

Effects of Type and Quantity of Organic Carbon on the Bioaccessibility of Polychlorinated Biphenyls in Contaminated Sediments

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Abstract: Organic carbon principally controls sorption and desorption of hydrophobic organic compounds in sediments. We investigated the effects of organic carbon type and quantity on compound bioaccessibility. The desorption of 21 polychlorinated biphenyl (PCB) congeners was determined in spiked sediments amended with black carbon, humic acid, and sawdust at either 3 or 6% organic carbon. Desorption parameters were determined using Tenax sequential extractions and then modeled as operationally defined rapid, slow, and very slow fractions and rate constants. The effects of the amendments on PCB bioaccumulation were also evaluated using *Lumbriculus variegatus*. The lowest and highest PCB bioaccessibilities were observed in the black carbon and sawdust amendments, respectively. The total amount of PCBs desorbed ranged from 3 to 27% for the black carbon amendments, 12 to 55% for humic acid amendments, 16 to 80% for sawdust amendments, and 35 to 89% for controls. The results also showed that desorption of PCBs was slower in 6% amendments than 3% amendments, and this finding was most evident in humic acid and black carbon amendments. Overall, the trend in PCB bioaccumulation was similar to what was found for compound desorption in that the highest PCB bioaccumulation was observed in controls and sawdust amendments, whereas humic acid and black carbon amendments showed lower bioaccumulation. Finally, the 24-h single-point Tenax and bioaccumulation data were fit to a Tenax regression model. The PCB bioaccumulation was effectively predicted by the model, with 80% of the data falling within the 95% confidence intervals. *Environ Toxicol Chem* 2018;37:1280–1290. © 2018 SETAC

Keywords: Desorption; Tenax; Organic carbon; Bioaccessibility; Polychlorinated biphenyl

INTRODUCTION

Once released into the environment, different hydrophobic organic contaminants (HOCs), such as polychlorinated biphenyls (PCBs), pesticides, polycyclic aromatic hydrocarbons (PAHs), and other persistent organic pollutants, bind to the organic carbon, which acts as a natural sorbent in sediments (Ghosh et al. 2003; Pehkonen et al. 2010; Zhu et al. 2005). The overall sorption of a compound in sediments is controlled, in part, by the molecular interactions between the organic carbon and the sorbates, and these interactions determine whether the compound is loosely or strongly sorbed to the organic carbon (Birdwell et al. 2007; Pehkonen et al. 2010; Zhu et al., 2005). The sorption process has important consequences

for transport, degradation, bioaccessibility, and bioaccumulation of HOCs in the environment (Cornelissen et al. 2005; Kukkonen et al. 2003; Pehkonen et al. 2010; Pignatello and Xing 1996). For instance, part of the sorbed compound may be desorbed into the sediment porewater and hence become bioaccessible to organisms with the possibility of toxic effects (Kukkonen et al. 2003; Pehkonen et al. 2010). In this regard, bioaccessibility has been defined as the portion of the total concentration of a compound that is or can become available to organisms and degradation processes, while certain compound fractions remain bound to the sediment (Reichenberg and Mayer 2006). Previous studies using various chemical techniques have reported a correlation between bioaccessibility and bioaccumulation and toxicity of HOCs in sediment (Lydy et al. 2015; You et al. 2011). Tenax has been commonly used as a sorbent for estimating bioaccessible concentrations (Lydy et al. 2015) because of its high affinity and rapid clearance rates for desorbed HOCs (Sinche et al. 2017).

This article includes online-only Supplemental Data.

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Published online 9 January 2018 in Wiley Online Library (wileyonlinelibrary.com).

DOI: 10.1002/etc.4073

To mechanistically explain the release of a compound from sediment organic carbon, a conceptual desorption model based on polymer theory has been proposed (Birdwell et al. 2007). Sediment organic carbon can be thought of as 2 compartments classified by their composition and sorptive capacity (Cornelissen et al. 2005; Pignatello and Xing 1996). These compartments have been described as an amorphous, rubbery organic matter from which HOCs rapidly desorb and as a condensed, glassy organic matter from which desorption was much slower (Lydy et al. 2015). Sorption of HOCs in the rubbery compartment follows the laws of diffusion, and desorption from this compartment constitutes the rapidly desorbing fraction of the compound (Birdwell et al. 2007; Carroll et al. 1994). In contrast, sorption to the glassy compartment follows both diffusion and hole-filling mechanisms, and desorption from this compartment constitutes the slow desorbing fractions of the compound (Cornelissen et al. 2005; Pignatello and Xing 1996). To measure the desorbing fractions hypothesized in the desorption model for a given HOC, sorption isotherms have been proposed (Allen-King et al. 2002). In this regard, the Tenax technique has been used to measure compound desorption over hours or days utilizing sequential Tenax extractions of contaminated sediments (Cornelissen et al. 1997). As a result, the apparent sorption capacity of different compartments represented by a rapidly desorbing fraction (F_{rap}), a slow desorbing fraction (F_s), and a very slow desorbing fraction (F_{vs}) along with their respective desorption coefficients can be estimated (Leppänen and Kukkonen 2006).

Several studies have indicated that sorbent characteristics, such as aromaticity and hydrophobicity, are the main cause for differences in the sorption process (Bucheli and Gustafsson 2000; Jonker and Koelmans 2002; Peng et al. 2015). For example, reports indicate that the aromatic moieties in black carbon or soot-like materials control the sorption and distribution of PAHs in sedimentary organic matter (Bucheli and Gustafsson 2000; Jonker and Koelmans 2002). Likewise, Peng et al. (2015) studied sorption and desorption kinetics and thermodynamics of PCB congener 77 to humic acid and concluded that the affinity of humic acid for PCB 77 was mainly attributable to physical sorption rather than chemisorption. Although some of these studies were conducted to investigate the bioavailable potential of HOCs from contaminated or amended sediments, the influence of sediment organic carbon from different origin on the desorption, and hence bioaccessibility, of the same HOCs has not been reported.

In the present study, 3 types of organic carbon (black carbon, humic acid, and sawdust) that represent different environmental sources with widely different physical and chemical properties were used to determine the effect of the organic carbon type and quantity on the bioaccessibility of PCBs. Control sediment was amended at either 3 or 6% organic carbon with individual types of organic carbon. To measure the compound desorption from the amendments, Tenax was used as a simple, rapid, and inexpensive extraction technique. The overall goal was to determine whether Tenax concentrations could be used as a measure of compound bioaccessibility across contaminated sediments varying in organic carbon type and quantity. The specific objectives were to 1) determine the changes in PCB

bioaccessibility among the amendments based on the desorbing fractions, 2) assess the effects of the organic carbon type and quantity on Tenax concentrations, and 3) correlate the observed effects of the organic carbon type and quantity on Tenax PCB concentration with bioaccumulation using concurrent *Lumbriculus variegatus* bioassays.

EXPERIMENTAL PROCEDURES

Reagents and chemicals

A commercial PCB mix (C-WNN, Mix#5; purity >99%) containing 21 congeners in *iso*-octane (10 mg L^{-1}) was purchased from AccuStandard. The following PCB congeners were screened: International Union of Pure and Applied Chemistry number 8 (dichlorobiphenyl); 18, 28 (trichlorobiphenyl); 44, 52, 66, 77 (tetrachlorobiphenyl); 101, 105, 114, 126 (pentachlorobiphenyl); 128, 138, 153 (hexachlorobiphenyl); 170, 180, 187 (heptachlorobiphenyl); 195, 196 (octachlorobiphenyl); 206 (nonachlorobiphenyl); and 209 (decachlorobiphenyl). Surrogates 4,4'-dibromooctafluorobiphenyl (DBOBF) and PCB-186 were purchased from Supelco and AccuStandard, respectively. Internal standard ^{13}C -labeled PCB congeners (^{13}C -PCB-15, ^{13}C -PCB-52, ^{13}C -PCB-141, and ^{13}C -PCB-209) were purchased from Cambridge Isotope Laboratory. All materials and solvents (pesticide grade), including anhydrous Na_2SO_4 , hexane, acetone, and dichloromethane, were purchased from Fisher Scientific. Nitrogen (purity 99.99990%) and helium (99.999990%) were supplied by Airgas. Black carbon (4–14 mesh, CAS no. 7440-44-0) and humic acid (CAS no. 1415-93-6) were purchased from Sigma-Aldrich. The pine sawdust was collected from a local sawmill. Tenax-TA beads (60/80 mesh) were purchased from Scientific Instrument Services.

Organic carbon cleanup

Black carbon and humic acid were separately ground in a mortar and passed through a 63- μm ASTM International sieve (Gilon). The sawdust was ground to break larger particles using a commercial-grade electrical grinder (Cabela's). The ground particles were then passed through the same ASTM International sieve. The sieved black carbon and humic acid particles were washed with a 0.01 M CaCl_2 solution by shaking the suspension in a benchtop orbital shaker (Thermo Fisher Scientific) with daily solution changes (Jonker and Koelmans 2002; Pehkonen et al. 2010; Shaffer and von Wandruszka 2015). The washing procedures were repeated daily for 14 d or until the supernatants of the suspensions were clear. Likewise, the sawdust particles were washed to remove impurities and rinsed thoroughly with distilled water. All of the clean particles were air-dried at room temperature for 1 wk. After drying, the particles were used to prepare the treatments as described in *Sediment amendment preparation*.

Sediment amendment preparation

LaRue-Pine Hills sediment ($3.26 \pm 0.22\%$ organic carbon; Shawnee National Forest, IL, USA) was used as uncontaminated

control sediment. The LaRue-Pine Hills sediment has been used in other studies (Mackebach et al. 2012, 2014). The LaRue-Pine Hills sediment was first passed through a 2-mm ASTM International sieve to remove large debris, then sieved through a 500- μm ASTM International sieve, hand-homogenized, and stored at 4 °C in clean glass jars until use. Prior to preparing the amendment treatments, the organic carbon content in each of the amendment sources was determined (Supplemental Data, Table S1). The LaRue-Pine Hills sediment was then amended with black carbon, humic acid, and sawdust by adding individual sources (by dry wt) to achieve either 3 or 6% organic carbon amendments. Note that the organic carbon amendments were in addition to the native organic carbon present in the LaRue-Pine Hills sediment. The percentage of organic carbon present in amendments and controls is shown in Supplemental Data, Table S2. The final treatments consisted of black carbon amendments at 3 or 6%, humic acid amendments at 3 or 6%, and sawdust amendments at 3 or 6%. There was a gap of 2 wk between preparing 3 and 6% amendments because of logistics. As part of the quality assurance and quality control, a control was included during each preparation.

The amendments were homogenized daily by hand for 15 min, followed by a gentle rolling of the glass jar for an additional 15 min. This homogenization procedure was conducted for 2 wk. After the daily homogenization, the jars were capped and stored at 23 ± 2 °C in the dark. The controls were treated similarly as the amended sediments during the present study. In preliminary studies monitoring the organic carbon content distribution in the amended sediments, we found that a 2-wk period was sufficient to achieve uniformity of the organic carbon within the amended sediments.

Sediment amendment spiking

The controls and amendments were homogenized for 14 d to ensure proper incorporation of the organic carbon amendments and then spiked with a 21-congener PCB mix based on mass (nanograms per gram dry wt). A nominal sediment concentration of approximately 333 ng/g dry weight was added for each PCB congener. After spiking, all samples were homogenized by hand for 15 min, and then the jars were rolled for an additional 15 min. Mixing and rolling of samples were performed daily, and then the jars were capped and stored at 23 ± 2 °C in the dark. The samples were allowed to equilibrate for 30 d to provide time for sorbent/sorbate interactions. After the aging period, these sediments were used for sequential Tenax extractions, single-point Tenax extractions, exhaustive chemical extractions, and bioaccumulation tests as described in the *Extraction Procedures and Analyses* section.

EXTRACTION PROCEDURES AND ANALYSES

Exhaustive chemical extractions

The PCB congener concentrations in controls and amendments were determined via accelerated solvent extractions

(Dionex 200; Thermo Fisher Scientific) and followed methods outlined in Sinche et al. (2017). Details of the procedure are included in Supplemental Data.

Single-point Tenax extraction

Prior to use, the Tenax beads were cleaned using one rinse of acetone (30 mL) and 2 rinses with a mixture of acetone:hexane (1:1, v/v; 30 mL each) and sonicated for a total of 3 h (each solvent rinse included 1 h of sonication). The Tenax was allowed to dry at room temperature (25 ± 1 °C) for a few days (Cornelissen et al. 2001). The procedure for the 24-h single-point Tenax extractable concentrations has been described (Sinche et al. 2017). Additional details of the test methods can be found in the Supplemental Data.

Sequential Tenax extractions

Sequential Tenax extractions were conducted with the spiked controls and amended sediments to determine the desorption parameters (F_{rap} , F_{s} , and F_{vs}). The methods for conducting the sequential Tenax extractions have been published elsewhere (Cornelissen et al., 2001). Briefly, 3 g dry weight of each sediment was added to 50-mL vials, as well as 3 mg of HgCl_2 , 45 mL of moderately hard reconstituted water (Smith et al. 1997), and 0.500 g of Tenax. The vials were then briefly shaken and rotated as described above for 3, 6, 12, 24, 48, 96, 168, 336, and 672 h. At each time point, the Tenax was removed from the vial, transferred to a clean scintillation vial containing 10 mL of acetone, and replaced with 0.500 g of fresh Tenax; and the vials were rotated again until the next time point. Three replicates of each amended sediment sample, blank (control sediment), and matrix spiked control sediment were extracted at each time point.

Bioaccumulation and tissue extractions

Standard bioaccumulation tests using the oligochaete *L. variegatus* were conducted following modified US Environmental Protection Agency protocols (US Environmental Protection Agency 2000). The modification includes the use of a 14-d time frame rather than the 28-d bioaccumulation outlined in the original protocol. The alternative time frame was established because of reproduction of *L. variegatus* that begins at 14 d, which could influence bioaccumulation (Leppänen and Kukkonen 2006). Details for the bioaccumulation tests and tissue extractions can be found in the Supplemental Data.

Sample cleanup

The acid cleanup of all samples (Tenax, exhaustive chemical, and tissue extractions) was conducted following a published procedure (Trimble et al. 2008). Briefly, concentrated sulfuric acid (1 mL) was added to the 2-mL extract in the acid-resistant vial, vortexed for 5 min at 2000 rpm, and centrifuged for 5 min at 2000 rpm. The solvent layer was removed, passed through anhydrous Na_2SO_4 -packed columns (≈ 1 g) to remove acid residuals and any remaining water, and collected in a clean

scintillation vial. Next, hexane (2 mL) was added to the remaining sulfuric acid layer in the vial, vortexed, centrifuged, and filtered as described above 2 additional times. The combined filtered extract was concentrated to 1 mL under a steady nitrogen stream prior to analytical quantification.

Lipid analysis

Lipid content in *L. variegatus* was analyzed using individuals from each bioaccumulation test. Two individuals from each experimental replicate were randomly chosen, blotted dry, weighed, placed in a glass culture tube, and extracted with chloroform and methanol (1:1, v/v) as described (Van Handel 1985). A vanillin/phosphoric acid reagent was added, and transmittance was read at 525 nm using a spectrophotometer (Spectronic 20 GenesysTM; Spectronic Instruments). A 5-point calibration curve was constructed using 3-fold dilutions of vegetable oil and treated the same as tissue samples. Three independent samples were prepared, and a mean concentration and standard deviation were obtained for each sample.

Organic carbon analysis

The organic carbon content of the black carbon, humic acid, sawdust, controls, and 3 and 6% amendments were determined by Midwest Laboratories using hydrochloric acid digestion, followed by combustion using standard methods (ASTM International D-5373). The organic carbon analyses were conducted in triplicate, unless otherwise specified; and the data were reported as percentage of organic carbon.

Gas chromatography-mass spectrometry

Quantification of the 21 PCB congeners was completed on a gas chromatograph–mass spectrometer (GC–MS; Agilent 6890 GC and 5973 Network Mass Selective Detector). A 2.0- μ L sample was injected in pulsed splitless mode at 50 psi. Analytes were separated on a DB-*XLB* column (30 m \times 180 μ m \times 0.18 μ m; Agilent). Helium was the carrier gas, and column flow was 0.9 mL min⁻¹. A temperature ramp for the column was set at 100 °C and held for 2 min, heated to 160 °C at 10 °C min⁻¹, then heated to 280 °C at 6 °C min⁻¹, and held for 7 min. The inlet, ion source, and quadrupole temperatures were 260, 230, and 150 °C, respectively. Electron ionization/selected-ion monitoring mode was used. The molecular ion (M⁺) of each PCB congener was used as the target ion for quantification. To confirm the identification of each PCB congener, the ratios of qualifier ions ([M+2]⁺ and [M-70]⁺) to the target ion (<20%) and the retention time in comparison to the standard analyte (<0.1%) were used. Quantification of the samples was performed via internal standard calibration. Eleven calibration standards were prepared at levels of 1, 2, 5, 10, 25, 50, 75, 100, 200, 250, and 500 ng/mL of each PCB congener and surrogates in hexane, along with the 4 internal standards (each at 20 ng/mL).

Quality assurance and quality control

For extraction purposes, a surrogate mix of DBOFB and PCB-186 was used. The surrogate mix (50 ng) was added to all controls, amendments, tissues, blanks, matrix spike samples, and exhaustive chemical samples prior to extraction to determine the recovery of PCBs during the extraction and cleanup processes. Two matrix spike and 2 blank samples using control sediment were included with every experiment being conducted and extraction procedure.

For quantification purposes, a calibration check standard (50 ng/mL) was analyzed every 8 samples on the GC–MS. The relative differences of the reported concentration of all PCB congeners and surrogates had to fall within 20% for the results to be deemed acceptable; otherwise, the samples were reanalyzed. Reported concentrations were listed by individual congeners.

Statistical analysis and desorption parameter determination

All experiments and tests were conducted using triplicate sampling of matrices. A significance level of $\alpha = 0.05$ was chosen to determine whether statistically significant differences existed within and among samples ($p < 0.05$). For pairwise comparisons, the software SAS 10.0 (SAS Institute) was used. To this end, a one-way analysis of variance was used to conduct multiple comparisons among desorption parameters, PCB tissue concentrations, organic carbon content, biomass, and lipid content. An *F* test was used to determine the statistical significance among comparisons given the set of samples being analyzed. A post hoc test using Tukey's procedure was conducted to further investigate which pair of treatments or samples was statistically different.

Desorption of PCB congeners from controls and amendments to Tenax was modeled following a published procedure (Cornelissen et al. 1997). The empirical desorbing fractions (F_{rap} , F_s , and F_{vs}) and the corresponding desorption rate constants (k_{rap} , k_s , and k_{vs}) were fit to the following 3-compartment model with first-order equations

$$\frac{S_t}{S_0} = F_{rap} (e^{-k_{rap}t}) + F_s (e^{-k_s t}) + F_{vs} (e^{-k_{vs} t}) \quad (1)$$

$$F_{rap} + F_s + F_{vs} = 1 \quad (2)$$

where S_t and S_0 represent the amount of sediment-sorbed chemical at time *t* (hours) and time zero, respectively. F_{rap} , F_s , and F_{vs} are the rapidly, slowly, and very slowly desorbing fractions at time zero, respectively. The rate constants for desorption of the rapidly, slowly, and very slowly fractions are designated k_{rap} , k_s , and k_{vs} (per hour), respectively. The 3-compartment model was chosen based on the fit of the data using the coefficient of determination. For determination of desorption parameters, the software SigmaPlot 10.0 (Systat Software) was used. All desorption parameters are shown in Supplemental Data, Table S3.

RESULTS

Quality assurance and quality control

No target analytes were quantified above background levels in blank samples from any of the tests. The percentage of recoveries of PCB congeners and surrogates from matrix spiked samples from the exhaustive chemical extractions, sequential Tenax extractions, 24-h single-point Tenax extractions, and bioaccumulation tests ranged from 65 to 130% for the matrix spiked samples and from 79 to 108% for DBOFB and 71 to 106% for PCB-186. Surrogate recoveries from exhaustive chemical extractions, sequential Tenax extractions, 24-h single-point Tenax extractions, and bioaccumulation tests ranged from 75 to 104% for all tests for DBOFB and 79 to 112% for PCB-186. Relative standard deviations remained within the acceptable range (<25%) for all metrics including blank and matrix spiked samples. The percentage of recoveries reported in the present study were within values previously reported for PCBs in similar metrics (Mackenbach et al. 2012, 2014; Sinche et al. 2017).

Role of organic carbon type and quantity in defining desorption

A 3-compartment model (Cornelissen et al. 1997; Lydy et al. 2015) fit the desorption data for the proportion bound (S_t/S_0) versus time for the PCB congeners (Figure 1; Supplemental Data, Table S3). All 21 PCB congeners followed the same triphasic desorption pattern (Figure 1; Supplemental Data, S1). Desorption of PCBs from the amendments was strongly influenced by the organic carbon type and quantity added to the sediments. The total extent of desorption of all congeners after 672 h relative to the time zero concentrations was 4 to 27% for black carbon-3%, 3 to 24% for black carbon-6%, 15 to 55% for humic acid-3%, 12 to 45% for humic acid-6%, 25 to 80% for sawdust-3%, 16 to 70% for sawdust-6% amendments, and 35 to 90% for the controls. These results showed a greater congener bioaccessibility for the controls and sawdust amendments than in the humic acid and black carbon amendments. Note that the organic carbon in the controls was lower from the

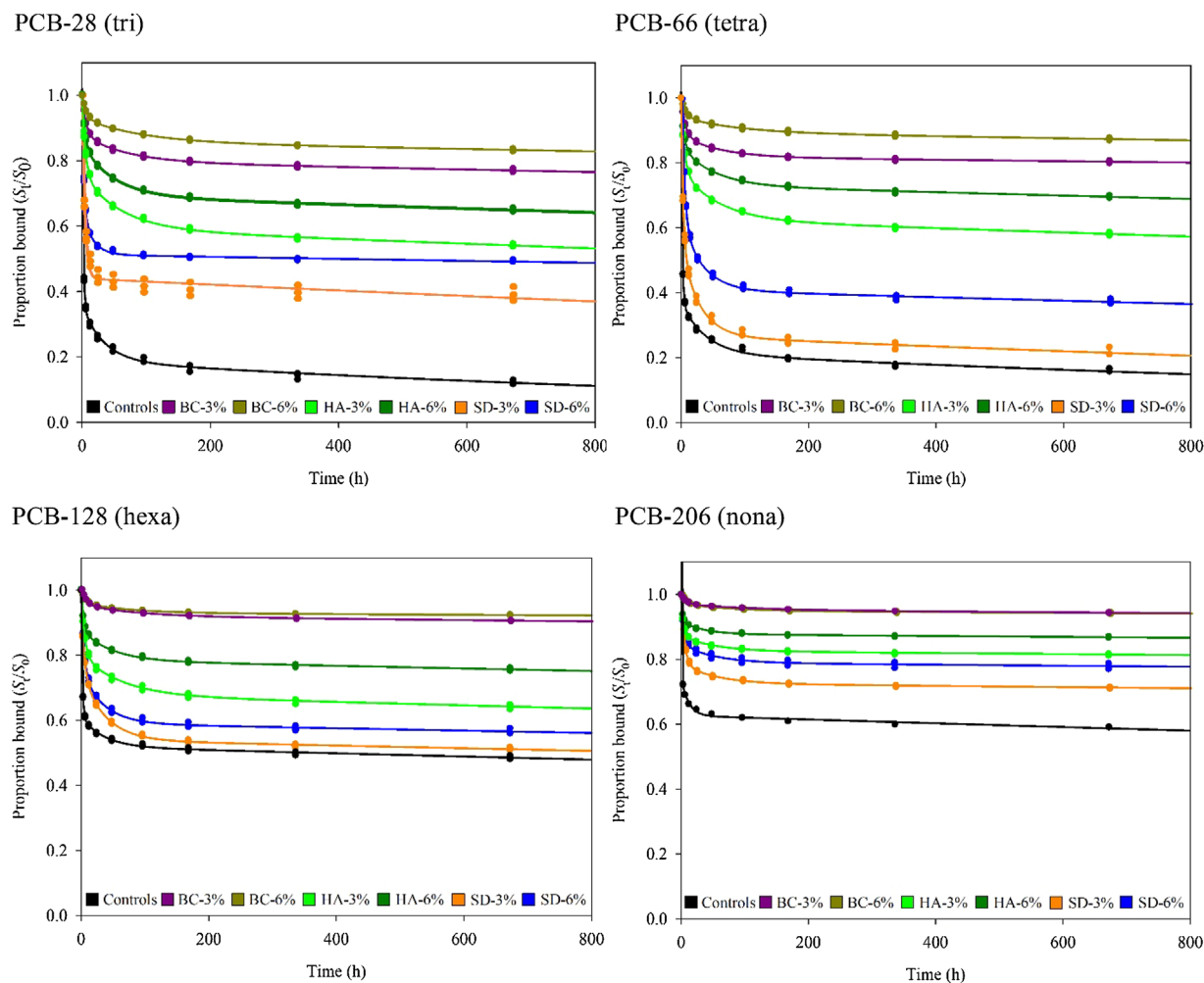


FIGURE 1: Desorption of representative polychlorinated biphenyl (PCB) congeners from the controls and 3 and 6% amendments. Data points from 3, 6, 12, 24, 48, 96, 168, 336, and 672 h of the desorption lines are shown for all treatments. The PCB homolog groups are in parentheses. Desorption lines were estimated from the 3-compartment model calculated using SigmaPlot 12.0. S_t and S_0 represent the amount of sediment-sorbed chemical at time (hours) and time zero, respectively. Desorption parameters are given in Supplemental Data, Table S3. BC = black carbon; HA = humic acid, SD = sawdust.

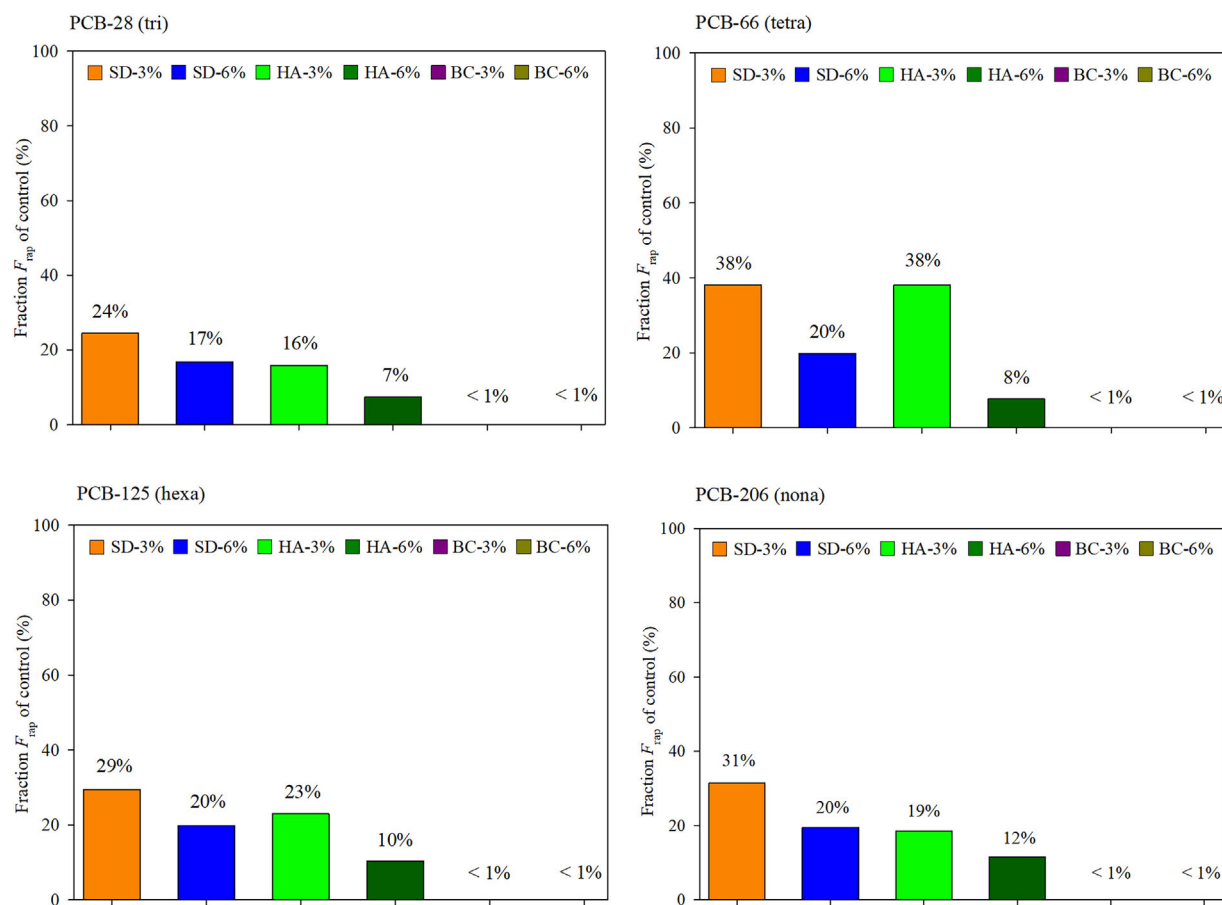


FIGURE 2: Comparison of rapidly desorbing fractions (F_{rap}) among amendments for selected polychlorinated biphenyl (PCB) congeners. Bars represent the reduction (percentage) of F_{rap} for amendments. Note that all F_{rap} percentages were calculated relative to the F_{rap} measured in controls; thus, by definition the F_{rap} in controls represented 100%. The PCB homolog groups are in parentheses. BC = black carbon; HA = humic acid, SD = sawdust.

amended organic carbon types. Furthermore, the F_{rap} values were consistently higher than the amendments (Supplemental Data, Table S3); thus, the changes in bioaccessibility among amendments were measured relative to F_{rap} from the controls (Figure 2). The results showed that F_{rap} in the black carbon amendments was significantly lower than in the humic acid and sawdust amendments. In contrast, PCBs in sawdust-3% were the most bioaccessible among the amendments (Figure 2). Furthermore, the increase from 3 to 6% organic carbon resulted in reduction in F_{rap} across all amendments. Considering the desorption data alone, these results indicate that bioaccessibility is expected to decrease with increasing quantity of organic carbon in the sediment. However, other factors, such as the organic carbon type and feeding behavior, also played a role in the PCB bioaccessibility, as discussed later in the *Discussion* section.

The organic carbon type and quantity also resulted in changes in the residual fractions (i.e., F_s and F_{vs}) in the amendments compared to the controls. For example, in controls the F_s values were consistently greater than in the black carbon amendments and smaller than in the humic acid and sawdust amendments (Supplemental Data, Table S3). When black carbon was present in the sediment at either 3 or 6% organic carbon, the

F_s values were the smallest across amendments. Furthermore, the F_{vs} values for black carbon and humic acid amendments were significantly greater than those observed in the sawdust amendments and controls ($p < 0.001$; Supplemental Data, Table S3). Moreover, the sum of both F_{rap} and F_s values in the controls and the sawdust amendments were significantly larger than their F_{vs} values ($p < 0.001$). This demonstrates that most of the compound was already desorbed mainly to the more bioaccessible fraction as indicated by the larger F_{rap} sizes in the controls and sawdust amendments.

The desorption rate constants (k_{rap} , k_s , and k_{vs}) of PCBs were the highest in the controls, followed by the sawdust, humic acid, and black carbon amendments (Supplemental Data, Table S3). For example, the k_{rap} in the controls and sawdust amendments ranged from 0.57 to 0.70 and 0.16 to 0.70 h^{-1} , respectively, whereas the k_{rap} in the humic acid and black carbon amendments ranged from 0.19 to 0.39 and 0.12 to 0.17 h^{-1} , respectively. These results indicate that the PCBs were more rapidly desorbed in the controls and sawdust amendments (Supplemental Data, Table S3). In contrast, the PCBs remained in the black carbon and humic acid amendments because of the slow rate constant values observed in these 2 amendments. Overall, the presence of sawdust in the sediments substantially

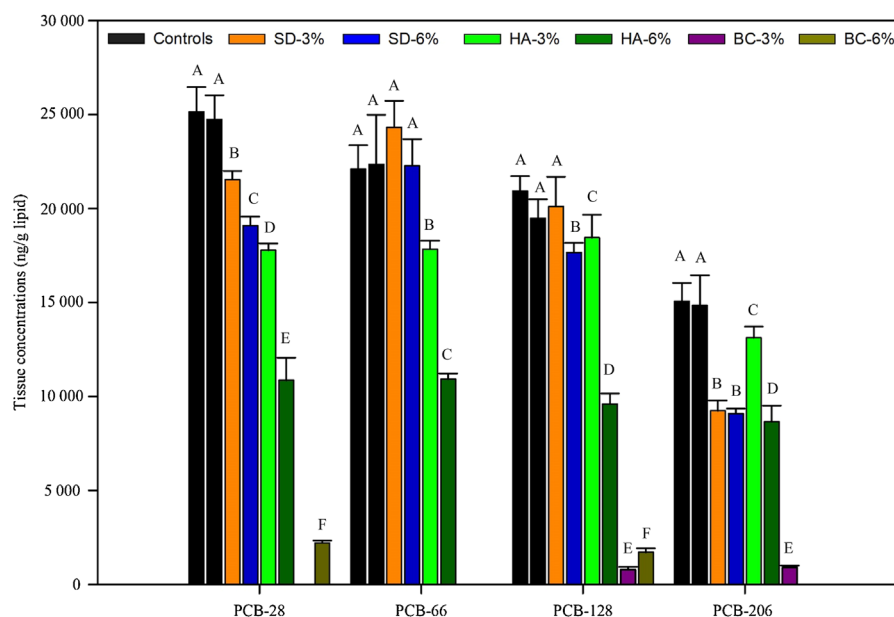


FIGURE 3: Distribution of selected polychlorinated biphenyl (PCB) congeners in tissue concentrations of *Lumbriculus variegatus* across treatments. Each bar represents the mean ($n = 3$) + 1 standard deviation. The PCB homolog groups are in parentheses. Bars with the same letter are not significantly different among treatments for a particular congener ($p < 0.05$). BC = black carbon; HA = humic acid; SD = sawdust.

contributed to desorption of PCBs, whereas black carbon in the sediments had the strongest binding effect that resulted in a reduced desorption of PCBs.

Effects of carbon amendments on bioaccumulation

The *L. variegatus* lipid-normalized PCB concentrations (nanograms per gram lipid) for selected PCB congeners across all amendments are shown in Figure 3 and Supplemental Data, Figure S2. No significant differences ($p > 0.05$) were found in the average PCB tissue concentrations in *L. variegatus* between the 2 controls. For the amendments, the PCB tissue concentrations were greater in the controls, followed by the sawdust, humic acid, and black carbon amendments. These results indicate that the PCB tissue concentrations directly reflected desorption of PCBs determined by the Tenax extractions (Supplemental Data, Table S3).

The lipid content in the *L. variegatus* was lower in the black carbon and humic acid amendments than in the controls and sawdust amendments (Supplemental Data, Figure S3). No significant differences were found in the lipid content in the organisms between the 3 and 6% amendments ($p > 0.05$; Supplemental Data, Figure S3). The wet weight of the organisms exposed to black carbon and humic acid amendments was significantly lower than the wet weight of organisms in the control ($p < 0.001$), whereas the wet weight of the organisms exposed to sawdust amendments was significantly higher than the wet weight of organisms in the control ($p < 0.001$; Supplemental Data, Figure S4). Overall, no significant differences were found in organism wet weights between 3 and 6% amendments within the same type of organic carbon ($p > 0.05$). No significant differences in percentage of survival were found between controls and amendments.

The PCB tissue concentrations and Tenax concentrations from controls and amendments were fit to the literature-based bioaccumulation Tenax model (Mackenbach et al. 2012). Overall, 80% of the data points fit within the 95% confidence limits of the bioaccumulation Tenax model. Note, all of the data from the control sediments fell well within the confidence intervals. For the amendments, 98, 93, and 89% of the data from black carbon, humic acid, and sawdust amendments fit within the 95% confidence limits of the bioaccumulation Tenax model, respectively (Figure 4).

DISCUSSION

The addition of organic carbon amendments to uncontaminated control sediment decreased PCB bioaccessibility as measured with 24-h Tenax, full desorption experiments, and bioaccumulation in *L. variegatus* (Figures 1–3; Supplemental Data, Figures S1 and S2). The reductions in bioaccessibility and bioaccumulation were attributable to changes in the type of organic carbon used to amend the sediment. Different decreases were observed because of the differences in the interactions between PCB congeners and the type of amendment. Black carbon decreased PCB bioaccessibility to the greatest extent of the amendments tested (Figure 2). Black carbon is characterized by high aromatic carbon content (up to 100% as measured by solid-state ^{13}C nuclear magnetic resonance spectroscopy; Simpson and Hatcher 2004; Zhu et al. 2005). Pi bond (π - π) interactions are an important binding force between the aromatic structures of black carbon and sorbate surfaces (Zhu et al. 2005) and significantly contribute to the high sorption capacity of HOCs for black carbon (Bucheli and Gustafsson 2000). Consequently, black carbon has been reported to effectively reduce the bioaccessibility of a variety of compounds including PCBs,

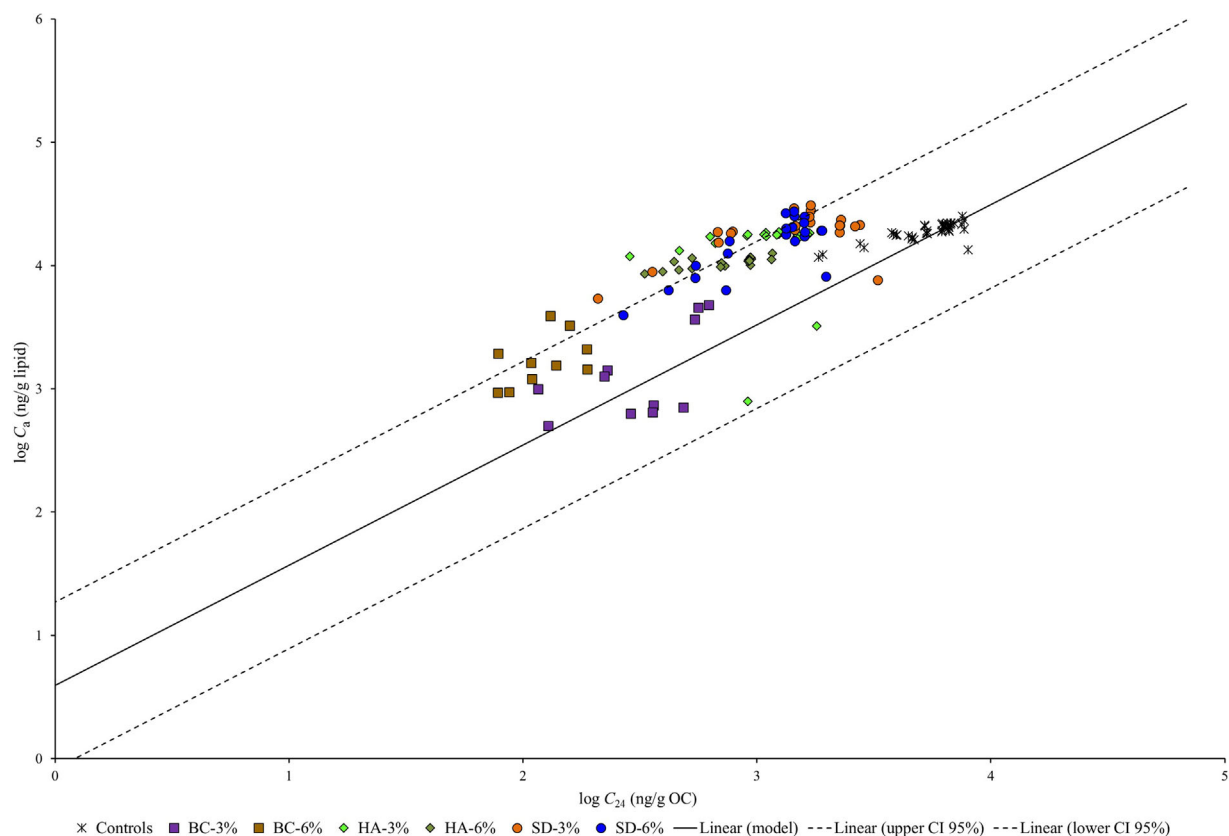


FIGURE 4: Relationship between 24-h single-point Tenax extractable concentrations (C_{24}) and tissue concentrations (C_a) of PCB congeners fit to the bioaccumulation Tenax model (BTM; Mackenbach et al. 2012). Data points represent the C_a and C_{24} concentrations from the controls and amendments. The 95% confidence intervals of the bioaccumulation Tenax model are represented by the dotted lines. From the total points ($n = 144$), 80% of the data fit within the within 95% confidence intervals of the bioaccumulation Tenax model. BC = black carbon; CI = confidence interval; HA = humic acid; OC = organic carbon; SD = sawdust.

polychlorinated dibenzodioxins, and PAHs (Cornelissen et al. 2005; Ghosh et al. 2003; Oen et al. 2006; Zhu et al. 2005). Oen et al. (2006) observed small amounts of total PAHs (<9%) in the rapidly desorbing fractions in field-contaminated sediments, attributable to the presence of black carbon (0.085–1.7%, dry wt) in the sediment. The authors concluded that there was a redistribution of the compound among the desorbing fractions that resulted in a large slowly desorbing fraction and a minimal rapidly desorbing fraction (Oen et al. 2006). In the present study, the PCB bioaccessibility was extensively reduced in black carbon amendments as shown by the larger residual fractions and the slow rate constants reported in Supplemental Data, Table S3. Furthermore, the extent of sorption is expected to increase with chemical surface area, as a greater sorbate surface becomes available for molecular interactions (Zhu et al. 2005). The present results support this concept because the more chlorinated PCB congeners were more strongly sorbed to the black carbon amendments, attributable to the possibility for more pi bond interactions and hydrophobic bonding (Figure 1; Supplemental Data, Figure S1).

Another potential mechanism of sorption for PCBs with black carbon is hydrophobic attractions within pores of the black carbon (Jonker and Koelmans 2002). The pores (i.e., micro- and meso-pores) provide high-energy sorption sites that can interact

with the sorbate (Jonker and Koelmans 2002). It has been suggested that smaller pores result in stronger binding of low-molecular weight molecules and planar compounds (Cornelissen et al. 2005). Similarly, Millward et al. (2005) concluded that a reduction in bioaccumulation in 2 marine organisms for more lipophilic PCB congeners from activated carbon-treated sediment (3–8%, dry wt) was attributable to the greater mass transfer resistance and the slower uptake for the higher K_{OW} congeners. We did not observe such effects in the present study because we employed freshly manufactured black carbon, which may have contained more available binding sites and unoccupied pores that could interact with the PCBs compared with weathered black carbon with saturated pores. Consequently, our target PCB congeners might have been equally able to interact with black carbon and fill the pore structures.

The presence of black carbon also had effects on the biomass and lipid content of *L. variegatus* (Supplemental Data, Figures S3 and S4). It has been reported that strong sorbents, such as activated carbon, can sequester lipids, carbohydrates, and proteins, which represent essential nutrients for organisms (Eretsky 2000). Hence, the lower biomass and lipid content in *L. variegatus* in the black carbon-amended sediments were likely attributable to the sequestration of nutrients by the black carbon amendments. It is important to note that no sediment

avoidance was observed in the black carbon amendments. Moreover, the application of activated carbon might have resulted in shorter and less dense microvilli in *L. variegatus*, which could have hindered the absorption of food and nutrients (Nybom et al. 2015). Therefore, the differences in biomass and lipid content of *L. variegatus* could be attributed to the sequestration of nutrients and physiological changes in the black carbon amendments.

Humic acid decreased PCB bioaccessibility to a lesser extent than black carbon but to a greater extent than the sawdust amendments and controls (Figures 1–3; Supplemental Data, Figures S1 and S2). The reduction of compound bioaccessibility has been attributed to an adsorption of the compounds with the humic substances (Peng et al. 2015). This sorption is facilitated by the aromaticity and branched structures of the molecules that constitute humic substances (Chin et al. 1994). The percentage of aromaticity of NaOH-extractable humic acid in soil ranged from 14.7 to 23.4% (Wang et al. 2016), whereas Traina et al. (1990) found that aromaticity ranged from 24 to 43% in humic acids extracted from 4 soils collected from Ohio. Similarly, Chin et al. (1994) found that percentage of aromaticity ranged from 13 to 27.4% in sediments from the Suwannee River in Georgia, the Yakima River in Oregon, the Ohio River, Coal Creek in Colorado, the Missouri River, and Lake Fryxell in Antarctica. The lower percentage of aromaticity of humic acid in comparison to black carbon is likely a cause of the reduced strength of sorption for humic acid-amended sediments.

It should be noted that the intermolecular interactions between PCBs and humic acid are likely a combination of pi bond interactions and induced dipole–dipole and dipole–dipole interactions (Peng et al. 2015). It is likely that weaker van der Waal interactions play a role in the relative decrease in bioaccessible and bioaccumulated PCBs in humic acid-amended sediments. These interactions were accentuated for the higher-molecular weight PCBs because larger reductions in F_{rap} and bioaccumulation were observed in *L. variegatus* for these congeners (Figure 2). The higher-molecular weight congeners may have offered greater hydrophobicity that increased binding strength. The reduction of PCB bioaccessibility/bioaccumulation by humic acid has also been reported in other studies (Freidig et al. 1998; Ramos et al. 1998). Ramos et al. (1998) reported that the bioaccumulation of pentachlorobenzene and PCB-77 in *Daphnia magna* was reduced because of the presence of humic acids in the treatment solutions. Similarly, the bioaccumulation of tetra-, penta-, and hexachlorobenzene in guppies decreased because of a reduction of 15 to 30% of the chemicals in exposure media containing humic acid (Freidig et al. 1998). We report similar findings in the present study with overall reductions in bioaccumulation in humic acid-amended sediments that ranged from 20 to 40% compared to controls (Figure 3; Supplemental Data, Figure S2).

The presence of sawdust in the sediment decreased PCB bioaccessibility the least of the 3 amended organic carbon sources but still decreased bioaccessibility compared to the controls (Figures 1–3; Supplemental Data, Figures S1 and S2). A wood-derived organic carbon, such as sawdust, is mainly composed of cellulose and lignin, with a structure of hexose

ring carbons with carboxyl groups and anomeric carbon typical of polysaccharides (Rutherford et al. 2005). Consequently, the weak interaction between PCBs and sawdust can be attributed to the lower percentage of aromaticity in the chemical structure of sawdust, which limits its intermolecular interactions to less favorable electrostatic forces (i.e., induced dipole–dipole interactions). Indeed, the aromaticity of pine wood, similar to that used in the present study, ranged from 16.8 to 35.1% (Rutherford et al. 2005). These observations are consistent with the low sorption capacity of cellulose and lignin for HOCs reported in other studies (Ghosh et al. 2003; Salloum et al. 2002). In a study comparing several sedimentary organic matter components, Salloum et al. (2002) reported that the sorption of phenanthrene to cellulose and lignin was minimal compared with kerogen and humic acid. Sawdust (i.e., lignin and cellulose) likely contributed to the type of organic carbon associated with the theoretical rubbery compartment, and hence the PCBs could be rapidly desorbed from sawdust-amended sediment, which makes the PCBs more bioaccessible to organisms.

Bioaccumulation of PCBs by *L. variegatus* was similar in the sawdust-amended sediments compared to the controls, with a few congeners even showing elevated tissue concentrations compared to the controls (Figure 3; Supplemental Data, Figure S2). This response was likely attributable to increased digestibility of the matrix by the oligochaetes (Figure 3; Supplemental Data, Figure S2). Other studies have indicated that sedimentary plant-derived organic carbon can be used as a source of nutrients by benthic organisms and microorganisms (Kukkonen et al. 2005; Laine et al. 1997). For example, various HOCs, including 2 PCB congeners from the tetra- and hexahomolog groups, sorbed to plant-derived carbon material less tightly and therefore were more bioaccessible to *L. variegatus* and *Diporeia* spp. compared with native black carbon present in the sediments (Kukkonen et al. 2005). The authors reported that this type of material was more likely to be ingested and used as a source of energy by benthic organisms (Kukkonen et al. 2005). Similarly, in a study of sawmill areas contaminated with polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and chlorophenols, it was reported that 30 to 40% of the carbon in chlorophenols was built into the bacterial biomass (Laine et al. 1997). The present results suggest that the use of the sawdust as a food source could have led to the greater PCB bioaccessibility found for the sawdust amendments than would be expected based on the organic carbon type.

The reductions in bioaccumulation between 3 and 6% amendments are shown in Figure 3 and Supplemental Data, Figure S2. The higher the percentage of black carbon in the sediment, the lower the bioaccumulation. However, we also observed a slightly higher PCB tissue concentration in the black carbon-6% amendment for some of the congeners tested (e.g., 28, 105, 126, 128, 138, 187, and 196; Supplemental Data, Figure S2). The present results showed that digestion likely played a role during desorption of these congeners, making them more bioaccessible in black carbon-6% than in black carbon-3%. For the humic acid amendments, a large reduction in bioaccumulation was observed with increasing humic acid content from 3 to 6% in the sediment and was consistent for

most congeners. The 2 exceptions were congeners 8 and 180, for which no significant differences were found between the humic acid-3% and humic acid-6% amendments (Supplemental Data, Figure S2). This implies that higher humic acid content provided additional binding sites for PCBs, and hence a reduction of bioaccessible PCBs that could potentially bioaccumulate. For the sawdust amendments, minimal statistical differences were found in PCB tissue concentrations between sawdust-3% and sawdust-6% amendments, with only congeners 101, 114, and 153 being slightly higher in the sawdust-3% compared to sawdust-6% amendments (Supplemental Data, Figure S2). This suggests that the high desorption of PCBs from sawdust amendments and the ability of oligochaetes to use the sawdust as a source of energy greatly contributed to the bioaccessibility of PCBs in the sawdust amendments. Finally, the highest bioaccumulation was observed in the controls (Supplemental Data, Figure S2). However, it is important to mention that there were no statistical differences between the PCB tissue concentrations in controls and sawdust amendments for the majority of the congeners tested (Supplemental Data, Figure S2), despite the greater organic carbon in sawdust amendments compared to controls.

Finally, to illustrate the relationship between PCB tissue concentrations and 24-h single-point Tenax extractable concentrations, the data from each of the amendments were fit to the bioaccumulation Tenax model. Overall, 80% of the data fit within the 95% confidence intervals of the bioaccumulation Tenax model. The data points that fell outside the model corresponded to the highly chlorinated homolog groups (hepta, octa, nona, and deca) for all 3 of the organic carbon amendments, and the model underpredicted the PCB tissue concentrations for these groups (Figure 4). This implies that other potential mechanisms, such as feeding behavior, may have increased the bioaccessibility.

CONCLUSIONS

The overall objective of the present study was to investigate the effects of the organic carbon type and quantity on PCB bioaccessibility. To this end, we employed 3 types of organic carbon differing in their physical and chemical properties, and hence different binding affinities for HOCs. Black carbon was chosen to represent a ubiquitous organic carbon source in sediments with a predominantly aromatic carbon chemical structure. The addition of black carbon to the sediment greatly reduced PCB bioaccessibility, and the strong binding affinity of black carbon for PCBs is thought to be responsible for such a reduction. Humic acid was chosen to represent a source of organic carbon derived from decayed animal and plant debris with polar moieties but also with a significant aromatic character. A lower PCB bioaccessibility was observed in the humic acid amendments compared with sediments amended with the same amount of organic carbon from sawdust. Thus, the aromaticity in the humic acid structure was likely important in decreasing PCB bioaccessibility in the humic acid amendments. Finally, sawdust was chosen to represent a source of organic carbon with more polar moieties and less aromatic carbon. The addition of sawdust in the sediment had a minimal

effect on compound sorption because the highest PCB bioaccessibility was observed in the sawdust amendments, despite the greater organic carbon compared to controls. Thus, areas with large quantities of residual wood material (e.g., paper mills), contaminated with PCBs and like compounds, could potentially become recurrent sources of bioaccessible compounds to the benthic biota inhabiting those environments. We also investigated the effect of increasing the percentage of organic carbon in the sediment to reduce PCB bioaccessibility. Desorption of PCBs was slower in 6% amendments compared to 3% amendments. This trend was more evident in humic acid and black carbon amendments, which had a greater influence on desorption of the PCBs. Thus, a decrease in PCB bioaccessibility was not only a function of increasing the percentage of organic carbon in the sediment but also a function of the type of organic carbon added to the sediment. This suggests that the prediction of PCB bioaccumulation in nonselective deposit feeders exposed to contaminated sediments, with varying organic carbon types and quantities as described in the present study, could be accomplished using 24-h single-point Tenax extractable concentrations because the present results demonstrated a close relationship between F_{rap} and 24-h Tenax concentrations. Finally, Tenax and PCB tissue concentrations were fit to the bioaccumulation Tenax model, and the overall results showed that 80% of the data fit within the 95% confidence interval of the model. This provides evidence of the robustness and reliability of the bioaccumulation Tenax model in predicting compound bioaccessibility across a wide range of sediment characteristics, which can aid in the initial assessment of organic pollutants in contaminated sediments.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4073.

Acknowledgment—The authors thank the Dissertation Research Assistant Award and Center for Fisheries, Aquaculture and Aquatic Sciences, for providing partial funding for this project. The authors also thank Alstat Wood Products for their assistance with the collection of sawdust materials and P. Hitchens and A. Coursey for their assistance with the commercial-grade electrical grinder.

Data availability—Data, associated metadata, and calculation tools are available from the corresponding author (mlydy@siu.edu).

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