ERDC/TN EEDP-02-31
April 2003

LONG-TERM EFFECTS OF DREDGING OPERATIONS PROGRAM

Development of Sediment Extracts for Rapid Assessment of Organic Contaminant Bioavailability

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PURPOSE: This technical note describes the development of sediment extraction procedures for evaluating the bioaccumulation potential of non-polar organic contaminants from dredged material. Potential approaches are described and the experimental procedure of the sediment extraction approach for assessing bioavailability is outlined.

BACKGROUND: Federal regulations (Clean Water Act 404b1 and Marine Protection Research and Sanctuaries Act 103) require that biological evaluations be conducted to determine the suitability of dredged material for placement in open water. These evaluations include assessing the biological effects and the potential levels of bioaccumulation that could result from the presence of chemical contaminants in the sediments. In the tiered approach that is used, the complexity of the evaluations increases as the tier number increases. Sediment bioassays, which are performed in Tier III and IV evaluations, are time-consuming and expensive. Even more time and costs will be added when methods for chronic sub-lethal testing are eventually agreed upon and adopted by the regulatory agencies. However, these bioassays are considered definitive for deciding among disposal options for contaminated dredged sediments. Existing approaches can be improved by implementing methods that distinguish the bioavailable fractions of contaminants in dredged materials from the total amount of the contaminant present.

INTRODUCTION: Total sediment chemical analysis is often used to estimate the bioavailable fraction of non-polar organic contaminants in dredged material. Total chemical analysis, however, often overestimates the fraction of bioavailable contaminant in sediment (Weston and Mayer 1998, Schuler and Lydy 2001). This results in the use of time-consuming, expensive bioassays to determine contaminant bioavailability in dredged materials. In-vitro (sediment extract) bioassays have the potential of providing rapid, low-cost, mechanistically interpretable alternatives to these in vivo, whole sediment tests, but are presently severely limited by the inability of sediment extracts to accurately reflect contaminant bioavailability. The use of solvent extracts in in-vitro assays consistently produces false positives. In-vitro tests that could provide results that consistently and accurately forecast the results of the definitive in-vivo bioassays would greatly decrease the expense of dredged material evaluation.

Bioavailability of non-polar organic contaminants in dredged materials is affected by geochemical factors such as soot concentration (Gustafsson et al. 1997), organic carbon aromaticity (Grathwohl 1990; Brannon et al. 1998), and other factors that are not as clearly defined. The research presented in this note focuses on development of extractants that respond to changes in sediment geochemistry known to affect the bioavailability of polyaromatic hydrocarbons (PAHs) from whole sediments. Chemical extractants that respond to changes in sediment bioavailability will remove less contaminant as sediment bioavailability decreases. Soot amendment was used to change sediment contaminant bioavailability and to determine if contaminant extractability was related to changes in sediment bioavailability. Soot in sediment has been shown to affect availability of PAHs to exposed

organisms (Gustafsson et al. 1997). Soot preferentially absorbs non-polar organic contaminants such as PAHs more than they are absorbed by organic carbon. However, the resulting adsorbed PAH is much less bioavailable from soot. Spiked sediment was subjected to extractants of different concentrations and compositions to determine which of them respond to changes in sediment soot concentration and the corresponding changes in PAH bioavailability. This technical note presents results of sediment extraction and bioaccumulation tests with different levels of soot and identifies the extractants most likely to predict PAH bioavailability.

MATERIALS AND METHODS

Adsorption Testing. Sediment was obtained from a freshwater lake (Brown's Lake) located at the ERDC, Vicksburg by using a grab sampler. The sediment is a silty loam comprised of 11 percent sand, 78 percent silt, and 11 percent clay with a total organic carbon content of 0.83 percent. Adsorption tests were conducted in triplicate in 25-ml centrifuge tubes containing 4 g (oven dry weight (ODW)) of sediment or 0.01 g soot in the form of diesel particulate matter (Standard Reference Material 2975) (Standard Reference Materials, Gaithersburg, MD). Tests were spiked with 16 ml of distilled deionized water containing the PAH phenanthrene at concentrations of 0.5, 0.4, 0.3, 0.2, or 0.1 mg/L. Solutions of phenanthrene were composed of 1-percent radiolabeled (^{14}C-phenanthrene, specific activity 13.1 mCi/mmol; Sigma Chemical Co., St. Louis, MO) to 99-percent unlabeled phenanthrene. Following addition of the solutions, the sediment tests were shaken for 24 hr on a reciprocating shaker at 180 excursions/minute. Samples were centrifuged at 8,000 rpm for 30 min. One ml of supernatant was removed and counted in 15 ml of Ultima Gold Liquid Scintillation Cocktail (Packard Instruments, Meridan, CT) on a Packard Tricarb 2500 TR Liquid Scintillation (LS) Counter (Packard Instruments, Meridan, CT).

Selection of Sediment Extraction Technique. The available methods for extraction of hydrophobic compounds from sediments were investigated ((Kelsey et al. 1997; Tang and Martin 1999; Schuler and Lydy 2001; Van Hoof and Hsieh 1996; U.S. Environmental Protection Agency (USEPA) 1996)). Methods primarily described sediment extraction using sonication and soxhlet extraction techniques.

Preliminary investigations focused on sonication methods because soxhlet extraction is very vigorous and less likely to reflect changes in sediment bioavailability. Sediment samples were spiked at a concentration of 10 ug/g ODW using the same concentration ratio of labeled and unlabeled phenanthrene as described previously. Tests were shaken for 24 hr, then aged for a 5-day period at room temperature (~ 24 °C).

After the 5-day incubation, 5 g ODW of the spiked sediment was added to 25-ml Corex glass centrifuge tubes. A standard solvent mixture recommended for sediment extraction of PAHs was used for initial extraction testing (USEPA 1996). The solvent mixture consisted of 75 percent methylene chloride : 25 percent acetone solution; 15 ml was added to each 5-g sample. Six different sonication techniques were applied to the sediment samples. Duplicate samples were extracted using the following methods: extraction in a Branson Model 8510 sonic bath for 30 min (Technique 1, Chuang (1996)); 60 min (Technique 2, Chuang (1996)); 18 hr (Technique 3, USEPA (1996)); and 60 min with an 18-hr rest period followed by another 60-min sonication (Technique 4, Van Hoof and Hsieh (1996)). A sonic probe (Fisher Scientific Model 550 Suwanee, GA) was also

used and extractions were made for 1 min (Technique 5, Schuler and Lydy (2001)) and 3 min (Technique 6, SW-846 USEPA (1996)) with 15-sec pulses.

Selection of Solvents. Several solvents and solvent mixtures were evaluated to determine their response to changes in sediment phenanthrene extractability as a function of soot concentration (Table 1). Two Brown's Lake sediment samples were spiked with both unlabeled (9.9 ug/g ODW) and radiolabeled phenanthrene (0.1 ug/g ODW) using methods previously described. The samples were allowed to age for 5 days prior to extraction. Soot was added to one of the sediment samples at a concentration of 0.1 percent ODW. Extractions were conducted in triplicate with each of the extractants. First, 3 g (ODW) sediment was added to 25-ml glass centrifuge tubes. Then, 10 g of sodium sulfate was added to each test and mixed with the sediment to promote free-flow of the sediment and uniform extraction. Next, 15 ml of extractant was added and tubes were shaken to mix sediment and solvent. Tests were placed into a sonic water bath for 60 min, allowed to sit for 18 hr with no sonication, and sonicated for an additional 60 min. Tests were centrifuged at 8,000 rpm for 30 min. As previously described, 1 ml of extract was counted by LS.

Table 1
Percent Phenanthrene Extracted from Sediment With and Without Soot, Mean (standard error)

Solvent	0% soot	0.1% soot
Methylene chloride	107 (10)	78 (2.5)
Hexane	100 (5.8)	77.6 (2.1)
1:1 methylene chloride:hexane	109 (3.2)	68.5 (7.8)
Soft water[1]	1.63 (.34)	0.93 (.16)
1:1 methanol:DDI water[2]	81.7 (2.1)	67.2 (1.4)
9:1 methanol:DDI water	113 (0.58)	105 (4.1)
Propanol	107 (0.88)	92 (2.2)
Pressurized fluid extraction (comparable to soxhelet)[3]	100 (7.1)	100 (3.1)
Acetonitrile	102 (5.1)	95.6 (1.5)
Methanol	94.5 (1.6)	91 (1.0)
1:1 methanol:methylene chloride[3]	92.6 (2.4)	89.7 (4.8)
50:46:4 methylene chloride:hexane:acetonitrile	84.5 (8.7)	83.1 (5.8)
Moderately hard water[3,4]	0.78 (.10)	1.18 (.33)
Hard water[3,5]	0.96 (.17)	1.35 (.67)
75:25 hexane:acetone[3]	93.8 (.21)	95.4 (5.7)
Octanol[3]	80.5 (.13)	81.9 (3.5)
Acetone[3]	89.5 (8.2)	93.6 (3.5)
50:50 acetone:methylene chloride[3]	82.1 (8.2)	92.8 (3.5)
80:20 acetone:water[3]	73.9 (1.1)	93.4 (1.3)
75:25 methylene chloride:acetone[3]	73.6 (1.7)	82.7 (11)
50:50 acetone:hexane[3]	96.9 (3.3)	126 (1.5)
50:50 hexane:acetonitrile[3]	73.8 (2.2)	100 (6.8)
75:25 hexane:acetonitrile[3]	81.8 (3.7)	95.4 (.44)

[1] Soft water – reconstituted synthetic water with a hardness of 40 mg/L.
[2] DDI – distilled deionized water.
[3] No decrease in phenanthrene extractability as a function of soot.
[4] Moderately hard water – reconstituted synthetic water with a hardness of 77 mg/L.
[5] Hard water – reconstituted synthetic water with a hardness of 122 mg/L.

Sediment Extractions as a Function of Soot Concentration. The effects of soot concentration and short-term aging on phenanthrene extractability from sediment were investigated using four of the seven tested extractants. Four separate samples of Brown's Lake sediment were spiked with both unlabeled and radiolabeled phenanthrene at the same concentration using methods identical to those previously described. Soot was added to three of the sediment samples at concentrations of 0.1, 0.25, and 0.5 percent ODW of each sediment sample. One sample was not spiked with soot so that extractability of phenanthrene could be compared between treatments. Triplicate sample extractions were conducted 5, 10, 15, and 20 days after phenanthrene and soot were added.

Biological Exposures. Adult *Lumbriculus variegatus* were obtained through Carolina Biological Supply (Burlington, NC), and third instar *Chironomus tentans* through Aquatic Research Organisms (Hampton, NH). The organisms were maintained in aged tap water for at least 5 days prior to exposures and fed daily during this acclimation period; *L. variegatus* was fed the food supplied by the vendor, and *C. tentans* was fed TetraMin fish food as recommended by the EPA (USEPA 1999). Cultures were kept at 21 ± 1 °C.

Exposures were conducted in Brown's Lake sediment spiked with 10-ug/g phenanthrene (0.1 ug/g ODW radiolabeled phenanthrene and 9.9 ug/g unlabeled) using methods previously described. Three separate soot concentrations (0.1, 0.25, and 0.5 percent ODW) were also tested. After the initial 5-day incubation period, exposures were conducted in 22-ml glass scintillation vials, with 7 g of sediment (~1 cm depth) and 10 ml of aged tap water. Vials were aerated by inserting a pipette through a polyurethane foam plug, which was in turn connected to an air pump (Figure 1). Water levels and airflow were checked daily, with water being added when the levels dropped. Temperature was kept at 21 ± 1 °C. For each sediment treatment, six replicate vials for each species were set up with either four adult *L. variegatus* or three third instar *C. tentans* per container.

After 10 days of exposure, the organisms were retrieved from the vial by sieving the sediment. Organisms were placed in 1 ml of aged tap water for a 2-hr period to purge sediment from the intestinal tract. Excess water was removed by placing the organisms on a paper towel before transferring them to a clean, pre-weighed scintillation vial. Tissue weight from each replicate was recorded before addition of 0.25 ml of NCS II tissue solubilizer (Amersham Biosciences, Piscataway, NJ). After overnight solubilization, 15 ml of UltimaGold liquid scintillation cocktail (Packard, Boston, MA) was added to each vial. Samples were vortexed, then allowed to sit for 24 hr before scintillation counting to reduce interference from chemiluminescence.

RESULTS AND DISCUSSION

Adsorption Testing. The soot exhibited a partitioning coefficient (K_d) of 1,880 L/Kg compared to 65 L/Kg for the unamended Brown's Lake sediment, a factor of 29 difference. Increasing sediment soot concentrations should, therefore, result in decreasing contaminant extractability and consequently decreasing bioavailability if the extractant is removing primarily bioavailable contaminants.

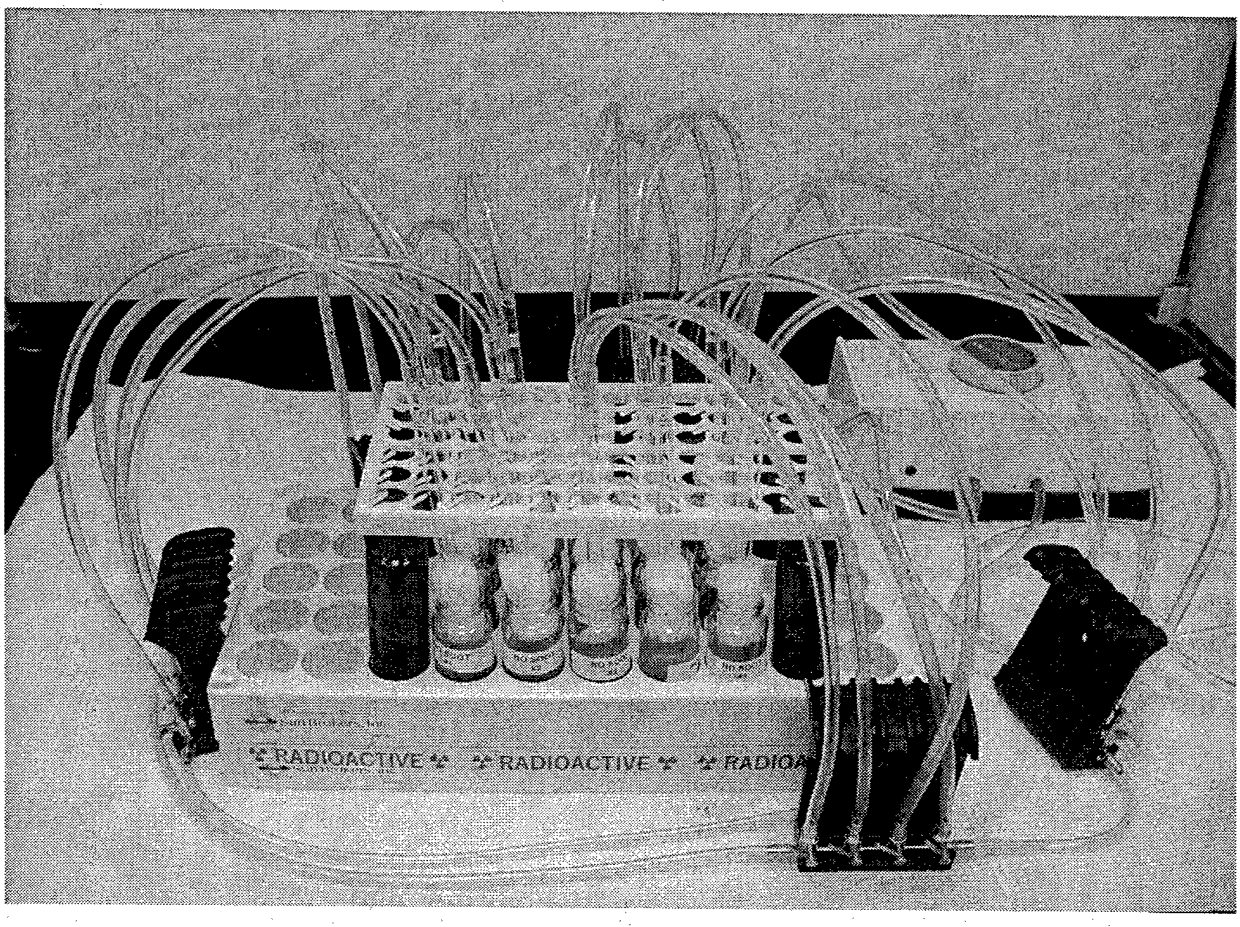

Figure 1. Experimental design of biological exposure laboratory test

Sediment Extraction Technique. Based on the lowest standard deviation between replicates (ensuring reproducible results) and high recovery of sonic bath extraction techniques, the sonication method using two 60-min sonication extractions with an 18-hr rest period between was selected as the technique to use for extracting sediment with various solvents and solvent combinations (Table 2).

Table 2
Percent Recovery of Radiolabeled Phenanthrene and Standard Deviation Among Replicates for Each Sonication Technique

Sonication Technique	Percent Recovery	Standard Deviation
Sonic Bath – 30 min	50	8.1
Sonic Bath – 60 min	45.9	2.83
Sonic Bath – 18 hr	66.4	6.86
Sonic Bath – 60 min/18 hr rest/60 min	54.8	1.13
Sonic Probe – 1 min	12.3	5.16
Sonic Probe – 3 min, 15-sec pulses	11	1.77

Selection of Solvents. Sediment samples with added soot were compared to those not containing soot; solvents exhibiting a decrease in phenanthrene recovery were selected for further investigation (Table 1). The solvents selected included 50:50 methylene chloride:hexane, hexane, methylene chloride, soft water, 1:1 methanol:distilled deionized water (DDI), 9:1 methanol:DDI water, and propanol.

Phenanthrene Extractability as a Function of Soot Concentrations. Hexane and methylene chloride extracts generally removed less phenanthrene as sediment incubation time increased regardless of soot concentration (Figure 2). Decreases in extractability over time were not as clear for soft water and 50:50 methylene chloride:hexane. However, all extractants were lower in phenanthrene concentration for sediment containing 0.5 percent soot.

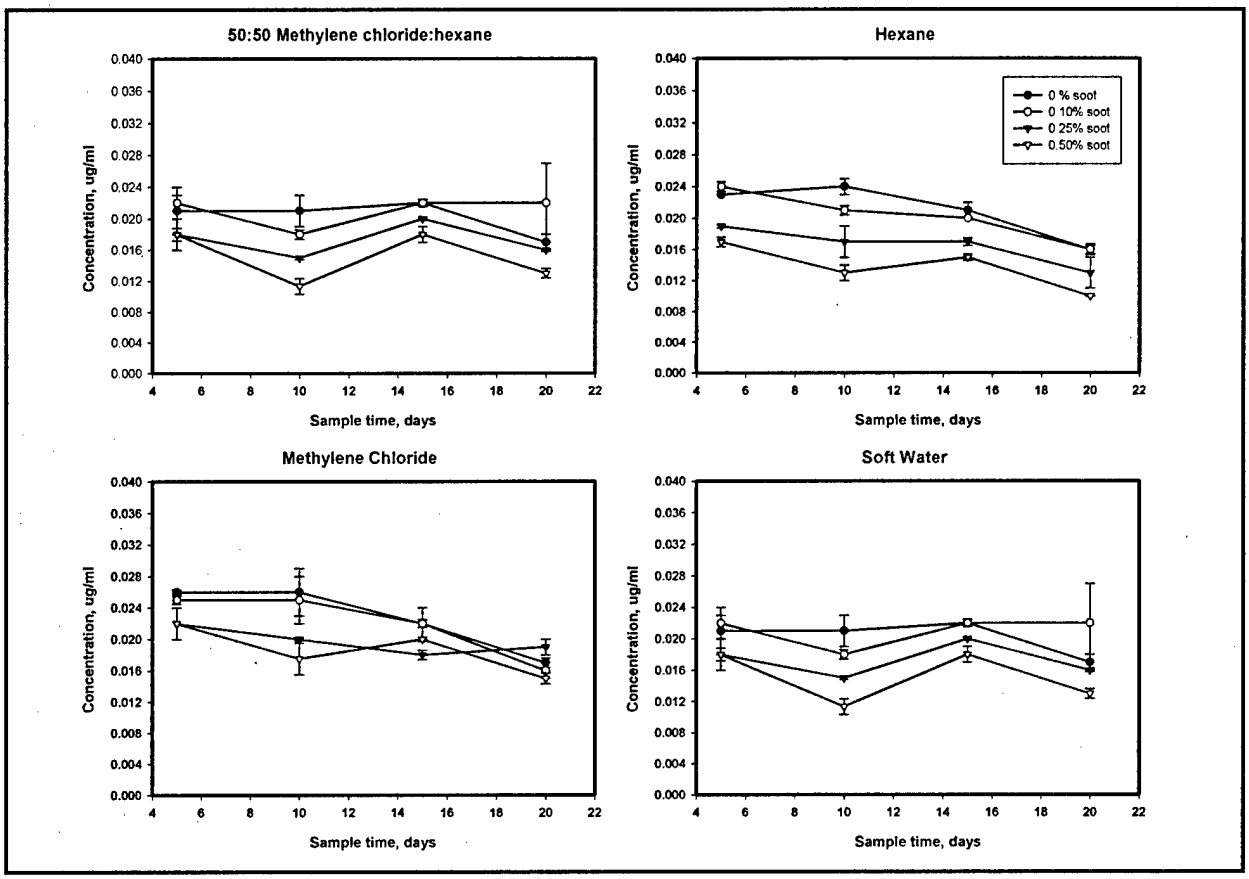

Figure 2. Phenanthrene concentrations over time at each soot concentration for each solvent test

To compare sediment bioavailability to contaminant extractability, day 15 samples for the four solvents evaluated as a function of sediment incubation time were used to correspond to the sediment contaminant contact time of the bioassay testing (5-day pre-incubation and 10-day bioassay). The extraction data for the sediment samples incubated for 5 days and extracted with 1:1 methanol:DDI water, 9:1 methanol:DDI water, and propanol were also compared to bioassay results. Concentrations of phenanthrene decreased in all seven extractants as sediment soot concentrations increased, except for a small increase at 0.5 percent soot with methylene chloride

(Figure 3). Soft water and 1:1 methanol:DDI water showed the largest percent decreases of 79 and 71 percent, respectively, in phenanthrene concentrations between sediment samples amended with 0 and 0.5 percent soot. A 50.6- and 52.5-percent decrease in phenanthrene extractability between the 0 and 0.5 percent soot treatments was seen in the 9:1 methanol:DDI water and propanol extracts, respectively. Phenanthrene concentration decreases of 20 and 28 percent from 0 to 0.5 percent soot amendments were noted for 50:50 methylene chloride:hexane and hexane, respectively. Phenanthrene concentrations in methylene chloride extracts decreased only 8 percent between the highest and lowest soot amendments.

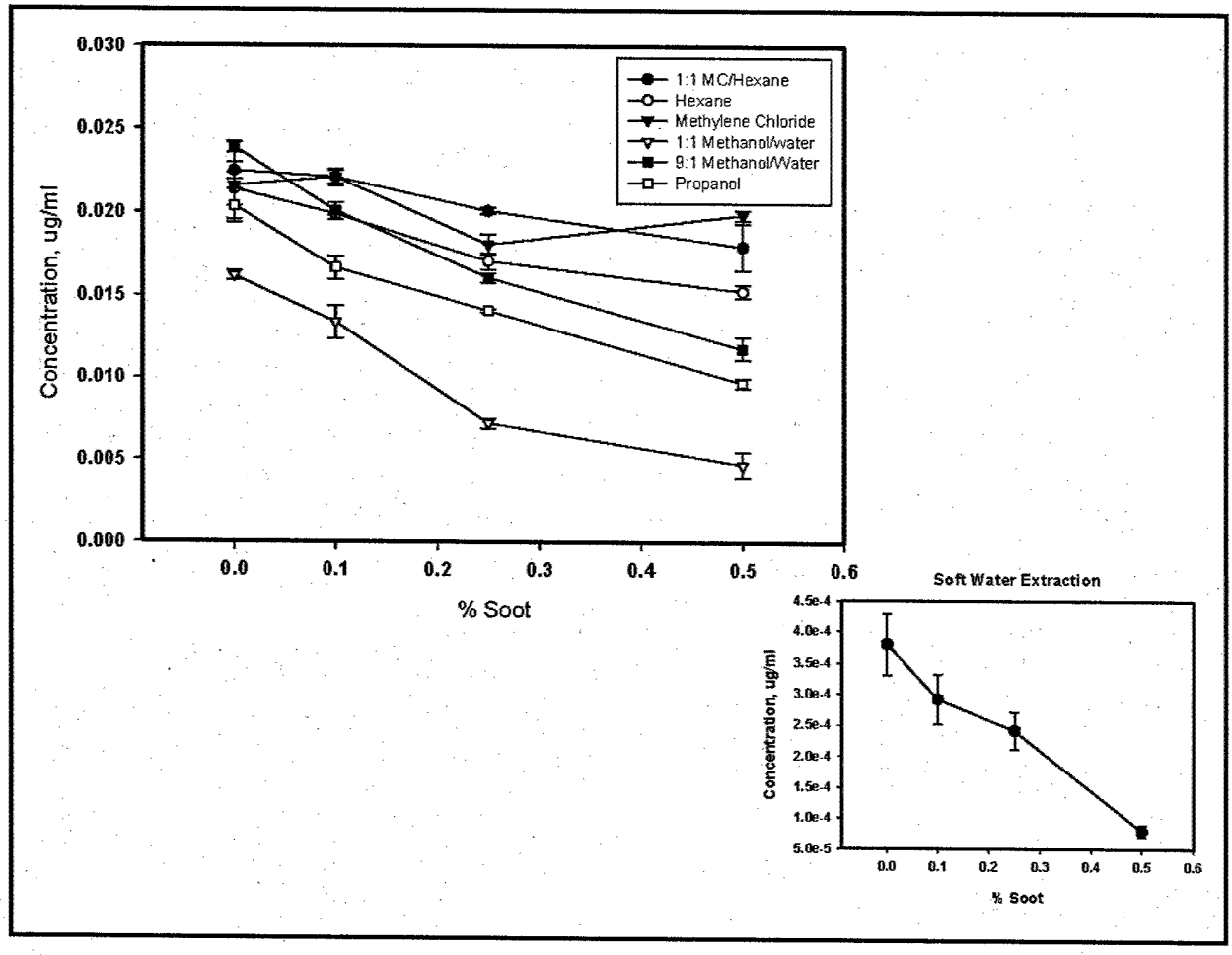

Figure 3. Phenanthrene solvent concentrations 5 and 15 days after addition of phenanthrene and soot

Phenanthrene Bioavailability as a Function of Soot Concentrations. Bioavailability of phenanthrene to both organisms decreased with increasing soot content (Figure 4). Organism radiolabeled carbon content decreased to approximately the same concentration (milligrams wet weight tissue basis) for both species in the 0.5-percent soot sediment treatments. *Lumbriculus* showed the largest percent decrease in bioavailability (66.4 percent) of phenanthrene between sediment samples amended with 0- and 0.5-percent soot as compared to a 55.6-percent decrease in *Chironomus*.

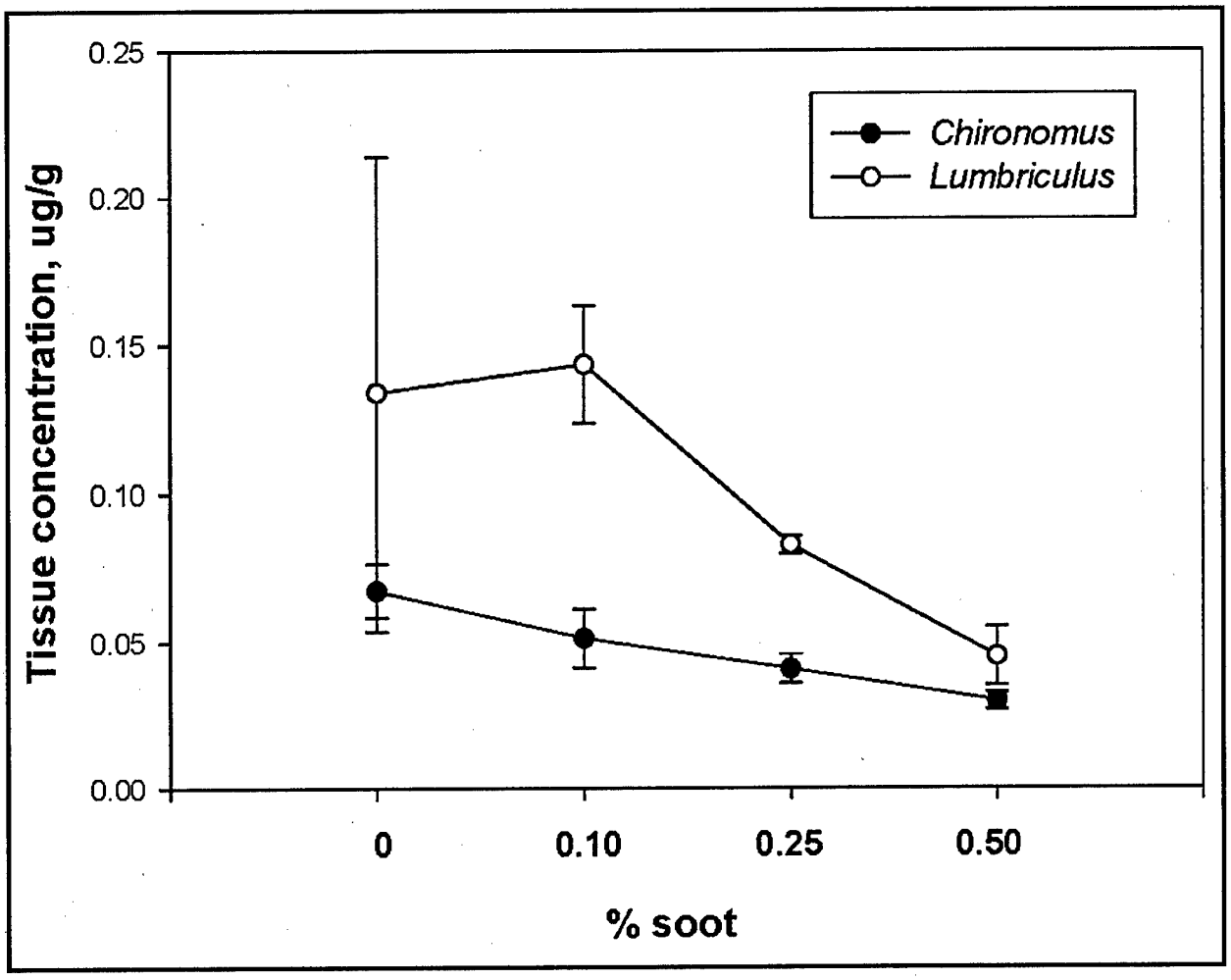

Figure 4. Bioavailability of phenanthrene to two organisms, *Chironomus* and *Lumbriculus*, as a function of soot concentration in sediment

The relationship between bioavailability and extractability of phenanthrene was examined using linear regression analysis with the data representing the amount of compound taken up by the organism or extracted by the solvents. The bioavailability of phenanthrene, in sediment spiked at four soot concentrations, to *Chironomus* and *Lumbriculus* was correlated to phenanthrene extractability for the seven selected extractants (Table 3). Except for methylene chloride, the r values were greater than 0.80. The data show that six of the sediment extractants demonstrate changes in contaminant concentrations that correspond to changes in sediment bioavailability. Both the availability to *Chironomus* and *Lumbriculus* and the amount of phenanthrene recovered by the extractants declined with increasing soot concentrations in the sediment. Despite the fact that the extent of reduction in bioavailability varied with the test organism and the degree of reduction in phenanthrene extractability also varied with solvent, reasonable correlation between the two assay systems was evident.

Table 3
Correlation Between Uptake of Phenanthrene by *Chironomus* and *Lumbriculus* and the Extractability of Phenanthrene by Organic Solvents and Water

Extractant	r Value	
	Lumbriculus	Chironomus
50:50 Methylenechloride:hexane	0.97	0.87
Hexane	0.90	0.96
Soft water	0.81	0.92
Methylene chloride	NS[1]	NS[1]
1:1 Methanol:DDI water	0.89	0.95
9:1 Methanol:DDI water	0.86	0.98
Propanol	0.82	0.98

[1] No significant correlation.

These results proved that simulation of organism bioavailability by solvent extraction of sediments is feasible. The six selected extractants will be further tested with contaminated sediments from field environments and an array of toxicity and bioaccumulation tests.

SUMMARY: An extraction procedure was first identified that gave good recovery and reproducibility. Using this procedure, 23 different solvents were screened to determine their potential usefulness for evaluating changes in bioavailability of phenanthrene. Seven solvents that showed promise were evaluated against phenanthrene bioaccumulation by two different burrowing organisms in sediment with soot concentrations ranging from 0 to 0.5 percent. Bioaccumulation of phenanthrene by the two organisms decreased as sediment soot concentration increased. These trends in organism bioavailability were mirrored by changes in extracted phenanthrene concentrations in six of the extractants. These results proved that simulation of organism bioavailability by solvent extraction of sediments is feasible. The six selected extractants will be further tested with contaminated sediments from field environments and an array of toxicity and bioaccumulation tests.

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> Price, C. Inouye, L., Brannon, J., McFarland, V., and Hayes, C. (2003). "Development of sediment extracts for rapid assessment of organic contaminant bioavailability," *EEDP Technical Notes Collection*, ERDC/TN EEDP-02-31, U.S. Army Engineer Research and Development Center, Vicksburg, MS. *http://www.wes.army.mil/el/dots/eedptn.html*

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