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Diffusion Sampler for Compound Specific Carbon Isotope Analysis of Dissolved Hydrocarbon Contaminants

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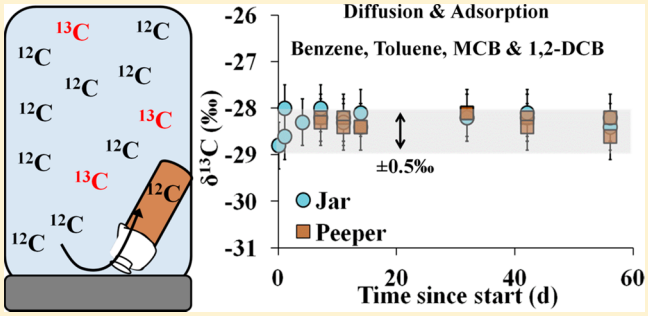
Supporting Information

ABSTRACT: Compound Specific Isotope Analysis (CSIA) is widely utilized to study the fate of organic contaminants in groundwater. To date, however, no method is available to obtain CSIA samples at a fine (cm) spatial scale across the sediment−surface water interface (SWI), a key boundary for discharge of contaminated groundwater to surface water. Dissolved contaminants in such discharged zones undergo rapid temporal and spatial changes due to heterogeneity in redox conditions and microbial populations. The compatibility of a passive sediment pore water sampler ("peeper") to collect 40 mL samples for CSIA of benzene, toluene, monochlorobenzene, and 1,2-dichlorobenzene at field-relevant concentrations (0.1−5 mg L^{-1}) was evaluated in laboratory experiments. Results demonstrate that physical diffusion across the polysulfone membrane does not alter the carbon isotope values (±0.5‰). Measured $\delta^{13}C$ values also remain invariant despite significant adsorption of the compounds on the peeper material, an effect which increased with higher numbers of chlorine atoms and sorption coefficient (K_{oc}) values. In addition, isotope equilibrium between the peeper chamber and the sediment pore water occurred in less than a day, indicating the peeper method can be used to provide samples for CSIA analysis at fine spatial and temporal sampling resolutions in contaminated sediments.



INTRODUCTION

Aromatic and chlorinated aromatic hydrocarbons have been frequently quantified worldwide in many groundwater and surface waters.[1−3] Serious health effects of these contaminants on the kidney, liver, nervous system, and possible carcinogenic properties have been recognized, leading to increased concern for human and ecosystem health.[3−5]

When contaminated groundwater discharges into surface water, aromatics and chlorinated aromatics tend to accumulate in the sediment−water interface (SWI) due to their fairly high sorption potential. Knezovich and Harrison[6] showed that sediment-laden chlorinated benzenes can be assimilated by benthic organisms and further up in the food web at a rate that is proportional to sediment pore water concentrations, thereby presenting a direct risk for aquatic life. Desorption of chlorinated benzenes to surface water acts as a direct vector of pollution toward aquatic ecosystems.[7]

In sediments, differences between the transfer and consumption rates for oxygen and other electron acceptors can affect chemical and microbial transformation reactions.[8] Kurt et al. showed that microbial activity in both the oxic and anoxic zones of sediment−water interfaces can protect the overlaying surface water by supporting biodegradation of substituted aromatics.[9,10] Similarly, in wetland sediments, Lorah and Olsen showed evidence for anaerobic degradation of trichloroethylene (TCE) and 1,1,2,2-tetrachloroethane (PCA) coming from a contaminated aerobic sand aquifer.[11] Understanding the rapidly changing geochemical, redox, and microbiological parameters across the SWI will provide important constraints on the potential for organic contaminants to biodegrade, or, in contrast, to persist and discharge into overlaying water and sediments.

The investigation of contaminant removal processes in sediment−water interfaces is critical to ensure the protection of surface waters. Passive equilibrium dialysis samplers, commonly known as "peepers," consist of several horizontal chambers that enable a discrete sampling of the sediment pore water with a high spatial resolution along the vertical gradient. The first peeper designs were proposed by Hesslein and Mayer[12,13] and were later optimized[14,15] and employed in many sediment−water interfaces.[16−18] Peepers can be made of different lengths depending on the application (e.g., 30−90 cm). Besides increased depth resolution, the major advantages of peepers are their ease of use, minimal disturbance of the sediment environment, maintenance of sample integrity, and compatibility with ecotoxicology evaluation studies.[19] Before

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deployment in the field, peeper chambers are initially filled with oxygen-free deionized water and covered with a membrane.[19] The functioning principle of peepers used for determination of contaminant concentrations is based on equilibration between ambient interstitial pore water and peeper chamber water. The time required for concentrations to equilibrate is a minimum estimate for deployment time in the field, which depends on membrane properties, peeper chamber geometry, and contaminant physical−chemical characteristics.[19]

Implementing mass balance approaches in interstitial sediment pore water using concentration measurements is a first step to evaluate contaminant removal. However, it does not distinguish between transfer (nondegradative) and transformation (degradative) processes. Whereas the latter involves complete elimination of the parent contaminant, the former only redistributes it to the environment (e.g., via dilution, diffusion, adsorption). There is considerable interest in integrating peeper technology with a technique that can identify if biotransformation is actually occurring. Compound Specific Isotope Analysis (CSIA) has proven effective for differentiating between degradative and nondegradative processes for petroleum hydrocarbons, fuel additives, and chlorinated ethenes and ethanes. Earlier papers have demonstrated that fractionation of stable carbon isotopes is generally either within analytical uncertainty (±0.5‰) for equilibrium adsorption,[20,21] dissolution,[22,23] volatilization,[21,24,25] and gas[26,27] and aqueous phase diffusion or small compared to isotopic fractionation that occurs when chemical bonds are broken in transformation reactions.[28] During biodegradation, significant isotope fractionation can occur due to differences in reaction rates of ^{12}C- versus ^{13}C-bearing contaminants. This kinetic isotope effect (KIE) can be identified by determining the contaminant stable isotope delta value ($\delta^{13}C$ for carbon isotopes), expressed as

$$\delta^{13}C = \frac{R_s - R_{std}}{R_s} \quad (1)$$

where R_s and R_{std} are the isotope ratios ($^{13}C/^{12}C$) for a sample and an international standard, respectively. Typically, over the course of degradation, the remaining parent contaminant becomes progressively enriched in ^{13}C, leading to a less negative $\delta^{13}C$ value. These results have permitted the use of CSIA as a line of evidence for *in situ* biodegradation of many hydrocarbons and chlorinated solvents.[28] For carbon stable isotopes, a 0.5‰ total instrumental error encompassing accuracy and reproducibility is generally accepted,[29] whereas a minimum of a 2‰-difference between two samples is recommended for data interpretation.[28,29]

To date, CSIA has been mainly applied to contaminated groundwater[30−33] and some wetland systems.[34−36] Only one group has integrated CSIA and peeper technology for carbon isotope analysis of dissolved methane using a 75-μm-thick Teflon membrane.[37−39] However, aromatics and chlorinated aromatics have significantly different sorption, volatilization, and reactivity properties from methane, preventing extrapolation of these results[37,38] to these contaminants. Similar to peepers, High Resolution Multi-Level Well (HR-MLW) groundwater sampling every 3 cm was able to identify biogeochemical gradients and sulfate reduction processes combining concentration and isotope analysis for sulfur and oxygen.[40] Contrary to peepers, HR-MLW sampling is adapted to groundwater wells and requires more material and cost than peepers installed in surface water−sediment interfaces. In order to ensure that samples in the peeper chambers will permit accurate and precise measurements of the isotopic signatures of target contaminants dissolved in pore water in the field, peeper design parameters (e.g., membrane type, peeper material) and standard operating procedures (e.g., deployment period) must be carefully selected and tested for their suitability for CSIA before deployment in the field.

The primary objective of this paper was to develop and validate a peeper design that is compatible with sample collection for CSIA of aromatics (benzene, toluene) and chlorinated aromatics (monochlorobenzene (MCB), 1,2-dichlorobenzene (1,2-DCB)) present at realistic concentrations typically found at a contaminated field site (0.1−15 mg L^{-1}).[34,41,42] Tests carried out to verify the peeper and membrane design and functionality for CSIA fulfilled a secondary objective: providing data that could confirm the absence of carbon isotope fractionation effects associated with contaminant diffusion through and adsorption on the peeper membrane.

MATERIAL AND METHODS

Peeper Design. The peeper tested in this study was 39.5 cm long, 20.4 cm wide, and 3.3 cm thick (Supporting Information (SI) Figure S1). The peeper body was made on a 3D stereolithography machine using polycarbonate material. It consists of four parts held together with stainless steel rods: two complementary main body components and two end pieces. Within each main body component, 11 chambers are distributed along the length of the peeper. Each chamber fits one 40 mL EPA VOA vial, hereby called a "peeper chamber." The peeper was therefore composed of a total of 22 chambers, each 3 cm apart. The peeper's external edge design includes a lip on each chamber to maintain the vials in place on the outward facing edge. Oxygen concentration in saturated sediments is usually below detection below the top few millimeters or centimeters. Therefore, to be representative of *in situ* redox conditions, before use, the vials were filled with oxygen-free deionized water and covered with a membrane. A polysulfone membrane was selected due to its resistance to biodegradation when incubated in sediments.[43] The membrane is placed over the mouth of the vial, and a Viton O-ring is used to securely hold the membrane around the vial neck. Another Viton O-ring was placed behind each bottle in the chamber to prevent them from sliding out. The exposed surface area of the membrane on each VOA vial opening was 3.1 cm^2. The two main advantages of using 40 mL vials are the simplicity in sampling the peeper vials and the possibility to combine two peeper vials from the same row (same depth) for preconcentration, if lower detection limits needed to be reached.

Peeper Laboratory Experiment Setup. Three stock solutions (125 mg L^{-1}) of (i) benzene, (ii) 1,2-DCB, (iii) toluene and MCB were prepared in Nanopure water (18.2 MΩ-cm). All isotopic working standards were characterized by continuous flow isotope ratio mass spectrometry (IRMS, see description below). Characterized $\delta^{13}C$ values were −28.5 (benzene), −28.5 (1,2-DCB), −28.2 (toluene), and −28.7 ‰ (MCB) with a total uncertainty of ±0.5‰ accounting for both accuracy and reproducibility.[29] A 20 L volume of O_2-free Nanopure water was prepared in a polypropylene tank (Nalgene, Thermo Scientific) by continuously sparging O_2 with a flow of argon until all jars and peepers were prepared. Oxygen concentrations were monitored throughout the whole preparation process to ensure concentrations were lower than 5% saturation. The peeper chamber vials were filled with O_2-free water, and each was covered, under water, with a 142 mm diamater polysulfone membrane (HT Tuffryn 450 membrane disk filters polysulfone 0.45 μm, VWR). The membrane was maintained in place on the

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vial mouth using a 20 cm long nylon fishing line. The vials were left submerged in the tank until use.

Three peeper experiments were conducted: one with benzene, one with 1,2-DCB, and one with both toluene and MCB together. The three stock solutions were distributed into individual 40 mL vials with no headspace and placed in the fridge until use. A series of twenty-four 940 mL glass sacrificial jars with Teflon-lined lids were prepared for each experiment (Figure S2). Jars were submerged in O_2-free Nanopure water in a large tank, and a 40 mL vial (the peeper chamber), prepared as described above, was placed inside the larger jar (Figure S2). While the jars were submerged, a 40 mL stock solution of benzene, 1,2-DCB, or a mixture of toluene and MCB was then added through the jar mouth opening to the bottom of the jar using a 50 mL Micro-Mate Luer-Lock syringe (Popper & Sons, Inc., New Hyde Park, NY) with a 30 cm long needle. The cold stock solution enabled it to sink to the jar bottom during introduction. After introduction of the vial and stock solution, the jar lid was quickly secured while under water, before removal from the water tank. The jar was then vigorously shaken for 20 s and then stored upside down (to limit losses outside the jar's lid) in the fume hood until sampling. Initial concentrations ranged from 2.35 ± 0.54 (MCB) to 4.47 ± 0.95 mg L^{-1} (1,2-DCB; SI, Table S1). The sealed jars simulate the groundwater aquifer and ensure no loss (or changes in $\delta^{13}C$ value) of the target compound other than diffusion across the membrane into the 40 mL vial.

Duplicate blanks were prepared for each experiment with O_2-free water and a peeper vial, without the addition of any stock solution. At each of the nine sampling times over two months (0, 1, 2, 4, 7, 10, 14, 28, 42, 56 days since the start of the experiment), duplicate jars and vials were sacrificially sampled. The jars were vigorously shaken for 20 s before opening. A 40 mL subsample of the jar solution was taken to determine concentration and isotope value ($\delta^{13}C$) of contaminant remaining in the solution. The 40 mL VOA vials had the membrane removed and were securely capped. Samples were then kept at 4 °C until analysis.

Control Experiments. In each of the "peeper experiments" described above, two duplicate control jars were prepared and sampled after 14 and 42 days following the start of the experiment. These control jars only contained the solution of the contaminant with no peepers.

In addition, two "sorption control" experiments were conducted to test the effects of sorption of the compounds onto the various materials used in the experimental setup. The first included benzene, toluene, and MCB, whereas the second included 1,2-DCB alone. Polysulfone membrane (0.72 g, 316.7 cm^2), polycarbonate material (two pieces tested with two shapes: 1.90 g, 11.6 cm^2; 7.21 g, 62.1 cm^2), Viton O-rings (two pieces tested with two shapes: 0.73 g, 6.2 cm^2; 1.45 g, 9.8 cm^2), and fishing line (0.11 g, 6.7 cm^2) were each added to a jar (940 or 137 mL) filled with O_2-free Nanopure water and a volume of the stock solution as described previously. These samples were prepared and sacrificially sampled over a period of three weeks. Samples were kept at 4 °C before concentration and stable isotope analysis.

Analytical Methods. Benzene, toluene, and MCB concentrations and stable isotopes were determined by headspace analysis (300-μL (concentrations) and 1 mL (CSIA) injections) after the addition of 5 g of NaCl to enhance partitioning to the gas phase, after the method of Slater et al.[23] For 1,2-DCB, a preconcentration step, consisting of a liquid−liquid extraction, was conducted. For concentration analysis of 1,2-DCB, 250 μL of hexane was added to a 6.8 mL aliquot of sample in a vial (no headspace); the mixture was shaken using a wrist action shaker for 15 min and then allowed to rest for 15 min before collecting the 250-μL extract, now containing 1,2-DCB after the method of Dempster et al.[22] A 2-μL subsample of the extract was injected into the gas chromatograph (GC). For isotope analysis of 1,2-DCB, the purge-and-trap technique was used as described in Zwank et al.[44] A Teledyne Tekman purge-and-trap sample concentrator was used with a type K trap (Tekman Purge Trap K, Vocarb 3000). After an 11 min purge with helium, the contaminants were desorbed from the trap for 6 min at 220 °C. The trap was then baked at 230 °C for 10 min.

For all four compounds, concentrations were determined using a Varian 3380 or 3400 GC equipped with a flame ionization detector (FID) and a VOCOL capillary column (Supelco, 30 m × 0.25 mm, 1.5 μm film thickness) from Supelco. Injector and detector temperatures were 200 °C. Stable isotopes were determined by GC-C-IRMS (GC Varian 3400 connected via a combustion interface (C) to an IRMS Finnigan MAT 252 or Delta XP). A VOCOL (Supelco, 60 m × 0.32 mm, 1.8 μm film thickness) GC column was used for all four molecules, and the injector temperature was 180 °C. Isotope ratios are reported as $\delta^{13}C$ values relative to the international standard V-PDB, with a total uncertainty incorporating both accuracy and reproducibility of 0.5‰ (see above).[28,29] In this study, differences in $\delta^{13}C$ values of at least 1‰ are considered significant, as recommended in ref 28. Details on sampling, injection, and temperature programs are provided in Table S2.

Modeling. A simplified model was applied to the jar and peeper vial concentration data. The purpose of the model was to describe 1D transport of the four contaminants from the jar into the peeper vial. Instantaneous mixing and steady state conditions in both the jar and peeper vial sides are assumed. For each system (i.e., a jar containing a peeper vial), the volume of the jar into which a peeper vial was immersed was large compared to that of the peeper vial (the latter accounting for 4.7% of jar volume). In the field, the decrease in concentrations in the sediment pore water adjacent to a peeper chamber membrane is compensated by replenishment of solute due to diffusion, desorption, and dissolution of contaminants from surrounding areas. Contaminant transfer through the membrane is often considered the rate-limiting step, as compared with contaminant diffusion to water boundary layers adjacent to the membrane. We therefore assumed that the membrane exerted the highest resistance to contaminant diffusion. The expression for the flux of contaminants (J) being transported from the jar to the peeper vial can be written for the jar side as

$$J_j = -\frac{DH}{l}(C_j(t) - C_p(t)) \quad (2)$$

For the peeper vial side, the concentration increase can be written as

$$J_p = -\frac{D}{l}(C_p(t) - C_j(t)) \quad (3)$$

where D and H are two coefficients used to parametrize the model, l is the membrane thickness, and $C_j(t)$ and $C_p(t)$ are the jar and peeper vial concentrations, respectively, at time t. In this model, D is a transfer coefficient of the contaminants across the membrane, which includes contaminant diffusion into the jar and peeper solutions, the jar and peeper boundary layers on each side of the membrane, and the membrane itself. Parameter D is also affected by possible losses of contaminants due to volatilization

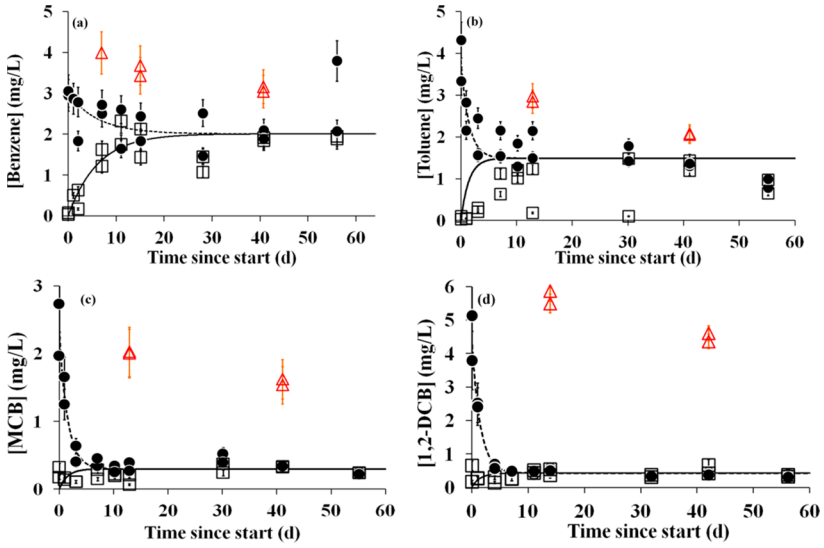

Figure 1. Jar (●), peeper vial (□), and control jar (△) concentrations over time since the start of the peeper experiments, for (a) benzene, (b) toluene, (c) MCB, and (d) 1,2-DCB. The error bars represent the total error on concentration measurements (2 standard deviations): 13% (benzene), 10% (toluene), 18% (MCB), and 23% (1,2-DCB). The initial concentration points were determined from samples taken right after the stock solution was introduced in the jar, and the jar was shaken vigorously for 20 s.

or adsorption onto the membrane or jar material. Hence, D cannot be considered a true diffusion coefficient, and the general term "diffusion-like coefficient" will be employed to describe it. Similarly, H is comparable to a partitioning (adsorption) coefficient of the contaminants between the solutions and membrane material. Though it is not exactly a sorption coefficient, the term "adsorption-like coefficient" will be used throughout the paper. Parameters D and H were computed in order to provide a quantitative means to compare contaminants' transport through the membrane.

The flux is defined as a unit of contaminant mass diffusing per unit area of the membrane (A) and per unit of time. The system of homogeneous linear differential equations can be written as

$$\frac{d}{dt}\begin{bmatrix} C_j(t) \\ C_p(t) \end{bmatrix} = \begin{bmatrix} -\frac{DAH}{lV_j} & \frac{DAH}{lV_j} \\ \frac{DA}{lV_p} & -\frac{DA}{lV_p} \end{bmatrix} \cdot \begin{bmatrix} C_j(t) \\ C_p(t) \end{bmatrix} \quad (4)$$

where V_j and V_p are the jar and peeper volumes, respectively. Using initial conditions of null peeper concentration ($C_p(t=0) = 0$) and $C_j(t=0) = C_{j,0}$, the system's solution is

$$C_j(t) = \frac{C_{j,0}}{V_j + V_pH} \cdot [V_j + V_pH \times e^{-DA/l(\frac{H}{V_j}+\frac{1}{V_p})t}] \quad (5a)$$

$$C_p(t) = \frac{V_jC_{j,0}}{V_j + V_pH} \cdot [1 - e^{-DA/l(\frac{H}{V_j}+\frac{1}{V_p})t}] \quad (5b)$$

When concentration equilibrium is reached between the jar and peeper vial (i.e., when $t \to \infty$), $C_j(t \to \infty) = C_p(t \to \infty) = C_{eq}$ (equilibrium concentration), an expression for H can be found from the above solution:

$$H = \frac{V_j(C_{j,0} - C_{eq})}{C_{eq}V_p} \quad (6)$$

eq

The error on H was calculated using the error propagation on eq 6 and is presented as the expanded uncertainty for a coverage factor of 2 (95% level of confidence).

An estimate for parameter D was obtained for each contaminant by fitting the expression for $C_j(t)$ to the data with the nonlinear least-square method using the Gauss−Newton algorithm in the R software,[45] with initial values of 7.4×10^{-9} (benzene, toluene), 7.4×10^{-10} (MCB), and 7.4×10^{-8} cm^2 s^{-1} (1,2-DCB).

RESULTS

Controls and Blanks. Blanks showed no background contamination from the experimental setup or material (data not shown). In Figure 1, concentrations for the four contaminants in the controls (no peepers) showed a decrease over the course of the experiment. For instance, after 42 days, concentrations decreased by 46 ± 8 (toluene) and 33 ± 17% (MCB) compared to the initial concentration, likely due to volatilization out of the jar due to poor Teflon-lined lid sealing. Values of δ^{13}C in all controls remained within ±0.5‰ of the known δ^{13}C values for each contaminant working standard, indicating no changes in δ^{13}C of the contaminants in the controls (Figure 2).

Sorption Controls on Peeper Material. Sorption control tests were conducted with pieces of each of the peeper materials (polysulfone membrane, polycarbonate material, Viton O-rings, fishing line) in contact with solutions of dissolved benzene, toluene, MCB, and 1,2-DCB (Figures S3−S6). Over 21 days, no significant changes in concentration or isotope values were observed in the experiments using a piece of fishing line and the Viton O-rings (Figures S5, S6). All four compounds showed a decrease in concentration over time on the polysulfone membrane (Figure S3) and polycarbonate material (Figure S4), consistent with adsorption to these materials (see compounds' adsorption coefficients in Table S1). For the polysulfone membrane, this concentration decrease was more significant for the two chlorinated compounds than for the nonchlorinated ones. For instance, initial concentrations were 3.26 ± 0.43 (benzene), 3.62 ± 0.38 (toluene), 2.69 ± 0.48 (MCB), and 2.53 ± 0.99 mg L^{-1}

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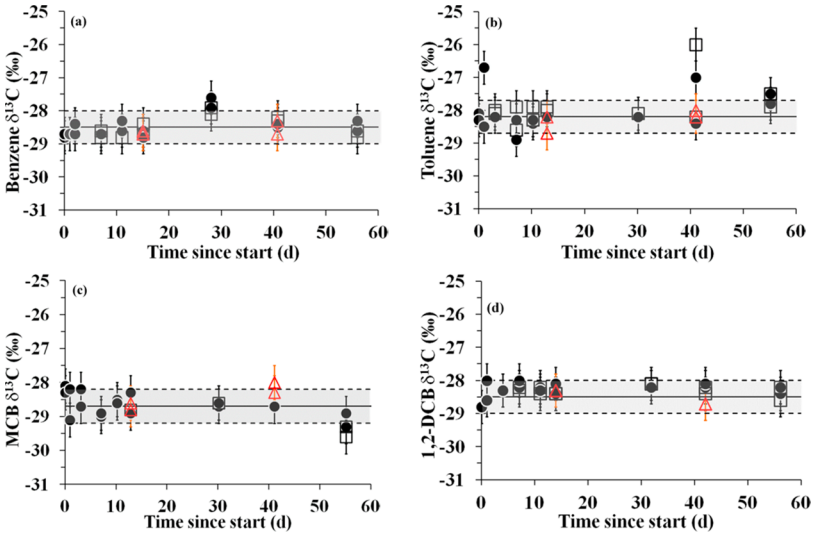

Figure 2. Jar (●), peeper vial (□), and control jar (△) isotope $\delta^{13}C$ values over time since the start of the peeper experiments, for (a) benzene, (b) toluene, (c) MCB, and (d) 1,2-DCB. The black horizontal lines correspond to the compounds' characterized values consisting of the average $\delta^{13}C$ value resulting from several injections over different split settings, concentrations, and injected volumes, as described in Ward et al.[63] and Gray et al.[64] The horizontal dotted lines and data point error bars represent a ±0.5‰ total error encompassing both accuracy and reproducibility after Sherwood Lollar et al.[29]

(1,2-DCB), whereas after 14 days, concentrations were 3.56 ± 0.55 (benzene), 1.97 ± 0.48 (toluene), 0.58 ± 0.03 (MCB), and 0.25 ± 0.06 mg L^{-1} (1,2-DCB; Figure S3). Concentrations decreased rapidly to equilibrium in 2 to 4 days for all compounds. Overall, no significant changes in $\delta^{13}C$ values were observed in these experiments with the possible exception of benzene and toluene which showed enriched $\delta^{13}C$ values although always within ±1‰ of their characterized values. Benzene and toluene being fairly volatile (Henry's coefficients, K_H = 5.55 × 10^{-3} (benzene) and 6.64 × 10^{-3} atm m^3 mol^{-1} (toluene)), the observed ^{13}C enrichment might be due to small leaks outside the jar.

Peeper Experiments. *Isotope Equilibrium.* At each sampling point, the $\delta^{13}C$ values of all compounds in both the jar and associated peeper vial did not change within ±0.5‰ (Figure 2). Most importantly, the $\delta^{13}C$ values for all four contaminants in the jar and peeper vials remained constant (±0.5‰) throughout the experiment. For three toluene data points, $\delta^{13}C$ values fell outside the ±0.5‰ range of its characterized value. Compared to the overall data, it is clear (Figure 2b) that there was no constant change in $\delta^{13}C$ of toluene through the experiment, and these points may be outliers.

Concentration Equilibrium. While the $\delta^{13}C$ values remained constant, the contaminant concentrations in the jars decreased, and those in the VOA vials correspondingly increased until equilibrium was reached. Jar and peeper concentrations equilibrated after 11 days for MCB and 1,2-DCB, and after 14 days for benzene and toluene (Figure 1). Equilibrium concentrations were calculated as the average of jar and peeper measured concentrations at and after equilibrium, determined graphically. Equilibrium concentrations were 2.01 ± 0.57 (benzene), 1.49 ± 0.28 (toluene), 0.30 ± 0.08 (MCB), and 0.43 ± 0.10 mg L^{-1} (1,2-DCB) (Table S1).

Model Parameters. Table S1 summarizes experimentally determined initial and equilibrium concentrations and model-derived H and D parameters. The diffusion-like coefficients obtained via modeling indicated that toluene transferred at the fastest rate (1.28 × 10^{-4} cm^2 s^{-1}), followed by benzene (5.14 × 10^{-5} cm^2 s^{-1}), MCB (3.94 × 10^{-5} cm^2 s^{-1}), and 1,2-DCB (3.05 × 10^{-5} cm^2 s^{-1}). This order is similar to contaminants' diffusivities in water (Table S1), except for toluene. Values for H ranged from 11 ± 6 (benzene) to 202 ± 73 (1,2-DCB), a good correlation with the established sorption coefficients (K_{oc}) for these compounds (Table S1 and Figure S7). Equation 5a was applied for the hypothetical case where H = 1, in order to determine equilibrium concentrations in the case where no adsorption of the contaminant would have occurred ($C_{eq\_calc\_H=1}$). These were higher than those actually measured: $C_{eq\_calc\_H=1}$ were 2.87 ± 0.37 (benzene), 3.66 ± 0.37 (toluene), 2.24 ± 0.40 (MCB), and 4.27 ± 0.98 mg L^{-1} (1,2-DCB; Table S1). The poor fit of the model for H = 1 can be observed in Figure S8. The great improvement of the model fit to the data when parameter H is not forced to unity is evident from Figure 1 and suggests the importance of accounting for adsorption-like processes to explain the transport of contaminants from the jar through the membrane into the peeper vial.

DISCUSSION

Sediment Pore Water Sampling Technique for CSIA. While our experimental and modeling results established that contaminant diffusion through and adsorption onto the membrane likely occurred, they also demonstrated that neither of these two processes caused significant fractionation of stable carbon isotopes. Peepers with a polysulfone membrane are therefore compatible with CSIA application on collected sediment pore water samples. Over the 56-day time period of the experiment, no temporal trends were discernible in $\delta^{13}C$ for any of the studied contaminants, at the sampling time scale at which the experiments were conducted. A minimum of 1 day separated two sampling events. The absence of isotopic fractionation at this sampling time scale suggests that 1 day is sufficient for isotopic equilibrium to be reached.

The results presented here also help establish the most appropriate deployment time for the peepers in the field. Typically, peepers are deployed long enough so that concentrations equilibrate between the sediment pore water and the peeper chambers (the "vials" in the herein presented design).

As a result, this concentration equilibrium time controls the peeper deployment time in the field. In these laboratory experiments without sediments, concentrations equilibrated after 11 to 14 days. As suggested in former studies, a two- to four-week deployment time can be recommended if concentrations are to be determined.[12,16−19,37] Here, we showed that at the time when concentrations equilibrated, the jar and peeper isotope values were identical. More importantly, jar and peeper vial $\delta^{13}C$ values are the same ($\pm 0.5‰$) at any time while contaminants diffuse from the jar artificial sediment pore water to the peeper vial. If CSIA alone is to be implemented, peepers could therefore be deployed for a reduced time period. It has to be noted that the deployment period should allow for collecting sufficiently high concentrations in the vials that equal or exceed CSIA detection limits (5−10 $\mu g\ L^{-1}$ for stable carbon isotopes[22,44,46]). Stable isotope analysis is rarely conducted without preliminary concentration analysis. However, if the extent of contamination of the sediment pore water at a given site is well characterized and CSIA alone is applied, this peeper technique has the potential to provide a fine temporal and vertical sampling resolution.

Absence of Significant Diffusion- and Sorption-Induced Isotopic Fractionation. While the jar concentrations decreased over the course of the experiments, those in the peeper vials increased. Besides an initial manual mixing of the jar solutions after their preparation, no other advective processes supported contaminant transport from the jar to the peeper vials (e.g., no stirring of the jar solution). However, jar and peeper concentration differences were likely the controlling factor on contaminant transport through the polysulfone membrane via diffusion. For the four contaminants, results showed that the $\delta^{13}C$ value of each jar was identical to that of its associated peeper vial within a $\pm 0.5‰$ accuracy and reproducibility error. This demonstrates that diffusion through the membrane is isotopically conservative (i.e., not fractionating).

The diffusion-like coefficients estimated herein all agreed within an order of magnitude with the water diffusivities found in the literature (Table S1). For all four contaminants, the slight offsets in the estimated diffusion coefficients, as compared with published water diffusivities, were all in the same direction, i.e., all estimated diffusion-like coefficients were larger than previously published water diffusivities. Apart from toluene, the results also showed that with an increasing number of chlorine atoms on the benzene ring, the concentration decrease over time was more pronounced. In addition, the modeling tests in the hypothetical case where $H = 1$ resulted in a bad fit of the model to the data. These observations support the role for partitioning of the contaminants between the solution and the membrane, likely due to adsorption.

Decreases in contaminant concentrations in the presence of different material also demonstrated the potential for adsorption (Figures S3−S6), especially for chlorinated aromatics on the polysulfone membrane (Figure S3). Nevertheless, some of these losses might also be attributable to leaking through the jar's Teflon-lined lid, as confirmed by the control jars in the peeper experiments whose concentrations decreased over time (Figure 1). However, even when some enrichment in ^{13}C was observed, it did not exceed $\pm 1‰$. It is interesting to note that MCB and 1,2-DCB concentrations decreased rapidly from 2.69 ± 0.48 (MCB) and 2.53 ± 0.99 (1,2-DCB) to 0.29 ± 0.09 (MCB) and 0.14 ± 0.29 mg L^{-1} (1,2-DCB) in the first four days, thus emphasizing the potential of the membrane to adsorb chlorinated contaminants. However, despite the large concentration decreases, their isotopic composition remained stable ($\pm 0.5‰$). The surface area of the polysulfone membrane available for adsorption in these sorption control experiments was 100 times larger (316.7 cm^2) than the area actually exposed in the field (3.1 cm^2). The concentration decreases in the jars during the peeper experiments, for all contaminants, were therefore likely strongly affected by the presence of the large surface area of the membrane. For example, during the first two days, MCB concentrations decreased by 2 mg L^{-1} in the sorption control experiment with the polysulfone membrane, and by 0.5 mg L^{-1} with polycarbonate, corresponding to a total mass decrease rate for these materials of 0.67 mg day^{-1} m^{-2}. This would be expected to be replenished due to MCB diffusion from surrounding areas in the sediment pore water. Indeed, in the field, a diffusion of 76 $\mu g\ day^{-1}\ m^{-1}$ can be estimated using the MCB diffusion coefficient in sediments assuming a tortuosity of 1.59 and a concentration gradient of 2.54 mg L^{-1}, equivalent to that used in the sorption control experiment.

Electrostatic attractive forces that occur through aromatic stacking interactions are well established in biological[47] and synthetic systems.[48] Since each repeating unit of the polysulfone polymer contains four aromatic benzyl groups capable of electrostatic interactions with the aromatic contaminants used in this study, the adsorption of the contaminants to the membrane likely results from aromatic stacking interactions. The largest decreases in concentration were observed for 1,2-DCB and MCB. Less significant concentration changes were observed for benzene and toluene. The observation that a stronger interaction exists between the chlorinated benzenes and the polysufone membrane corresponds with the expected increase in the strength of interactions expected from substituent effects, as reported by Cockroft et al.[48,49] These authors showed that aromatic stacking interactions are influenced by substituents that alter the electronic properties of the aromatic ring via an inductive electron withdrawing/releasing effect.[50] Consistent with the results in Figure 1 and Figure S3, stronger stacking interactions are expected from electron withdrawing substituents (e.g., chlorine in MCB and 1,2-DCB), whereas electron releasing substituents (e.g., methyl group in toluene) are expected to produce weaker stacking interactions. Since these are noncovalent interactions, negligible isotopic fractionation was observed (Figure 2 and Figure S3). Our results on aromatic and chlorinated aromatic contaminants are thus in agreement with former studies that showed negligible isotopic fractionation of stable carbon isotopes under equilibrium sorption conditions.[20,51] The adsorption coefficients of the herein studied four compounds are similar to or higher than those of other common groundwater contaminants such as chlorinated ethenes and ethanes, suggesting applicability to a broader range of target compounds. The strongest stacking interaction is expected to occur between the membrane and contaminant. Additional interactions between contaminants bound to the membrane are not expected. Therefore, once the available binding sites on the membrane are saturated with a contaminant, the large pore size relative to the size of the contaminant molecules is not expected to affect diffusion across the membrane upon implementation in the field.

Diffusion is a mass-dependent mechanism that can theoretically discriminate between light and heavy isotopes.[52] Van Breukelen and Rolle[53] observed up to a 2‰ difference in groundwater PCE $\delta^{13}C$ values between the plume center and fringes, by measurements and computer simulations conducted every 20 cm, which is within the 2‰ minimum difference that the U.S. EPA recommends for reliable interpretations to be made.[28] Diffusion-related isotope fractionation will unlikely be observed in real field situations, given classically implemented

sampling space scales (0.25 to a few meters minimum per well), mixing of the water during well sampling, sampling time scales (from days to years), groundwater flow processes (rarely dominated by diffusion alone), currently available analytical method detection limits (>5 to 10 μg/L),[22,23,44,46] and analytical error (±0.5‰).[29] In addition, no more diffusion-related isotope fractionation would be observed once concentrations equilibrate between two zones, which is the case when peepers are retrieved from sediments. Hence, if peepers are collected when isotope equilibrium is reached, there is potential for CSIA of elements other than carbon (e.g., H, Cl). This should be verified in future studies (see SI Table S3).

Implications for Field Investigations at Sediment/Water Interfaces. Peepers have been used for a long time to collect sediment pore water to quantify inorganic and organic compound concentrations along vertical gradients.[12,13] For instance, using peepers with a polysulfone membrane installed in contaminated wetland sediments, Lorah and Olsen observed vertical gradients of TCE and PCA concomitantly to lower chlorinated ethenes and ethanes, thus providing preliminary evidence of *in situ* natural attenuation of TCE and PCA.[11] In the field, in addition to adsorption on the peeper and membrane materials, and diffusion through the membrane, contaminants can also adsorb on sediment particles, diffuse through the sediment pores, and be subject to microbial degradation. Among these processes, aerobic and anaerobic biodegradation are the only ones to have the potential to significantly affect contaminant δ^{13}C values. Because former studies have demonstrated the resistance of polysulfone membranes to biofouling, membrane-associated microbial communities are not expected to produce significant isotope effects,[43] although future additional studies would be a worthwhile confirmation. Enrichment factors were established in laboratory microcosm experiments for anaerobic degradation of benzene (−1.5 ± 0.1‰ to −3.6 ± 0.3‰),[54−56] toluene (−0.5 to −1.8 ± 0.3‰),[57−59] and dechlorination of MCB (−5.0 ± 0.2‰) and 1,2-DCB (−0.8 ± 0.1‰).[60] Depending on the reaction mechanism, aerobic degradation can also generate carbon isotope fractionation ranging from −0.7 ± 0.1‰ to −4.3 ± 0.4‰ for benzene[55] and from −0.4 to −3.3 ± 0.3‰ for toluene.[31,58,59,61,62] Conversely, no significant isotopic fractionation was observed for degradation of MCB under aerobic conditions.

Large variations in oxidation−reduction conditions prevail in sediments and can impact contaminant isotope ratios. The fact that diffusion and adsorption are isotopically conservative in this study indicates that δ^{13}C values can be used to distinguish between contaminant degradation and transfer (nondegradative) processes in the sediment pore water using the proposed peeper technique. Isotope results from peeper-collected pore water samples in the field are in preparation for publication.

■ ASSOCIATED CONTENT

Supporting Information

Gas chromatography temperature programs for concentration and stable isotope analysis are provided in Table S2. Diagrams of the peeper and lab experimental setup are presented in Figures S1 and S2, respectively. Results from the sorption control experiments are in Figures S3−S6. Modeling results are summarized in Table S1 and Figures S7 and S8. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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