

Dissolved Organic Matter Sources and Consequences for Iron and Arsenic Mobilization in Bangladesh Aquifers

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Iron (Fe) and dissolved organic matter (DOM) cycling have been implicated in arsenic mobilization via microbially mediated Fe oxide reduction. To evaluate the sources and multiple roles of DOM in Bangladesh aquifers, we conducted spectroscopic analyses on various types of surface water and groundwater samples from a site representative of aquifer chemistry and hydrology. Surface water contained humic substances with oxidized quinone-like moieties and high concentrations of labile microbially derived DOM. In contrast, in shallow groundwater where dissolved iron and arsenic concentrations were high, the quinone-like moieties of humic substances were more reduced, with less abundant labile DOM than that of surface water. Instead, DOM at these depths was characterized by terrestrial (plant/soil) signatures. A sediment microcosm experiment demonstrated that Fe(II) and terrestrially derived DOM were released from sediment over time. The results provide new evidence to support a dual role of natural DOM in Bangladesh aquifers (1) as a labile substrate for Fe- and humic-reducing bacteria and (2) as an electron shuttle via humic substances to enhance microbial iron reduction. Fluorescence index, amino acid-like fluorescence, and redox index may serve as useful indicators of the type of DOM likely to be involved in Fe solubilization and potentially As mobilization reactions.

Introduction

Iron (Fe) and dissolved organic matter (DOM) cycling have been implicated in the mobilization of arsenic in aquifers worldwide and in Bangladesh, where dissolved arsenic

concentrations, [As], frequently exceed the World Health Organization limit of $10 \mu\text{g L}^{-1}$ (Figure S1 of the Supporting Information). Recently, it was proposed that microbially mediated Fe oxide reduction and subsequent dissolution, fueled by the presence of labile DOM, resulted in the release of arsenic bound to sedimentary Fe oxides (1–4). It has also been demonstrated that DOM is able to complex dissolved Fe and As (3, 5) and directly reduce As(V) to As(III) (6).

Biological Fe reduction is coupled to DOM chemistry in two ways. First, some labile DOM can serve as a source of energy (electrons) for Fe- and humic-reducing microbial communities, leading to reduction of Fe oxides (7). In Bangladesh aquifers, potential sources of labile DOM include terrestrial (plant/soil) organic matter such as peat dispersed throughout the aquifer sediment (2) and surface water DOM brought to depth by natural flow or irrigation pumping (4, 8, 9). Second, Fe reduction and DOM are related via the electron shuttling ability of humic substances (HS). Quinones are among the redox active chemical moieties responsible for electron shuttling by HS (10–12). It is generally accepted that sediment-bound arsenic, possibly sorbed to iron(III) oxyhydroxides, has been transported as particles by rivers in Bangladesh and subsequently deposited when the aquifers formed. To degrade labile DOM in this setting, it has been shown that bacteria transfer electrons outside of the cell to use Fe(III) as an electron acceptor, and that mobilization of As(III) from sediments containing Fe(III) ensues (13). In laboratory experiments, oxidized HS have been shown to enhance this electron transfer and Fe reductive dissolution (14–17) (Figure S2 of the Supporting Information). In the presence of Fe(III) and other compounds that can accept electrons from HS, the reduced HS can cycle back to their oxidized form and serve as electron shuttles multiple times (14, 15).

Although HS-enhanced electron shuttling has been shown to stimulate Fe reduction rates (14), this process and its potential for As mobilization may be influenced by other environmental factors. For example, the kinetics of electron shuttling from bacteria to HS to Fe oxides has been found to be limited by the amount of dissolved HS (16) and may also depend on the mineralogy of the Fe(III) compounds (17). Also, secondary precipitation of iron phases during Fe reduction has been shown to cause As retention, although As release ensued with greater reaction time (7). In addition to reductive Fe dissolution, other mechanisms have been shown to influence the mobility of solid-phase As, such as As complexation by natural organic matter (NOM), competitive sorption by NOM, and As redox transformation by reduced and oxidized HS (3, 5, 6).

Understanding the chemical nature of DOM under reducing conditions can provide new insights into the dominant processes influencing As mobilization in Bangladesh aquifers. DOM is particularly well-suited to spectroscopic characterization (UV–vis absorbance and fluorescence) because of its ability to absorb light and fluoresce. Specific UV absorbance (SUVA) (18) can fingerprint aromatic DOM such as DOM from plant and soil sources and is used in water quality monitoring as an indicator of the humic fraction of DOM (18). Fluorescence spectra scanned at a single excitation wavelength have been used to describe DOM quality. The fluorescence index (FI) has been found to be a stable and robust indicator of terrestrial and microbial DOM sources (19), even under long periods of sample storage (20). Fluorescence spectra, obtained as three-dimensional excitation–emission matrices (EEMs) provide more information about DOM sources and redox states. For example, parallel factor analysis (PARAFAC) modeling of EEMs allows for

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quantification of the contribution of fluorescent DOM components, including reduced and oxidized quinone-like fluorophores (11, 21).

The release of As and other toxic metals by HS-enhanced microbial Fe reduction has broad implications for groundwater quality, yet direct chemical characterization of DOM and the humic redox state in groundwater has not been conducted. Therefore, our goals were to conduct a field investigation in an environmentally relevant setting with well-documented aquifer chemistry and hydrology in order to (i) characterize the sources of groundwater DOM at depths corresponding to different groundwater age and dissolved As content and (ii) evaluate the role of DOM in humic-enhanced Fe reduction and other processes that may result in Fe and As mobilization.

Materials and Methods

This study focuses on Site B (also called the "Bay Site" in ref 5) located in Araihasar, Bangladesh. Shallow tube wells at Site B have average [As] of $272 \pm 96 \mu\text{g L}^{-1}$. Here, the water is characterized by three distinct ages and flowpaths as evidenced by three distinct stable oxygen and deuterium isotope signals of water (22). The water of the shallow Holocene aquifer at 8 m depth has an age of 1.6 years, with [As] of $\sim 30 \mu\text{g L}^{-1}$ (Figure 1a), and has not traveled far from its recharge source at the surface. The water from 11 and 14 m is about 19 years old, with [As] up to $550 \mu\text{g L}^{-1}$, and has traveled along a longer flowpath with a different surface water source than water at 8 m (22). In the deep Holocene aquifer, the water at depths >40 m is separated from the shallow Holocene aquifer by a clay layer and is at least 50 years old and possibly hundreds of years old, with [As] of $\sim 20 \mu\text{g L}^{-1}$ (23). Evidence from oxygen and deuterium stable isotope analyses further indicates that groundwater at 40 and 52 m originated from different surface water sources than groundwater at shallower depths (22).

Sampling and Field Measurements. To evaluate the chemical character of surface water DOM, we sampled a range of surface water sites (Table S1 of the Supporting Information) in January 2006 and March 2008. We sampled ponds at Sites B (SW-4) and F (SW-5, SW-6), a canal at Site E (SW-2), and a downstream location of the Old Brahmaputra River (SW-3).

Groundwater samples were collected from 8, 11, 14, 40, and 52 m depths at Site B in December 2006 and March 2008 and were filtered using in-line GFF filters. Conductivity, temperature, pH, and redox potential (ORP) were monitored using a pH/Eh meter (Orion 210A) and a conductivity/temperature meter (Orion 105A+) with waterproof probes that were calibrated on the day of sampling. Alkalinity of groundwater was determined by Gran titration (24) in March 2008 in the field.

Chemical Analyses. Total dissolved As and Fe concentrations were determined on acidified 2008 samples by ICP-MS following U.S. Geological Survey protocol. Dissolved Fe(II) concentrations [Fe(II)] were determined by ferrozine colorimetry (25) immediately onsite in 2006 and in the laboratory in 2008 on filtered groundwater. Dissolved organic carbon (DOC) concentrations [DOC] were measured on acidified samples using a TOC-5000 analyzer (Shimadzu), with a confidence interval of 0.02 mg C L^{-1} . For spectroscopic (UV-vis absorbance and fluorescence) analyses, groundwater samples were handled under anoxic conditions, and surface water samples were handled under ambient open-air conditions.

UV-vis absorbance was measured using an Agilent 8453 UV-vis spectrophotometer with a 1 cm path length cell, with a confidence interval of 5%. Specific UV absorbance (SUVA) was calculated as the UV absorbance (measured at 254 nm) normalized to the [DOC] and reported in units of $\text{m}^{-1} \text{ mg}^{-1} \text{ L}$.

Three-dimensional fluorescence excitation emission matrices (EEMs) were generated by scanning filtered acidified water samples over an excitation range of 240–500 nm at 10 nm increments and an emission range of 350–550 nm at 2 nm increments on a JY-Horiba/Spex Fluoromax-3 spectrofluorometer. All EEMs were fit to a PARAFAC model (11), and the relative amounts (percent of total fluorescence) of each component were measured (Tables S1 and S2 of the Supporting Information) as well as the FI. From PARAFAC modeling, the redox index (RI) and hydroquinone-like fluorescence (%HQ) were determined, which provide information regarding the redox state of quinone-like moieties (21).

Microcosm Sediment Amendments. In March 2008, a sediment core was collected from 11 m depth at Site B and homogenized inside a nitrogen filled glovebag. Microcosms were prepared in duplicate by placing approximately 2 g of mixed sediment in 15 mL amber serum vials and immediately amending the sediments with the following: (A) approximately 12 mL of groundwater from an 11 m depth well and (B) groundwater + 5 mM of 2,6-anthroquinone disulfonate (AQDS).

Results and Discussion

DOM in Surface Water. Surface water in Araihasar was characterized by low [As] and [Fe] (Figure 1a; Table S1 of the Supporting Information) and high [DOC], ranging from 4.5 to 7.4 mg L^{-1} . Its relatively high FI and amino acid-like fluorescence (Figure 1b for Site B pond and Table S1 of the Supporting Information for other sites) indicated substantial microbial influence, and these chemical characteristics are consistent with wastewater influenced environments (26). In surface water near the monitoring wells (Site B pond) in 2006, the low RI and HQ content and positive ORP (Figure 1c) indicated that a high percentage of quinone-like fluorophores (humic quinones) were in an oxidized state.

Processes Occurring in Young, Shallow Groundwater. In young, shallow 8 m groundwater, the FI was similar to that of surface waters (Figure 1b), supporting an interpretation that DOM arrived at the 8 m depth along a short flowpath after recharge. In particular, the substantially higher FI and amount of amino acid-like fluorophores (% AA) observed in 2008 than in 2006 is in the range reported for surface water (FI of 1.50–1.63 and % AA of 6–12%; Table S1 of the Supporting Information) and is consistent with young (<2 years old) groundwater that responds to variable and more rapid surface water inputs to this depth (27). The lower SUVA and [DOC] compared to surface water (Figure 1b,c) suggests that the more aromatic and humic DOM was lost to preferential sorption along the flowpath from the surface to 8 m. This type of preferential loss of aromatic DOM along groundwater flowpaths has been observed in laterally transported groundwater of a recharge wetland (28). The shift to reducing conditions at 8 m, as reflected in the lower ORP, higher RI, and HQ content and higher sediment FeII/Fe ratio, in combination with high dissolved [Fe] and lower [DOC] than in surface water (Figure 1a,c; Table S2 of the Supporting Information), supports scenarios in which labile DOM is used as an energy source for bacteria to either directly reduce Fe or to reduce humics that shuttle electrons. In the latter case, the HS serve as shuttles to enhance Fe oxide reduction and solubilization (Figure S2 of the Supporting Information). If the high dissolved [Fe(II)] at 8 m (Figure 1a) is driven by an electron shuttling process as described above, then our results suggest that the [DOC] necessary for HS to participate in HS-enhanced electron shuttling and Fe(III) mineral reduction in the field may be lower than proposed by Jiang and Kappler (16). Alternatively, dissolved humics may be more concentrated at or near mineral surface sites with higher [DOC] than the bulk [DOC] we measured. Both

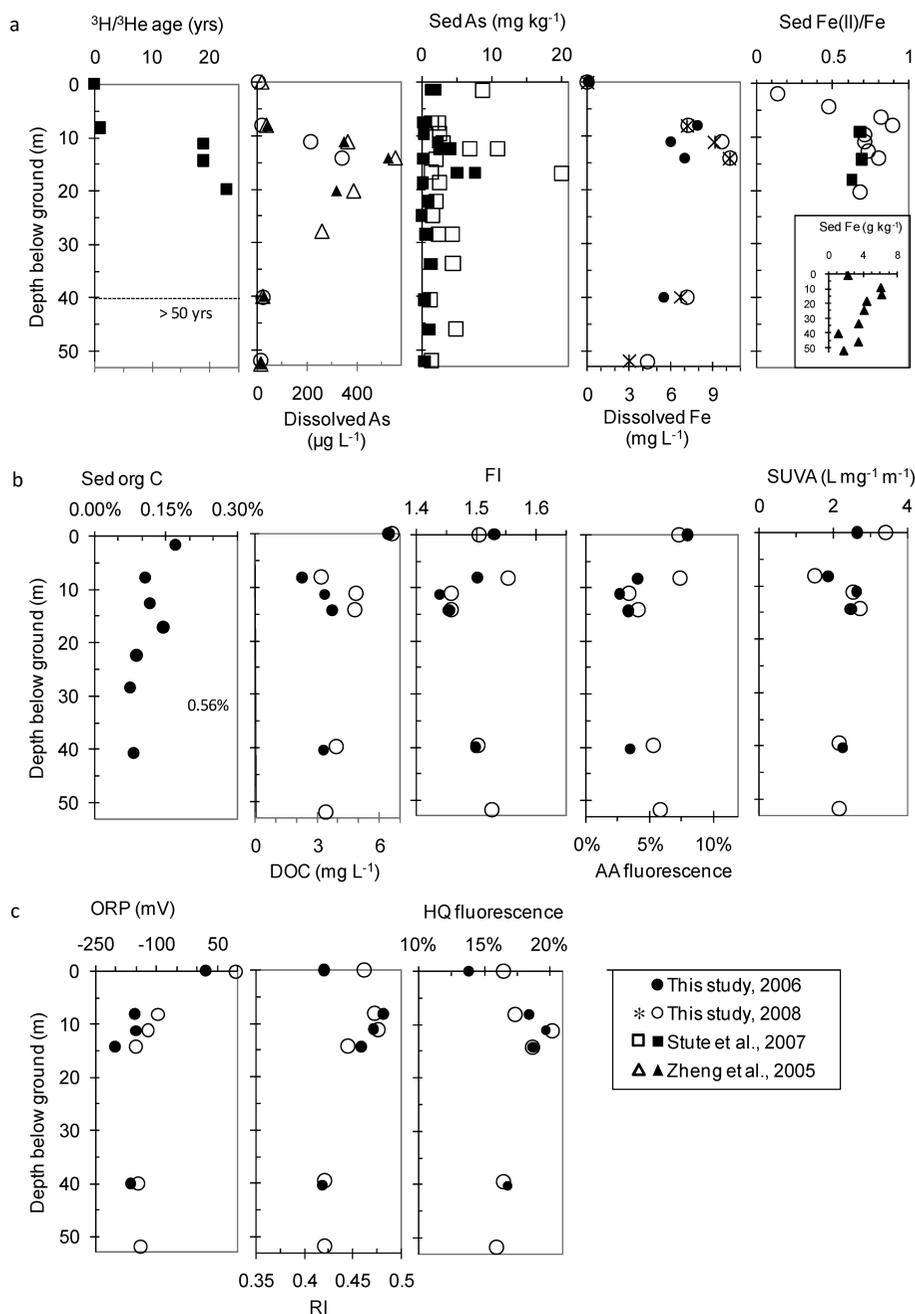


FIGURE 1. Depth profiles at Site B. (a) Aquifer inorganic chemistry: $^3\text{H}/^3\text{He}$ age, dissolved As concentrations [As III (filled \blacktriangle) and total dissolved As (open \triangle and open \circ)], sediment As content [bulk As (filled \blacksquare) and P-extractable As (open \square)], dissolved Fe(II) (filled \bullet and \ast) and total dissolved Fe (open \circ) concentration, sediment Fe(II)/Fe ratios (open \circ and filled \blacksquare) with inset of sediment Fe content [hot HCl-extractable total Fe (filled \blacktriangle)]. (b) Aquifer organic chemistry: sediment organic C content, DOC concentration, fluorescence index (FI), amino acid-like (AA) fluorescence [sum of tryptophan and tyrosine-like components (model components 8 and 13)], and specific UV absorbance at 254 nm (SUVA). (c) Redox conditions: redox potential (ORP), redox index (RI), and hydroquinone-like (HQ) fluorescence (model component 4). Surface water samples (0 m depth) are from a pond next to Site B. The age of the 40 m groundwater sample was determined to be >50 years by radiocarbon dating (23). Sedimentary As is heterogeneous and 0–10 m depths are typically low in sediment As while 10–20 m depths have highest average sediment As content. Leachable sediment Fe(II)/Fe content was constant at 0.61 – 0.65 between 9 and 20 m belowground (22). Sediment organic C at 34 m depth was approximately 0.56% (scale shortened for resolution). Depictions of the sediment profile for Site B can be found in Zheng et al. (23) and Dhar et al. (27). Dates of sample collection and references for published data are denoted with different symbols and shown in the legend.

mechanisms described above reflect the need for a better understanding of microbiological processes and their relationship to As mobilization under field conditions. Recent results indicate that shallow Arahazar sediments (between 2 and 6 m depth) harbor close relatives of Fe-reducing bacteria from the *Geobacter* genus and the *Shewanellaceae* family (Legg, personal communication). In addition to Fe-reducing bacteria, many bacteria, including fermenters, sulfate reduc-

ers, and even methanogens, have been shown to reduce humic substances (29).

DOM in Older, Shallow Groundwater. At 11 and 14 m, the [DOC] (Figure 1b) was substantially higher than at 8 m even though the average age of these waters is much older than 19 years, and DOC is expected to be lost to microbial respiration as the water ages. Because the flowpath of water at 11 and 14 m is distinct from that of water at 8 m (22), the

DOM at these depths is unlikely to have evolved directly from the DOM at 8 m. The lower FI compared to 8 m (Figure 1b) reflects a different DOM source at these depths than at 8 m or in the variety of surface waters present in the Araihaaz area (Table S1 of the Supporting Information). Together with the higher SUVA and lower % AA at 11 and 14 m than those at 8 m and in surface water (Figure 1b; Tables S1, S2 of the Supporting Information), these spectroscopic signatures are representative of lignaceous and aromatic DOM, similar to those observed in plant-dominated DOM environments (30) and reduced peat soils (31). These results suggest that a contribution of DOM from sediment peat deposits or other types of dispersed sedimentary organic carbon has occurred over time. Indeed, others have documented the presence of 3000 to 5000 year old DOM in a similar Holocene aquifer in southern Bangladesh (8, 32).

Groundwater DOM Mobilized from Shallow Sediment.

Dispersed sedimentary organic matter has been suggested as a source of labile DOM to fuel microbial reactions in the reducing aquifers of Bangladesh (2) and Cambodia (33), although the process of mobilization of sedimentary organic matter has not been directly demonstrated in these environments. Organic-coated mineral oxides have been shown to undergo reductive dissolution in situ, releasing aromatic and high molecular weight DOM to wetland sediment pore water (34). Similarly in our study, sorbed organic material, including HS, may be released into solution during the reductive dissolution of iron oxides. Klapper et al. (35) interpreted the fluorescence spectra of weakly-bound HS extracted from marine sediments as indicating that the HS had been microbially reduced under anoxic conditions. This biotic process may be another important mechanism for the dissolution and release of sedimentary HS.

To test whether DOM could be released from aquifer sediment, we performed two microcosm experiments, one with sediment amended with 11 m depth groundwater and one with sediment + 11 m groundwater + AQDS. An increase in [DOC] from 4.84 mg C L⁻¹ in the original 11 m water to 6.10 mg C L⁻¹ in the sediment + groundwater treatment was observed. In both experiments a decrease in FI from 1.46 to 1.41 and 1.35, respectively (Table S1 of the Supporting Information) was also observed, indicating that terrestrially derived DOM was released from the aquifer sediment. Further evidence for the mobilization of DOM from sediments as a potential source of terrestrially derived DOM stems from the radiocarbon (¹⁴C) signature of groundwater from the 11 m depth. Our calculations (Supporting Information) indicate that at least 10% of the groundwater dissolved inorganic carbon (DIC) appears to originate from bacterial respiration of sediment-derived, old DOM at 11 and 14 m. The microcosm experiments and DIC calculations were not performed for other depths discussed in this study. Given that the exact mechanism of DOM release from aquifer sediment is not known, it is difficult to speculate on whether this process occurs at other depths. Understanding the potential for release of sedimentary HS by abiotic leaching, direct microbial reduction, or other mechanisms is relevant for understanding the quality of DOM in Bangladesh aquifers with variable [As] and warrants further investigation.

Redox Cascade for Fe Oxide Dissolution. The combination of low redox potential and humic quinones in a reduced state (represented by high RI and HQ content; Figure 1c) represent a necessary step to support an active redox cascade at 11 and 14 m (Figure S2 of the Supporting Information). In this scenario, labile DOM is used as a microbial substrate, which would explain why DOM with amino acid-like fluorescence does not accumulate at this depth (Figure 1b). Here, humic DOM also plays a role as an electron shuttle to enhance Fe reductive dissolution and As mobilization (Figure S2 of the Supporting Information). Whereas Fe(III) is most suited to forming aqueous complexes

with DOM (ref 3 and references therein), the dominant form of dissolved Fe at these depths is Fe(II), representing >90% of the total dissolved Fe (Figure 1a). Therefore, the high [Fe(II)] at 11 and 14 m is probably not a result of Fe-DOM complexation but more likely explained by the reductive dissolution hypothesis. To further support this, our microcosm experiments showed a substantial increase in dissolved [Fe(II)], from 7.22 mg L⁻¹ in the initial water to 17.5 mg L⁻¹ in the sediment + water experiment and to 17.2 mg L⁻¹ in the sediment + water + AQDS experiment (Table S3 of the Supporting Information). In the sediment amended only with 11 m depth water, the substantial increase in dissolved [Fe] was accompanied by an increase in RI (Table S3), which is consistent with humics shifting to a more reduced state during the 12 month incubation. In contrast, the sediment amended with 5 mM AQDS (>10 times the natural HS concentration at 11 m) did not show an increase in RI after 12 months, suggesting that all the added oxidized quinones probably could not be reduced before labile DOM was depleted.

Although sediment microcosm experiments performed here were useful to show Fe and DOM mobilization from sediments, it is important to recognize the limitations of incubation experiments (36). They are performed on a small, albeit homogenized, sediment sample collected from a very heterogeneous aquifer. Moreover, Radloff et al. (36) demonstrated that the inability to reproduce true field conditions such as groundwater flow and high sediment:water ratio can confound incubation experiments, particularly in relation to As mobilization. For example, the lack of flow means that only locally occurring processes were represented and that solutes from "upstream" along the flowpath were not replenished as they would be in the field. In both of our experiments, dissolved [As] decreased substantially, when under field conditions dissolved [As] at 11 and 14 m remain high. This may result from As sulfide precipitation, if sulfate reduction occurred in this reducing environment, or from an excess of Fe(III) minerals leading to secondary Fe mineral formation and As sequestration (7). Therefore, the removal of As from solution is a likely consequence of the no flow conditions and underscores the importance of reactions occurring along the flowpath for As mobilization.

DOM in Deep Groundwater. In the deepest and oldest groundwater at 40 and 52 m, the chemical quality of DOM is very similar to that of water at 8 m, with slightly lower [DOC] and SUVA, higher FI and AA-like fluorescence, and lower RI than water at 11 and 14 m. Whereas this combination of high FI and amino acid-like fluorescence in Araihaaz surface water and young groundwater at 8 m may reflect pollution by sewage (26), in the very old groundwater at 40 and 52 m these signatures may be due to contributions from senescent bacteria (26). The higher content of oxidized quinones at 40 and 52 m (Table S2 of the Supporting Information) under reducing conditions (low ORP; Figure 1c) suggests that the redox cascade may have been driven to a steady state by a limited supply of electron donor (labile DOM), possibly occurring well before the water reached the 40 m depth. Slightly lower dissolved [Fe] at 40 m (4 to 6 mg L⁻¹; Figure 1b) than at 11 and 14 m and even less at 52 m (approximately 3 mg L⁻¹) may be due to low Fe content at this depth or the presence of minerals from which Fe cannot be easily mobilized.

DOM and As Mobilization. The variation in DOM sources and [As] from surface water to shallow and deep groundwater in the GBD aquifer is strongly linked to the amount of labile DOM and redox state of quinone-like moieties (Figure 1). The significant relationship ($R^2 = 0.79$; $p < 0.01$; $n = 10$) between FI and amino acid-like fluorescence (Figure 2a) is consistent with the known association of amino acid-like fluorescence with microbial precursor material (26), which typically has a high FI (19). The shallow groundwater at 8 m had the lowest

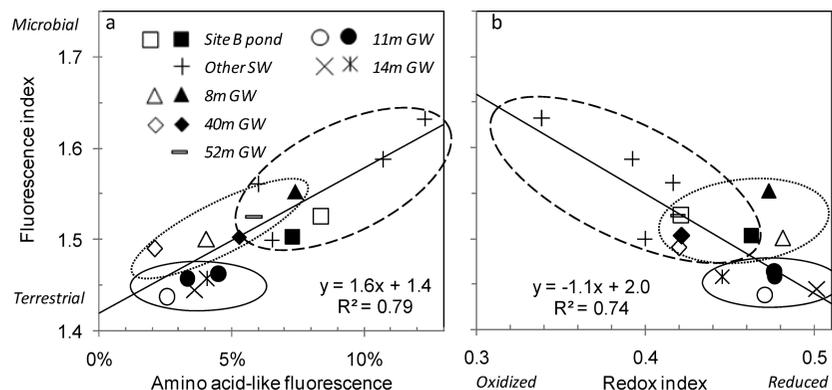


FIGURE 2. Relationships between fluorescence index and (a) amino acid-like fluorescence [sum of amounts of components C8 (tryptophan-like) and C13 (tyrosine-like)] and (b) redox index for representative surface water (SW) and groundwater (GW) samples. Trendlines and regression equations represent only samples collected in 2006, denoted by empty symbols, X, and +. All other symbols denote samples collected in 2008. Other surface water samples are listed in the Supporting Information. Larger circles group together the samples that contain high dissolved [As] and a nonlimiting supply of electron donor and acceptor (solid line), low-moderate dissolved [As] and limiting supply of electron donor and/or acceptor (dotted line), and low dissolved [As] and oxidizing surface water environment (dashed line). * $p < 0.01$.

[As] and the highest FI and AA-like fluorescence of all groundwater samples (Figures 1b, 2a). The low [As] may be explained by low sediment As content, potentially due to repeated flushing of As from the sediment at 8 m (23). It is also possible that secondary precipitation of iron phases during Fe reduction decreased As mobility (7). In contrast, at 11 and 14 m, the reactions occurring along the flowpath generated DOM with low FI and high SUVA values and water with high dissolved [Fe] and [As]. While Fe reductive dissolution likely represents the dominant reaction to solubilize Fe along the flowpath, As mobilization may be enhanced via complexation by the more aromatic DOM released from sediments during dissolution, resulting in high [As(III)] at 11 and 14 m (Figure 1a). The carboxyl, hydroxyl, and other functional groups of HS in DOM can form stable complexes with metals and strongly bind As anions, in particular As(III), which favors aqueous complexation of DOM when compared to As(V) ((3) and references therein). Along these lines, low [As] at 8, 40, and 52 m may also be attributed to the less aromatic and reactive character of DOM at those depths. HS have also been shown to abiotically reduce As(V) to the more mobile As(III) species (6), and this may further contribute to the high concentrations of dissolved As(III) observed at 11 and 14 m (Figure 1a).

The significant relationship ($R^2 = 0.74$; $p < 0.01$; $n = 10$) between FI and RI (Figure 2b) suggests that groundwater with a plant/soil fluorescence signature is likely to reflect the reducing conditions necessary for electron shuttling leading to Fe reduction. This pattern has been observed in groundwater of a recharge wetland, where dissolved [Fe] was highest under low FI, high RI, and low microbial quinone conditions (28). Moreover, the samples in this study were grouped on the basis of electron shuttling ability, represented by sufficient [DOC] (electron donor and shuttle), high RI, and high sediment Fe content (electron acceptor), and arsenic concentration (Figure 1a,b), with the highest dissolved [As] groundwater consistently displaying low FI, low % AA-like fluorescence, and high RI (Figure 2). These fluorescence properties, therefore, may be useful indicators of the type of DOM that is likely to participate in HS-enhanced Fe reduction and As mobilization.

Environmental Implications. Our data show that characteristics of DOM at a site in Bangladesh containing high and low groundwater As concentrations are distinct, with DOM from depth intervals with elevated groundwater [As] characterized by terrestrial DOM signatures, potentially derived from solubilization of sedimentary organic matter. These findings provide an alternative perspective for evaluating sources and transformations of DOM in Bangladesh aquifers. Direct evidence for the drawdown of labile DOM into aquifers by irrigation

pumping has eluded investigators (27, 37). To reconcile this, it has been suggested that the As mobilization rate may be highest in near surface oxic–anoxic transition zones where As and labile carbon are high, such that groundwater As can be largely traced to this near surface source (38). However, large amounts of labile DOM similar in chemical character to surface water DOM were not observed at 11 and 14 m, where dissolved [As] is highest. Instead, the terrestrial (plant/soil) quality of DOM at 11 and 14 m and the release of DOM in 11 m microcosm experiments support the previous notion that dispersed sedimentary organic matter such as peat adds labile substrate to fuel bacteria and maintain reducing conditions in the aquifer (2). What mechanism can reconcile the lack of labile DOM and the terrestrial quality of DOM at depths where [As] is highest? Our data suggests that electron shuttling by sediment-derived HS facilitates the microbial reduction of Fe, actively using labile DOM as a substrate, and may explain why there is not a large pool of labile DOM present at depths where dissolved [Fe] and [As] are high. However, the importance of alternative mechanisms of As mobilization such as DOM–As complexation and direct As reduction by HS relative to that of HS-enhanced electron shuttling and Fe reduction merits further study. Nevertheless, the fluorescent properties, FI, RI, and amino acid-like content may serve as indicators of the type of DOM present in reducing environments with strong tendencies for Fe reduction and As mobilization. Our study further underscores the need to better understand the biogeochemical reactions involving DOM, Fe, and As along groundwater flowpaths.

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Supporting Information Available

Methods details, description of ferric and ferrous iron effects on absorbance, and radiocarbon calculation for dissolved inorganic carbon resulting from DOM respiration; tables showing fluorescence properties of Arai-hazar surface water and groundwater, sediment microcosm results, and absorbance of ferric and ferrous iron; figures showing map of sampling sites, schematic of electron shuttling cascade, and fluorescence

excitation–emission matrix spectra of surface water and groundwater samples. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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