

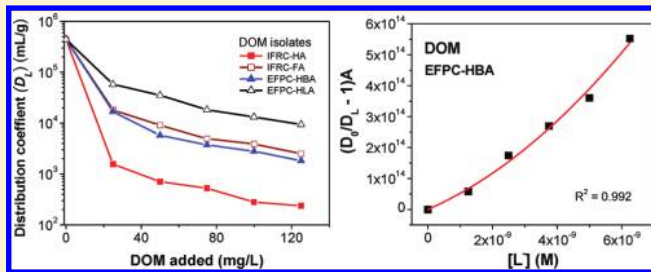
Binding Constants of Mercury and Dissolved Organic Matter Determined by a Modified Ion Exchange Technique

Wenming Dong,[†] Yongrong Bian,[‡] Liyuan Liang,^{*} and Baohua Gu^{*}

Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, United States

Supporting Information

ABSTRACT: Ion-exchange techniques have been widely used for determining the conditional stability constants ($\log K$) between dissolved organic matter (DOM) and various metal ions in aqueous solution. An exception is mercuric ion, Hg^{2+} , whose exceedingly strong binding with reduced sulfur or thiol-like functional groups in DOM makes the ion exchange reactions difficult. Using a Hg-selective thiol resin, we have developed a modified ion-exchange technique which overcomes this limitation. This technique allows not only the determination of binding constants between Hg^{2+} and DOM of varying origins, but also the discrimination of complexes with varying coordination numbers [i.e., 1:1 and 1:2 Hg:thiol-ligand (HgL) complexes]. Measured $\log K$ values of four selected DOM isolates varied slightly from 21.9 to 23.6 for 1:1 HgL complexes, and from 30.1 to 31.6 for 1:2 HgL_2 complexes. These results suggest similar binding modes that are likely occurring between Hg^{2+} and key thiolate functional groups in DOM particularly at a relatively low Hg to DOM ratio. Future studies should further elucidate the nature and precise stoichiometries of binding between Hg^{2+} and DOM at environmentally relevant concentrations.



INTRODUCTION

Dissolved organic matter (DOM) is one of the most important aqueous species influencing mercury (Hg) speciation, transformation, and bioavailability in aquatic ecosystems.^{1–6} This is due to strong complexation between mercuric ion, Hg^{2+} , and reduced sulfur (S) functional groups in DOM.^{7–11} As a soft metal ion, Hg^{2+} shows strong Lewis acid characteristics and a high tendency to coordinate, particularly with S-containing organic and inorganic ligands such as thiols ($-\text{SH}$).^{7–11} Because DOM occurs in all natural sediments and water, usually at concentrations much higher than Hg, understanding of Hg–DOM complexation is essential for predicting not only Hg speciation but also its bioavailability and transformation in the environment.

The ability of DOM to bind Hg^{2+} is commonly expressed by a conditional stability constant ($\log K$), which measures the binding strength between Hg^{2+} and the DOM ligand (L) in a given aqueous system at equilibrium.^{12–20} Unfortunately, the reported $\log K$ values vary widely from about 20 to 47^{1–3,12–20} and are subject to considerable uncertainty due to the differing methodologies and experimental conditions used. This large variation in $\log K$ values makes it difficult to select a reliable stability constant for calculating Hg speciation and estimating the dominant Hg species in processes such as biological uptake and methylation under environmental conditions.³

Among the many techniques that have been developed to determine the binding constant between Hg^{2+} and DOM, solid-phase extraction with competitive ligand exchange is considered

one of the most reliable.¹² This ion exchange technique has been widely used to determine complexation between DOM and other metal ions,^{21–24} except for Hg^{2+} ion, because Hg^{2+} forms exceptionally strong Hg–DOM complexes with $\log K \gg 14$, exceeding the detection window of the method.¹² Ion exchangers used in these previous studies were mostly sulfonate or carboxylic chelating resins, and the aim was to exchange metal ions from the abundant carboxyl and hydroxyl functional groups in DOM.^{21–24} At relatively high ionic concentrations, conventional ion exchange resins are sufficiently strong to competitively exchange metal ions such as Cu^{2+} , Pb^{2+} , Cd^{2+} , Eu^{3+} , and Am^{3+} from carboxyl and hydroxyl functional groups in DOM. However, these resins cannot exchange Hg^{2+} in the presence of strong complexing ligands such as thiols. As a result, conventional ion exchange methods are well suited for complexation between metal ions and carboxylic/phenolic groups, but are ineffective for complexation between Hg^{2+} and thiol ligands.¹² However, specific ion exchange resins with surface functionalized thiols have been developed and are now commercially available. They are often used in treatment of Hg-contaminated water and waste waters.²⁵ We therefore hypothesized that, with appropriate selection of the ion exchangers, the technique could be adapted to determine the binding constant between Hg^{2+} and thiols in DOM.

Received: December 15, 2010

Accepted: March 3, 2011

Revised: February 14, 2011

Published: March 18, 2011

Here we report a modified ion-exchange technique that allows not only the determination of binding constants between Hg^{2+} and DOM of varying origins, but also the discrimination of complexes with varying coordination numbers [i.e., 1:1 or 1:2 $\text{Hg(II)}:\text{thiol-ligand (HgL)}$ complexes]. Future studies are recommended to improve understanding of the nature and precise stoichiometries for binding between Hg^{2+} and DOM at environmentally relevant concentrations.

MATERIALS AND METHODS

DOM Isolates. Four DOM isolates of both aquatic and terrestrial origins were selected for the sorption and ion exchange reactions with Hg(II) . Soil humic acid (IFRC-HA) and fulvic acid (IFRC-FA) were isolated from topsoil at the Integrated Field Research Challenge (IFRC) site located at the Y-12 National Security Complex (NSC) in Oak Ridge, Tennessee. Details of the extraction and purification procedures and characteristics of these humic materials are given elsewhere.²⁶ The aquatic hydrophobic (HBA) and hydrophilic (HLA) DOM isolates were obtained following established procedures²⁷ from a contaminated East Fork Poplar Creek (EFPC) site, about 0.9 km from its headwater at the Y-12 NSC. In brief, the surface water was collected, filtered through a 0.45- μm glass fiber filter, acidified to pH 2, and then passed through a series of resin columns of XAD-8 and XAD-4. The HBA fraction was retained on the XAD-8 resin, and HLA fraction was retained on the XAD-4 resin. The columns were then back-eluted separately with 0.1 M NaOH, from which the EFPC-HBA and HLA effluents were obtained. These DOM isolates were further purified and converted to H^+ -form, desalted, freeze-dried, and stored in a desiccator until use. All DOM stock solutions (1000 mg/L) were prepared by dissolving a given amount of freeze-dried DOM in deionized water, and pH was adjusted to ~ 7 with 0.1 M NaOH. The solution was filtered through a 0.2- μm cellulose nitrate filter before use.

Ion Exchange Experiments. A Hg-selective cation exchange resin, Ambersep GT74 in H^+ -form (Rohm & Hass, Philadelphia, PA), was used for Hg sorption and ion-exchange reactions. The resin is a polystyrenic, weak acidic cation exchanger with surface functionalized thiol groups at an exchange capacity of 1.3 eq/L. The resin was prewashed thoroughly with ethanol followed by deionized water and subsequently air-dried before use. The experiment was initiated first by equilibrating the resin (30 mg dry weight) with the background electrolyte solution (0.1 M NaCl at pH 8 buffered with 1 mM NaHCO_3) for 24 h. DOM (0–125 mg/L) in the same background solution was then added to the resin suspension and equilibrated for an additional 2 h, during which pH was adjusted as necessary. Finally, Hg^{2+} was added to give a final concentration of 6 nM, and the volume was made up to 30 mL in 40-mL amber-glass vials. The samples were subsequently equilibrated on a reciprocating shaker at room temperature (~ 23 °C) for 5 days, after which the pH was measured, and the resin was separated by centrifugation at 3000 rpm for 15 min. An aliquot of clear supernatant was taken and analyzed for aqueous Hg and DOM concentrations. The amount of Hg sorbed on resin was calculated by the difference between the amounts of Hg added and that found in the final equilibrium solution. Preliminary kinetic experiments (up to 7 days) indicated that the 5-day reaction time was sufficient for Hg^{2+} adsorption to reach equilibrium. All containers used were acid-washed and all glassware was ashed

at 500 °C for 8 h before use. All experiments were performed in duplicate with resin-free controls to determine potential loss of Hg onto reaction containers, and with Hg-free controls to determine the amount of Hg initially associated with the DOM.

Equilibrium Dialysis Ligand Exchange (EDLE) Experiments. The EDLE technique is also widely used for determining conditional stability constants between DOM and metal ions^{16,28–30} and thus was adapted for Hg–DOM complexation studies. Here the dialysis membrane (cellulose ester) with a nominal molecular weight cutoff (MWCO) at 100–500 Da (Spectrum Laboratories) was used for determining equilibrium distribution of Hg inside and outside the dialysis bag. This membrane was selected based on its minimal sorption for Hg,¹⁶ its ability to both retain DOM inside the dialysis bag and allow Hg^{2+} and other inorganic Hg(II) species to diffuse freely through the membrane. Experimentally, the dialysis bag contained 10 mL of the DOM solution (10 mg/L), which was equilibrated with 90 mL of the Hg reactant solution in a 200-mL Teflon bottle. The final Hg concentration varied from 0.1 to 750 $\mu\text{g/L}$. All solutions were buffered at $\text{pH } 8.0 \pm 0.1$ using 1 mM NaHCO_3 , and the ionic strength was maintained at 0.1 M NaCl. Samples were then equilibrated on a reciprocating shaker for 3 days, although preliminary experiments indicated that a 2-day reaction time was sufficient to reach equilibrium at ~ 23 °C. Finally, aliquots from both inside and outside the dialysis bag were taken and determined for total Hg and DOM concentrations.

Chemical Analyses. Total dissolved Hg concentration was measured by cold vapor atomic fluorescence spectrometry (CVAFS) (Tekran model 2600 Mercury Analysis Systems) using U.S. EPA Method 1631.^{3,5,31} Duplicate and Hg standards were analyzed frequently to assess precision and accuracy of the method. In general, a recovery of 90–110% of Hg was achieved, and errors were usually within $\pm 10\%$. All Hg^{2+} stock solutions were prepared from a reference standard (1000 $\mu\text{g/mL}$ in 3% HNO_3 (Ricca Chemical Company), and other chemicals were either ACS reagent grade or better.

Total organic carbon (TOC) in equilibrium solutions was analyzed using a Shimadzu TOC-5000 Total Organic Analyzer (Shimadzu Co., Japan). For the ion-exchange reactions, supernatant solutions before and after sorption were also analyzed by UV–visible spectrophotometry at 280 nm (Agilent 8453 spectrophotometer) to evaluate potential sorption of DOM onto the resins.

Determination of Conditional Stability Constants. In the absence of DOM, aqueous Hg(II) species partitioning onto ion exchange resins is determined with a distribution coefficient, D_0 (mL/g), defined as

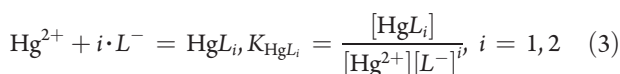
$$D_0 = \frac{[\text{Hg(II)}]_{\text{R}}}{[\text{Hg(II)}]_{\text{aq}}} \quad (1)$$

where $[\text{Hg(II)}]_{\text{R}}$ is the total adsorbed Hg(II) species on the resin (nmol/g), and $[\text{Hg(II)}]_{\text{aq}}$ is the equilibrium Hg(II) concentration (nmol/mL) in solution. Note that subscript “aq” denotes aqueous, whereas subscript “R” denotes the resin or the solid phase. Because the experiment was performed in 0.1 M NaCl solution at pH 8, $[\text{Hg(II)}]_{\text{R}}$ represents the sum of sorbed Hg(II) species including free Hg^{2+} and its complexes with Cl^- and OH^- . Equation 1 is thus rewritten as

$$\begin{aligned}
 D_0 &= \frac{[\text{Hg}^{2+}]_R + \sum_j [\text{HgCl}_j]_R + \sum_k [\text{Hg}(\text{OH})_k]_R}{[\text{Hg}^{2+}]_{\text{aq}} + \sum_j [\text{HgCl}_j]_{\text{aq}} + \sum_k [\text{Hg}(\text{OH})_k]_{\text{aq}}} \\
 &= \frac{\lambda_{\text{Hg}^{2+}} [\text{Hg}^{2+}]_{\text{aq}} + \sum_j \lambda_j [\text{HgCl}_j]_{\text{aq}} + \sum_k \lambda_k [\text{Hg}(\text{OH})_k]_{\text{aq}}}{[\text{Hg}^{2+}]_{\text{aq}} + \sum_j [\text{HgCl}_j]_{\text{aq}} + \sum_k [\text{Hg}(\text{OH})_k]_{\text{aq}}} \quad (2) \\
 &= \frac{(\lambda_{\text{Hg}^{2+}} + \sum_j \lambda_j \beta_{\text{HgCl}_j} [\text{Cl}^-]_{\text{aq}}^j + \sum_k \lambda_k \beta_{\text{Hg}(\text{OH})_k} [\text{OH}^-]_{\text{aq}}^k)}{(1 + \sum_j \beta_{\text{HgCl}_j} [\text{Cl}^-]_{\text{aq}}^j + \sum_k \beta_{\text{Hg}(\text{OH})_k} [\text{OH}^-]_{\text{aq}}^k)} \\
 &= \frac{B}{A}, \quad j = 1, 2, 3, 4 \text{ and } k = 1, 2, 3
 \end{aligned}$$

where $A = 1 + \sum_j \beta_{\text{HgCl}_j} [\text{Cl}^-]_{\text{aq}}^j + \sum_k \beta_{\text{Hg}(\text{OH})_k} [\text{OH}^-]_{\text{aq}}^k$, $B = \lambda_{\text{Hg}^{2+}} + \sum_j \lambda_j \beta_{\text{HgCl}_j} [\text{Cl}^-]_{\text{aq}}^j + \sum_k \lambda_k \beta_{\text{Hg}(\text{OH})_k} [\text{OH}^-]_{\text{aq}}^k$, and $\lambda_{\text{Hg}^{2+}}$, λ_j , and λ_k are the distribution coefficients of corresponding Hg(II) species. β_x is the cumulative stability constant of corresponding aqueous complexes of Hg(II). These values were obtained from the critically reviewed thermodynamic data^{3,32} and corrected to an ionic strength (I) of 0.1 M using Davies equation.³³ Here A is called the side reaction coefficient and calculated to be 6.52×10^{11} at $I = 0.1$ M and $\text{pH} = 8$. At fixed concentrations of Cl^- and OH^- , $\lambda_{\text{Hg}^{2+}}$, λ_j , and λ_k can be treated as constants (i.e., linear adsorption isotherms). As a result, D_0 is independent of Hg^{2+} concentration (eq 2).

The presence of DOM leads to the formation of aqueous complexes between Hg^{2+} and reactive functional groups (L) as follows:



where $K_{\text{Hg}L_i}$ represents the conditional cumulative stability constant for complexes of $\text{Hg}L$ (1:1 when $i = 1$) and $\text{Hg}L_2$ (1:2 when $i = 2$). Here the stability constants cannot be obtained using the activity correction methods because the DOM is ill-defined in terms of chemical and structural characteristics.^{1,12} Because DOM macromolecules are negatively charged over a wide pH range, we neglect any sorption of DOM or DOM-associated Hg(II) species by the negatively charged resin. Therefore, in the presence of DOM, the aqueous Hg(II) partitioning onto ion exchange resins is modified and determined with a distribution coefficient, D_L (mL/g), given by

$$\begin{aligned}
 D_L &= \frac{[\text{Hg}^{2+}]_{\text{aq}} \cdot B}{[\text{Hg}^{2+}]_{\text{aq}} \cdot A + \sum_i [\text{Hg}L_i]_{\text{aq}}} \\
 &= \frac{[\text{Hg}^{2+}]_{\text{aq}} \cdot B}{[\text{Hg}^{2+}]_{\text{aq}} (A + \sum_i K_{\text{Hg}L_i} [L^-]_{\text{aq}}^i)} = \frac{B}{A + \sum_i K_{\text{Hg}L_i} [L^-]_{\text{aq}}^i} \quad (4)
 \end{aligned}$$

where $[L^-]_{\text{aq}}$ is the free concentration of reactive functional groups in DOM. Combining eqs 2 and 4 yields

$$\left(\frac{D_0}{D_L} - 1\right) \cdot A = K_{\text{Hg}L} [L^-]_{\text{aq}} + K_{\text{Hg}L_2} [L^-]_{\text{aq}}^2 \quad (5)$$

where D_0 , D_L , and $[L^-]_{\text{aq}}$ are measured experimentally. Values of the corresponding $K_{\text{Hg}L}$ and $K_{\text{Hg}L_2}$ are obtained by plotting

$(D_0/D_L - 1) \cdot A$ versus $[L^-]_{\text{aq}}$ and fitting the experimental data to eq 5. If a single complex, either $\text{Hg}L$ or $\text{Hg}L_2$, is dominant, we can neglect one of the terms on the right-hand side of eq 5. A plot of $\log[(D_0/D_L - 1) \cdot A]$ versus $\log[L^-]_{\text{aq}}$ will thus yield a straight line with a slope of either 1 or 2, with corresponding intercepts of either $\log K_{\text{Hg}L}$ or $\log K_{\text{Hg}L_2}$. If both $\text{Hg}L$ and $\text{Hg}L_2$ complexes are formed, the slope will fall between 1 and 2.

Reactive thiols in DOM are now widely recognized to be responsible for complexing Hg^{2+} at relatively low Hg/DOM ratios, as evidenced by analysis of extended X-ray absorption fine structure spectroscopy (EXAFS).^{8–11} This reactive thiol content was determined by the EDLE method to be ~ 4 nmol/mg DOM for both EFPC HBA and HLA isolates (see section EDLE below). This value agrees well with the previously reported value of 5 nmol thiols/mg DOM.¹⁶ In this study, an average thiol content of 5 nmol/mg and a $\text{p}K_a$ value of 10 were used for calculating the deprotonation of thiols in all DOM,^{3,34} so that the free, aqueous concentration of thiolate $[L^-]_{\text{aq}}$ is given by

$$[L^-] = \frac{[\text{Thiols}]_{\text{total}}}{1 + 10^{\text{p}K_a} [\text{H}^+]} \quad (6)$$

RESULTS AND DISCUSSION

Determination of Conditional Binding Constants by Modified Ion Exchange. The distribution coefficient (D_0) on GT74 resin in the absence of DOM was first evaluated to determine if the methodology is suited to determination of the stability constants between Hg^{2+} and DOM. D_0 has to satisfy the condition that it is independent of the aqueous Hg(II) concentration, as defined in eq 2. This is illustrated in Figure 1a, in which a plot of Hg(II) sorption against $[\text{Hg}(\text{II})]_{\text{aq}}$ yielded a linear isotherm at a constant pH. In these experiments, a relatively high concentration of Cl^- was used as a competitive ligand so that solution concentrations of Hg(II) species such as HgCl_3^- and HgCl_4^{2-} were sufficient to facilitate the analysis.¹² The negatively charged HgCl_4^{2-} and HgCl_3^- ions also prevent Hg sorption onto the resin, as shown in previous studies.¹² Here Cl^- ion, instead of Br^- or I^- , was used because of its known stability constant, as critically reviewed and recommended by IUPAC.³²

The distribution coefficient (D_L) in the presence of different DOM isolates at a fixed Hg concentration (6 nM) was subsequently determined at varying DOM concentrations (0–125 mg/L) (Figure 1b). As expected, the equilibrium concentration of $[\text{Hg}(\text{II})]_{\text{aq}}$ increased (or D_L decreased) with increasing DOM concentrations (Figure 1b). With the addition of 125 mg/L IFRC-HA, about 95% of Hg remained in solution (Figure 1b and Figure S1). In contrast, only about 10% of Hg was left in solution after the addition of 125 mg/L EFPC-HLA, indicating that these different DOM isolates vary in their ability and capacity to bind with Hg^{2+} . For IFRC-HA, the D_L decreased from about 1560 to 240 mL/g when the DOM increased from 25 to 125 mg/L, whereas D_L decreased from about 58 100 to 9400 mL/g in the presence of EFPC-HLA (Figure 1b). Similarly, D_L values for IFRC-FA and EFPC-HBA decreased from about 18 100 to 2500 and from about 16 850 to 1800 mL/g, respectively, when the DOM increased from 25 to 125 mg/L. Among the four DOM isolates studied, IFRC-HA is the most effective in binding Hg^{2+} and forming stable Hg–DOM complexes, and the relative effects of these DOM's on Hg^{2+} sorption follow the order: IFRC-HA > EFPC HBA \approx IFRC-FA > EFPC HLA.

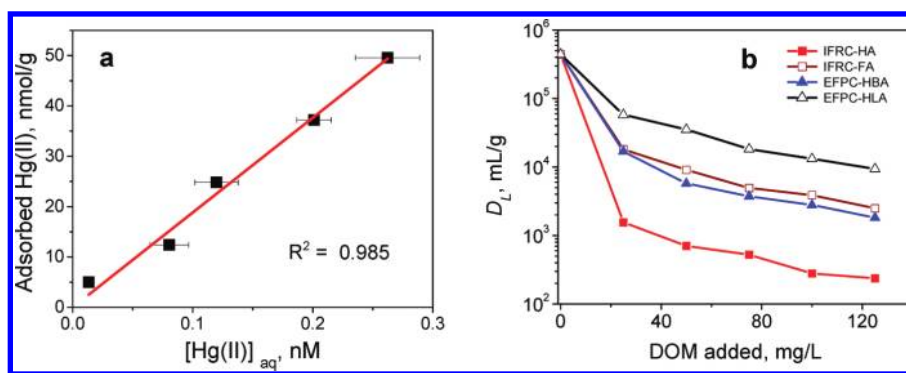


Figure 1. (a) Hg(II) adsorption isotherm on Ambersep GT74 resin in the absence of DOM. The solid line is the linear fitted line with a slope (D_0). (b) The distribution coefficient (D_L) of Hg as a function of DOM concentration during equilibrium reaction between Hg^{2+} and the resin. The initial Hg concentration was 6 nM. DOM isolates of both aquatic (EFPC-HBA, EFPC-HLA) and terrestrial origins (IFRC-HA and IFRC-FA) were obtained from Oak Ridge Reservation in Tennessee (see text for details). All experiments were performed at an ionic strength of 0.1 M NaCl, pH of 8.0 ± 0.1 , and P_{CO_2} of $10^{-3.5}$ atm at room temperature.

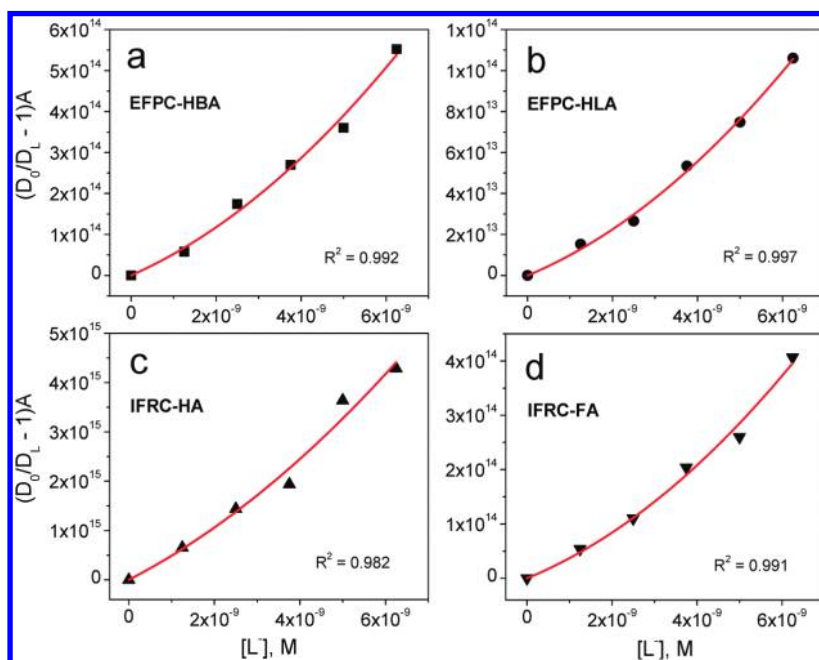


Figure 2. Plots of $(D_0/D_L - 1)A$ vs aqueous $[L^-]$ for Hg^{2+} interactions with four DOM isolates of both aquatic and terrestrial origins obtained from Oak Ridge Reservation in Tennessee. Each data point or symbol represents an average of duplicate measurements; lines are the best-fits to eq 5, from which binding constants of either $\log K_{\text{HgL}}$ or $\log K_{\text{HgL}_2}$ for complexes of HgL and HgL_2 are calculated. R^2 is the square of correlation coefficient.

Plots of $[(D_0/D_L - 1) \cdot A]$ against the thiol concentration $[L]$ in DOM show a positively curved correlation between $[D_0/D_L - 1]$ and $[L]$ (Figure 2). The lines in Figure 2 are the best-fits to experimental data using eq 5, from which the binding constants of both 1:1 and 1:2 complexes of HgL and HgL_2 are calculated. The estimated $\log K_{\text{HgL}}$ values varied between 21.9 and 23.6, and $\log K_{\text{HgL}_2}$ between 30.1 and 31.6 among the four DOM isolates studied (Table 1). The IFRC-HA gives the highest $\log K_{\text{HgL}}$ and $\log K_{\text{HgL}_2}$ values, whereas the EFPC HLA shows the lowest, as expected from the sorption and partitioning of Hg(II) in the system (Figure 1b).

The conditional stability constants ($\log K_{\text{HgL}}$) for 1:1 complexes determined in this study compared well to those reported by Lamborg et al.¹⁷ and Benoit et al.,¹³ but are somewhat lower than those (up to 28.5) by Haitzer et al.¹⁶ (Table 1). Black et al.²⁰

reported even higher $\log K_{\text{HgL}}$ values between 29.9 and 33.5 for DOM. The $\log K_{\text{HgL}_2}$ values for 1:2 complexes obtained in this work are slightly higher than that (28.7) obtained by Haitzer et al.¹⁵ but much lower than those (38.2–40.4) obtained by Khwaja et al.¹⁹ These differences in $\log K_{\text{HgL}}$ and $\log K_{\text{HgL}_2}$ values are usually attributed to different methodologies and/or binding models; the latter are limited by our understanding of the chemical structure of, and binding stoichiometry with, DOM. However, if we assume that reactive thiols in DOM are the principal binding sites for Hg^{2+} , particularly at relatively low Hg/DOM ratios,^{8–10,16,35} then the bonding environment of DOM for Hg^{2+} should essentially be similar regardless of the origins of the humic materials.¹² Small variations in $\log K_{\text{HgL}}$ and $\log K_{\text{HgL}_2}$ values obtained in this study support this argument (Table 1).

Table 1. Comparisons between Measured Conditional Stability Constants ($\log K$) in This Study and in Literature for the 1:1 and 1:2 Complexes between Hg^{2+} and Thiol Functional Groups in DOM

DOM	$\log K_{\text{HgL}}$	$\log K_{\text{HgL}_2}$	pH	I (M)	method ^a	reference
EFPC-HBA	22.7 ± 0.1	30.8 ± 0.1	8.0 ± 0.1	0.1	ion exchange	this work
EFPC-HLA	21.9 ± 0.1	30.1 ± 0.1	8.0 ± 0.1	0.1	ion exchange	this work
IFRC-HA	23.6 ± 0.1	31.6 ± 0.2	8.0 ± 0.1	0.1	ion exchange	this work
IFRC-FA	22.5 ± 0.1	30.7 ± 0.1	8.0 ± 0.1	0.1	ion exchange	this work
DOM	21–24		7.5	na	reducible Hg	17
HBA, HLA	21.4–23.8		4–6	0.06	CLE-SSE	13
HBA	28.5	28.7 ± 0.1	7.0	0.1	EDLE	15,16
DOM	29.9–33.5		7.4–7.8	na	CLE-SPE	20
HA and peat		38.2–40.4	3–5	~ 0.5	CLE-SE	19

^a CLE = competitive ligand exchange, SSE = solvent–solvent extraction, EDLE = equilibrium dialysis ligand exchange, SPE = solid phase extraction, SE = sorption equilibrium modeling, na = not available.

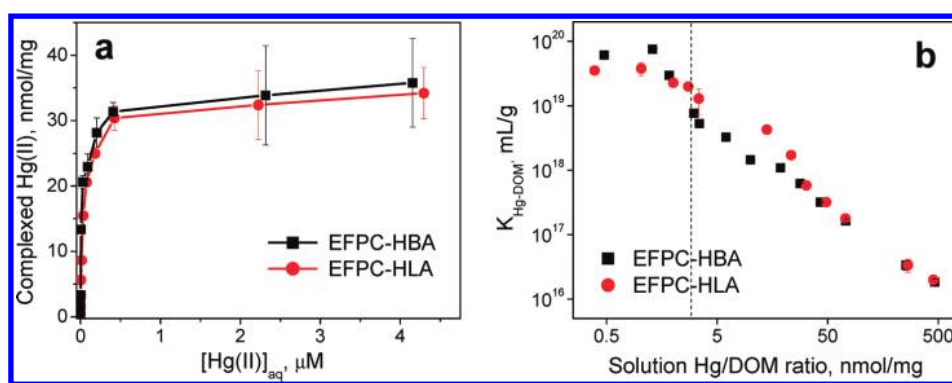


Figure 3. Equilibrium dialysis ligand exchange (EDLE) results. (a) Hg(II) complexation with EFPC-HBA and EFPC-HLA and (b) dependence of the conditional distribution coefficient $K_{\text{DOM}'}$ (eq 7) on the Hg/DOM ratio in solution. Error bars show one standard deviation, and some are smaller than the symbol size. The pH was 8.0 ± 0.1 buffered with 1 mM NaHCO_3 , and ionic strength maintained at 0.1 M NaCl .

This study further indicates that both HgL (1:1) and HgL_2 (1:2) complexes formed under our given experimental conditions (Figure 2 and Table 1). If only a single species of HgL or HgL_2 is dominant, a plot of $\log[(D_0/D_L - 1) \cdot A]$ vs $\log[L]$ (eq 5) would yield a straight line with a slope of either 1 or 2. Instead, the calculated slope ranged from 1.18 to 1.35 (Figure S2 and Table S1), suggesting the existence of mixed HgL and HgL_2 complexes in the system. Therefore, the modified ion exchange technique allows simultaneous determination of both $\log K_{\text{HgL}}$ and $\log K_{\text{HgL}_2}$ constants, as opposed to that of either 1:1 or 1:2 complexes between Hg^{2+} and thiols in DOM determined in previous studies. For example, only HgL_2 complex was considered by Khwaja et al.,¹⁹ and other studies^{13,16,17,20} considered only the HgL complex as a simplification in calculating the binding constants.

Equilibrium Dialysis Ligand Exchange (EDLE). To determine the reactive thiol contents and compare the conditional binding constants determined by the modified ion-exchange technique, the EDLE method^{15,16} was also used to quantify the $\log K$ values for the EFPC-HBA and EFPC-HLA isolates. The conditional distribution coefficient ($K_{\text{DOM}'}$) for reactions between Hg(II) and DOM is defined in eq 7

$$\text{Hg}^{2+} + \text{DOM} = \text{HgDOM}, \quad K_{\text{DOM}'} = \frac{[\text{HgDOM}]}{[\text{Hg}^{2+}][\text{DOM}]} \quad (7)$$

where $[\text{HgDOM}]$ is the concentration of Hg –DOM complexes (1:1) at equilibrium inside the dialysis bag. $[\text{DOM}]$ is the free concentration of DOM and is approximately equal to the initial DOM concentration (10 mg/L) because $[\text{DOM}] \gg [\text{Hg(II)}]$. At equilibrium, the total Hg(II) concentration outside the dialysis bag is measured and given by

$$C_{\text{outside}} = [\text{Hg}^{2+}] + \sum[\text{HgCl}_i] + \sum[\text{Hg}(\text{OH})_k] \\ = [\text{Hg}^{2+}](1 + A) \quad (8)$$

The total Hg(II) concentration inside the dialysis bag is

$$C_{\text{inside}} = [\text{Hg}^{2+}](1 + A) + [\text{HgDOM}] \quad (9)$$

where A is defined in eq 2, and $[\text{HgDOM}]$ is determined by the difference between Hg(II) concentrations inside and outside the dialysis bag.

Figure 3 shows the amount of Hg^{2+} complexed by DOM and the calculated conditional distribution coefficient ($K_{\text{DOM}'}$) at varying Hg/DOM ratios. Here $K_{\text{DOM}'}$ is defined similarly to the conditional stability constants (K_{HgLi}) in eq 3, except that the mass concentration (g/L) of DOM is used in the calculation of $K_{\text{DOM}'}$ instead of the molar concentration (mol/L) of the reactive thiols in the computation of K_{HgLi} . Sorption and complexation of Hg(II) by both EFPC-HBA and EFPC-HLA showed initial sharp increases with increasing Hg concentration (Figure 3a) and then leveled off at higher concentrations. The

initial steep slope of the isotherm (Figure 3a) is indicative of strong binding of Hg(II) for reactive thiols in DOM. The binding strength, as reflected in $\log K$, is highest at relatively low Hg/DOM ratios (<4 nmol/mg, Figure 3b), and decreased thereafter with increasing Hg/DOM ratios. The highest $\log K$ value is in close agreement with those observed by Haitzer et al.,¹⁶ who hypothesized that, at a Hg/DOM ratio of <5 nmol/mg, Hg^{2+} is mostly bound to the reactive thiol groups in DOM. This number, 5 nmol thiols/mg DOM, has thus been used in calculating the conditional stability constant (K_{HgLi}).^{15,16} Using the same approach, we determine mean values of the conditional stability constants, $\log K_{\text{HgLi}}$, being equal to $\sim 22 \pm 2$ for complexes between Hg^{2+} and reactive thiols in EFPC-HBA and EFPC-HLA. These values are in close agreement with the $\log K_{\text{HgLi}}$ values determined by the ion exchange technique (Table 1), demonstrating that the modified ion exchange technique is well suited to determine the conditional binding constants between Hg^{2+} and reactive thiols in DOM.

The continuous distribution of $K_{\text{Hg-DOM}}$ in Figure 3b is expected considering the heterogeneous nature, the complex steric arrangement, and bonding environments of reactive functional groups in DOM.³⁶ The sulfur functional groups (e.g., R-SH, R-S-S-) are the most important for Hg(II) binding, and the oxygen (COOH, OH) containing functional groups are much weaker in comparison.^{14,16} The observed distribution coefficients (Figure 3b) are therefore consistent with previous studies, which show that binding of Hg^{2+} by DOM depends on the Hg/DOM ratio.^{9,10,16} Once the strong binding sites (i.e., thiol functional groups) are saturated, Hg^{2+} would bind to functional groups with decreasing binding strengths under the conditions of increasing Hg/DOM ratios.

Limitations of the Complexation Model and Implications. Accurate determination of Hg-DOM stability constants is needed in order to predict mercury speciation, transformation, and transport in natural aquatic environments.^{2,3,12} It is also critically important to understand the Hg species that are potentially available for biological methylation. However, to date, there is no universal approach that can generate an agreed stability constant for Hg-DOM complexation. The reported $\log K$ values vary widely from about 20 to 33.5 for the 1:1 Hg-ligand complexes and from 28 to 47 for the 1:2 complexes.^{1-3,12-20} The present study demonstrates a modified ion-exchange technique that uses a Hg-specific resin to determine the binding constants between Hg^{2+} and DOM. The technique overcomes limitations in the conventional ion-exchange techniques, such as limited detection windows, and allows simultaneous determination of the $\log K$ values for both 1:1 and 1:2 complexes. However, like any other technique, the method remains limited by our incomplete understanding of the chemical and structural properties of DOM, and inability to determine and characterize both heterogeneous binding sites and Hg-ligand binding stoichiometries, particularly at environmentally relevant concentrations (i.e., ng levels of Hg). Future studies are recommended as detailed below.

First, the chemical and structural heterogeneities of DOM and the presence of small quantities of strong binding sites make it difficult to accurately determine the specific reactive sites involved in bonding Hg^{2+} . DOM is a mixture of natural organic compounds with different types of binding sites; as a result, meaningful thermodynamic expressions do not exist.^{1,12} It is not surprising that complexation of Hg^{2+} with DOM varies greatly with the ratio of Hg to DOM, as demonstrated in this and other studies.^{9,10,16} The wide range of stability constants reported in the literature merely reflect an average binding strength of Hg^{2+} with a specific set of functional groups under given experimental conditions.

Second, the uncertainty of stoichiometry for reactions between Hg^{2+} and different reactive functional groups in DOM at varying Hg to DOM ratios contributes to a wide range of reported $\log K$ values. This is further complicated by the presence of the auxiliary competitive ligands such as Br^- ,⁷ penicillamine,¹⁹ diethyldithiolcarbamate,²⁰ and OH^- or Cl^- ,¹³ since these ligands can potentially interact with DOM and Hg-DOM complexes, forming ternary complexes in solution or on solids. Many studies have assumed the formation of either 1:1 or 1:2 complexes between Hg^{2+} and DOM.^{7,12,14,15,19,20} However, simultaneous formation of 1:1 and 1:2 complexes, as well as mixed complexes of Hg^{2+} with one thiol and one other ligand (e.g., carboxyl), cannot be ruled out. Techniques such as sulfur K-edge and EXAFS spectroscopy may be the best tools currently available to provide the coordination chemistry and stoichiometry. However, these techniques involve the use of concentrations of Hg(II) and DOM that are orders of magnitude higher than those in natural environments, and the results may therefore not be realistic. Furthermore it is difficult to distinguish organic-thiols from organic-sulfide compounds such as R-S-S-R in DOM. Techniques and methodologies for accurately quantifying reactive functional groups and active coordination sites at low concentrations are therefore critically needed for further understanding the complexation between Hg^{2+} and DOM.

Third, reactive functional groups, such as organic thiols, usually exist as a minute fraction in DOM, and their acidity could be largely masked by a much greater fraction of acidic functional groups, such as carboxyls and phenols. The uncertainty in determination of the DOM thiol deprotonation constants ($\text{p}K_{\text{a}}$ in eq 6) by conventional acid-base titration technique propagates errors into the Hg-DOM binding constants. For example, assumption of a $\text{p}K_{\text{a}}$ value of 4–10 for thiol-like groups in DOM can result in several orders of difference in calculated binding constants.

Lastly, the range of measurable stability constants is also set by analytical limitations for any given method.¹² Due to strong bindings between Hg^{2+} and DOM, ultrasensitive detection of aqueous Hg concentrations (e.g., at pg/L or fg/L) is highly desirable. Although a known auxiliary ligand may be used to maintain sufficiently high Hg concentration in solution, it can complicate the determination of binding constants, as described earlier. Furthermore, Hg(II) species are prone to sorption onto surfaces such as container walls and membranes, and also readily undergo reduction (either dark or photochemically induced).^{6,37} A mass balance is thus critical to obtaining reliable binding constants between Hg^{2+} and DOM.

■ ASSOCIATED CONTENT

S Supporting Information. The Hg(II) concentration as a function of DOM concentration (Figure S1); a plot of $\log[(D_0/D_L - 1) \cdot A]$ vs $\log[L]_{\text{aq}}$ (Figure S2), and its fitting parameters (Table S1). This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: (865)-574-7286; fax: (865)-576-8543; e-mail: gubl@ornl.gov (B.G.) or phone: (865)-241-3933; fax: (865)-576-8646; e-mail: liangl@ornl.gov (L.L.).

Present Addresses

[†]Earth Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California.

[‡]State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Science, Nanjing, China, 210008.

ACKNOWLEDGMENT

We thank X. Yin and C. Miller for technical assistance in CVAFS analysis of Hg. This work is part of the mercury Science Focus Area (SFA) research program at Oak Ridge National Laboratory sponsored by the Subsurface Biogeochemical Research (SBR) Program, Office of Biological and Environmental Research, U.S. Department of Energy (DOE). ORNL is managed by UT-Battelle, LLC, for the U.S. DOE under Contract DE-AC05-00OR22725.

REFERENCES

- (1) Ravichandran, M. Interactions between mercury and dissolved organic matter - a review. *Chemosphere* **2004**, *55*, 319–331.
- (2) Skyllberg, U. Competition among thiols and inorganic sulfides and polysulfides for Hg and MeHg in wetland soils and sediments under suboxic conditions: Illumination of controversies and implications for MeHg net production. *J. Geophys. Res. Biogeosci.* **2008**, *113*, G00C03.
- (3) Dong, W.; Liang, L.; Brooks, S. C.; Southworth, G.; Gu, B. Roles of dissolved organic matter in the speciation of mercury and methylmercury in a contaminated ecosystem in Oak Ridge, Tennessee. *Environ. Chem.* **2009**, *7*, 94–102.
- (4) Gorski, P. R.; Armstrong, D. E.; Hurley, J. P.; Krabbenhoft, D. P. Influence of natural dissolved organic carbon on the bioavailability of mercury to a freshwater alga. *Environ. Pollut.* **2008**, *154*, 116–123.
- (5) Miller, C.; Southworth, G.; Brooks, S. C.; Liang, L.; Gu, B. Kinetic controls on the complexation between mercury and dissolved organic matter in a contaminated environment. *Environ. Sci. Technol.* **2009**, *43*, 8548–8553.
- (6) Gu, B.; Bian, Y.; Miller, C. L.; Dong, W.; Jiang, X.; Liang, L. Mercury reduction and complexation by natural organic matter in anoxic environments. *Proc. Natl. Acad. Sci., U.S.A.* **2011**, *108*, 1479–1483.
- (7) Skyllberg, U.; Xia, K.; Bloom, P. R.; Nater, E. A.; Bleam, W. F. Binding of mercury(II) to reduced sulfur in soil organic matter along upland-peat soil transects. *J. Environ. Qual.* **2000**, *29*, 855–865.
- (8) Xia, K.; Skyllberg, U. L.; Bleam, W. F.; Bloom, P. R.; Nater, E. A.; Helmke, P. A. X-ray absorption spectroscopic evidence for the complexation of Hg(II) by reduced sulfur in soil humic substances. *Environ. Sci. Technol.* **1999**, *33*, 257–261.
- (9) Hesterberg, D.; Chou, J. W.; Hutchison, K. J.; Sayers, D. E. Bonding of Hg(II) to reduced organic sulfur in humic acid as affected by S/Hg ratio. *Environ. Sci. Technol.* **2001**, *35*, 2741–2745.
- (10) Skyllberg, U.; Bloom, P. R.; Qian, J.; Lin, C. M.; Bleam, W. F. Complexation of mercury(II) in soil organic matter: EXAFS evidence for linear two-coordination with reduced sulfur groups. *Environ. Sci. Technol.* **2006**, *40*, 4174–4180.
- (11) Manceau, A.; Nagy, K. L. Relationships between Hg(II)-S bond distance and Hg(II) coordination in thiolates. *Dalton Trans.* **2008**, 1421–1425.
- (12) Gasper, J. D.; Aiken, G. R.; Ryan, J. N. A critical review of three methods used for the measurement of mercury (Hg²⁺)-dissolved organic matter stability constants. *Appl. Geochem.* **2007**, *22*, 1583–1597.
- (13) Benoit, J. M.; Mason, R. P.; Gilmour, C. C.; Aiken, G. R. Constants for mercury binding by dissolved organic matter isolates from the Florida Everglades. *Geochim. Cosmochim. Acta* **2001**, *65*, 4445–4451.
- (14) Drexel, R. T.; Haitzer, M.; Ryan, J. N.; Aiken, G. R.; Nagy, K. L. Mercury(II) sorption to two Florida Everglades peats: Evidence for strong and weak binding and competition by dissolved organic matter released from the peat. *Environ. Sci. Technol.* **2002**, *36*, 4058–4064.
- (15) Haitzer, M.; Aiken, G. R.; Ryan, J. N. Binding of mercury(II) to aquatic humic substances: Influence of pH and source of humic substances. *Environ. Sci. Technol.* **2003**, *37*, 2436–2441.
- (16) Haitzer, M.; Aiken, G. R.; Ryan, J. N. Binding of mercury(II) to dissolved organic matter: The role of the mercury-to-DOM concentration ratio. *Environ. Sci. Technol.* **2002**, *36*, 3564–3570.
- (17) Lamborg, C. H.; Tseng, C. M.; Fitzgerald, W. F.; Balcom, P. H.; Hammerschmidt, C. R. Determination of the mercury complexation characteristics of dissolved organic matter in natural waters with “reducible Hg” titrations. *Environ. Sci. Technol.* **2003**, *37*, 3316–3322.
- (18) Han, S. H.; Gill, G. A. Determination of mercury complexation in coastal and estuarine waters using competitive ligand exchange method. *Environ. Sci. Technol.* **2005**, *39*, 6607–6615.
- (19) Khwaja, A. R.; Bloom, P. R.; Brezonik, P. L. Binding constants of divalent mercury (Hg²⁺) in soil humic acids and soil organic matter. *Environ. Sci. Technol.* **2006**, *40*, 844–849.
- (20) Black, F. J.; Bruland, K. W.; Flegal, A. R. Competing ligand exchange-solid phase extraction method for the determination of the complexation of dissolved inorganic mercury(II) in natural waters. *Anal. Chim. Acta* **2007**, *598*, 318–333.
- (21) Stevenson, F. J. Stability-constants of Cu²⁺, Pb²⁺, and Cd²⁺ complexes with humic acids. *Soil Sci. Soc. Am. J.* **1976**, *40*, 665–672.
- (22) Bertha, E. L.; Choppin, G. R. Interaction of humic and fulvic acids with Eu(III) and Am(III). *J. Inorg. Nucl. Chem.* **1978**, *40*, 655–658.
- (23) Maes, A.; Debrabandere, J.; Cremers, A. A modified Schubert method for the measurement of the stability of europium humic-acid complexes in alkaline conditions. *Radiochim. Acta* **1988**, *44–5*, 51–57.
- (24) Dong, W.; Li, W.; Tao, Z. Use of the ion exchange method for the determination of stability constants of trivalent metal complexes with humic and fulvic acids II. Tb³⁺, Yb³⁺ and Gd³⁺ complexes in weakly alkaline conditions. *Appl. Radiat. Isot.* **2002**, *56*, 967–974.
- (25) Lloyd-Jones, P. J.; Rangel-Mendez, J. R.; Streat, M. Mercury sorption from aqueous solution by chelating ion exchange resins, activated carbon and a biosorbent. *Process Safety Environ. Prot.* **2004**, *82*, 301–311.
- (26) Gu, B.; Yan, H.; Zhou, P.; Watson, D.; Park, M.; Istok, J. D. Humics impact uranium bioreduction and oxidation. *Environ. Sci. Technol.* **2005**, *39*, 5268–5275.
- (27) Aiken, G. R.; Mcknight, D. M.; Thorn, K. A.; Thurman, E. M. Isolation of hydrophilic organic-acids from water using nonionic macroporous resins. *Org. Geochem.* **1992**, *18*, 567–573.
- (28) Vanloon, L. R.; Granacher, S.; Harduf, H. Equilibrium dialysis-ligand exchange - a novel method for determining conditional stability-constants of radionuclide-humic acid complexes. *Anal. Chim. Acta* **1992**, *268*, 235–246.
- (29) Glaus, M. A.; Hummel, W.; Loon, L. R. V. Stability of mixed-ligand complexes of metal ions with humic substances and low molecular weight ligands. *Environ. Sci. Technol.* **1995**, *29*, 2150.
- (30) Amirbahman, A.; Reid, A. L.; Haines, T. A.; Kahl, J. S.; Arnold, C. Association of methylmercury with dissolved humic acids. *Environ. Sci. Technol.* **2002**, *36*, 690–695.
- (31) U.S. EPA. *Method 1631, Revised E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry*; EPA-821-R-02-019: Washington, DC, 2002.
- (32) Powell, K. J.; Brown, P. L.; Byrne, R. H.; Gajda, T.; Hefter, G.; Sjoberg, S.; Wanner, H. Chemical speciation of environmentally significant heavy metals with inorganic ligands - Part 1: The Hg²⁺-Cl⁻, OH⁻, CO₃²⁻, SO₄²⁻, and PO₄³⁻ aqueous systems - (IUPAC technical report). *Pure Appl. Chem.* **2005**, *77*, 739–800.
- (33) Langmuir, D. *Aqueous Environmental Chemistry*; Prentice-Hall: New York, 1997.
- (34) Martell, A. E.; Smith, R. M.; Motekaitis, R. J. *NIST Critically Selected Stability Constants of Metal Complexes; NIST Standard Reference Database 46*; 6.0 ed.; National Institute of Standards and Technology: Gaithersburg, MD, 2001.

(35) Yoon, S. J.; Diener, L. M.; Bloom, P. R.; Nater, E. A.; Blear, W. F. X-ray absorption studies of CH_3Hg^+ -binding sites in humic substances. *Geochim. Cosmochim. Acta* **2005**, *69*, 1111–1121.

(36) Xia, K.; Weesner, F.; Blear, W. F.; Bloom, P. R.; Skyllberg, U. L.; Helmke, P. A. XANES studies of oxidation states of sulfur in aquatic and soil humic substances. *Soil Sci. Soc. Am. J.* **1998**, *62*, 1240–1246.

(37) O'Driscoll, N. J.; Lean, D. R. S.; Loseto, L. L.; Carignan, R.; Siciliano, S. D. Effect of dissolved organic carbon on the photoproduction of dissolved gaseous mercury in lakes: Potential impacts of forestry. *Environ. Sci. Technol.* **2004**, *38*, 2664–2672.