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Particle-Scale Understanding of the Bioavailability of PAHs in Sediment

JEFFREY W. TALLEY,[†] UPAL GHOSH,[‡]
SAMUEL G. TUCKER,[§]
JOHN S. FUREY,[§] AND
RICHARD G. LUTHY*,[‡]

Department of Civil Engineering and Geological Sciences, University of Notre Dame, Notre Dame, Indiana 46556-0767, Department of Civil and Environmental Engineering, Stanford University, Stanford, California 94305-4020, and Environmental Laboratory, U.S. Army Engineer Research and Development Center, Vicksburg, Mississippi 39180

This study reports results of sediment bioslurry treatment and earthworm bioaccumulation for polycyclic aromatic hydrocarbon (PAH) contaminants found in sediment dredged from Milwaukee Harbor. A significant finding was that bioslurry treatment reduced PAHs on the sediment clay/silt fraction but not on the sediment coal-derived fraction and that PAH reduction in the clay/silt fraction correlated with substantial reduction in earthworm PAH bioaccumulation. These findings are used to infer PAH bioavailability from characterization of particle-scale PAH distribution, association, and binding among the principal particle fractions in the sediment. The results are consistent with work showing that the sediment comprised two principal particle classes for PAHs, coal-derived and clay/silt, each having much different PAH levels, release rates, and desorption activation energies. PAH sorption on coal-derived particles is associated with minimal biodegradation, slow release rates, and high desorption activation energies, while PAH sorption on clay/silt particles is associated with significant potential biodegradability, relatively fast release rates, and lower desorption activation energies. These characteristics are attributed to fundamental differences in the organic matter to which the PAHs are sorbed. Although the majority of the PAHs are found preferentially on coal-derived particles, the PAHs on the clay/silt sediment fraction are more mobile and available, and thus potentially of greater concern. This study demonstrates that a suite of tests comprising both bioassays and particle-scale investigations provide a basis to assess larger-scale phenomena of biotreatment of PAH-impacted sediments and bioavailability and potential toxicity of PAH contaminants in sediments. Improved understanding of contaminant bioavailability aids decision-making on the effectiveness of biotreatment of PAH-impacted sediments and the likelihood for possible reuse of dredged sediments as reclaimed soil or fill.

Introduction

Approximately 400 million cubic yards of sediment are dredged from United States ports, harbors, and waterways each year to maintain the nation's navigation system. It is estimated that approximately 10% of these dredged materials are impacted by organic and inorganic contaminants (*1*). Contaminated dredged materials are generally discharged into diked, confined disposal facilities (CDFs). Many CDFs are filled or reaching their design capacity, and economic treatments are sought to extend the life of CDFs by reclaiming dredged material for reuse as fill or soil.

The reuse of dredged material as reclaimed soil will extend the operational life of CDFs while benefiting the environment through reclamation of topsoil and fill material. However, dredged harbor sediments contaminated with polycyclic aromatic hydrocarbons (PAHs) face very stringent cleanup standards prior to reuse. The reuse of contaminant-impacted dredged material is restricted to terrestrial application; therefore, the material generally must meet either state soil or solid waste cleanup criteria. This is a serious impediment for reuse of dredged materials. In Wisconsin, for example, the use of contaminated dredged material for topsoil is governed by suggested generic soil cleanup levels (*2*). If the contaminated dredged material is reused as fill material, it falls under Wisconsin's regulation for the beneficial use of industrial byproducts (*3*). As shown in Table 1, for use in nonindustrial (topsoil) or residential (fill material) applications, certain PAH contaminants such as benzo[*a*]pyrene and dibenz[*a*,*h*]anthracene must be cleaned up to as low as 8.8 ppb (*2, 4*). These levels are practically unachievable using bioreclamation technologies. Thus, relatively low-cost, natural cleanup processes such as composting may not meet cleanup standards. These low-level cleanup standards assume that the PAHs are readily available and may result in environmental exposure. While there is growing awareness that contaminant availability and risk should determine cleanup levels, adequate tools to evaluate and understand contaminant availability in dredged materials are lacking. As is the case with contaminated soils (*5*), conservative, low-level cleanup standards are invoked, wherein sediment cleanup is driven by total contaminant mass removal not bioavialability (*6*). Consequently, the adherence and slow release of PAHs from geosorbents is both an obstacle to remediation (*7, 8*) and a challenge to concepts about cleanup standards and risks (*5, 9*).

Bioremediation of PAH-contaminated sediments often results in high residual concentrations that do not satisfy the minimum standards for reuse as soil (*10*). It is believed that residual PAH concentrations are caused by the limited bioavailability of PAHs. Operational, physicochemical tests are being developed to assess contaminant bioavailability and to evaluate the potential feasibility of biological treatments to meet cleanup objectives. Often such tests are based on the determination of readily available PAHs as measured by release into the water phase. These tests may involve long-term, batch rate of release experiments with the uptake of dissolved PAHs onto a solid sorbent (*11, 12*) and the data evaluated using an empirical first-order or a two-site model consisting of two first-order expressions (*13*). Thermal techniques may be useful in conjunction with aqueous desorption tests to characterize the available fraction (*14*).

Study Objectives. The primary question addressed in this work was whether the macroscopic bioavailability behavior of PAHs in dredged sediment could be explained by particle-scale characterizations of PAH distribution, association, and

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* Corresponding author phone: (650)725-9170; fax: (650)723-3164; e-mail: luthy@stanford.edu.

[†] University of Notre Dame.

[‡] Stanford University.

[§] U.S. Army Engineer Research and Development Center.

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TABLE 1. Comparison of PAH Concentrations (mg/kg) in Untreated Milwaukee CDF Sediment and Wisconsin Required Cleanup Levels (mg/kg) for Reuse of Dredged Material

		WI generic soil cleanup levels			WI solid waste cleanup levels	
compd	concn	GW pathway	nonindustrial	industrial	unrestricted	restricted
naphthalene[a]	1.2	0.4	20	110	600	6000
2-methylnaphthalene	2.1	20	600	40000	na	na
acenaphthylene	0.1	0.7	18	360	8.8	88
acenaphthene	1.9	38	900	60000	900	9000
fluorene	2.3	100	600	40000	600	6000
phenanthrene[a]	15.4	1.8	18	390	0.88	8.8
anthracene	3.1	3000	5000	300000	5000	50000
fluoranthene	21.8	500	600	40000	600	6000
pyrene	17.7	8700	500	30000	500	5000
chrysene[a]	9.8	37	8.8	390	8.8	88
benzo[*a*]anthracene[a]	6.9	17	0.088	3.9	0.088	0.88
benzo[*b*]fluoranthrene[a]	7.6	360	0.088	3.9	0.088	0.88
benzo[*k*]fluoranthrene[a]	6.3	870	0.88	39	0.88	8.8
benzo[*a*]pyrene[a]	7.0	48	0.0088	0.39	0.0088	0.08
indeno[1,2,3-*c*,*d*]pyrene[a]	5.6	680	0.088	3.9	0.088	0.88
dibenz[*a*,*h*]anthracene[a]	1.1	38	0.0088	0.39	0.0088	0.088
benzo[*g*,*h*,*i*]perylene[a]	5.1	6800	1.8	39	0.88	8.8

[a] Denotes PAHs that exceed at least one of the cleanup levels required for reuse of dredged materials. Generic soil cleanup levels are for reuse as topsoil or near-surface soil. Nonindustrial and industrial soil cleanup levels are based on direct contact pathways. Groundwater (GW) pathway cleanup levels assume reuse will be in areas where the material will come into contact with groundwater. Restricted solid waste cleanup levels limit the reuse to capped, roadfill, or nonresidential applications. Unrestricted solid waste cleanup levels are based on unrestricted reuse as fill material. Compiled from refs *2* and *3*.

binding with sediment. To this end, the research comprised aerobic bioslurry testing and earthworm PAH uptake studies before and after bioslurry treatment. These data were interpreted in view of recent reporting (*14*, *15*) of particle-scale measurements of PAH distribution, organic matter association, release rates, and desorption binding energies for this sediment.

Milwaukee Confined Disposal Facility (CDF). The sediment used in this study was obtained from the Jones Island Confined Disposal Facility (CDF), commonly known as the Milwaukee CDF and operated by the Milwaukee Harbor Port Authority. Shown in Figure 1 is the 44-acre CDF located in the South Milwaukee Harbor. Constructed in 1975 with a maximum thickness of 10 m, it serves as a disposal facility for maintenance-dredged materials unsuitable for open-lake disposal. The majority of the CDF was filled via hydraulic dredging and consists of consolidated, fine-grained material. Borings from the Milwaukee CDF indicate 50 mg/kg total PAH near the surface (within 2 m), 275 mg/kg at 5 m, and 100 mg/kg at 7 m (*16*). Samples for this work were collected in fall 1996 by backhoe at a depth of 4 m, placed in a 55-gal drum and sealed on site, shipped via refrigerated truck to the Environmental Laboratory, U.S. Army Engineer Research and Development Center, and stored at 4 °C for 2 yr prior to use.

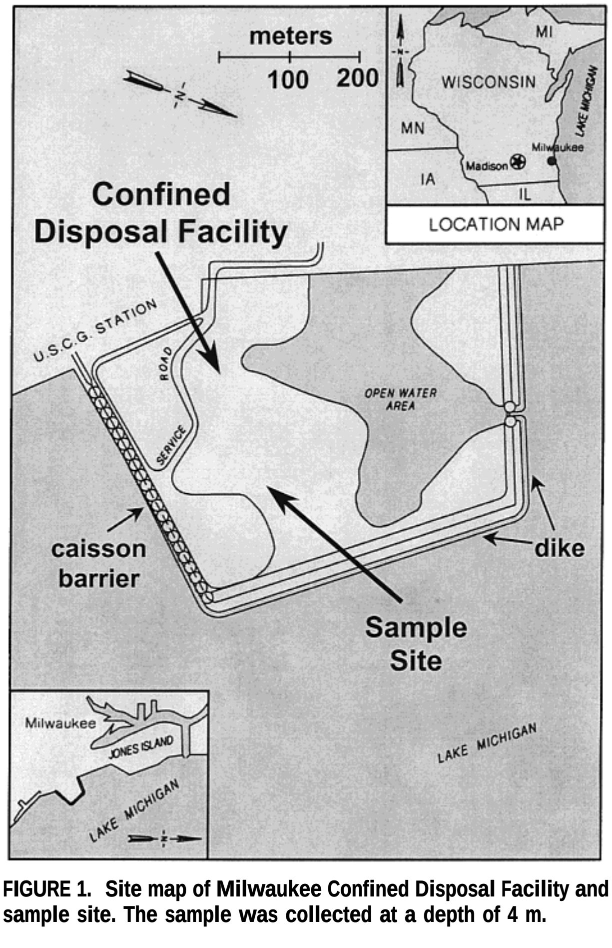

FIGURE 1. Site map of Milwaukee Confined Disposal Facility and sample site. The sample was collected at a depth of 4 m.

Experimental Methods

Homogenization of Dredged Materials. Drummed sediment was placed in an aluminum trough and homogenized for 6 h using a heavy-duty mixer. Wet sieving removed material larger than 2 mm. Sievings were poured into a 55-gal drum and allowed to settle for 1 month at 4 °C, and then the overlying water layer was removed using a peristaltic pump. The sediment was homogenized again prior to sampling and loading the bioslurry reactors.

Bioslurry Reactor System. The bioslurry reactor system comprised six 5-L reactors. Two reactors were controls (anaerobic and poisoned), and four were active (aerobic) reactors. Each bioreactor received 5.3 kg (wet weight) of dredged material, which consisted of 56.5% solids. To produce a 30% (by volume) slurry, 3.5 L of modified Stanier's basal media (*17*) was added to each reactor. The media did not contain any carbon source. The reactors had two 3.8-in impellers mounted to a mixing shaft. The lower impeller was positioned about 1 in. from the bottom of the reactor, and the upper impeller was mounted about 3 in. above the lower impeller. Each reactor had three diffuser stones in the bottom that bubbled either argon (control reactors) or air (aerobic reactors). The gas was fed from compressed gas cylinders and passed through double charcoal filters before entering the bioreactors. The reactors were continuously stirred at 350 rpm. The reactors were vented to a fume hood. Off-gas monitoring in related tests showed no significant loss of PAHs by volatilization.

The anaerobic reactors were poisoned with 500 ppm each of sodium azide and mercuric chloride. Initial biomass analyses indicated that the poisoning was not effective in killing the microbial community for longer than a few days. Thus another 500 ppm of both compounds was added 18 d into the experiment, 1000 ppm 1 week later, and 750 ppm every 3 weeks thereafter. This maintained poisoned, anaerobic controls, as shown by streaking the slurry onto nutrient agar plates and incubating for 72 h. The bioreactors were tested daily for pH, dissolved oxygen, oxidation−reduction potential, and temperature and for nitrate and phosphate initially every 2 days and then weekly after the first month. Carbon, nitrogen, and phosphorus ratios were maintained at 100:10:1, with the carbon content based on the sediment total organic carbon content. Dissolved oxygen was maintained in the range of 8−9 mg/L for the aerobic reactors with pH 7.0.

Each month sediment was sampled using 50-mL pipets after allowing the reactors to settle for 1 h. Samples were collected from five different locations and at three different depths (top, middle, and bottom) within each reactor. The collected slurry was placed in sterile screw-top containers and centrifuged at 8000 rpm for 20 min, and the supernatant was decanted; the solids were placed in a pan lined with sterile aluminum foil and mixed thoroughly and placed into sample vials. At the end of the test, the reactor was emptied, and a composite sample was collected. All sampling tools were autoclaved prior to use.

Physical Separation. The whole sediment was separated into four size fractions (>1000, 1000−250, 250−63, and <63 μm) for purposes of determining PAH presence and availability among sediment fractions. The larger size fractions (>63 μm) comprised primarily sandy grains, coal-derived particles, and woody material. It was possible to wash off the lighter fractions (coal and wood) from the heavier sand by swirling with water in a beaker and draining off the entrained lighter particles. Materials in the fine fraction (<63 μm) were density separated using a cesium chloride solution having a specific gravity of 1.8 (*15*). Five grams of wet sediment and 40 mL of cesium chloride solution were centrifuged at 200 rpm for 10 min in 50-mL glass centrifuge tubes. The fine coal-derived and wood particles floated, were decanted, and were collected on filter paper and rinsed with water several times. The heavy clay and silt fractions were similarly washed several times to remove cesium chloride. Each of these density-separated fractions was placed in cold storage.

Desorption Analyses. Room temperature, batch, desorption kinetic studies evaluated the mass transfer of the PAHs from the whole and separated fractions of sediment to Tenax beads (*15*). Duplicate rate of release tests followed procedures reported previously by Ghosh et al. (*15*) and others (*18*, *19*). Contaminated sediment (2.0 g) and Tenax beads (0.5 g, 40−60 mesh, Suppelco, PA) were added to 15-mL glass vials containing 10 mL of deionized water and continuously mixed on a rotator. Mercuric chloride (1.0 mg) was added to the mixture to prevent biological growth. At sampling times, the Tenax beads were harvested by allowing the sediment to settle, and the Tenax beads to float up. The Tenax beads were removed from the glass vials, and fresh Tenax beads were added. PAHs were extracted from the Tenax beads by agitating the beads in 10 mL of hexane and acetone (50:50 mixture) for 12 h and repeating twice. The extracts were then combined and concentrated, cleaned using silica gel, and analyzed by GC-FID.

Thermal program desorption (TPD) measurements employed a gas chromatograph quadrupole mass spectrometer (GCQ-MS) with a direct insertion probe manufactured by Thermoquest. These tests evaluated binding activation energies, as interpreted from TPD rate responses, for PAHs from untreated and bioslurry treated sediment. TPD tests followed procedures described by Talley (*20*), which showed that the release of PAHs is dependent both on PAH molecular weight and the character of the sorbent material. Milligram-level samples of whole sediment and separated fractions were placed into the mass spectrometer via a 1 mm diameter glass vial inserted in the probe. The temperature of the probe containing the sample glass vial was then increased at 10 °C/min from 30 to 400 °C. As the probe heated, the volatilized compounds were ionized, selectively filtered in the quadrupole, and detected in the mass spectrometer. The high sensitivity of the GCQ-MS enabled sample size down to the particle level. The thermogram data reduction techniques are described in Ghosh et al. (*14*).

Chemical Analyses. PAH concentrations were determined by analysis of four replicate samples from the sieved and mixed sediment prior to placing in the bioslurry reactors. After the bioslurry reactors were loaded, samples were collected monthly from each reactor and analyzed for PAHs by Soxhlet extraction and GC/MS analysis for PAHs using EPA Method 3540. GC-FID was used for PAH analyses on whole sediment and separated size fractions to determine PAH distribution by particle size and density. These analyses followed EPA Method 3550B with ultrasonic extraction and silica gel cleanup by EPA Method 3630C (*15*). Samples were analyzed for the 16 PAHs on the EPA Priority Pollutant List as well as for methyl naphthalene. Transmission FTIR analysis was performed with a Mattson ATI FTIR−NIR spectrometer using sediment particles ground and formed into KBr pellets.

Microbial Analyses. Microcosm studies used radiolabeled phenanthrene, pyrene, chrysene, and benzo[*a*]pyrene to confirm that viable PAH-degrading microorganisms were present in the sediment. The aerobic systems were evaluated for acetate, phenanthrene, pyrene, chrysene, and benzo[*a*]-pyrene mineralization. Anaerobic systems were tested for acetate and phenanthrene mineralization. Microcosm studies were conducted on sediment collected from each bioslurry reactor after 18 h of mixing and then monthly thereafter. One gram (wet weight) of slurry material was placed into 15-mL Teflon-lined screw-cap test tubes to which was added 2.7 mL of the modified Stanier's basal media (*17*) and 20 000 DPM amount of ^{14}C-labeled substrate in triplicate for each substrate. A modified version of the method described by Fulthorpe et al. (*21*) assessed microbial activity in which glass fiber filters (Whatman, Maidstone, U.K.), 10 mm diameter and saturated in 1 M barium hydroxide, were placed into the caps of screw-top test tubes to trap evolved $^{14}CO_2$ (*31*). The filters were placed in 15-mL scintillation cocktail (Ultima Gold, Packard Instruments Co., Downers Grove, IL) and counted twice with a top count microplate scintillation counter (Packard Instrument Co., Downers Grove, IL). Counts were corrected for background and counting efficiency using the external standard method described by the manufacturer.

Polar lipid fatty acid analyses (PLFA), as described by White and Ringelberg (*22*), identified the level of biomass present in the sediment and provided information about the microbial community structure. The total lipid extractant was separated into neutral, glyco-, and polar lipid fractions (*23*) and prepared for gas chromatography/mass spectrometry (GC/MS) by mild alkaline methanolic transesterification (*31*). Peak identities were confirmed using a gas chromatograph-mass selective detector (Hewlett-Packard GC6890-5973 MSD) with electron impact ionization at 70 eV. DNA analysis targeted the presence of select genes known to be present during active PAH degradation. Total DNA was isolated from triplicate 500-mg slurry samples using a mini-bead beater system as described by Borneorneman et al. (*24*) and purified using Fast DNA SPIN Kit (FastPrep7 Instrument FP120; BIO 101, Vista, CA). Total DNA was suspended in 50 μL of molecular biology grade H_2O (Five prime-Three prime, WI) and stored at −20 °C (*31*).

Earthworm Bioassays. Earthworm acute toxicity and bioaccumulation bioassays evaluated the adverse effects of the sediment PAHs to *Eisenia fetida*. In as much as dredged sediment for the Milwaukee CDF was disposed on land and reuse of dredged sediment would involve terrestrial application, earthworm bioassays were determined to be most appropriate test for toxicity end point assessments. The use of these organisms as indicators of environmental pollution is well-documented (*25*, *26*). Acute toxicity was assessed by earthworm survival in 14-day tests following EPA procedures (*27*, *28*). Earthworms were exposed in a static system of the untreated sediment mixtures and artificial soil (70% sand, 20% kaolin, and 10% peat). Ten concentrations of sediment (100%, 75%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78%, and 0%) were prepared in triplicate, where the percent concentration refers to the amount of contaminated sediment present in the test with 100 g dry weight total in covered 250-mL flasks with air holes. The 0% concentration (100% artificial soil) served as the control. After being mixed, the test sediment was hydrated to 75% of water-holding capacity with deionized water. Ten earthworms were placed into each jar on the surface of the sediment. The jars were capped and stored in a humidity-controlled room at 25 °C. Test results were based on earthworm survival.

Earthworm bioaccumulation tests followed a modified ASTM method E1676-97 (*29*) where 500 g of sediment was collected from each reactor at the conclusion of the bioslurry tests. After the sample was homogenized, three test containers were filled with 160 g each. Sixteen earthworms were added to each test container. The sediment and artificial soil were hydrated to 75% of water-holding capacity, covered with cotton muslin cloth to allow the passage of air, and stored in a humidity-controlled room for 28 d. A few flakes of powdered oatmeal were added to the surface of each test container to attract the earthworms to the surface, resulting in greater earthworm mobility and activity. At the end of the 28 d, the earthworms were rinsed with dechlorinated water and placed in glass vials lined with filter paper. The worms remained in the vials (2 per vial) for 1 d in order to allow their gullet to cleanse. The worms were then placed in a −70 °C freezer. On the day of chemical analysis, the worms were removed from the freezer and freeze-dried in a lyophilizer, producing a dry earthworm powdery material for Soxhlet extraction (EPA Method 3540) and analysis by GC/MS. Bioaccumulation tests were performed on untreated and final biotreated sediment.

Results and Discussion

Sediment Composition. Composite sediment samples were separated into two density fractions: (i) specific gravity <1.8 comprising mainly coal-derived material with some plant and woody matter and (ii) specific gravity >1.8 comprising clay, silt, and sand. Elemental analyses using SEM with wavelength dispersive X-ray showed silica the most abundant material in the heavier, >63 μm size fraction, and clays dominant in the heavier, <63 μm size fraction (*15*). The second most abundant particle class within all size fractions comprised high-carbon content black particles, principally coal-derived with some coal as determined by petrography microanalysis under reflected light of polished particles (ASTM standard methods for coal analysis: D2797, D2798, D2799). Historic coal coking, coal gasification, and coal shipping operations are believed to be the source of this material (*30*). The sediment mass distribution by size and density showed clay and silt comprised about 60% of the sediment by weight and sand particles greater than 63 μm about 35% of the sediment. Thus, the heavy sediment fraction constituted 95% of the sediment by weight, while the light fraction, comprising coal-derived, wood material, and plant debris constituted the remaining 5% of the sediment weight.

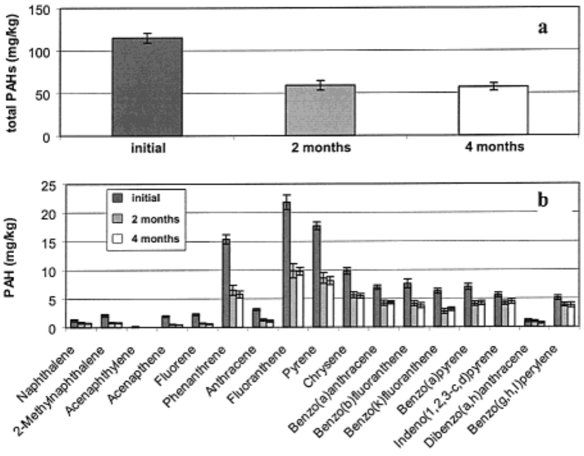

FIGURE 2. Bioslurry treatment results: (a) total PAH concentrations for initial sample, second and fourth month; (b) individual PAH concentrations for initial sample, second and fourth month. There was a 49% reduction of total PAHs by month 2 and a 50% reduction by month 4. Error bars indicate ± 1 SD.

Distribution of PAHs in Sediment. Total PAHs were 115 ± 5.7 mg/kg as determined by Soxhlet extraction and GC/MS analysis of composite samples prior to bioslurry treatment. The distribution of PAHs in whole sediment is shown in Figure 2, among which fluoranthene had the highest concentration at 21.8 mg/kg. Ten of the 17 PAH compounds exceeded either the Wisconsin suggested generic soil cleanup levels (*2*) or the solid waste cleanup levels for the beneficial use of industrial byproducts (*3*, *4*), as indicated in Table 1.

The coal-derived/wood material and debris contained 62% of the total PAHs, with 38% of the total PAHs associated with the sand, clay, and silt in the sediment (*15*). Although the clays and silt were the most abundant component in the sediment at about 60 wt %, this size fraction accounted for only about one-third of the total PAHs. A significant observation is that the coal-derived/wood material and debris comprised only 5% of the sediment mass but contained more than three-fifths of the total PAHs. The clay/silt fraction contained about 52 mg/kg total PAHs as compared to as much as several thousand mg/kg total PAHs in the light <63 μm size fraction. Additional analysis involving separation of wood-derived particles from coal in the lighter density fraction showed more than 95% of PAHs in the lighter fraction were associated with coal-derived particles. Thus, we refer to this lighter density fraction as coal-derived in the rest of the paper.

Reduction of PAHs by Bioslurry Treatment. Bioslurry treatment was conducted for 4 months with one reactor sacrificed after 2 months. There was a small amount of reduction of PAHs for the control reactors in which samplings at 2 and 3 months showed average PAH levels of 100 mg/kg as compared to 115 mg/kg initially. This slight decrease in total PAH concentration was judged not significant and was most likely a consequence of the difficulty of sampling representatively from the reactors versus sampling from the initial whole sediment composite prior to filling the reactors.

Average concentrations of total and individual PAHs for the active bioreactors for the initial sample before biotreatment and at months 2 and 4 after biotreatment are shown in Figure 2. No significant change in PAH concentrations occurred between 2 and 4 months of treatment, and thus the

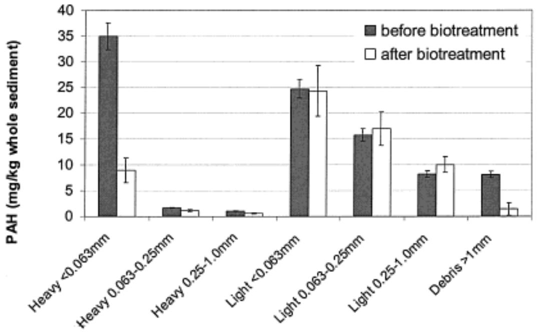

FIGURE 3. PAH reduction in sediment fractions before and after bioslurry treatment. These values are based on the contribution of each fraction to the total PAH concentration of 90 mg/kg as measured by GC-FID. Error bars indicate ± 1 SD based on analysis of four reactors.

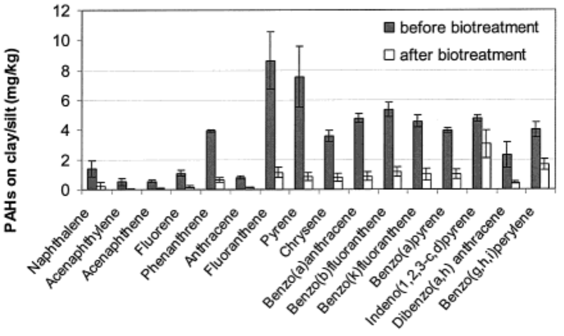

FIGURE 4. PAH concentrations for the Milwaukee CDF clay/silt fraction (heavy, $<$63 μm) before and after bioslurry treatment. Error bars indicate ± 1 SD based on analysis of four reactors.

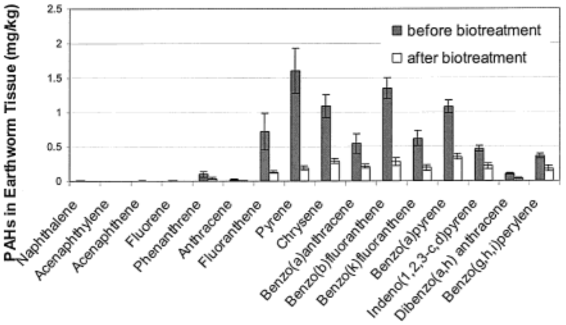

FIGURE 5. Measurement of the bioaccumulation of PAHs by earthworms (*Eisenia fetida*) from Milwaukee CDF sediment before and after biotreatment. Error bars indicate ± 1 SD.

2-month reactor data were included in an overall average for determining final PAH reduction.

The aerobic bioreactors showed a net reduction in total extractable PAH of approximately 50% over the 4-month study from an average of 115 mg/kg to 57 mg/kg. As shown in Figure 2b, all PAH compounds decreased in concentration. The greatest decrease was 64% that occurred for PAHs in the 128−178 MW range, followed by 51% reduction in the 202−228 MW range. The higher molecular mass compounds in the 252−278 MW range showed an average 38% decrease.

A total of 500 g of sediment samples was collected from each of the four bioslurry reactors after 2 or 4 months of treatment to assess PAH biodegradation by examination of sediment particle size and type. Average PAH reductions by size fraction for all reactors are shown in Figure 3, and these data reveal a significant difference in the extent of PAH biodegradation among the sediment particle types. A marked distinction in the biotreatability of PAHs among the heavy and light density fractions was evident. PAH biodegradation was achieved mainly within the $<$63 μm heavy fraction comprising the clays and silt, for which there was 75% reduction in PAHs. The coal-derived material, which was the principal PAH-containing component in $>$63 μm to 1 mm size fractions, showed no reduction in PAH concentrations. The apparent reduction in the PAHs in the $>$1 mm light fraction, which included more friable woody material and debris, is believed to be due attrition rather than biodegradation because the particle mass decreased in this size fraction during bioslurry treatment.

The data in Figure 3 suggest that the PAHs on the $<$63 μm clay/silt fractions are largely available for biodegradation and that the PAHs on the coal-derived $<$63 μm to 1 mm size fractions are not available to the microorganisms during bioslurry treatment. The lack of change of PAHs in the coal-derived fraction clearly indicates that the PAHs on this fraction are not bioavailable to the microorganisms. In contrast, data in Figure 4 show for the $<$63 μm clay/silt fraction that all PAHs monitored in this study were reduced as a result of biotreatment of the sediment. The largest percent reduction ($>$85%) was achieved for fluoranthene and pyrene, which were the most prominent PAHs in the clay/silt fraction.

Biodegradation Capability of Indigenous Microorganisms. Radiolabeled microcosm studies were performed with material from bioslurry bioreactors to assess whether Milwaukee CDF sediment contained indigenous microorganisms capable of degrading PAHs. These results showed that viable PAH-degrading organisms were present in the sediment throughout the study and that mineralization rates for each of the PAHs examined was greatest at 1 and 2 months.

PLFA analyses revealed that microbial biomass was relatively low in the control reactors averaging less than 8000 total pmol of PLFA g^{-1} through 3 months. Biomass in the active reactors ranged from slightly less than 10 000 total pmol of PLFA g^{-1} initially to almost 50000 total pmol of PLFA g^{-1} at 2 months, decreasing to about 30 000 total pmol of PLFA g^{-1} by 4 months (*31*). Biomass increased during the first 2 months of the study during the time that the total PAH concentration showed a concomitant decrease in concentration. These data provide supporting evidence of a microbial link to the PAH degradation and that the aerobic reactors contained viable PAH-degrading microorganisms.

The DNA analyses of the sediment at the start of the study showed gene encoding enzymes associated with aromatic degradation (naphthalene dioxygenase, biphenyl dioxygenase, catechol 2,3-dioxygenase, toluene monooxygenase, and alkane hydroxylase) were present in copy numbers below the detection limit of the assay ($<$1 gene copy per gram). By the first and second month of bioslurry treatment, the copy numbers for each of the genes assayed increased to the maximum detectable number—10 000 copies g^{-1}. This correlates strongly to the microbial biomass increase and to the decrease in PAH concentrations. The increase in the copies of these genes and increase in microbial biomass is consistent with observed mineralization response for phenanthrene, pyrene, and chrysene detected in the microcosm studies.

Earthworm Toxicity and Bioaccumulation. Earthworm acute toxicity tests with sediment prior to biotreatment showed a 100% survival rate, indicating that the PAHs present initially in the sediment were not toxic to induce death. Earthworm bioaccumulation tests were performed on sediment before bioslurry treatment and after 4 months of bioslurry treatment. The bioaccumulation results for individual PAHs are shown in Figure 5. These data comprise an average of the results from 192 earthworms exposed to sediment before treatment and 144 earthworms exposed to sediment after 4 months of bioslurry treatment. Each reactor was evaluated in triplicate with 16 earthworms per subsample for extraction and analyses. The uptake of total PAHs in earthworm tissue from the untreated sediment was 8.1 mg/kg. Earthworm uptake after bioslurry treatment was reduced to 2.0 mg/kg. This represents a 75% reduction in total PAH uptake. Seven PAHs (naphthalene, 2-methylnaphthalene, acenaphthene, fluorene, fluoranthene, pyrene, and benzo[*b*]fluoranthene) showed reductions in bioaccumulation over 80% as a result of bioslurry treatment. Every PAH showed at least 56% reduction in uptake in the earthworm tissue.

When comparing Figures 4 and 5, a similar trend is apparent. Both the clay/silt fraction (Figure 4) and the earthworm uptake (Figure 5) showed 75% PAH reduction as a result of bioslurry treatment, while the coal-derived fraction showed no reduction in PAHs. Thus, a strong correlation exists between the individual PAHs biodegraded and a beneficial effect on lowered bioaccumulation as measured by earthworm tissue uptake. This observation suggests that only the PAHs in the clay/silt fraction comprise the readily available fraction to both microorganisms and earthworms.

PAH Rate of Release and Availability. The binding and association of PAHs with the silt/clay and coal-derived particles were assessed by PAH rate of release experiments at room temperature, PAH binding activation energy assessments using thermal programmed desorption, and sorbent organic matter characterizations using infrared microspectroscopy.

Desorption kinetic studies were conducted with the whole sediment and separated fractions to investigate the availability of PAHs (*15*). The 100-d desorption kinetic tests with whole sediment showed that approximately 40% of the PAHs were released quickly within about 1 month, while the remaining approximately 60% were strongly bound and not released over 100 d. Thus, approximately 40% of the PAHs in the whole sediment may constitute a readily available fraction. An important finding from tests with separated particles was that the clay/silt and coal-derived fractions showed much different PAH release rates. The PAHs associated with the coal-derived fraction appear strongly bound, as only about 8% of the PAHs in the 63−250 μm coal-derived fraction were released in 100 d. In contrast, desorption data from the clay/silt fraction indicate a higher availability with nearly 80% of the PAHs in this fraction readily desorbing within two months. These trends mirror the results from the bioslurry tests, wherein the majority of the PAHs in the clay/silt fraction apparently are bioavailable and degraded in comparison to the coal-derived material. The majority of the PAHs in the coal-derived material apparently are not bioavailable during bioslurry treatment nor desorbed in long-term tests. Thus, a supposition from these data is that the majority of the PAHs associated with the coal-derived particles may not be bioavailable for degradation nor exhibit a highly toxic response as inferred from the bioslurry and bioaccumulation tests.

Thermal Programmed Desorption Analysis of PAH Release from Untreated Sediment. The thermodynamics of the desorption process for the $<63\ \mu$m clay/silt fraction and the 63−250 μm coal-derived fraction was investigated by the effect of temperature on PAH desorption, as measured by thermal programmed desorption MS. Room temperature desorption data for individual PAH mass homologues was used to determine the fast and slow desorbing PAH fraction and the respective apparent first-order or effective diffusive rate for four PAH mass homologues in each particle class (*14*). The effect of temperature on the desorption rate was modeled as an activated first-order or diffusion process with the activation energy determined by fitting the thermal programmed desorption data. These results showed that the coal-derived and clay/silt particles had much different PAH desorption activation energies. PAH binding with the coal-derived particles was associated with high activation energies, i.e., in the range of 115−139 kJ/mol. Activation energy values greater than 60 kJ/mol may be associated with diffusion through polymeric materials (*32*, *33*). In contrast, PAHs associated with the clay/silt material had much lower desorption activation energies, i.e., in the range of 37−41 kJ/mol. Low values of desorption activation energies in the range of 10−50 kJ/mol have been reported for pore diffusion (*33*, *34*). TPD analysis of coal-derived particles after bioslurry treatment showed little or no change in response, while TPD analysis of clay/silt particles showed marked decrease in mass ions, consistent with PAH reduction in this fraction.

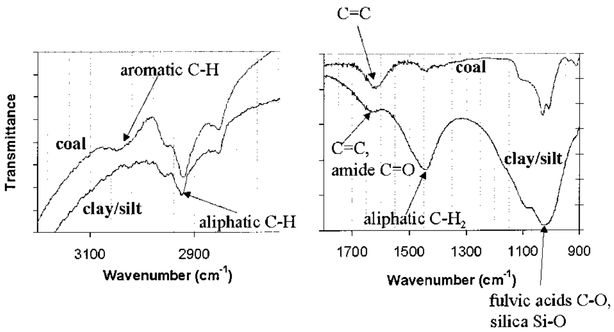

FIGURE 6. Comparison of infrared absorbance spectra of coal-derived particles in sediment and clay/silt particles. The coal-derived particles show higher absorbance of aromatic $\mathrm{C{-}H}$ stretch and a higher absorbance for $\mathrm{C{=}C}$ indicating a much more aromatic nature of the coal−carbon as compared to the organic matter associated with the sediment clay/silt matrix. The clay/silt material also showed higher absorbance for oxygen-containing groups.

Infrared Analysis of Sorbent Organic Matter. Infrared (IR) absorption analysis investigated the differences in organic matter character between the two primary classes of sediment particle types in the Milwaukee Harbor sediment. Separated clay/silt particles and coal-derived particles from the sediment were ground and mixed with KBr to form a pellet for transmission IR analysis. As shown in Figure 6, the IR absorption spectra of the coal-derived particles and the clay/silt particles have marked differences. The coal-derived particles show a higher absorbance of aromatic $\mathrm{C{-}H}$ stretching vibrations and $\mathrm{C{=}C}$ stretching vibrations indicating a much more aromatic nature of the coal−carbon as compared to the organic matter associated with the sediment clay/silt matrix. The clay/silt material also showed higher IR absorbance associated with oxygen-containing groups and aliphatic $\mathrm{C{-}H_2}$ bending vibrations. Thus, the coal-derived particles are more aromatic and nonpolar and provide a substrate that is more conducive to the binding of the highly aromatic PAH molecules. For example, it has been shown that carbon-normalized partition coefficients (K_{oc}) of phenanthrene and TCE for highly aromatic sorbents such as soot and coal are 2−3 orders of magnitude higher as compared to that for natural organic matter (*35*−*37*). The differences in release rates and desorption activation energies of PAHs may thus be attributed to fundamental differences in the organic matter to which the PAHs are bound. These characteristics are then manifested in larger-scale phenomena of PAH biodegradability and bioaccumulation.

Relevance to Sediment Management. We show in this work that the PAHs associated with the fine clay/silt fraction are potentially the PAH contaminants of greatest concern. PAHs associated with the clay/silt fraction comprise about one-third of the total PAHs in this sediment, and this is the PAH fraction that is much more mobile and biodegradable, and if reduced in total mass results in substantial improvement with respect to earthworm bioaccumulation. In contrast, PAHs associated with coal-derived materials are much less available, not biotreatable, and apparently are not significant with respect to bioaccumulation by earthworms. Thus, an hypothesis from our work is that PAHs in the coal-derived fraction are potentially much less of a concern for protection of the environment, even though the PAHs in this fraction comprise the majority of the PAHs in the sediment. Consequently, decisions about dredged sediment quality criteria and treatment and material reuse should focus on the PAHs associated with the available fraction, which in this case comprises those PAHs in clays and silt. Determining which sediments pose greatest risk and which do not is of greatest concern in deciding whether sediments need active management. The work presented here provides support for the hypothesis that total concentrations of a particular hydrophobic organic contaminant in sediment does not translate directly to risk.

The approach presented in this work, which included both particle-scale mechanistic characterizations and larger-scale bioassay tests, provides a framework to improve decision-making regarding the management and treatment of PAH-impacted sediments. We demonstrate that PAH behavior at the particle-scale is relevant to understanding larger-scale phenomena of PAH contaminant availability and bioaccumulation. By recognizing that both labile and residual fractions of PAH contamination may exist and that these fractions are associated with identifiable sediment components, it is possible to justify treatments that focus on the fraction of concern. This will increase the likelihood for reuse of dredged and deposited sediment and suggest alternatives for in situ management, while being protective of the environment.

Some suggestions on how the new knowledge in this study might be applied include the following. Biotreatment to a low bioavailability end point may be a reasonable objective. An intensive treatment process to separate the high- and low-risk sediment fractions might be economical in some instances. Addition of coal-derived carbon to sediments as a treatment approach may promote in situ stabilization of organic contaminants. More broadly, using information from this work to understand and explain low bioavailability of PAHs and other hydrophobic organic contaminants in sediments and perhaps to justify low-intensity management approaches will be an important benefit.

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