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Binding Constants of Divalent Mercury (Hg^{2+}) in Soil Humic Acids and Soil Organic Matter

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Distribution coefficients (K_{OC}) for Hg^{2+} binding by IHSS Pahokee peat humic acid (PHA) and humic acids separated from O-horizons and peats in a northern temperate forest were determined using a competitive ligand-exchange method. All measurements were made at low ratios of added Hg^{2+} to reduced S. The commonly used chelating agents, EGTA and DTPA, were found to be ineffective competitive ligands; thus, we used DL-penicillamine, a synthetic amino acid with a thiol group. Calculated free [Hg^{2+}] at equilibrium is very low, ranging from $10^{-26.4}$ at pH 1.9 to $10^{-36.9}$ at pH 5.8. Corresponding log K_{OC} values ranged from 22.6 to 32.8. The slope of the plot of pH versus log K_{OC} was 2.68, suggesting that two or more protons are released when each Hg^{2+} is bound. This is consistent with binding of Hg^{2+} to bidentate thiol sites with some participation of a third weak-acid group, presumably a thiol. The 1:2 stoichiometry is consistent with X-ray spectroscopy data for Hg^{2+} bound to HA and with other pH-dependency results showing release of two protons with the binding of each Hg^{2+}. Our K_{OC} values are much greater than indicated by the data from most previous studies.

Introduction

Natural organic matter (NOM) plays important roles in the environmental fate and transport of mercury. For example, photochemical reduction (*1*) and bioaccumulation (*2*−*4*) in aquatic systems are greatly influenced by the presence or absence of NOM. Adsorption of Hg^{2+} in soils is correlated with the soil organic matter content (*5*), and this process has implications for the transport of Hg^{2+} from watersheds to pristine lakes in runoff and interflow (*6*−*8*).

Mercuric ion (Hg^{2+}) is a soft Lewis acid, and according to Pearson's hard and soft acid−base theory, it should complex strongly with reduced S-containing ligands (e.g., see ref *9*). Soils and aquatic humic substances have S contents of ∼0.1−3.6%. Using X-ray absorption near-edge structure spectroscopy (XANES), Xia et al. (*10*) showed that 15−50% of the total S in aquatic and soil humic substances is in the most reduced forms, thiol, sulfide, and disulfide. Some of these sites are available for bonding with Hg^{2+} (*11*).

About half of the carbon-bonded S in soils occurs as S-amino acids, methionine, and cysteine, with the latter, a thiol amino acid, accounting for the larger fraction (*12*). The source of cysteine in soils is plant residues that are precursors to soil organic matter (SOM). Much of the S in organisms is accounted for by thiol groups of cysteine in peptides and proteins (*13*). Cysteine is involved in disulfide linkages in proteins, and it provides the thiol groups involved in the binding of Hg and other soft metals by phytochelatins in plants (*13*) and metallothioneins in animals (*14*). Cysteine thiol groups likely are active in metal binding in SOM and other sources of NOM.

Xia et al. (*11*) used extended X-ray absorption fine-structure spectroscopy (EXAFS) to demonstrate that both reduced S groups (S_{red}) and oxygen-containing groups, presumably carboxyl groups, can participate in binding Hg^{2+} to soil organic matter. Lin (*15*) and Hesterberg et al. (*16*) used EXAFS to show that two-coordinate binding of Hg^{2+} to reduced S groups occurs when Hg levels are well below the saturation limit of these groups. As more Hg^{2+} is bound, oxygen-containing ligands participate increasingly in the binding.

Reported association constants for Hg^{2+} with NOM vary greatly. Lövgren and Sjöberg (*17*) reported a formation constant of 10^{10} for Hg^{2+} complexed with aquatic dissolved organic matter (DOM). They concluded that Hg^{2+} is bound to two carboxyl groups; however, they used very high loadings of Hg^{2+} that equaled or exceeded the concentrations of carboxylic groups. When Hg^{2+} is added at much less than molar equivalence to reduced S, much stronger binding is found, but there is no unanimity on binding constants. Haitzer et al. (*18*) used EDTA as a competitive ligand to determine conditional distribution coefficients of Hg^{2+} with an aquatic humic extract and reported a partition coefficient (K_{DOM}) of 10^{23} (pH 7.0), where K_{DOM} = {sorbed Hg, mol $kg^{-1}$of DOM}/[Hg^{2+}, mol L^{-1}]. In a subsequent study (*19*), they determined the pH dependence of K_{DOM} and found that complexation with Hg^{2+} releases two protons. They determined a formation constant of $10^{28.7}$ for Hg^{2+} binding to an assumed bidentate thiol site. The competitive ligand EDTA was used in these studies because strong binding of Hg^{2+} to reduced S groups in NOM decreased free aquo Hg^{2+} to unmeasurable levels. Hsu and Sedlak (*20*) used diethyldithiocarbamate as a competitive ligand to determine binding constants of Hg^{2+} for the hydrophilic fraction of Suwannee River humic acid. They estimated conditional constants in the range of $\sim 10^{26}$ to 10^{32} assuming a monodentate mercury−thiol model at pH 7.4. In a study of intact organic soil samples, Skyllberg et al. (*21*) used Br^- as a competitive ligand at native Hg^{2+} concentrations and reported partition coefficients (K_{OC}) of 10^{22} to 10^{23} at pH 3, where K_{OC} = {sorbed Hg, mol kg^{-1} C}/[Hg^{2+}, mol L^{-1}]. All these studies suggest that in oxic freshwaters and well-drained soils, where [HS^-] is very low, Hg^{2+} is predominantly bound to NOM.

In the present study, we determined K_{OC} and formation constants for Hg^{2+} in IHSS Pahokee peat humic acid and humic acids extracted from organic surface soils from a forest in northern Minnesota. Partition coefficients were determined in the pH range of 2−6, typical of O-horizons and

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TABLE 1. Organic C and S, Fraction of Reduced S, and Hg Content of Soil and Peat HA (*10*, *15*)

sample ID	carbon content (%)	total organic sulfur (mmol kg^{-1})	reduced sulfur (% of S in reduced form)	Hg (μmol kg^{-1})
S3-1 humic acid	52.9	150	48	5.0
S3-2 humic acid	53.6	140	48	3.7
S3-3 humic acid	53.1	130	51	4.4
S3-3 soil organic matter	15.1	22	56	0.69
S3-4 humic acid	52.5	220	57	2.3
S3-5 humic acid	52.0	280	61	3.1
S2-5 humic acid	53.9	180	57	4.2
IHSS peat humic acid	56.9	110	50	2.1

surficial peats in northern temperate forests. We also compared the binding in an intact surface organic soil with the extracted humic acid from that soil. The objectives were to determine stoichiometry and strength of binding of Hg^{2+} by SOM. We used a competitive-ligand exchange method with DL-penicillamine, a synthetic thiol amino acid that has Hg^{2+} binding abilities similar to that of cysteine (*22*). The studies were conducted in 0.167 M Ca^{2+} ($I = 0.5$) to flocculate the humic acids and keep dissolved organic carbon concentrations low.

Materials and Methods

Sample Collection and SOM Isolation. Pahokee peat humic acid, IR103H-2 (PHA), was purchased from the International Humic Substances Society (IHSS), St. Paul, MN. Surface soil samples (high organic matter O-horizons and peat) were taken along an upland-to-wetland transect in a watershed of wetland S3 (a fen) in the Marcell Experimental Forest (47°32′N, 93°28′W), north of Grand Rapids, MN. The sampling was designed to obtain organic soil materials from soils that range in wetness from well-drained to wet much of the year. The upland sample was denoted S3-1; the wetland sample was denoted S3-5; samples S3-2 to S3-4 were intermediate in the transect. In addition, a surface soil sample, S2-5, was taken from a nearby bog (S2). The soil humic acids were extracted using NaOH according to the IHSS method used to prepare PHA (*23*). The S, C, and Hg contents of the samples used in the study are given in Table 1.

The organic S contents were determined by Huffman Laboratories (Golden, CO) using a combustion method. The fraction of reduced S was determined by Lin (*15*) using XANES. Mercury was determined by cold-vapor atomic fluorescence (method described next) after digestion with 1:1 concentrated HNO_3 and concentrated H_2SO_4 in a 70 °C water bath for 12 h. Organic C in the soil and soil humic acids was determined by total combustion using a Skalar Primacs carbon furnace (Skalar Inc., Norcross Georgia).

Binding of Hg^{2+} by Soil NOM. All experiments were conducted in solutions of DL-penicillamine (2 amino-3-mercapto-3- methylbutanoic acid, Sigma-Aldrich) in 50 mL Teflon centrifuge tubes. A known amount of 1000 mg L^{-1} $Hg(NO_3)_2$ solution was added to 100 mg of HA or NOM samples to yield a known molar ratio of Hg^{2+} to S_{red} (1:120 in most of the PHA experiments). Ultrapure water was added to the centrifuge tubes so that the total volume of solution added was 5.0 mL. The centrifuge tubes were double bagged in polyethylene and equilibrated for 6 days at 30 °C to allow for equilibrium adsorption of Hg^{2+}. After adsorption was complete, the pH was adjusted by adding solid CaO. Preliminary experiments were conducted, and a trial and error approach was used to determine the amount of solid CaO needed to reach the desired pH. Then, we added 15 mL of penicillamine in 0.33 M $Ca(NO_3)_2$ and 10 mL of ultrapure water. After 13 days (except for an initial kinetic experiment in which samples were equilibrated for 1, 2, 5, 6, 10, and 13 days), the tubes were centrifuged at 10 000 rpm (24 000*g*) for 15 min. Unfiltered supernatant was removed and analyzed for total soluble [Hg_T], and the pH was measured. DOC and [Hg_T] also were analyzed in controls, without penicillamine.

To evaluate the effect of penicillamine concentration on binding, six different penicillamine concentrations were used at Hg^{2+}/S_{red} = 1:120. To evaluate the effect of Hg/S_{red} on binding, the concentration of added Hg^{2+} was adjusted to provide Hg/S_{red} of 1:1370, 1:910, 1:270, 1:150, and 1:120. The effect of pH was evaluated by adding varying quantities of solid CaO to attain pH values up to 6. Binding in soil HA extracts from the forest surface soils and one whole soil was determined with Hg^{2+}/S_{red} molar ratios in the range of 1:27 to 1:730 at pH 4−5 (pH adjusted with CaO). Both humic acid extract and the whole soil were included for sample S3-3 to determine whether extraction of HA changed its ability to bind Hg^{2+}.

Dissolved organic carbon (DOC) was determined using EPA method 415.1 (*24*) with UV-persulfate oxidation on a Tekmar Dorhman Phoenix 8000 TOC analyzer. Solution pH was measured with a glass electrode calibrated at pH 7 and 4.

Total mercury [Hg_T] in solution was determined using a Brooks Rand Model III cold vapor atomic fluorescence spectrometer by the method of Bloom and Fitzgerald (*25*), as modified by Claas (*26*). This method is similar to EPA 1631. Samples were first digested with 1% BrCl overnight at 70 °C to destroy dissolved organic matter. After destruction of excess BrCl with NH_2OH−HCl and reduction by $SnCl_2$, Hg^0 was sparged from solution with N_2 and collected on gold-coated glass beads. The traps were heated, and Hg^0 in the N_2 gas stream was determined by fluorescence at 254 nm. Analyses were done in a positive-pressure clean room with HEPA filtered air. QA/QC procedures included using acid-cleaned Teflon centrifuge tubes/bottles and glassware, following clean room techniques and analysis of standard apple leaves (NIST-SRM 1515). Recoveries of Hg for the SRM varied from 91 to 112%.

Calculation of Binding Constants. At equilibrium, [Hg_T] represents the sum of Hg bound to penicillamine and DOC. We measured DOC in zero penicillamine control treatments and found concentrations ranging from 25 to 50 mg L^{-1} across the pH range of the experiments. The Hg associated with DOC was small as compared to the Hg bound to penicillamine (e.g., see the difference between penicillamine treatments and control in Table 2). The Hg associated with penicillamine was calculated as the difference between [Hg_T] and DOC-Hg in the control. Control treatments were conducted across the pH range to provide unique DOC-Hg control values for each pH. The quantity of Hg sorbed by the soil NOM was calculated as the difference between the quantity of Hg added (includes the small quantity of native Hg in HA of soil) and the quantity in solution.

Free aquo Hg^{2+} concentrations were calculated using Visual MINTEQ (*27*). Formation constants for penicillamine complexes were from Casas and Jones (*28*). Overall formation constants, β_1 and β_2, of Hg^{2+} are given by eqs 1 and 2

$$Hg^{2+} + L^{2-} \leftrightarrow HgL \quad (1)$$

$$Hg^{2+} + 2L^{2-} \leftrightarrow HgL_2^{2-} \quad (2)$$

where $\log \beta_1 = 38.3$ and $\log \beta_2 = 44.4$ (both at 25 °C; ionic strength = 0.1 M). The acidity constants ($pK_1 = 10.6$, $pK_2 = 7.9$, and $pK_3 = 1.9$) are from Martell and Smith (25 °C; ionic strength = 0.1 M) (*29*). All constants were corrected to zero ionic strength using the Davies equation. The predominant

TABLE 2. Partition Coefficients (K_{OC}) and Bidentate Thiol Formation Constants (K_{HgL}) of Hg^{2+} Bound to IHSS Pahokee Peat Humic Acid over a Range of Penicillamine Concentrations[a]

penicillamine (mM)	pH	Hg^{2+} added (μmol kg^{-1} of C)	soluble Hg_T (μM)	free Hg^{2+} (M)	sorbed Hg (μmol kg^{-1} of C)	K_{OC}	K_{HgL}
3.75	2.98	794	1.29	1.42×10^{-29}	111	7.80×10^{24}	2.4×10^{38}
2.5	3.00	781	1.22	1.83×10^{-29}	146	7.97×10^{24}	2.3×10^{38}
1.25	2.95	779	1.16	4.42×10^{-29}	179	4.05×10^{24}	1.4×10^{38}
0.5	3.01	779	0.783	5.60×10^{-29}	372	6.63×10^{24}	1.8×10^{38}
0.25	3.03	789	0.541	7.02×10^{-29}	503	7.17×10^{24}	1.8×10^{38}
control	2.99	796	0.00863		790		
					mean	6.7×10^{24}	1.9×10^{38}
					standard deviation	1.6×10^{24}	0.48×10^{38}
					log K (mean)	24.8	38.3

[a] $Hg/S_{red} = 1{:}120$.

complex between Hg^{2+} and penicillamine is HgL for all penicillamine concentrations in the pH range of 2−6.

We first calculated a distribution coefficient (K_{OC}) for free Hg^{2+} bound to SOM or humic acid, defined as

$$K_{OC} = \{\text{sorbed Hg}\}/[Hg^{2+}] \quad (3)$$

where{sorbed Hg} is in units of mol of Hg^{2+} per kg of C and K_{OC} has units of L kg^{-1}. This definition of K_{OC} is similar to K_{DOM} defined by Haitzer et al. (*18*) for Hg^{2+} binding to aqueous NOM, except that they calculated sorbed Hg per unit mass of dissolved organic matter, not per mass of organic C. Both K_{OC} and K_{DOM} are conditional constants.

We calculated formation constants for HA and SOM assuming Hg^{2+} bonds to two thiol groups at bidentate sites. This is consistent with EXAFS studies of Hg binding that show two-coordinate S sites for Hg^{2+} (*15*, *16*) and with a study on the pH dependence of Hg^{2+} binding to aquatic humic substances (*19*). The assumed reactions in solid-phase HA are

$$RS_2H_2 \leftrightarrow RS_2H^- + H^+ \quad (4)$$

$$RS_2H^- \leftrightarrow RS_2^{2-} + H^+ \quad (5)$$

$$RS_2^{2-} + Hg^{2+} \leftrightarrow HgRS_2 \quad (6)$$

where $\{RS_2H_2\}$, $\{RS_2H^-\}$, $\{RS_2^{2-}\}$, and $\{HgRS_2\}$ are in units of mol per kg of C and $\{HgRS_2\}$ is equivalent to {sorbed Hg}. The site density of bidentate thiol sites was assumed to equal 0.2 times the concentration of reduced S calculated from the data in Table 1 because the EXAFS study of Lin (*15*) showed that bidentate binding is predominant when the sorbed Hg is ≤20% of the reduced S. In PHA, this yields a site density of 11 mmol kg^{-1}. The equilibrium constants for eqs 4 and 5 are designated K_{a1} and K_{a2}, respectively. We assumed that K_{a1} and K_{a2} both have values of $10^{-8.4}$, the expected pK_a of cysteine thiol groups in peptides if no nearby groups are interacting with thiol (*30*). The apparent constant (K_{HgL}) for binding of Hg^{2+} is given by

$$K_{HgL} = \{\text{sorbed Hg}\}/\{RS_2^{2-}\}\gamma_{Hg}[Hg^{2+}] \quad (7)$$

This K, which has units of L mol^{-1}, can be calculated from K_{OC} using the following relationship:

$$\log K_{HgL} = \log K_{OC} - \log \gamma_{Hg} - \log 0.2[S_{red}] - 2\log[(\{H^+\}/K_a) + 1]] \quad (8)$$

Results and Discussion

Effectiveness of Penicillamine in Competing with PHA for Hg^{2+}. With 3.75 mM penicillamine at pH 3.0, Hg^{2+} desorbed from PHA to reach equilibrium in less than 6 days (Figure 1). To ensure equilibrium, we used a 13 day equilibration time in subsequent experiments. In all experiments, Hg_T concentrations were greatly elevated over concentrations in controls (Table 2).

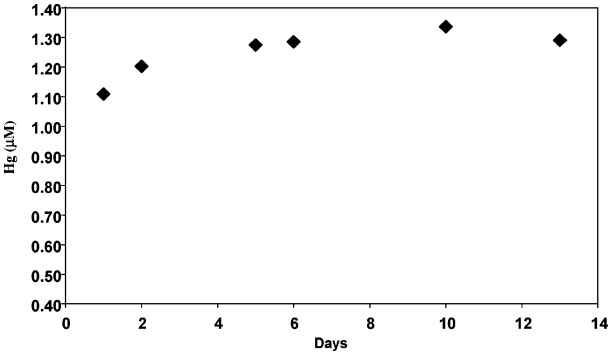

FIGURE 1. Kinetics of mercury [Hg_T] desorption from IHSS Pahokee peat humic acid at pH 3.0, $Hg/S_{red} = 1{:}120$ and [penicillamine] = 3.25 mM.

We investigated the effectiveness of diethylenetriaminepentaacetic acid (DTPA), which has a formation constant of $10^{26.4}$ for the HgL complex (*29*), and ethylene glycol-bis-(2-amino-ethyl ether) *N*,*N*,*N*′,*N*′ tetraacetic acid (EGTA), which has a formation constant of $10^{22.9}$ (*29*), but we did not observe any desorption. We also considered using EDTA, but its HgL formation constant is only $10^{22.1}$ (*29*). We used 0.1 M concentrations of DPTA and EGTA at pH 3.7−3.9 with 30 μM kg^{-1} of adsorbed Hg^{2+}. After 16 days, soluble [Hg_T] in both chelate treatments did not exceed that of the controls (zero penicillamine), despite the fact that both DTPA and EGTA reduced the Hg^{2+} activity to $<10^{-20}$ M.

With increasing concentrations of penicillamine and the constant addition of Hg^{2+} at pH 3.0, more Hg was desorbed from PHA (Table 2). In 0.25 mM penicillamine, 36% of the added Hg was desorbed and in 3.75 mM penicillamine, 86% of the Hg was desorbed. In the zero penicillamine control treatment essentially all added Hg was retained by PHA, and soluble [Hg_T] associated with DOC in the supernatant was only 1.5% of that for lowest [$Hg^{2+}{}_T$] in the penicillamine treatments (Table 2). K_{OC} values were constant across the penicillamine concentrations (mean $K_{OC} = 6.7 \times 10^{24}$ and log $K_{OC} = 24.8$; coefficient of variation (CV) = 24%). Calculated free Hg^{2+} concentrations in the penicillamine treatments were in the range of 10^{-39} to 10^{-30} M, values too low to represent real concentrations of ions. Rather, the calculated concentrations represent the very low chemical potential (activity) of Hg^{2+} at equilibrium. At these low activities of free Hg^{2+}, essentially all Hg(II) is associated with ligands, and desorption can occur only by bimolecular reaction of penicillamine with Hg on PHA adsorption sites.

TABLE 3. Partition Coefficients (K_{OC}) and Bidentate Thiol Binding Constants (K_{HgL}) of Hg^{2+} Bound to IHSS Pahokee Peat Humic Acid at Different Hg/S_{red} Ratios[a]

Hg/S_{red}	pH	Hg^{2+} added (μmol kg^{-1} C)	soluble Hg_T (μM)	free Hg^{2+} (M)	sorbed Hg (μmol kg^{-1} C)	K_{OC}	K_{HgL}
1:1370	3.58	75	0.112	7.2×10^{-32}	11	1.5×10^{26}	2.9×10^{38}
1:910	3.54	109	0.167	1.3×10^{-31}	17	1.3×10^{26}	3.0×10^{38}
1:270	3.55	356	0.495	3.6×10^{-31}	90	2.5×10^{26}	5.5×10^{38}
1:150	3.58	645	1.06	6.8×10^{-31}	72	1.1×10^{26}	2.1×10^{38}
1:120	3.49	803	1.08	1.1×10^{-30}	220	2.1×10^{26}	6.3×10^{38}
					mean	1.7×10^{26}	3.8×10^{38}
					standard deviation	0.58×10^{26}	1.8×10^{38}
					log K (mean)	26.2	38.5

[a] Penicillamine = 3.75 mM.

With increasing quantities of Hg added at constant penicillamine concentration (3.75 mM) and pH = 3.5, the concentration of both adsorbed and soluble Hg^{2+} increased (Table 3), but K_{OC} remained constant (mean $K_{OC} = 1.7 \times 10^{26}$; log $K_{OC} = 26.2$; CV = 34%) and about 40 times greater than the mean at pH 3.0 (Table 2).

The accuracy of the calculated free aquo Hg^{2+} concentrations and constants derived from them depends on the accuracy of the constants used for formation of the mercury−penicillamine complexes. Measurement of formation constants for metal−ligand complexes is difficult when the interaction is strong, and published mercury−penicillamine formation constants vary over many orders of magnitude. The value for HgL, the predominant complex at our pH and penicillamine concentrations, reported by Martell and Smith in the NIST Standard Reference Database (*29*) is $10^{18.8}$, which is much lower than value of 10^{38} from Casas and Jones (*28*) that we used. The NIST value is from Strand et al. (*31*), who determined the constant by proton potentiometric titration. However, so few protons displace Hg^{2+} from penicillamine, even at very low pH, that it is not possible to determine this constant accurately by proton titration (*28*, *32*). Casas and Jones (*28*) used Hg^{2+} potentiometry with an Hg electrode to measure Hg^{2+} activity directly in 6 mM penicillamine and assumed a 1:1 HgL stoichiometry. Their results were substantially confirmed by Koszegi-Szalai and Paal (*32*), who used an equilibrium partition method, forming a dithizone complex in 1−6 mM penicillamine and partioning it into CCl_4. We evaluated our results using their constants and found that the calculated free [Hg^{2+}] at 3.75 mM penicillamine was 50-fold lower than that given by the Casas and Jones constants. Nonetheless, the Koszegi-Szalia and Paal (*32*) constants would not result in different interpretation of our results (Table 2).

pH Dependence Hg^{2+} Binding in PHA. We observed a linear increase in log K_{OC} in the pH range of 1.9−5.8 (Figure 2). The slope of the linear regression line, 2.68, is greater than the value of 2.0 we expected from our hypothesis that Hg^{2+} is bound predominantly to two thiol groups. The high slope corresponded with a decrease in solution mercury−penicillamine with increasing pH. Because two protons are released when Hg^{2+} binds to penicillamine, we expected the mercury−penicillamine concentration to be constant with pH. These results suggest that some adsorbed Hg^{2+} binds to more than two weak acid groups in PHA. EXAFS data for Hg^{2+} bonding in fully protonated S3-3 HA showed that when Hg^{2+} was added to achieve Hg/S_{red} = 1:4, the predominant species was a linear two-coordinate complex with two inner shell S atoms (*15*). However, our data suggest that at lower concentrations of Hg^{2+}, and higher pH, a third weak acid group is coordinated to Hg. Likely, this is a three-coordinate complex with thiol. Thiol complexes involving more than two cysteine residues are common in metal-binding proteins (*14*).

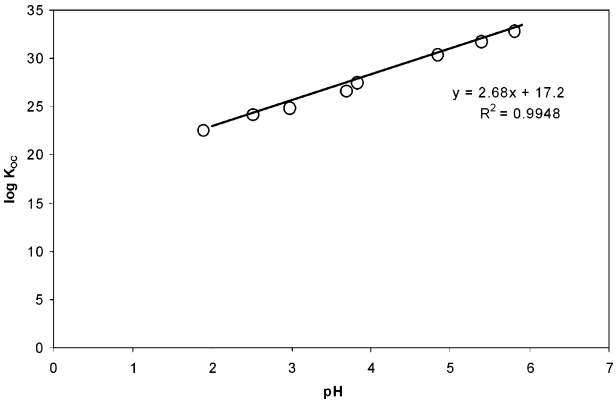

FIGURE 2. Log of distribution coefficients (K_{OC}) vs pH for Hg^{2+} bound to IHSS Pahokee peat humic acid. Hg/S_{red} ratio = 1:120 and [penicillamine] = 3.75 mM.

Binding Hg^{2+} to Forest Soil and Extracted HA. Partition coefficients (K_{OC}) were determined in 3.75 mM penicillamine for the S3 forest soil HAs and one intact forest soil in the pH range of 4−5 with Hg/S_{red} ranging from 1:27 to 1:730 (Table 4). Comparison of these values (Table 4, column 7) with those for PHA (estimated from the regression line in Figure 2; Table 4, column 8) shows that Hg^{2+} sorption to HA from a northern temperate forest is similar to that for peat HA from Florida. Except for S2-1 HA, values for PHA are within a factor of 10 of the Minnesota HA values at the same pH, suggesting that the binding sites must be similar in all samples of this study. In addition, the similarity of K_{OC} for S3-3 HA and intact S3-3 soil shows that extraction of HA with NaOH and treatment with HCl and HF did not change its ability to adsorb Hg^{2+}. Studies with HA thus can be used to interpret interactions of Hg^{2+} with SOM.

***K_{HgL}* for Binding to Bidentate Thiol Sites.** We calculated K_{HgL} for a bidentate site using eq 8 to compare our results to those of Haitzer et al. (*19*), who found log K = 28.1 for binding at a bidentate site in an aquatic humic extract. Our log K_{HgL} in Tables 2−4 are in the range of of 38−40, and because the calculated K_{HgL} underpredicts the effect of protons, the values increase with pH. The linear regression equation for relationship of pH and log K_{HgL} is $K_{HgL} = 0.682$pH + 36.7 (R^2 = 0.92).

Comparison with Other Studies. Comparison of formation constants for Hg^{2+} complexes in NOM determined by different investigators is difficult because of the different approaches used and different models for binding sites assumed in the calculation of K values. Many of the studies have been conducted with NOM in solution, assuming it behaves similarly to monomeric ligands. The study of Skyllberg et al. (*21*) is most similar to our study. They used Br^- as a competitive ligand to determine binding strengths in organic soils collected along dry-to-wet soil transects in

TABLE 4. Partition Coefficients (K_{OC}) and Apparent Binding Constants (K_{HgL}) of Hg^{2+} with Humic Acids and a Soil from a Dry to Wet Transect of O-Horizons and Peats in a Forest in Northern Minnesota[a]

sample	Hg/S_{red} ratio	pH	soluble Hg_T (μM)	free Hg^{2+} (M)	sorbed Hg (μmol kg^{-1} C)	K_{OC}	K_{OC} of PHA[b]	K_{HgL}
S3-1HA	1:310	4.08	1.32	1.2×10^{-31}	98.7	8.3×10^{26}	1.4×10^{28}	1.6×10^{38}
S3-2HA	1:280	4.99	0.11	1.0×10^{-35}	740	7.5×10^{30}	3.7×10^{30}	2.2×10^{40}
S3-3HA	1:260	4.80	0.47	1.0×10^{-33}	551	5.3×10^{29}	1.2×10^{30}	3.7×10^{39}
S3-3 SOM	1:27	4.82	1.0	2.1×10^{-33}	260	1.3×10^{29}	1.3×10^{30}	8.1×10^{38}
S3-4HA	1:480	4.51	0.097	8.3×10^{-34}	738	8.9×10^{29}	1.9×10^{29}	2.4×10^{40}
S3-5HA	1:730	4.40	0.64	9.1×10^{-33}	456	5.0×10^{28}	9.8×10^{28}	2.3×10^{39}
S2 HA	1:430	4.30	1.1	2.4×10^{-32}	231	9.6×10^{27}	5.3×10^{28}	6.9×10^{38}

[a] 800 μmol kg^{-1} of Hg^{2+} was added; penicillamine concentration = 3.75 mM. [b] Extrapolated from Figure 2 for pH in column.

the Marcell Forest in Minnesota, where we obtained our samples. Their results were called into question by Bloom et al. (*33*), who found evidence for the formation of mixed-ligand complexes of Hg^{2+} with SOM and bromide, which implies that the actual constants may be lower than reported. Despite the likely formation of mixed-ligand complexes, it may be useful to compare our data with theirs. In one of their experiments, they reacted forest soil in 0.38 M Br^- without adding Hg^{2+} to the native Hg^{2+}. Equilibrium pH values ranged from 3.0 to 3.5, and the log of calculated free $[Hg^{2+}]$ was in the range of −29.1 to −27.8. In comparison, we found log $[Hg^{2+}] = -28.9$ at pH 3.0 for the treatment in Table 1 with the lowest adsorbed Hg. They reported log K_{OC} values of 22.4−23.5, not greatly different from our results for Hg in PHA (Table 2). Their data suggest that a competing ligand must lower the activity of Hg^{2+} to $<10^{-25}$ to desorb enough Hg^{2+} into solution and achieve ligand-bound Hg concentrations in solution significantly greater than mercury−DOC.

Drexel et al. (*34*) determined constants for Hg^{2+} binding to DOC and peat using two freeze-dried Florida Everglades suspended in 0.01 M $NaNO_3$. They added Hg^{2+} and determined the Hg adsorbed to peat and bound to DOC, similar to our zero penicillamine control samples (e.g., Table 2) with pH fixed at 6.0. They used an iterative procedure to estimate Hg^{2+} binding constants for DOC (≈1 mg L^{-1}) and peat. At low loading of Hg^{2+}, corresponding to levels used in our study, they reported a log K_{peat} of 22.0 and 21.8, where K_{peat} is like our K_{OC} except the Hg concentration in peat was calculated per unit mass of peat, not C (assuming 50% C, K_{peat} is 0.3 log units less than K_{OC}). Our log K_{OC} at pH 6.0 for PHA (Figure 2) is 33.3. They calculated log K_{DOM} values of 22.8 and 23.2. They also calculated K_{HgL} for monodentate Hg^{2+} binding to thiol sites and reported values of 25.8 and 26.0 for the peat and 27.2 and 26.8 for DOM. Drexel et al. (*34*) did not define the convergence criteria they used, and we are not certain that the constants they obtained represent the only possible solutions.

Haitzer et al. (*18*) used a 48 h equilibrium−dialysis ligand-exchange method with 0.01 M EDTA to determine conditional partition coefficients, K_{DOM}, of Hg^{2+} with a hydrophobic DOM (mostly fulvic acid) extracted from peat water by XAD-8 resin. They used 3500 MWCO membranes, which did not completely retain the DOM, and corrected for the DOM that diffused through the membrane. Because EDTA is a poor competitor for Hg^{2+} at low loading, correction for binding of Hg^{2+} by the fraction of Hg that passed the membrane is most important at low loadings of Hg^{2+} (comparable to our loadings). At pH 7.0 with 1.0 mg L^{-1} DOM, the largest partition coefficient (log $K_{DOM} = 23.2$) was measured when Hg/DOM < 1 g Hg kg^{-1} (Hg/S_{red} < 1:64). This compares with log K_{OC} = 36.0 for PHA at pH 7. In a subsequent study, they determined that constants for low loading of Hg at pH 7 were similar for seven different sources of aquatic humus. They also investigated the pH dependence of binding for one humic extract and concluded that two protons are displaced during formation of the mercury−DOM complex (*19*).

All the studies discussed previously show that at environmentally realistic loadings of Hg^{2+} binding to NOM is very strong. The pH dependence of binding and EXAFS evidence (*15*, *16*) suggests that the binding involves at least two thiol groups. Given that the major source of thiol in the soils is cysteine (*12*), it is likely that the binding in soil HA is to cysteine in peptide fragments of HA. The question as to why K_{OC} values for soil and soil HAs are so different from K_{DOM} values for dissolved aquatic humic substances will be solved only with more research. One contributing factor may be an error in the assumption that K_{OC} values for soil HA are essentially equivalent to K_{DOM} for aquatic humic solutions. The partition of an adsorbate to a solid HA over a defined range is linear with concentration, but in solution, this can hold only if for each adsorbent molecule or ion the binding involves only one molecular unit. The molecular weight of aquatic humic substances studied by Haitzer (*19*) is small enough that on average there is less than one thiol S per molecular unit. Thus, unless thiol S is highly clustered, the bidentate assumption is not valid for aquatic humic substances, and two-coordinate binding with RS^- will involve two molecular units. In this case, K_{DOM} will increase linearly with concentration of humic substance, and the bidentate site assumption for calculating K_{HgL} may not be appropriate for relatively low molecular weight aquatic humic substances.

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