

*Environmental Chemistry*CHARACTERIZATION OF POLYCYCLIC AROMATIC HYDROCARBON
BIOAVAILABILITY IN ESTUARINE SEDIMENTS USING THIN-FILM EXTRACTION

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Abstract—It is well documented that the bioavailability of hydrophobic organic chemicals (HOCs) can vary substantially among sediments. This makes risk assessments based on total sediment concentrations problematic. The present study investigates the application of thin-film solid-phase extraction to measure bioavailable concentrations of phenanthrene in estuarine sediment by comparing concentrations of phenanthrene in the amphipod *Corophium colo* and in thin ethylene/vinyl acetate films at different concentrations in three geochemically different sediments. For all sediment types, concentrations of phenanthrene in sediments and thin films followed linear relationships, indicating first-order exchange kinetics. Organism/thin-film concentration ratios did not vary systematically among sediment types but dropped significantly with increasing phenanthrene concentration in the sediments. While at low phenanthrene concentrations in the sediment fugacities of phenanthrene in the amphipods approached the fugacities in the thin films, they were significantly lower than those in the sediments at higher concentrations. While phenanthrene concentrations in the three sediment types were identical, biota sediment accumulation factors and concentrations in amphipods and thin films were consistently lower in sediments enriched with black carbon than in sediments with sedimentary organic matter bearing a more diagenetic organic signature. It is concluded that, for the range of concentrations tested, thin-film solid-phase extraction can be a useful tool in the characterization of differences in bioavailability of HOCs among sediment types.

Keywords—Bioavailability Fugacity Polycyclic aromatic hydrocarbons Sedimentary organic matter Amphipods

INTRODUCTION

Hydrophobic organic compounds (HOCs) tend to bind to suspended particulate matter and to then accumulate in the sediments at the bottom of lakes, estuaries, and coastal regions following their discharge into the aquatic environment. Once in the sedimentary environment, HOCs generally enter the greater ecosystem via bioaccumulation by benthic invertebrates and deposit-feeding fish [1]. Contaminant accumulation in biological tissue requires the compound to be present at the animal–environment interface in the dissolved state, that is, either dissolved in the sedimentary pore or overlying waters prior to uptake or dissolvable from ingested particles by digestive fluids within the gut. Therefore, the total concentration of a sediment-associated contaminant may be poorly representative of what is actually bioavailable.

Sediment organic matter (SOM) heterogeneity plays a significant role in sorption processes and therefore in the concentration of HOC in the dissolved phase [2–5]. The SOM is composed of a number of compositionally and structurally different organic domains. A structurally amorphous, diagenetically immature SOM compartment has been identified as responsible for kinetically rapid and reversible partitioning behavior [5,6]. In contrast, a more structurally condensed, molecularly cross-linked and diagenetically mature SOM moiety has been characterized as a phase with which contaminants can, over time, become tightly sequestered and desorb from much more slowly [5]. A number of researchers have stressed that, in addition to diagenetic carbon, the existence of even small quantities of pyrogenic carbon in the form of acutely

hydrophobic soot or black carbon can enhance the sorption affinity of a sediment and therefore play a significant role in regulating HOC bioavailability [7].

As these SOM compartments can vary between sediments, the sorption and sequestration of HOCs can be complex and difficult to model or predict. It is therefore desirable to develop an empirical measurement for the bioavailability of sediment-associated contaminants. Mackay [1] and others have suggested that it may be more insightful to express the presence of a chemical in environmental media in terms of fugacity rather than concentration. Wilcockson and Gobas [8] and Mayer et al. [9] have proposed that a solid-phase extraction approach could be used to measure the fugacity of sediment-associated contaminants. The approach rests on the notion that in a multiphase system at equilibrium, the fugacity of a chemical in one medium is equal to the fugacity in the other. Therefore, by measuring the chemical's fugacity in a known reference medium in contact with contaminated sediment, the fugacity of the chemical in the sediment can be indirectly inferred. Fugacity can be viewed as concentration, C , normalized to the fugacity capacity, Z . In the present work, it is contended that fugacity can act as a useful measure of the bioavailability of sediment-associated HOCs. In this context, a low fugacity would indicate that the chemical was sorbed tightly to the sediment particle and would be less available for desorption into the pore-water phase from which it could be readily bioaccumulated.

Several conditions must be met in the selection of an appropriate extracting medium. First, apparent equilibrium must be achieved between the extracting medium and the sediment, preferably within a short, practical time scale [8,9]. Second, the solid-phase extraction material should remove an insig-

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nificant fraction of the mass of analyte from the environmental sample [8,9]. Finally, the Z value of the solid-phase extraction material for the chemical of interest must be known [8]. Wilcockson and Gobas [8] proposed the use of a thin film of ethylene vinyl acetate (EVA). The EVA film has a high ratio of surface area to volume, promoting fast exchange kinetics. This method was used to measure the fugacity of hydrophobic chemicals in biological tissue [8] and in air [10]. The method has also been adapted for use with sediments by S.V. Otton (2004, master's thesis, Simon Fraser University, Vancouver, BC, Canada) and L. Meloche (2004, master's thesis, Simon Fraser University, Vancouver, BC, Canada), and these researchers successfully applied the adapted approach to measure the fugacity of a range of semivolatile chlorobenzenes and polychlorinated biphenyl congeners associated with spiked and field-contaminated sediments. To date, however, the potential for the thin-film extraction method to characterize HOC bioavailability has not been investigated.

In the present study, the uptake of spiked phenanthrene, a common polycyclic aromatic hydrocarbon (PAH), from sediments of varying geochemical composition into, first, the EVA thin film and, second, the tissue of the Australian infaunal amphipod *Corophium colo* was measured. Three sediments were selected on the basis of characteristics that affect sorption kinetics (black carbon, aromatic SOM). Two objectives were identified for these experiments: first, to obtain a greater understanding of the geochemical and biological controls on sediment-contaminant bioavailability through the comparison of the patterns of phenanthrene uptake into biological tissue and film, and, second, to determine the extent to which a fugacity approach is useful for characterizing the bioavailability of HOCs in sediments.

THEORY

The purpose of the thin-film extraction is to determine the fugacity of the test chemical (phenanthrene in the present study) in sediments by measuring the concentration of the test chemical in the EVA thin film at apparent equilibrium where the fugacity (Pa) of the chemical in the thin film (f_E) equals that in the sediment (f_S):

$$f_S = f_E \quad (1)$$

The term f_E is determined from the measured concentration (mol/m³) of the chemical in the thin film (C_E) as C_E/Z_E , where Z_E is the fugacity capacity (mol/m³/Pa) of the thin film for the test chemical. The Z_E can be determined by various means (e.g., [8]). In the present study, we determined Z_E by measuring the EVA-octanol partition coefficient K_{EO} and then determined the K_{EA} from the product of K_{EO} and the octanol-air partition coefficient K_{OA} ; that is, K_{EA} equals $K_{EO} \cdot K_{OA}$. Since K_{EA} or C_E/C_A equals Z_E/Z_A and Z_A equals the inverse of the product of the gas constant R (8.314 J/mol/K) and temperature in Kelvin (i.e., $[RT]^{-1}$), Z_E can be determined as K_{EA}/RT , and the fugacity of the chemical in the sediment can hence be determined from the chemical concentration in the thin film as

$$f_S = C_E \cdot R \cdot T / K_{EA} \quad (2)$$

The purpose of the simultaneous exposure of thin films and biota to spiked sediments was to test whether the concentration and fugacity of the test chemical in the organism (specifically, in the lipid of the organism) is correlated to the fugacity in the thin films. The fugacity of the chemical in the organism's

lipid, f_{lip} , was determined from the concentration in the organism as

$$f_{lip} = C_{lip} / Z_{lip} \quad (3)$$

where Z_{lip} is the fugacity capacity of the organism's lipid for the chemical. The Z_{lip} was assumed to approximate the fugacity capacity of octanol, Z_O , which was derived as K_{OA}/RT .

MATERIALS AND METHODS

Sediments

Sediment samples (15 L) were collected from two sites in Lake Conjola and one site in Port Hacking, two estuaries on the southeastern Australian coast, using a specially built Birge-Ekman grab sampler. Solid state ¹³C-nuclear magnetic resonance (NMR) spectroscopy found the sediments to vary in organic matter content and character [11,12]. The SOM associated with Lake Conjola Terrestrial (LCT) sediment is derived from vascular plants and is rich in degradation-resistant aromatic components [11]. The character of SOM associated with Lake Conjola Marine (LCM) also bears a terrestrial signature; however, this is considerably diluted by the contribution of more marine, algae-derived aliphatic functional groups [11]. Experiments involving novel solid state ¹³C-NMR techniques established that the main difference between SOM in Port Hacking Marine (PHM) and SOM in LCT and LCM sediments is that SOM in PHM sediments contains a significant amount of black carbon resulting from regular bushfires in the catchment [11]. Sediments were passed through a 1.1-mm stainless-steel sieve, returned to the laboratory, and stored at 4°C until spiking. The total sedimentary organic carbon (SOC) fraction was measured using a Laboratory Equipment Corporation elemental analyzer (St. Joseph, MI, USA) and was 4.70, 5.23, and 6.31% for LCT, LCM, and PHM sediments, respectively.

Sediment spiking and analysis

Each sediment was spiked with phenanthrene (99% purity, Sigma Aldrich, Sydney, NSW, Australia) at nominal concentrations of 0.19, 0.70, and 1.4 μmol/g dry weight. Sediment concentrations were selected on the basis of screening tests in which low (<10% mortality), mid- (~50%), and high (>90%) toxicities to *C. colo* were induced. Spiking followed the jar-rolling method [13]. Briefly, between 0.64 and 10 ml of concentrated phenanthrene in acetone were delivered into several open-mouth 1-L jars, and this volume was topped up to 13 ml with acetone. For control treatments, 13 ml of unspiked acetone were used. Glassware had previously been cleaned with phosphate-free detergent (Extran MA03, Merck, Darmstadt, Germany), acetone, and nitric acid. The jars were placed, open, on a specially built rolling mill and rotated at 15 rpm until the acetone had evaporated to leave a uniform film of phenanthrene on the inner surface of the glass. Wet sediment (670–800 g) was then added as appropriate to achieve the dry-weight concentration corresponding to the added mass of phenanthrene. The jars were sealed with Teflon®-lined lids and returned to the rolling mill for 16 h over 2 d. Then sediments were returned to 4°C for 28 d. This is a duration that has been identified in sorption studies elsewhere as sufficient for spiked HOCs to establish apparent equilibrium between SOM and pore water (i.e., changes in solution-phase solute concentrations too small to measure over a reasonable period of time) [5,14,15]. It is important to note that PAH bioavailability is likely to decline even beyond the establishment of apparent equilibrium; there

is a substantial body of literature that demonstrates differences in the bioavailability of freshly added PAHs (as in our present study) and contaminants that are allowed to age beyond 28 d (e.g., [16]).

After equilibration, sediment subsamples were poured into acid-washed, solvent-rinsed 250-ml glass jars and delivered, on ice, to the accredited Australian Laboratory Services P/L (ALS; Sydney, NSW, Australia). Samples were analyzed by ALS for a suite of 16 PAHs (listed in Table S1, *Supplementary Data*; <http://dx.doi.org/10.1897/06-378.S1>). For the low-concentration treatment, four subsamples were prepared to determine the homogeneity of the phenanthrene concentration in the spiked sediments. A mass of 10 ± 0.05 g wet sediment was dried with Na_2SO_4 and extracted by tumbling for 1 h with 20 ml of 1:1 dichloromethane (DCM)/acetone. Analysis of extracts were conducted by gas chromatography/mass spectrometry (GC-MS) in the selected ion-monitoring mode using an HP6890 GC, HP5973 MS (Hewlett Packard Australia, Melbourne, VIC, Australia), and Chrompack CP-SIL GC column (Varian, Melbourne, VIC, Australia). For PAH analysis of sediments, a level of reporting (LOR) of $0.05 \mu\text{g/g}$ was applied. The laboratory quality control measures applied to sediment analysis are detailed in Table S1.

Measured phenanthrene concentrations in LCM, LCT, and PHM sediments were $78 \pm 13\%$, $65 \pm 12\%$, and $66 \pm 3\%$, respectively, of the nominal concentrations. In the PHM sediment, the PAHs pyrene and fluoranthene were detected in unspiked sample in the nmol/g dry-weight concentration range. We interpret the detection of these PAHs as being related to the sedimentary black carbon previously detected in this sediment [11,12].

Amphipod bioaccumulation experiments

The ecotoxicity of the contaminated sediments was primarily measured through amphipod bioaccumulation over 10 d. The same experiments allowed 10-d LC50 values (the concentration at which 50% of a population exhibited mortality) to be determined, and these could also be used to express the effect of sediment type on the apparent toxicity of sediments. Filtered seawater ($\sim 33\%$ salinity) pumped from 8-m depth outside the mouth of Port Hacking was used in bioaccumulation experiments. Seawater was diluted in the laboratory to $15 \pm 0.1\%$ salinity using Sydney mains water (which is chlorinated by the supplier). Two weeks prior to the beginning of the bioaccumulation experiments, 300 individuals of the Australian infaunal amphipod *C. colo* [17] were collected from the vertical wall of an intertidal creek feeding into the upper Hawkesbury River, New South Wales, and acclimated in the laboratory at $20 \pm 1^\circ\text{C}$, following Hyne et al. [18]. Amphipods had moisture and lipid contents of $82.5 \pm 2.2\%$ and $0.54 \pm 0.09\%$ wet weight ($3.04 \pm 0.49\%$ dry wt), respectively. Lipid contents were determined gravimetrically according to an approach modified from Fay et al. [19] (detailed in Table S1). Amphipods were exposed to contaminated sediment, following Hyne et al. [18], for durations of 6, 12, 24, 48, 120, and 240 h. This duration is typical for the common acute bioassay involving this species [18] and was selected to match the duration of the fast equilibrating EVA thin-film experiments. Twelve hours prior to the beginning of the exposure, the sediment for each concentration were combined in a 4-L beaker and thoroughly mixed. A mass of 200 g wet sediment was distributed into fresh 1-L jars that were then topped up with approximately 800 ml of 15% seawater and allowed to settle.

One jar was prepared for each time point with the exception of the 240-h time point, for which four jars were prepared to ensure sufficient tissue for analysis at the end of the experiment and to permit LC50 determination. Amphipods were carefully sieved from the acclimation trays, and 10 to 15 amphipods were added to each test chamber. The jars were lidded, aerated, and kept at constant illumination until the end of the exposure period. Temperature was maintained at $20 \pm 1^\circ\text{C}$, and no substantial increases in the salinity of overlying water were observed. Surviving amphipods were then removed from the test chambers and allowed to clear ingested sediment in 200 ml of test water for 6 to 8 h. Organisms were blotted dry and placed in polypropylene conical vials and stored in the freezer until extraction. Dead amphipods were discarded without being extracted, as the exact time of death of these individuals could not be identified. Frozen tissue from at least three individuals was weighed using a Perkin Elmer AD-4 autobalance (Perkin Elmer, Melbourne, VIC, Australia) before and after being lyophilized using a Labconco Freezone 6 freeze-drier (Labconco, Kansas City, MO, USA). It was necessary to pool all surviving amphipods from each treatment to ensure that tissue residues were above LORs for tissue extracts and to minimize the impact on the organisms' field collection site (it is presently not possible to culture *C. colo* in the laboratory [S. McCready, University of Sydney, NSW, Australia, personal communication]). To determine analytical reproducibility, the surviving amphipods exposed to the lowest phenanthrene concentration for 10 d were divided into four subsamples and extracted and analyzed separately. Analyses of blank samples were also conducted.

Amphipods were extracted using a microextraction technique [19] using sample sizes between 5 and 100 mg. Samples and approximately $150 \times 1\text{-mm}$ -diameter glass beads (Extech, Melbourne, VIC, Australia) were homogenized in 1.5 ml polypropylene conical vials (Extech) for 5 min at 2,800 rpm using a Disruptor Genie[®] tissue homogenizer (Lomb, Sydney, NSW, Australia). Then $400 \mu\text{l}$ 4 N NaOH, $100 \mu\text{l}$ methanol, and $500 \mu\text{l}$ iso-octane (spiked with 10 mg/L benzo[*a*]pyrene as a surrogate) were added, and samples were homogenized for another 20 min. Vials were centrifuged at 4,000 rpm for 20 min at 10°C , after which the iso-octane layer was removed. A further $500 \mu\text{l}$ ethyl acetate and $130 \mu\text{l}$ 12 N HCl were added to the vials, which were then homogenized and centrifuged as before. The ethyl acetate layer was then added to the iso-octane, and the combined extract was exchanged to iso-octane under N_2 . Mean (\pm standard deviation) surrogate recovery was $98 \pm 23\%$, $97 \pm 5\%$, and $87 \pm 7\%$ for amphipod tissue exposed to LCM, LCT, and PHM sediments, respectively. With regard to analytical reproducibility, relative standard deviations (%RSDs) were 5%, 35%, and 14% for the treatments analyzed in quadruplicate for LCM, LCT, and PHM, respectively. The LOR for PAHs was 0.1 mg/L extract.

Thin-film extractions

Before thin-film extraction, 21-ml glass scintillation vials (Extech) were cleaned with phosphate-free detergent, with hexane, and twice with ultrapure water and placed upside down to dry. A solution of 6.1 g/L EVA (28% vinyl acetate) (Bacto Laboratories, Sydney, NSW, Australia) in dichloromethane was prepared, with approximately 10 mg of Sudan IV dye (Sigma Aldrich) added to the solution. Then $150 \mu\text{l}$, or 0.92 mg of EVA, were added to each vial using an adjustable pipette (Eppendorf South Pacific, Sydney, NSW, Australia). Vials

were then manually rotated until the DCM had evaporated. This produced thin films with 0.05 μm thickness. The uniformity of the EVA coating was determined by visual observation of the distribution of the dye (Fig. S1, *Supplementary Data*; <http://dx.doi.org/10.1897/06-378.S1>). After each vial had been coated, a final hexane rinse was performed to remove the dye and other contaminants. Vials were capped with aluminum-lined lids and stored for no more than 3 d before extraction.

Thin-film extractions involved adding sediments to thin-film-coated vials at the beginning of the bioaccumulation experiment and sampling the film after 5 min, 30 min, and 1, 6, 12, 24, 48, 120, and 240 h of exposure. Uptake of phenanthrene in EVA from unspiked control sediment was tested only after 240 h. To sample the films, sediment was removed from the vials, after which the vials were washed with reverse-osmosis water to remove any particulate matter left in the vial. Each vial was then centrifuged for 2 min at 3,000 rpm in a Jouan CR412 centrifuge (Thermo Scientific, Milford, MA, USA) to remove leftover water. Phenanthrene was extracted from the EVA by adding 500 μl of hexane, tightly capping the vial, and mixing for 20 s using a vortex mixer (Selby Scientific, Sydney, NSW, Australia). The hexane was then removed using a fine-tipped Pasteur pipette and placed in a 2-ml GC-autosampler vial (Agilent, Melbourne, VIC, Australia). To ensure that concentrations exceeded the analytical LORs, extracts from the three vials prepared for each concentration were combined at this point. The combined EVA extracts were reduced to 500 μl under N_2 and analyzed by capillary GC-MS. Therefore, while concentrations reported in the present study each represented three replicates, it was not possible to determine standard deviations for each data point. To determine analytical reproducibility, thin-film extractions of 12 individual 120-h-exposed midconcentration sediments were carried out. Thin-film extracts of three samples were pooled to produce four independent thin-film extracts for analysis. From these, %RSDs were 16, 12, and 7% for LCM, LCT, and PHM sediments, respectively. These results indicate that the variation among replicate samples is relatively low, suggesting that the method is reproducible.

Determination of the K_{EO} value for phenanthrene

The EVA–octanol partitioning coefficient (K_{EO}) for phenanthrene, necessary for calculating Z_E and Z_O , was determined following the method described by S.V. Otton (2004, master of resource management thesis, Simon Fraser University, Vancouver, BC, Canada). A mass of 0.1 ± 0.004 g EVA beads was weighed into 2-ml GC-autosampler vials, which were then placed on a heating tray at a low heat setting for 0.5 h until the EVA beads had melted into an elongated mound. A solution of 6.7 g/L EVA was spiked at 0.10 g/L phenanthrene, and 50 μl of this solution were delivered into each vial using a 100- μl glass syringe (Hamilton Company, Reno, NV, USA). Each vial was filled with 1.65 ml of 1-octanol, capped, and allowed to equilibrate at $20 \pm 1^\circ\text{C}$ on a rotating tumbler. Equilibration was tested over 2 and 7 d. Control treatments were prepared in the same way, differing only in that no octanol was added. All treatments were carried out in duplicate. At the end of each experiment, the EVA was soaked in a 2-ml volume of DCM for 30 min. This was repeated six times with fresh volumes of DCM. The DCM was pooled in a glass scintillation vial with 100 μl of toluene. The combined extracts were then reduced under N_2 until only the toluene remained, at which

point 5 ml of methanol were added to precipitate the EVA. Vials were centrifuged at 3,000 rpm, and an aliquot of the methanol was taken for analysis by GC-MS. Phenanthrene concentrations in EVA and in octanol were determined by comparison of control and octanol-dosed treatments.

Data analysis

Phenanthrene uptake into EVA was analyzed using nonlinear regression (Graphpad Prism Ver 4.03, GraphPad Software, San Diego, CA, USA). The thin-film data were fit to a one-compartment first-order kinetic uptake model:

$$C_E = C_{E,eq}(1 - e^{-kt}) \quad (4)$$

where C_E is the observed concentration of the contaminant in the EVA film ($\mu\text{mol}/\text{cm}^3$), $C_{E,eq}$ is the film concentration at apparent equilibrium, and k is a first-order uptake rate constant (per hour). The correlation coefficient, R^2 , was used to indicate the quality of fit. Moreover, we found that the model fit was improved significantly when the data were fit to a two-compartment model that was similar to Equation 4 but comprised two Arrhenius functions. The time to 95% of apparent equilibrium was determined as $t_{95} = 3/k$.

Biota–sediment accumulation factors (BSAF) were determined from

$$\text{BSAF} = \frac{C_B \cdot f_{oc}}{C_S \cdot f_{lip}} \quad (5)$$

where C_B is the concentration in the organism ($\mu\text{mol}/\text{g}$ dry wt), C_S is the concentration in the sediment, and f_{oc} and f_{lip} are the fractions (g/g) of organic carbon and lipid, respectively. The BSAF based on the phenanthrene concentration in the organism after 10 d is referred to as BSAF_{10d} , while the maximum observed BSAF throughout the exposure is referred to as BSAF_{max} . In order to compare C_{lip} with C_E , C_{lip} was converted into volumetric units by multiplying by 0.827 g/cm^3 , the density of 1-octanol at 20°C . The 10-d acute mortality LC50s were calculated using the trimmed Spearman–Kärber method [20].

RESULTS AND DISCUSSION

Toxicity and observations of animal behavior

Amphipods began construction of U-shaped burrows immediately on addition to the test chambers. Differences in burrowing behavior between the control and the different concentration treatments for each sediment were not observed; in all treatments, turbidity in the water decreased within the first 48 h of the test, and this corresponded to the time when amphipods had completed constructing burrows. The 10-d acute LC50s for LCM, LCT, and PHM were 0.68 (95% confidence interval [CI] = 0.56–0.83), 0.61 (95% CI = 0.43–0.86), and 1.01 (95% CI = 0.88–1.17) $\mu\text{mol}/\text{g}$ dry weight, respectively. The LC50s in LCM and LCT sediments were not significantly different, but the LC50 of phenanthrene in PHM sediments was significantly greater than the LC50 in LCM and LCT sediments. This illustrates that sediment type can have a significant effect on the apparent toxicity of phenanthrene in sediments.

Phenanthrene uptake into amphipod tissue and into thin film

Figure 1a to c illustrates the bioaccumulation of phenanthrene over the 10-d exposure from the three sediments. Each

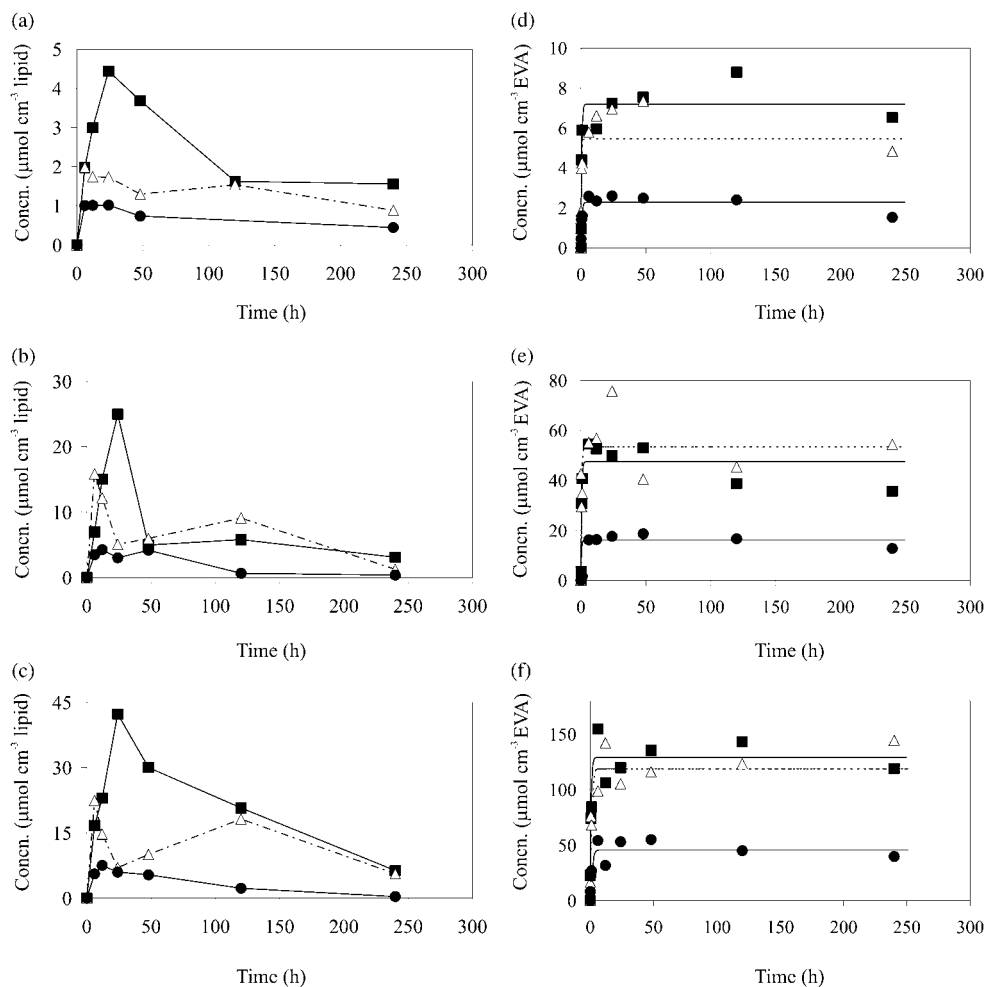


Fig. 1. Uptake of phenanthrene into (a–c) amphipod tissue and (d–f) ethylene vinyl acetate (EVA) thin film from three sediments contaminated at low, mid-, and high concentrations (top to bottom). Lines through the EVA data are best-fit regression curves (Eqn. 4), while lines through tissue data are not based on statistical analysis; these curves are for qualitative interpretation only. Uptake curves are dashed for Lake Conjola Terrestrial (LCT = Δ) sediment to aid the distinction with Lake Conjola Marine (LCM = \blacksquare) and Port Hacking Marine (PHM = \bullet).

uptake profile was characterized by a rapid, initial increase in animal body burden, which reached a maximum between 6 and 24 h. Then a decline in tissue concentration occurred. This observed bioaccumulation profile is similar to that described for other species of amphipod [21–23] and invertebrates [24,25]. Given that the thin-film concentrations did not show a decline of the exposure concentration during the bioaccumulation test (see the following discussion), it is possible that the observed decline of the phenanthrene concentration in the amphipods was due to metabolic transformation of phenanthrene in the amphipod, which appears to have initiated at the beginning of the uptake period because of enzyme induction. In all three treatments, phenanthrene concentrations in amphipods in LCM sediments were greater than those in LCT sediments, while concentrations in amphipods exposed to PHM sediments showed the lowest concentrations. This is further evidenced by the $BSAF_{max}$ (Table 1), which ranged between 1.5 and 2.3 kg/kg in LCM sediments, 0.61 and 1.3 kg/kg in LCT sediments, and 0.41 and 0.47 kg/kg in PHM sediments.

Concentrations of phenanthrene in EVA films exposed to the sediment (Fig. 1d–f) increased rapidly at the beginning of the exposure period and then achieved a maximum concentration that was maintained throughout the rest of the exposure

period. The one-compartment uptake rate constants into the film ranged between 1.1 and 2.6/h (Table 1), corresponding to 95% apparent equilibrium times of 1.2 to 2.8 h. Uptake rates did not show significant differences among the three sediment types or vary with increasing phenanthrene concentration. This suggests that thin-film extraction occurs within a reasonable time frame and, because uptake does not vary with sediment type, is independent of geochemistry. These uptake rates were more rapid than those measured for phenanthrene into other solid-phase extraction media, such as poly(dimethylsiloxane) solid-phase microextraction [9], and polyethylene semipermeable membrane devices [26].

Despite the fact that total phenanthrene concentrations in the three sediments were equal, thin-film concentrations were greatest in LCM and LCT sediments and smallest in PHM. This suggests that phenanthrene was substantially less available in PHM sediments than in LCM and LCT sediments despite the total phenanthrene concentration in the sediments being the same. Figure 2 illustrates that the apparent equilibrium concentration in the thin films followed linear relationships with the concentration in the sediments. This indicates that the exchange of phenanthrene between the sediments and the films follows first-order kinetics producing a doubling of the concentration in the thin film with every doubling of the

Table 1. Biota sediment accumulation factor (BSAF) values, ethylene vinyl acetate (EVA) uptake parameters (concentration of phenanthrene in EVA at apparent equilibrium [$C_{E,eq}$], uptake rate constant [k], estimated time to reach 95% of apparent equilibrium [t_{95}], and correlation coefficient [R^2]), and fugacity values (fugacity of phenanthrene in EVA [f_E] and lipid [f_{lip}], f_{lip}/f_E , and the fugacity capacity of sediment [Z_s]) for Lake Conjola Marine (LCM), Lake Conjola Terrestrial (LCT), and Port Hacking Marine (PHM) sediments

		BSAF		EVA uptake				Fugacity			
		10-d	Max	$C_{E,eq}$ ($\mu\text{mol}/\text{cm}^3$) ^a	k (per hour) ^a	t_{95} (h)	R^2	f_E (mPa) ^b	f_{lip} (mPa) ^b	f_{lip}/f_E ^b	Z_s (nmol/g/Pa) ^b
LCM	Low	0.53	1.5	7.2 (0.36)	1.8 (0.46)	1.7	0.93	0.065 ± 0.021	0.048 ± 0.017	0.74 ± 0.35	2.8 ± 0.91
	Mid	0.28	2.3	47 (2.6)	2.0 (0.61)	1.5	0.90	0.43 ± 0.14	0.10 ± 0.033	0.23 ± 0.11	1.6 ± 0.52
	High	0.29	1.9	130 (6.3)	1.4 (0.35)	2.2	0.92	1.2 ± 0.37	0.20 ± 0.068	0.17 ± 0.076	1.2 ± 0.38
LCT	Low	0.27	0.61	5.5 (0.68)	2.5 (1.9)	1.2	0.59	0.049 ± 0.016	0.027 ± 0.0091	0.55 ± 0.26	3.7 ± 1.2
	Mid	0.10	1.3	53 (6.5)	2.0 (1.4)	1.5	0.43	0.48 ± 0.15	0.039 ± 0.013	0.081 ± 0.037	1.5 ± 0.46
	High	0.23	0.91	120 (5.7)	1.3 (0.33)	2.3	0.92	1.1 ± 0.34	0.17 ± 0.060	0.15 ± 0.073	1.3 ± 0.42
PHM	Low	0.19	0.42	2.3 (0.14)	1.6 (0.50)	1.9	0.88	0.021 ± 0.007	0.014 ± 0.0048	0.67 ± 0.32	8.9 ± 2.9
	Mid	0.041	0.47	16 (1.7)	2.6 (0.73)	1.2	0.94	0.15 ± 0.047	0.012 ± 0.0040	0.080 ± 0.037	4.8 ± 1.5
	High	0.019	0.41	46 (3.1)	1.1 (0.39)	2.8	0.86	0.41 ± 0.13	0.011 ± 0.0037	0.027 ± 0.012	3.4 ± 1.1

^a Values in parentheses are standard errors.

^b Mean ± standard deviation.

concentration in the sediments within the concentration range tested. The slope of the film–sediment concentration relationships varies among sediments, illustrating that the PHM sediments contained a greater sorptive capacity than the LCM and LCT sediments. This result was consistent with the reported LC50 values, suggesting that sediment toxicity was least in the more sorptive PHM sediments. Toward the end of the exposure, the relative positions of thin-film uptake curves among sediment types (Fig. 1d–f) were comparable to the placement of tissue uptake curves (Fig. 1a–c), which also illustrated phenanthrene concentrations declining in the order LCM ≈ LCT > PHM.

Thin-film extraction as a measure of bioavailability

For each sediment, C_{lip}/C_E was variable during the early period of uptake but became relatively stable at a ratio between 0.05 and 0.35 by approximately 120 h. The C_{lip}/C_E did not stabilize earlier because the rate at which phenanthrene reached steady state in the organism (Fig. 1a–c) was slow relative to the rate of sediment–EVA equilibration (Fig. 1d–f).

The 240-h value of C_{lip}/C_E is plotted against sediment concentration in Figure 3. Two observations are important. First, for each phenanthrene dose, the corresponding values of C_{lip}/C_E for each sediment are similar despite differences in SOM geochemistry. This indicates that EVA thin-film extraction is unaffected by differences between sediments. Second, the $C_{lip}/$

C_E for the mid- and high-concentration treatments did not vary substantially from each other, although these were consistently lower than the C_{lip}/C_E for the low concentration treatments. By deduction, the decline in C_{lip}/C_E was due either to a decline in C_{lip} relative to C_E or to an increase in C_E not reflected by C_{lip} . However, C_E follows the desorbed concentration, which varies with sediment geochemistry, of which, as observed previously, C_{lip}/C_E is independent. Therefore, since C_E has been demonstrated to follow established models for phase transfer, the decline in C_{lip}/C_E is attributed to a concentration-dependent biological response. An attractive example of such a response might be the concentration-dependent induction of metabolic enzymes. Figure 1a to c illustrates that, for each of the three sediments, a more significant decline in C_{lip} was observed in amphipods exposed to the highest phenanthrene sediment concentration than in those exposed to lower concentrations. Therefore, EVA thin-film extraction was able to describe passive uptake processes but was unable to account for elimination routes that were biologically mediated.

Fugacity, fugacity capacity, and bioavailability

The mean (±standard deviation) K_{EO} value for phenanthrene was 2.18 ± 0.08 and 3.41 ± 0.15 after equilibrations of 2 and 7 d. Using the latter K_{EO} value and a literature value of $7.89 (\pm 2.31) \times 10^7$ for the octanol–air partitioning coefficient (K_{OA}) [27], an EVA–air partitioning coefficient (K_{EA})

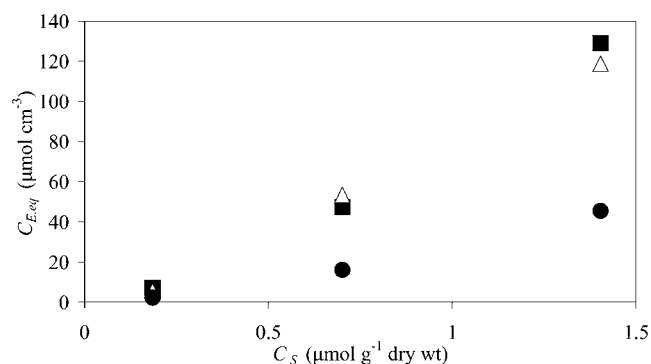


Fig. 2. Relationship between concentration in sediment and in the ethylene vinyl acetate film at apparent equilibrium for Lake Conjola Terrestrial (LCT = Δ), Lake Conjola Marine (LCM = \blacksquare), and Port Hacking Marine (PHM = \bullet) sediments.

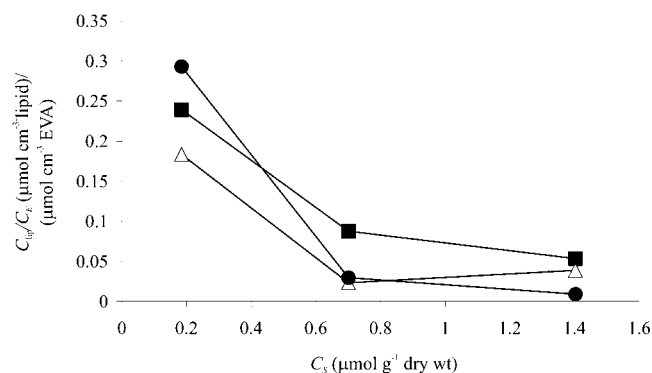


Fig. 3. Relationship between the ratio of lipid to ethylene vinyl acetate concentration and the concentration in sediment for Lake Conjola Terrestrial (LCT = Δ), Lake Conjola Marine (LCM = \blacksquare), and Port Hacking Marine (PHM = \bullet) sediments.

of $2.69 (\pm 0.86) \times 10^8$ was calculated for phenanthrene. This value compares well with the K_{EA} of 1.65×10^8 calculated from K_{OA} [27] and the $K_{OA}-K_{EA}$ relationship described by Wilcockson and Gobas [8]. Using the K_{EA} value from the current study, the Z_E value for phenanthrene was determined to be $1.10 (\pm 0.35) \times 10^5 \mu\text{mol}/\text{cm}^3/\text{Pa}$. From Z_E , Z_O was determined to be $3.23 (\pm 1.12) \times 10^4 \mu\text{mol}/\text{cm}^3/\text{Pa}$.

The $C_{E-\text{eq}}$ values estimated from the one-compartment uptake model were divided by Z_E to determine the fugacity of phenanthrene in the EVA films (Table 1). Similarly, the 10-d lipid-normalized tissue concentrations were divided by Z_O to determine the fugacity of phenanthrene in amphipod lipid. In situations where the bioaccumulation of a compound does not change with concentration, the ratio of the fugacity of the compound in the organism to the fugacity in sediment (f_{lip}/f_E) should be constant. Table 1 illustrates that the fugacities of phenanthrene in the organism approach those in the thin films (and therefore in the sediment); that is, f_{lip}/f_E ranges between 0.55 ± 0.26 and 0.74 ± 0.35 , for all three sediment types, but only for the low-concentration treatments. For mid- and high concentrations of phenanthrene in all three sediment types, the observed f_{lip}/f_E ratios are significantly lower than 1.0. Therefore, for the compound and species tested, f_{lip}/f_E was not constant but varied with concentration. This indicates that at low concentrations of phenanthrene in the sediment, first, the phenanthrene concentration in sediments and the amphipods were close to equilibrium, and, second, the thin-film concentrations were a reasonable surrogate for the concentration in the amphipods. At higher concentrations in the sediments, concentrations of phenanthrene in the amphipods were far below their equilibrium concentrations with the sediments. This may be due to a greater rate of enzyme induction and subsequently larger metabolic transformation of phenanthrene in the amphipods at higher phenanthrene exposures. At the lowest phenanthrene concentration in the present study, enzyme induction may have been small, causing a low rate of metabolic transformation and hence allowing the fugacity of phenanthrene in the amphipods to approach the fugacity in the sediments. If this is the case, nonmetabolizing chemicals can be expected to produce fugacities in the organisms that match (or exceed if the chemicals biomagnify) the fugacities in the sediments. The thin-film solid-phase extraction method that is designed to measure the fugacity in the sediment may therefore be a useful method to determine the exposure of benthic invertebrates to organic contaminants in the sediments.

The effect of SOM geochemistry on phenanthrene bioavailability

Phenanthrene was less bioavailable when associated with PHM sediments, and these sediments yielded consistently lower thin-film concentrations, fugacities, and BSAF values (Table 1) than the corresponding treatments from the Lake Conjola sediments. Higher Z_S values for this sediment relative to Lake Conjola are consistent with this sediment having a greater sorptive affinity (Table 1). Sediments rich in black carbon are likely to have a smaller pool from which organisms can accumulate hydrophobic contaminants, and several studies have identified that soils and sediments rich in this material can be less toxic than similar, although diagenetic carbon-rich, geosorbents [28,29].

Phenanthrene bioaccumulation from the LCM sediments was higher than from the LCT samples during the first 48 h (Fig. 1a-c). This suggests that phenanthrene associated with

the more marine Lake Conjola sediments was more bioavailable than that with the more terrestrially sourced substrate, with this distinction most marked early in the exposure. However, this difference was not reflected by the corresponding C_E values, which were not substantially different (Fig. 1d-f). This is an important result, as this difference between tissue and film concentrations implies that, early in the exposure, there were differences between the two sediments that affected bioavailability and that the EVA thin film could not account for these differences. For example, it may be that phenanthrene partitioning between sediment and water was affected by bioturbation during the initial period of the exposure. Agitation of the sediment during the organism's tube-habitat building behavior may have increased phenanthrene partitioning out of LCM sediment relative to that out of LCT sediment, making phenanthrene associated with LCM more bioavailable. This hypothesis would be consistent with the findings of a previous investigation [12] wherein both Lake Conjola sediments were identified as containing a pool of spiked phenanthrene that was desorption resistant, with LCT sediment containing a larger pool than LCM. The relative size of this pool was attributed to sedimentary aromatic carbon, which is generally more abundant in terrestrial-derived SOM than in marine-derived SOM. Several studies have recognized that aromatic carbon is often associated with structurally condensed SOM, which is more effective in sequestering HOCs than other types of organic matter [14,30,31]. Studies elsewhere have identified that this SOM functional group can play a role in controlling bioavailability to amphipods [32] and to microbial populations [33,34]. The latter result stresses an important limitation of abiotic media being used to characterize bioavailability, that is, that biotic processes that affect the exposure concentration (like metabolism, bioturbation, and tube building) cannot be accounted for by abiotic media.

Two results from the present study complicate interpretation of the sorptive behavior of the three sediments, specifically with respect to whether these sediments were limited in sorption capacity. First, the fugacity capacity of each sediment, broadly analogous to sorption capacity, declined with increasing solute concentration in sediment (Table 1). A decline in Z_S implies that phenanthrene sorption with SOM was limited by a finite number of binding sites; therefore, this result indicates that phenanthrene may follow a nonlinear, Freundlich-type isotherm in all three sediments. This finding supports models presented elsewhere that stress the importance of a finite-capacity SOM in HOC sorption [4]. However, it was also found in the present study that isotherms of film versus sediment concentration appear to be linear, at least within the concentration range investigated (Fig. 2). While it is possible that linearity may not be maintained at higher sediment concentrations, this finding complicates the previously mentioned interpretation of SOM sorptive behavior, as isotherm linearity would contradict the proposition that SOM is capacity limited. Further research is therefore warranted to assess how Z_S values can be applied in the characterization of sediments and associated organic matter.

Further potential for EVA exists as a solid-phase extraction medium in the assessment of PAH bioavailability in sediments. One application not explored in the current work involves the derivation of freely dissolved aqueous concentrations (activities) from measured thin-film concentrations and an EVA-water partition coefficient. The activities could be compared with established water quality criteria and used to establish

whether activities are a better indicator of chemical impact than total sediment concentration. Nevertheless, on the basis of the findings of the present study, we contend that the EVA thin-film approach qualifies as a promising predictive tool in bioavailability research.

SUPPORTING INFORMATION

Table S1. Polycyclic aromatic hydrocarbon (PAH) analytes and recoveries in laboratory control duplicate samples for Lake Conjola Marine (LCM), Lake Conjola Terrestrial (LCT), and Port Hacking Marine (PHM).

Figure S1. An ethylene vinyl acetate (EVA)-coated vial used for determining polycyclic aromatic hydrocarbon fugacity in sediment. The 0.05 μm thick EVA coating is colored pink by the dye Sudan IV.

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