a mean value of approximately 2, while the mean value for HCBP was approximately 6 with the maximum of nearly 10. Thus, the contaminants were on average approximately two to six times more available from Florissant soil than from the Lake Michigan sediment.

Discussion

The hypothesis that normalization by organic carbon will account for differences in bioavailability of non-polar organic contaminants among sediments was tested for a limited set of Lake Michigan sediments and one soil material. For the test of carbon normalization to be useful, the range of characteristics for both the compounds and the sediments should be reasonably broad. The compounds came from two chemical classes and had a range of log K_{ow} from 5.2 to 6.7. The organic carbon concentration in the sediments ranged by more than a factor of 10, but the maximum organic carbon content was only 3%. There was also a wide range of fine material content (<63 μm) among the sediments. Further, the source of material employed in the test came from two geographical regions. Therefore, the test should provide insights into the utility of carbon normalization.

The fraction of fine materials was greatest for the Florissant soil compared to that of the lake sediments, relative to the amount of organic matter. Thus, in a correlation between the fraction of fines and organic carbon content, the Florissant soil was an outlier. This probably results from the different component composition in the soil compared to the lake sediment. A more complete characterization of the sediment and soil materials would have likely provided greater insight into the differences both among the lake sediments, but specifically between the lake sediment and the Florissant material. Exactly which characteristics should be measured for bioavailability assessments remains to be defined. The question that remains is whether Florissant soil is a reasonable material to compare with lake sediments? Since a large part of sediment materials, particularly in riverine systems can come from surface runoff, it is not unreasonable to use this material.

As with previous studies (Landrum, 1989, Landrum and Faust, 1991), the PCB congeners were more bioavailable, based on larger k_s values, than the PAH congeners after accounting for the relative hydrophobicities of the compounds (Table 2). This occurred for all of the sediment materials examined. This may well result from both differential partitioning within the sediment matrix and feeding selectivity (Harkey *et al.*, in press). Upon examining the various materials, variability in k_s for any one compound ranges by a factor of 18.5 ± 3.9 in bioavailability, with station S-10 the most available and station S-45 the least available. On a dry sediment basis, bioavailability from the Florissant soil was somewhere in the middle.

The relationship between k_s values for the Lake Michigan sediments and organic carbon is curvilinear (Figure 2c). This suggests that organic matter in the lower organic carbon sediments has greater influence on bioavailability per gram of organic carbon than at higher organic carbon concentrations. The difference in the relative impact implies different compound organic carbon interactions, perhaps because of different compositions in the low organic carbon sediment. That Florissant material is an outlier in the relationship also suggests that composition is important in the compound sediment interaction and the bioavailability of these non-polar materials.

Once the k_s values are carbon normalized, the range of values declines to 1.9 ± 0.2 for all compounds if the Florissant soil is not considered. Thus, for materials from a similar source, normalization to organic carbon seems useful for reducing the variability between exposures to different sediments for non-polar compounds. However, if the Florissant soil is considered, the range varies depending on the compound class. The range of highest to lowest k_s values for the PAH congeners was 3.1–3.3, while for the chlorinated biphenyls, the range was greater by a factor of about two, 6.9–7.5 (Table 3).

After organic carbon normalization, there was no statistical increase in the variance across the range of compounds employed when only the Great Lakes sediments were used. However, the mean ratio of the $k_{s\text{-}oc}$ value for station S-10 divided by

the $k_{s\text{-}oc}$ of the other stations increased for compounds with larger log K_{ow} values (Figure 3). This suggests that the compositional changes in organic carbon may well be more important for more hydrophobic compounds. The mean ratio of the Florissant soil $k_{s\text{-}oc}$ divided by the $k_{s\text{-}oc}$ of the Great Lakes stations exhibits a strong increase with the log K_{ow} of the compound (Figure 4). This further supports the hypothesis that sediment composition beyond carbon normalization is important for determining the bioavailability of contaminants.

Overall, the bioavailability as measured by k_s for non-polar compounds varied greatly among sediments of differing composition. The variance was generally reduced by normalizing the bioavailability to the organic carbon content. Among the Great Lakes sediments, the variance was approximately a factor of two. This is thought to occur because the major components result from similar source types within the same system. However, when the bioavailability from the Florissant material was compared to that from the Great Lakes, the compounds sorbed onto the Florissant material were substantially more bioavailable. Further, the relative bioavailability was greater for the more hydrophobic compounds. This suggests that sediment composition beyond a simple measure of organic carbon contributes substantially to the bioavailability of hydrophobic organic compounds. The extent of the variance is sufficient so that simple normalization to organic carbon could inappropriately estimate bioavailability by a factor of ten for more strongly sorbed compounds.

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