Formation of Binary and Ternary Colloids and Dissolved Complexes of Organic Matter, Fe and As

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Natural organic matter can change As speciation via redox reactions and complexation influencing its mobility and toxicity. Here we show that binary and ternary colloids and dissolved complexes of As(V), Fe and organic matter (OM) form at environmentally relevant conditions and analyzed these colloids/complexes using ATR-FTIR- and Mössbauer-spectroscopy. Dissolved Fe−OM complexes and ferrihydrite-OM colloids were formed by reacting OM with ferrihydrite (Fe(OH)$_3$). Mössbauer-spectroscopy showed that 95% of the Fe in the Fe−OM fraction were present as ferrihydrite−OM colloids while the remaining 5% were in the dissolved fraction. In As(V) plus Fe−OM systems (containing both dissolved and colloidal Fe−OM), 3.5−8 μg As(V)/mg OC was bound to the Fe−OM complexes/colloids compared to <0.015 μg As(V)/mg OC in As-OM systems (without Fe). Upon filtration of As−Fe−OM complexes/colloids with a 3 kDa filter, ~6% As was found in the dissolved fraction and ~94% As in colloidal Fe−OM. This suggests that As(V) is associated with Fe−OM mainly via ferrihydrite-OM colloids but to a small extent also in dissolved Fe−OM complexes via Fe-bridging. Since As-contaminated soils and aquifers contain Fe(III) minerals and OM, colloids of As with OM-loaded ferrihydrite and complexes of As with dissolved Fe−OM have to be considered when studying As transport.

Introduction

Arsenic is a toxic element of significant global environmental concern due to its contamination of ground waters and soils (*1*). In Bangladesh alone, about 85 million people are facing a serious threat because of poisonous levels of arsenic in their drinking water, up to 1000 times the WHO recommended safety limit of 10 μg/L (*2, 3*).

Arsenic is present in inorganic and to a smaller extent in organic forms in natural waters. Under oxidizing conditions, inorganic As is usually present as arsenate [As(V)] and under reducing conditions as arsenite [As(III)]. The environmental behavior of arsenic, its toxicity, and the uptake by plants depend strongly upon its speciation (*2−4*). In aqueous pH-neutral solutions, arsenite exists as uncharged species with pK_a values of 9.2, 12.1, and 13.4, whereas arsenate has pK_a values of 2.2, 6.9, and 11.5 and exists as $H_2AsO_4^-$ and $HAsO_4^{2-}$ at circumneutral pH (*1*). The mobility of arsenic in the environment is primarily controlled by adsorption onto clay minerals and metal oxide surfaces but also by interactions with natural organic matter (NOM) (*4−9*).

Interactions of As with Fe (oxy) hydroxides, clay minerals, soils, and sediments have been widely studied (*6−10*). However, research on As−NOM interactions has been carried out only recently. Humic substances (or NOM) are formed during degradation of biopolymers, are polymeric and polydisperse, and are ubiquitous in nature. Humic substances interact not only with arsenic but also with other metal ions and minerals in soil via adsorption, complexation, and redox reactions (*5−7, 11−17*).

NOM competes with arsenic for sorption sites at mineral surfaces potentially increasing arsenic mobility (*1, 6−10, 18*). Additionally, it has been suggested that arsenic binds to NOM forming As−NOM complexes and that arsenic can undergo redox reactions with NOM changing its mobility and toxicity (*1, 12−14*). Due to their sizes that belong mostly to the colloidal size fraction (between 2 and 300 nm or even higher depending upon aggregation (*11*), most metal−humic complexes fall as well into the colloidal size range. Arsenic was found to bind less strongly to humic substances (by complexation) compared to binding with hydrous iron oxides (*1*). However, it is assumed that in particular in case of low iron oxide content in the environment, humic substances can have a significant effect on mobilization of arsenic (*1*).

The presence of ternary complexes of NOM, Fe ions, and As has been proposed recently (*6−8, 14−16*) and in particular binding of As to NOM via an iron metal bridge has been suggested (*8*). However, the structure(s) of these ternary complexes, the mechanisms of their formation as well as the role of such complexes in the behavior of As in the environment have not been identified yet.

The objectives of this study thus were (i) to demonstrate that the interaction of OM with Fe(III) oxides forms dissolved Fe−OM complexes and/or ferrihydrite−OM colloids similar to the potential formation of such aggregates in the environment (Figure 1), (ii) to demonstrate that the interaction of these Fe−OM complexes/colloids with dissolved As in groundwater leads to the formation of ternary As−Fe−OM complexes/colloids, and (iii) to determine to which extent these aggregates are dissolved and colloidal.

Materials and Methods

Reagents. Stock solutions of 1 mM arsenate (Na$_2$HAsO$_4$) and 1 M NaCl were prepared in deionized water. Pahokee peat humic acid (PHA) was purchased from the International Humic Substances Society. 0.1 mg/mL PHA solution was prepared by dissolving dry PHA in 5 mM NaCl, pH-adjustment to pH 7 (using 1 M NaOH), stirring for 1 h and filtration (0.45 μm, cellulose acetate, Millipore).

Synthesis of Ferrihydrite. Ferrihydrite (FH) was freshly synthesized before each experiment by hydrolyzing 0.2 M FeNO$_3$·9H$_2$O with 1 M KOH at circumneutral pH (7−7.5) (*19*). The precipitates were identified as 2-line FH by μ-XRD (Supporting Information (SI) Figure S1) with a surface area of 239.7 g/m^2 determined using single-point Brunau−Emmett−Teller (BET) N$_2$ adsorption isotherms (ASAP 2000, Micrometrics).

Synthesis of FH−OM Colloids and Dissolved Fe−OM Complexes. FH was added as a suspension (1 mM final concentration) to the PHA solution (0.1 mg/mL; 49.94 mg C/L) and shaken at 200 rpm on a horizontal shaker at room temperature in the dark (Figure 1). No pH change occurred after FH addition. After 72 h of incubation the suspension was filtered (0.45 μm, cellulose acetate, Millipore) to separate

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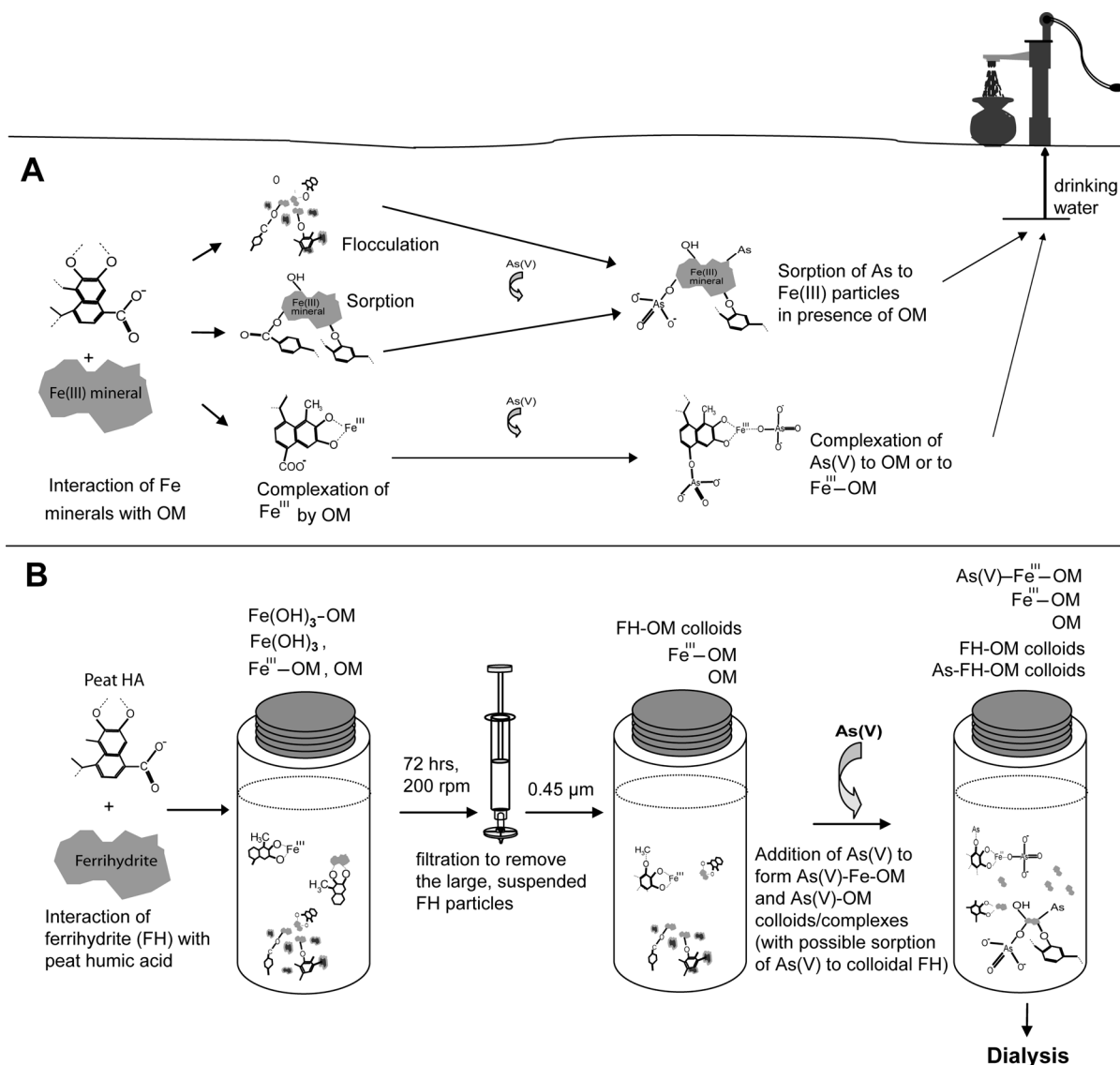**FIGURE 1. (A)** Illustration of how As(V)−OM and As(V)−Fe−OM colloids/complexes potentially form in nature and ultimately reach drinking water. **(B)** Lab experiment simulating the environmental process described in (A): Sequential process of formation of Fe−OM colloids/complexes via interaction of humic acid with Fe(III) minerals and in the second step the formation of As(V)−Fe−OM colloids/complexes via interaction of the Fe−OM colloids/complexes with dissolved As(V).

dissolved Fe−OM complexes and ferrihydrite−OM colloids from remaining FH particles. Dissolved organic carbon (DOC) and humic-bound Fe were quantified in the filtrate (see below).

To determine whether the Fe−OM aggregates are colloidal and/or dissolved by Mössbauer spectroscopy, ^{57}Fe−OM aggregates were prepared. First, a ^{57}FeCl$_2$ solution was prepared by dissolving ^{57}Fe(0) in 1 M HCl. 50 μL H$_2$O$_2$ (from a 35% H$_2$O$_2$ stock) was added to 1 mL of 0.53 M ^{57}FeCl$_2$ solution to oxidize Fe(II) to Fe(III). Simultaneously, 0.81 g of non-labeled Fe(NO$_3$)$_3$ was dissolved in 11.5 mL Millipore water and mixed with the oxidized ^{57}FeCl$_2$ solution. Under rigid stirring, 1 M KOH was added dropwise until the pH was circumneutral. After 2 h, the FH-suspension was washed four times with deionized water and at the end brought to a volume of 5 mL yielding a 0.5 M ^{57}Fe−FH suspension. ^{57}Fe−OM complexes/colloids were synthesized by incubating ^{57}Fe−FH and PHA using the same protocol as used for nonlabeled FH. For Mössbauer spectroscopy, the samples were freeze-dried.

Synthesis of Colloids and Dissolved Complexes of As(V)−OM and As(V)−Fe−OM. To synthesize As(V)−Fe−OM colloids/complexes, 100 μM As(V) was added to the Fe−OM

fraction. After As(V) addition, the pH was still 7. This mixture was incubated on a horizontal shaker (200 rpm) for 0−48 h in the dark. To synthesize As(V)−OM colloids/complexes, 100 μM As(V) was added to OM solutions and also incubated for 0−48 h. Since we used only As(V) in our experiments, throughout the paper we will use the terms As−OM and As−Fe−OM colloids/complexes.

Noncomplexed As was separated from As bound in As−OM and As−Fe−OM colloids/complexes using 1000 MWCO Regenerate Cellulose ester membrane dialysis bags (Roth, Germany). The dialysis bags were washed in deionized water (24 h) before usage to remove any leachable OC and then placed in 5 mM NaCl. Ten-mL aliquots of the As−Fe−OM and As−OM solutions were placed in the dialysis bag and the ends of the bag were closed with a PTFE clip. The As−Fe−OM and Fe−OM fractions were dialyzed for 5 days. Samples were taken from outside the dialysis bags every 24 h for As quantification. After sampling, the solution outside was replaced by fresh 5 mM NaCl solution. After 5 days, samples from inside the dialysis bags were analyzed for OM-bound Fe and OM-bound As. A 10-mL aliquot was taken separately from the As−Fe−OM fraction and subjected to centrifugal filtration with a nominal 3 kDa ($\sim$2 nm, see (20))

MWCO filter (Millipore) to separate dissolved As from As present in colloids (following (16)). The pH of the solution did not change during ultrafiltration. Arsenic sorption to dialysis bags was quantified by incubating bags with 100 μM As(V) followed by As quantification after 5 days. Each experiment was repeated twice and within each experiment three samples were analyzed per setup.

Analytical Methods. To quantify the amount of Fe in the Fe−OM and As−Fe−OM colloids/complexes, Fe was extracted by adding 500 μL of sample to 500 μL of 1 M HCl at 90 °C at 150 rpm in a thermomixer. After 30 min, the suspension (including precipitated humic substances) was centrifuged at 14 000 rpm for 15 min and the supernatant was analyzed for total Fe by the spectrophotometric ferrozine assay (21).

To quantify As, solution from inside and outside the dialysis bags as well as samples from the 3 kDa filtration were acidified by 1 M HNO_3, centrifuged for 3 min (14 000 rpm) and analyzed for total As by ICP-MS. DOC was quantified from filtered solutions (0.45 μm, cellulose acetate, Millipore) by a TOC analyzer. To analyze Fe−OM and As−Fe−OM colloids/complexes by ATR-FTIR spectroscopy, samples containing Fe−OM and As−Fe−OM colloids/ complexes were concentrated 10-fold using a vacuum centrifugation concentrator (SpeedVac, Bachhofer, Reutlingen, Germany) at 35 °C and 1500 rpm. Concentrating the samples did not change the pH. (ATR-)FTIR spectra were recorded using a Bruker IFS 113v FTIR-instrument (for details see SI S1).

The ratio of the absorbance at 465 and 665 nm (E4/E6) was determined for the organic matter to evaluate the preferential sorption of certain OM fractions to FH. The absorbance was measured in a plate reader (FlashScan 550, Jena Analytik, Germany).

Mössbauer spectra for the freeze-dried ^{57}Fe−FH and ^{57}Fe−OM samples were collected with a constant acceleration drive system in transmission mode and with a ^{57}Co source at room temperature. Samples were mounted in a close-cycle exchange-gas cryostat (Janis, U.S.) that allowed cooling of the sample to 5 and 77 K. An alpha-Fe metal foil was used for calibration at room temperature. Spectra calibration and fitting was performed with Recoil software (University of Ottawa, Canada) using Voigt based spectral lines.

Results and Discussion

Sorption of OM to Fe Minerals and Formation of Fe−OM Colloids and Dissolved Complexes. In order to simulate the interaction of organic matter (OM) with Fe(III) (hydr)oxides in the environment, we incubated ferrihydrite (FH) with Pahokee Peat Humic Acid (PHA) (Figure 1). During incubation of FH with PHA, a significant fraction of OM sorbed to the FH surface. In addition, FH particles in the colloidal size range associated with OM and became separated from the FH aggregates forming Fe−OM colloids. Simultaneously, some Fe ions leached from the Fe(III) mineral lattice leading to complexation of Fe by the PHA. Due to the 0.45 μm filtration step used in our study, the "dissolved" fraction (passing the filter) contained dissolved complexes but also colloids. Therefore, in a second step we separated truly aqueous dissolved from colloidal fractions to determine their respective importance (see below).

Quantification of DOC in ferrihydrite−PHA mixtures after incubation for 72 h revealed sorption of approximately 32% of the added OM to FH as indicated by the decrease in DOC from 49.9 to 33.9 mg C/L (Figure 2 and SI Figure S2) yielding a surface-normalized sorption of ~0.7 mg C/m^2 FH (calculated with the BET surface area of 239.7 m^2/g). Compared to a previous study with ferrihydrite and OM extracted from a Typic Haplorthod soil (22) with a surface-normalized sorption of 1.1 mg/m^2, OM sorption in our experiments was slightly
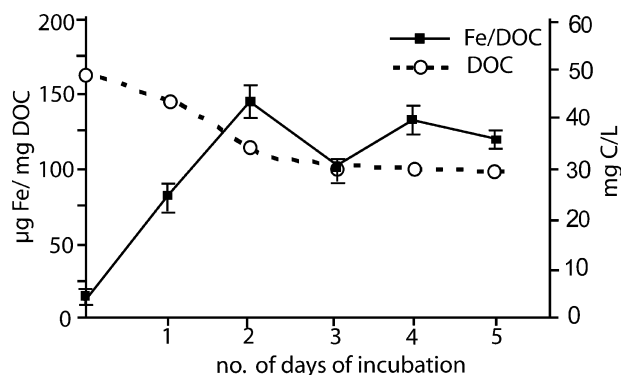

FIGURE 2. Complexation of Fe by Pahokee Peat Humic Acid (PHA) after incubation of the humic acids with poorly crystalline iron(III) hydroxide (ferrihydrite). DOC (defined by filtration through a 0.45 μm filter) and amount of total Fe bound to PHA are plotted against the number of days ferrihydrite and humic acids were incubated. The error bars indicate the standard deviation calculated from three independent samples each.

lower but in the same range. This difference could be due to the more acidic pH (pH 4.0) or the different humic material used in their experiments. UV absorption measurements of dissolved PHA before addition to FH showed slightly higher E4/E6 values compared to the remaining dissolved PHA after interaction with FH. Lower E4/E6 ratios typically represent a higher degree of aromaticity (23) suggesting that the nonsorbed PHA (including Fe−OM colloids/complexes) remaining after interaction of PHA with FH were slightly more aromatic than the original PHA (SI Table S3). This confirms a recent study in which a preferential sorption of some NOM fractions over others was suggested (24).

In addition to sorption to the Fe(III) mineral surface, humic compounds can leach and complex Fe ions from the Fe(III) mineral (25, 26). Additionally, nm- and μm-sized ferrihydrite particles can be coated by OM and separated from the remaining bulk ferrihydrite leading to flocculated colloidal ferrihydrite−OM aggregates (25). During the first days of incubation of PHA with FH, the DOC concentration decreased and in parallel the concentration of Fe associated with the PHA molecules (on a per DOC basis) increased (Figure 2). This suggests that the DOM covers the sorption sites at the FH mineral surface and at the same time Fe ions and/or Fe nanoparticles are leached and solubilized from the mineral phase by the DOM. After 3 days of incubation, DOC concentrations and Fe content reached a steady state suggesting that no more free sites at the FH particle surface were available for OM sorption (SI Figure S1). Based on these experiments, the incubation time for FH and PHA was set to 72 h followed by filtration to isolate Fe−OM colloids/ complexes for synthesis of ternary As−Fe−OM colloids/ complexes. Particle size measurements showed that the size range of the molecules in the original PHA solution was around 30 nm whereas for Fe−OM (and As−Fe−OM, see below) samples it was between 80 and 110 nm (SI Table S4) and thus in the colloidal size range.

The existence of Fe−OM colloids/complexes was demonstrated by ATR-FTIR spectroscopy (Figure 3). The two broad absorption bands at 1595 and 1390 cm^{-1} in the OM sample indicate asymmetric and symmetric vibrations for carboxyl (COO$^-$) groups (27−29). Complexation of COO$^-$ groups with metals causes a frequency shift and changes in the shape of the stretching bands of COO$^-$ (30). A frequency shift of the absorptions from 1595 to 1585 cm^{-1} and 1390 to 1340 cm^{-1} was indeed observed in the Fe−OM samples probably caused by the formation of Fe−OM colloids/ complexes and suggesting binding of Fe to the OM via COO$^-$ (28). The broad peaks in the Fe−OM spectrum at around
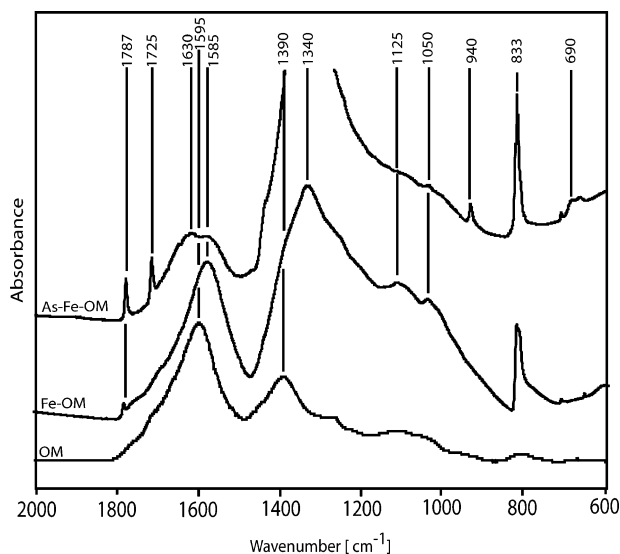
FIGURE 3. ATR-FTIR spectra comparing OM with Fe−OM and As−Fe−OM complexes.

1125 and 1050 cm^{-1} are caused by the bending mode of hydroxyl groups of Fe−OH (29–31). Due to the very low intensity of the 833 cm^{-1} peak in the OM-spectrum and the prominent signal at this wavenumber in the Fe−OM spectrum, we suggest that this peak represents most probably the ν(Fe−O) vibration in Fe−O−C or Fe−O−H. Interestingly, a weak signal at 833 cm^{-1} also exists for freeze-dried FH (SI Figure S3). However, we have to note that this peak has not been mentioned before as an iron-complexation related peak (for a detailed discussion of the peak at 833 cm^{-1} see below where the As−Fe−OM colloids/complexes are discussed). In addition, the FTIR spectra of the OM and Fe−OM samples show broad absorptions in the range of the ν(O−H) vibration (3700−3000 cm^{-1}) stemming from water, in the range of asymmetric carbonyl ν_{as}(CO$_2^-$) vibrations (1800−1550 cm^{-1}) and in the range of the symmetric ν_s(CO$_2^-$) vibration ($\sim$1400 cm^{-1}).

In summary, these results showed that interactions of humic substances with Fe(III) minerals can lead to the formation of dissolved Fe−OM complexes and Fe−OM colloids with a size of <0.45 μm. Similar processes are expected to occur in natural environments. It has been suggested that colloidal and dissolved Fe in groundwater is the result of weathering and leaching of Fe from different Fe-minerals by natural organic matter (NOM) (32). It has also been shown in lab experiments and field studies that the interaction of NOM with Fe-minerals can produce stable Fe−OM associations in aqueous systems (25, 32). Previous studies by Bauer and Blodau (16), where DOC ($\sim$2.5 mg C/L) was incubated with acidic solutions of FeCl$_3$ (Fe(total) <5 μM), also lead to the presence of Fe in both dissolved complexes and colloids although the Fe content in the dissolved fraction in our experiments was slightly higher than observed by these authors. The goal of our initial experiments was to reproducibly synthesize Fe−OM aggregates by simulating environmental conditions. While previous studies used acidic solutions of dissolved Fe^{3+} (e.g., FeCl$_3$) as Fe-source for studying the effect of Fe on As complexation by NOM (8), we chose FH and humic acids at neutral pH for our experiments for the following reasons: (1) FH is an Fe(III) mineral that is commonly found in the environment in contrast to free Fe^{3+} ions that are present only at very low concentrations at neutral pH without complexing ligands, (2) at circumneutral pH, humic acids are expected to interact with solid-phase Fe(III) minerals but when using dissolved Fe^{3+} at acidic pH, humic acids precipitate and the precipitated humic acids interact with dissolved Fe^{3+}, that is, just the reverse of what is seen

in the environment, and (3) starting with acidic solutions followed by readjustments of pH to neutral pH precipitates the Fe^{3+} as Fe(OH)$_3$ at pH values higher than 5, potentially coprecipitating part of the humic acids and in parallel solubilizing another part of the humic acids creating a highly artificial and not reproducible situation compared to the environmentally relevant scenario of reacting ferrihydrite with dissolved humic acids.

Identity of Fe−OM Colloids and Dissolved Fe−OM Complexes. In order to distinguish dissolved Fe−OM complexes from Fe−OM colloids containing FH nanoparticles, we carried out Mössbauer spectroscopy measurements of freeze-dried ^{57}Fe−OM aggregates in comparison to ^{57}Fe−FH. Spectra were recorded at 5 and 77 K (SI Figure S4). At 77 K, a Fe(III) doublet with high quadruple splitting (0.80) for ^{57}Fe−FH and 0.77 for ^{57}Fe−OM was observed (SI Table S5), which is typical for ferrihydrite ((33)). At 5 K, magnetically split sextets similar to values previously found for ferrihydrite were observed (SI Figure S5 and Table S5) (34). The hyperfine field decreased from 49 T for ^{57}Fe−FH to 47.5 T for ^{57}Fe−OM. A similar decrease was previously reported for FH−OM coprecipitates with increasing OM content (35). However, for ^{57}Fe−OM complexes, in addition to the sextet, we found also a Fe(III) doublet (center shift: 0.49, quadrupole splitting: 0.88). This doublet at 5 K does not represent any magnetically ordered structure and likely results from isolated Fe(III) ions associated with the OM suggesting the presence of complexed Fe(III) ions. Quantification of both signals yielded $\sim$95% of the total area for the sextet at 5 K meaning that 95% of the Fe in the Fe−OM fraction is in the form of ferrihydrite, whereas the remaining 5% is attributed to free Fe ions complexed by OM. The presence of complexed Fe(III) ions in Fe−OM (and As−Fe−OM) samples was also confirmed by analysis of As−Fe−OM samples obtained from the 3-kDa filtration step where $\sim$15 μM Fe(III) was measured in the filtrate, that is, the truly dissolved fraction (SI Table S6).

Formation of As−OM and As−Fe−OM Colloids/Complexes. Arsenic can bind to NOM directly in inner-sphere complexes via organic functional groups such as hydroxyl groups (1, 5) but it was also suggested that metal cations act as bridges between As to OM forming ternary complexes (6–8, 13–15). However, the existence of ternary As−metal−OM colloids/complexes has not yet been validated experimentally. To demonstrate the existence of the ternary colloids/complexes, to understand the mechanism of their formation, and to evaluate their relative importance in comparison to binary As−OM complexes/colloids, we investigated two different experimental setups: (1) As(V) plus OM and (2) As(V) plus Fe−OM systems.

In systems where As(V) was incubated with colloidal/dissolved OM and Fe−OM, we found significant concentrations of free (noncomplexed) As outside the dialysis bag in both systems after one day (approximately 160 μg/L) (Figure 4A and B). On the second day (the solution outside was changed after the first day), the As concentrations outside the dialysis bags dropped significantly and were hardly detectable on the third, fourth, and fifth day in both setups (Figure 4A and B). When we analyzed the As/DOC ratio in the solutions inside the dialysis bags (that contained the OM- and Fe−OM-bound As), As was found in considerable amounts ($\sim$3.5–8 μg As/mg DOC) in the As plus Fe−OM systems with slightly higher values for 24 and 48 h of incubation compared to 2, 6, or 12 h of incubation (Figure 4C). In contrast, in systems which consisted of As(V) plus OM (no Fe), the As per DOC ratio was much lower (<0.015 μg As/mg DOC) suggesting that only a minor fraction of As was bound to organic matter directly. This suggests that Fe plays an essential role in As binding to OM confirming a recent study that suggested that As binding to biomass also depends on the presence of Fe bridges (36).
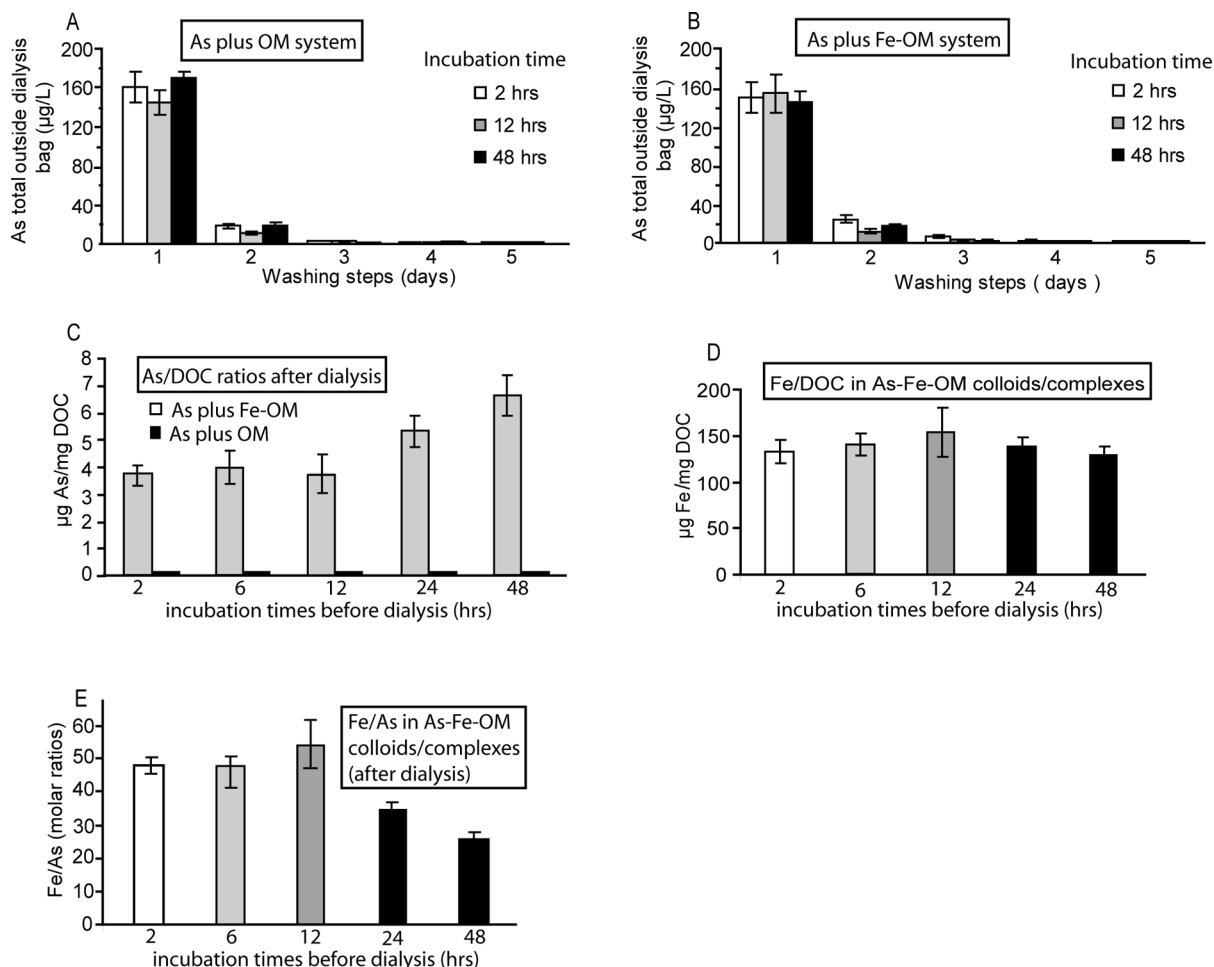
FIGURE 4. As and Fe content in As−OM and As−Fe−OM colloids/complexes after dialysis of mixtures of either As plus OM or As plus Fe−OM colloids/complexes. A and B show As concentrations in washing solutions outside of the dialysis bags for different washing steps for three different experiments with mixtures of As plus OM (A) and As plus Fe−OM (B) incubated before dialysis for either 2, 12, or 48 h. (C) shows total As content per DOC after dialysis in both the As plus OM and As plus Fe−OM systems for experiments with varying incubation times before dialysis. (D) shows Fe content per DOC in As−Fe−OM complexes for experiments with varying incubation times before dialysis. (E) shows the ratio of Fe to As in As−Fe−OM colloids/complexes for experiments with varying incubation times before dialysis. Samples for analysis of As and Fe content in complexes were taken from inside the dialysis bags at the end of the dialysis experiments. Error bars represent standard deviation calculated from three parallel samples.

Additionally to OM-bound As, we also determined the amount of Fe present in the As−Fe−OM colloids/complexes and calculated the Fe:As ratio. We found almost no variation of Fe/DOC ratios in the As−Fe−OM colloids/complexes for different predialysis incubation times between 2 and 48 h with 130−160 μg Fe/mg DOC for all incubation times (Figure 4D). This confirms our first results (Figure 2) that the Fe−OM colloids/complexes were in equilibrium before As(V) addition. From the Fe/DOC and As/DOC values we calculated Fe/As molar ratios of 26 to 55 with slightly decreasing values from 12−48 h of predialysis incubation (Figure 4E). These data suggest a significant excess of Fe bridges or Fe-nanoparticle surface sorption sites compared to the amount of As complexed, meaning that Fe could be responsible for all As complexation/binding with about every 20th to 50th Fe atom functioning as bridge between the OM molecules and the As(V) or as FH surface binding site for the As(V). Additionally, we analyzed the redox speciation of Fe and As in the complexes. However, no significant As and Fe redox reactions took place during the course of the experiment (SI Table S1 and Table S2) and therefore any effect of redox change on complexation of either Fe to OM or As to Fe−OM colloids/complexes can be ruled out.

In order to separate dissolved As−Fe−OM complexes from As−Fe−OM colloids and to quantify As and Fe in both fractions,

the As−Fe−OM sample that was incubated for 48 h and then dialyzed, was centrifuged with a 3 kDa filter (*16*). Before ultrafiltration, the As−Fe−OM fraction contained 167.1 μg/L As(V) and 58.3 μM Fe(III) (SI Table S6). After separating this As−Fe−OM sample into dissolved and colloidal fractions via 3 kDa filtration, the supernatant containing the dissolved complexes contained ∼10 μg/L As(V) (0.13 μM), ∼840 μg/L Fe(III) (15 μM), and ∼1 mg C/L DOC (SI Table S6); Fe(II) was below the detection limit of the ferrozine assay. This corresponds to about 6% of As(V) and 25% of Fe(III) in the dissolved As−Fe−OM complexes and 94% As(V) and 75% Fe(III) in the As−Fe−OM colloids suggesting that the fraction of complexed Fe (that was 5% in the Fe−OM fraction) increased to 25% as a consequence of the interaction of the Fe−OM fraction with the added As(V).

This data suggests that the majority of As(V) is bound to Fe−OM colloids, however, a small but still significant fraction of As(V) is complexed to OM via Fe(III) as a cation-bridge. These results are supported by a previous study by Bauer and Blodau (*16*) where comparable values for As distribution in colloidal and dissolved fractions were found upon reaction of DOC (∼2.5 mg C/L) with acidic solutions of FeCl$_3$ (Fe(total) <5 μM) and, As(V).

The result of almost no As complexation by Fe-free OM confirms a recent study where As sorption to biomass was

observed only in the presence of Fe-bridges (36) but was still rather surprising considering several recent reports on As-complexation by natural organic matter (13–15). However, Fe, Mn, or Al in their NOM samples could have acted as bridge in their system (significant amounts of Fe, Mn, and Al were measured in the compost extract used for their study). In contrast, the cation content in PHA from IHSS was found to be very low and close to the detection limit (SI Table S2). As a consequence of this lack of significant amounts of available PHA-bound cation bridges, complexation of As with PHA is close to negligible. For evaluating the two different As-binding scenarios (As−OM and As−Fe−OM), the charges of the three different components involved (As, Fe, and NOM) have to be considered. Arsenate and OM are negatively charged at neutral pH whereas the Fe(III) ions and FH colloids in the Fe−OM fraction provide a positive charge at neutral pH. Therefore, the negatively charged arsenate probably binds more strongly to the Fe-loaded OM, potentially even by electrostatic interactions forming outer-sphere complexes. In contrast, in As plus OM systems, the negatively charged carboxylic or hydroxyl groups will repel the negatively charged arsenate and inner-sphere complexes will be required for strong As(V) binding (13).

In order to demonstrate the presence of ternary As−Fe−OM colloids/complexes, ATR-FTIR of the As amended Fe−OM fraction (containing both colloids and dissolved complexes) were compared to Fe−OM samples and OM (Figure 3). Based on X-ray absorption spectroscopy studies of As-binding to biomass via Fe bridges (36), ternary complexation of Fe−OM with As is expected to produce a further frequency shift in the COO$^-$ stretching bands compared to the Fe−OM sample. And indeed, in As−Fe−OM samples, absorption peaks can be seen not only at 1585 cm^{-1} but also at 1630 cm^{-1}. The presence of both peaks in the As−Fe−OM sample is probably due to the presence of remaining Fe−OM colloids/complexes (signal at 1585 cm^{-1}) as suggested by the high Fe/As ratio in these samples (Figure 4) and the presence of the newly formed As−Fe−OM colloids/complexes (signal at 1630 cm^{-1}). To support that the 1630 cm^{-1} signal is indicative for the ternary complex formation, we collected FTIR spectra of As(V) sorbed to FH at different FH:As(V) ratios and from FH and As(V) alone (SI Figure S4). We found that pure FH shows a strong, broad signal at 1650 cm^{-1}. Additionally, with increasing As(V) concentrations no prominent peak at 1630 cm^{-1} (e.g., due to FH-sorbed As) is obvious. As(V) itself also gave a broad signal around 1650 cm^{-1} but only at very high As concentrations (1 mM), that is, concentrations much higher than present in our As−Fe−OM samples. Therefore, this data in combination with the fact that the 1630 cm^{-1} peak does not show up in the Fe−OM spectrum (Figure 3) but is present in the As−Fe−OM fraction (Figure 3) provides evidence that the 1630 cm^{-1} signal in the As−Fe−OM fraction is due to a peak shift from 1585 cm^{-1} to 1630 cm^{-1} from the As−Fe−OM fraction to the Fe−OM fraction, and is due to complexation of As to Fe−OM. The peak at 690 cm^{-1} can be attributed to the ν(As−OH) vibration further confirming the presence of As in these colloids/complexes (37).

UV absorption measurements of PHA in the As−Fe−OM fraction showed slightly lower E4/E6 values compared to the PHA in the Fe−OM samples (SI Table S3) suggesting a fractionation of the PHA by the loss of DOC during dialysis (leading to a slightly higher aromaticity in the As−Fe−OM fraction). This fractionation of OM could probably have influenced the FTIR spectra of the As−Fe−OM vs the Fe−OM fraction. However, such a fractionation (loss of DOC) is not expected to shift individual peaks (as has been observed after As addition) but rather to lead to the disappearance of certain signals. Therefore, the peak shift observed from the Fe−OM to the As−Fe−OM sample most likely provides evidence for

ternary complex formation and is not influenced by the fractionation of the OM.

Additionally to the peaks around 1630 cm^{-1}, some peaks were observed in the FTIR spectra of the As−Fe−OM samples. The signal at 3513 cm^{-1} (not shown) is due to the ν(O−H) vibration of As−OH (38). The peak at 940 cm^{-1} in the spectrum obtained for the As−Fe−OM sample (Figure 3) probably belongs to the asymmetric ν_{as}(As−O−Fe) vibration caused by As(V) binding to Fe−OM (reported at 938 cm^{-1} in refs 37, 39. This 938 cm^{-1} peak is not evident in any of the spectra of As(V) binding directly to ferrihydrite (SI Figure S4), therefore suggesting that OM is involved in the binding of the As(V) to the Fe. In the As−Fe−OM sample, the peak at 1125 cm^{-1} that was observed in the Fe−OM sample disappeared whereas the peak at 833 cm^{-1} is stronger than in the Fe−OM sample. This signal probably corresponds to the As−O stretching vibration suggesting that the 833 cm^{-1} peak involves not only Fe−O vibrations but also the symmetric ν_s(As−O−Fe) vibration that has been reported as an As−O signal (37, 40). The two sharp peaks at 1787 and 1725 cm^{-1} correspond to ν(C=O) vibrations in Fe(C=O) (41). In summary, the ATR-FTIR measurements demonstrate the formation of ternary As−Fe−OM colloids/complexes by interaction of Fe−OM colloids/complexes with dissolved arsenate.

Environmental Relevance of Dissolved and Colloidal As−Fe−NOM Complex Formation. In the present study we demonstrated the formation of ternary As−Fe−OM colloids and dissolved complexes under environmentally relevant conditions containing arsenate, (natural) organic matter and a Fe(III) mineral (ferrihydrite). The presence of other divalent and trivalent cations such as Ca^{2+}, $Mn^{2+/4+}$, Mg^{2+}, and Al^{3+} in groundwater suggests that there is also vast possibility of formation of ternary complexes of As with other metal−OM complexes as has been postulated and shown in some previous studies (8, 13–16). The inability of As to bind directly to OM (without metal bridges) means that As binding to FH−OM colloids and partially to dissolved Fe(III)−OM complexes are possibly the most common mechanisms of As association with NOM in the environment. The formation of binary or ternary complexes of As in flowing conditions as they are present in many aquifers (simulated in column experiments), the stability and mobility of the ternary complexes, and finally whether As(III) or As(V) is more likely to form ternary complexes with metal−OM complexes remains to be answered in further experiments.

Supporting Information Available
XRD of freeze-dried FH (Figure S1), sorption of PHA to FH (Figure S2), FTIR spectra of freeze-dried As−Fe−OM, Fe−OM, and OM and FH (Figure S3), FTIR spectra of As(V) only, FH only, and samples containing different As(V)−FH ratios (Figure S4), and Mössbauer spectra of freeze-dried ^{57}FH and ^{57}Fe−OM at 5 K (Figure S5). It also contains experimental details for ATR−FTIR analysis (S1), As speciation data for dissolved and colloidal As−Fe−OM (Table S1), speciation data of Fe in PHA, Fe−OM, and As−Fe−OM colloids (Table S2), E4/E6 ratios of PHA (Table S3), As(total), Fe(III) and DOC data for the three kDa fraction after centrifugal filtration

of As−Fe−OM complexes (Table S4), Mössbauer data for ^{57}Fe-labeled FH and ^{57}Fe-OM at 77 and 5 K (Table S5), and particle size data for PHA, and Fe−OM and As−Fe−OM colloids/complexes (Table S6). This material is available free of charge via the Internet at http://pubs.acs.org.

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