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Dissolved Organic Matter Quality in a Shallow Aquifer of Bangladesh: Implications for Arsenic Mobility

Natalie Mladenov,*,[†] Yan Zheng,[‡,§] Bailey Simone,[†] Theresa M. Bilinski,[‖] Diane M. McKnight,[†] Diana Nemergut,[†] Kathleen A. Radloff,[§] M. Moshiur Rahman,[‡,⊥] and Kazi Matin Ahmed[#]

[†]INSTAAR, University of Colorado, Boulder, Colorado 80309-0450, United States

[‡]School of Earth and Environmental Sciences, Queens College, City University of New York, Flushing, New York 11367, United States

[§]Lamont-Doherty Earth Observatory, Columbia University, Palisades, New York 10964, United States

[‖]School of Natural Sciences, St. Edward's University, 3001 S. Congress Ave, Austin, Texas 78704, United States

[⊥]Department of Environmental Science and Management, North South University, Bashundhara, Dhaka-1229, Bangladesh

[#]Department of Geology, University of Dhaka, Dhaka-1000, Bangladesh

S *Supporting Information*

ABSTRACT: In some high arsenic (As) groundwater systems, correlations are observed between dissolved organic matter (DOM) and As concentrations, but in other systems, such relationships are absent. The role of labile DOM as the main driver of microbial reductive dissolution is not sufficient to explain the variation in DOM−As relationships. Other processes that may also influence As mobility include complexation of As by dissolved humic substances, and competitive sorption and electron shuttling reactions mediated by humics. To evaluate such humic DOM influences, we characterized the optical properties of filtered surface water ($n = 10$) and groundwater ($n = 24$) samples spanning an age gradient in Araihazar, Bangladesh. Further, we analyzed fulvic acids (FA) isolated from large volume isolates ($n = 6$) for optical properties, C and N content, and ^{13}C NMR spectroscopic distribution. Old groundwater (>30 years old) contained primarily sediment-derived DOM and had significantly higher ($p < 0.001$) dissolved As concentration than groundwater that was younger than 5 years old. Younger groundwater had DOM spectroscopic signatures similar to surface water DOM and characteristic of a sewage pollution influence. Associations between dissolved As, iron (Fe), and FA concentration and fluorescence properties of isolated FA suggest that aromatic, terrestrially derived FAs promote As−Fe−FA complexation reactions that may enhance As mobility.
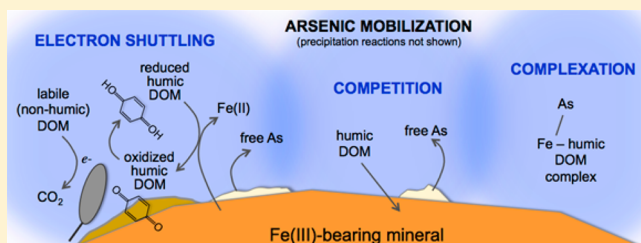
1. INTRODUCTION

In Bangladesh, it is estimated that ~45 million people are exposed to groundwater arsenic (As) concentrations that are above the World Health Organization guideline value of 10 μg L^{-1}.[1] Although it is generally agreed that microbially mediated reductive dissolution of As-bearing iron (Fe) minerals[2,3] is the dominant mechanism for enrichment of As in reducing groundwater in the Bengal Basin, the role of dissolved organic matter (DOM) remains enigmatic in part due to a lack of characterization of DOM composition. Compared to the well-recognized role of labile DOM fueling microbial reduction, demonstrated in several field studies of Bengal groundwater,[4−7] less is known about the role of humic DOM in As-laden groundwater. Although humic DOM is considered to be recalcitrant to biodegradation,[8] it is involved in both biological and chemical reactions that may influence the mobility of As.[9] For instance, microbial reduction of oxidized humic quinones has been shown to be an important step in the cascade that results in Fe reduction[10] and may consequently contribute to As mobilization. Quinone moieties in dissolved and solid-phase humic substances are able to shuttle electrons and accelerate microbial reduction of Fe[10−13] and other terminal electron acceptors and are able to regenerate and continue to serve as electron shuttles.[14]

In addition to the biological role for humic DOM, evidence for humic DOM influencing As mobility through abiotic mechanisms such as competitive sorption and complexation has emerged in laboratory studies. It was demonstrated that competition with As for sorption sites on hematite[15] and goethite[16] minerals resulted in As desorption. Humic DOM has also been shown to abiotically oxidize and reduce As from soils and sediments.[16,17] Of particular interest is the formation of complexes between humic substances, Fe, and As, which act to keep As in solution under reducing conditions. Direct complexation was initially suggested between dissolved humics

and arsenate[18,19] and arsenite.[20,21] More recently, Fe-bridging for ternary complex formation involving humic substances, Fe, either arsenate[22,23] or arsenite,[24] has been invoked as the mechanism responsible. Indeed, X-ray absorption spectroscopy results demonstrate that ternary As−Fe−humic DOM complexes form by inner-sphere binding of As(V) to Fe(III)-humic DOM complexes.[25]

Field observations also support that the quality of DOM exerts an influence on As mobility. DOM quality refers to the chemical characteristics of DOM that influence its biological reactivity such as how labile it is for microbial degradation and its chemical reactivity, which may be enhanced by reactive humic moieties and N- and S-containing functional groups. First, field observations of significant positive correlations,[26] significant negative correlations,[27] or no correlations at all[28] suggest that the role of DOM is more complex than previously thought due to the heterogeneous nature of DOM. Humic DOM, including dissolved humic and fulvic acids, participate in a potential suite of reactions, such as complexation, competition, and electron shuttling that likely also influence As mobility. Second, studies in the Bengal Basin have implicated the sediments including dispersed organic matter or peaty strata as a likely source of DOM in reducing groundwater.[29−32] Further, optical spectroscopic analyses of Bangladesh groundwater demonstrated that the DOM in groundwater with elevated As concentrations was largely derived from aromatic and lignaceous organic compounds already in the sediments.[33] Taken together, the role of the humic DOM pool in reducing aquifers of the Bengal Basin merits further exploration.

This study first elucidates the potential role that dissolved humic substances, namely, fulvic acids (FAs), the predominant humic fraction in the DOM pool of natural waters, play in As mobility through a characterization of DOM chemistry in groundwater of varying ages and As concentrations. Groundwater-dissolved fulvic acids have not previously been studied in As-rich reducing groundwater environments, although extracts of sediment organic compounds have recently been analyzed and provide some insight into sedimentary DOM character. In several regions with elevated groundwater As concentration, Reza et al.[34,35] performed absorbance and fluorescence analyses and ^{1}H- NMR, ^{13}C NMR, and Fourier transformed infrared spectral analyses of organic compounds extracted from sediments using an acid−alkanine method. These studies demonstrated that the extracted sedimentary organic compounds from the Meghna and Brahmaputra floodplains had higher aromaticity and fluorescence intensity than those from the Ganga floodplain and could be involved in complexation reactions with As. These studies of sedimentary organic compounds provide additional motivation for investigating the role of humic DOM in groundwater. Noting, however, that groundwater of the Bengal Basin likely has diverse DOM sources in addition to sedimentary organic matter, there is a need to evaluate the influence that both humic DOM, isolated directly from groundwater, as well as nonhumic DOM may have on As mobility.

Therefore, the goals of this study are to better characterize the DOM fractions and investigate the interactions between DOM fractions, As, and Fe in groundwater. Here, we combine FA isolation, elemental analysis, ^{13}C NMR spectroscopy, UV−vis absorbance spectroscopy, and fluorescence spectroscopy to characterize dissolved humic substances in groundwater of varying ages and varying dissolved As concentrations that represent the range of hydrogeological and biogeochemical conditions typical of the Holocene shallow aquifer of Bangladesh. The association between fulvic acid content, Fe, and As was also investigated to evaluate if the presence of humic DOM could help explain variability in DOC and As concentration correlations noted in previous field studies.

2. METHODS

Groundwater and surface water samples (Tables S1 and S2) were from Araihazar upazila, Bangladesh, located approximately 30 km northeast of the capital city Dhaka. It lies within the floodplain of the Old Brahmaputra River, an abandoned river channel that has been filled through recent sedimentation[36] and reduced to a small stream (Figure S1). The study area was chosen because the redox condition of the shallow Holocene aquifer, and in turn, groundwater As level and age, spans nearly the entire range found in Bangladesh.[37−41] The groundwater flow in the shallow aquifer is localized, with flow direction toward local rivers or streams except during the monsoon. The shallow aquifer comprises fine to medium sand sediments with less than 0.15% organic carbon, although the silty surficial sediment or dispersed clay lenses in aquifer displayed organic carbon concentration between 0.2% to 0.6%.[42,43] Only one peat layer containing 8.6% organic carbon has been identified, at nearby site A,[44] but there is no evidence of peat at Site K.

Groundwater samples were collected from monitoring wells installed at Site K described in Radloff[45] and Site B described in Zheng et al.[42] The two sites are approximately 2 km apart and are in a primarily rural setting with numerous ponds and irrigated rice and vegetable fields.

To isolate FA, a total of six large volume water samples (∼215 L) were collected in October 2009: one from a small stream next to the field where monitoring wells were installed (Figure S1), three from wells at Site K, and two from wells at site B. The three samples from Site K represented young groundwater with an ^{3}H/^{3}He age[45] of younger than 5 years old (K12 and K10) and old groundwater with an age of greater than 30 years old according to ^{3}H/^{3}He dating (K8). Two samples were also collected from Site B (B-11 and B-14) for which the groundwater was known to be about 19 years old[39] to ensure that the age range of 5−30 years was represented. The wells were pumped for 10 to 30 min, until readings of temperature, conductivity, pH,[45] and Eh[45] on multiprobes in a flow-through cell stabilized. Then, about 215 L of groundwater was collected into cubitainers and driven to the University of Dhaka, Bangladesh, where they were then acidified and filtered. Large volume filtered samples were shipped to the University of Colorado, where they were pumped through columns packed with cleaned XAD-8 resin.[46] After rinsing loaded columns with nanopure water, the FA was isolated by back-eluting with base and run through a cation exchange column. These "large volume FA isolates" were freeze-dried and analyzed for C and N content with an elemental analyzer and C functional group distribution with solid state ^{13}C-nuclear magnetic resonance (^{13}C NMR) spectroscopy. The surface water sample was collected and processed in the same way. Prior to isolation, an aliquot of the large volume isolates was reserved and analyzed for total dissolved As and Fe concentrations, DOC concentration, and UV−visible absorbance and fluorescence spectroscopy (see Supporting Information for details).

To provide the geochemical context for the FA isolate chemistry data, a total of 24 small volume groundwater samples were also collected from Site K, along with a total of 10 surface

Table 1. Characteristics of Small Volume Samples, Including Age, Depth, Solute Chemistry[b] and DOM Optical Spectroscopic Properties[c] Collected from Araihazar, Bangladesh, in March 2008[a]

	surface water $n = 10^d$	<5 year old groundwater $n = 10$	5−30 year old groundwater $n = 6$	>30 year old groundwater $n = 8$	t test results[e]
age (years)	−	1.9 ± 1.5	10.5 ± 5.0	34.7 ± 1.6	−
depth (m)	−	8.9 ± 2.2	11.1 ± 3.6	10.5 ± 3.2	G
As_T ($\mu g\ L^{-1}$)	24 ± 14	37 ± 29	158 ± 107	281 ± 112	B−F
% As(V)	66 ± 30	16 ± 33	1 ± 1	4 ± 4	G
Fe_T ($mg\ L^{-1}$)	0.11 ± 0.12	6.7 ± 5.3	10.3 ± 5.5	10.8 ± 2.0	E
SO_4 ($mg\ L^{-1}$)	19 ± 15	15 ± 23	4.4 ± 6.4	0.05 ± 0.07	C, E
Ca ($mg\ L^{-1}$)	38 ± 4.0	39 ± 25	41 ± 3.0	57 ± 17	C, E, F
Mn ($\mu g\ L^{-1}$)	149 ± 117	1885 ± 1054	2145 ± 1036	1252 ± 970	A−C
TDN ($mg\ L^{-1}$)	1.37 ± 1.11	0.62 ± 0.60	1.20 ± 0.62	1.76 ± 0.58	A, E
DOC ($mg\ L^{-1}$)	6.30 ± 2.98	2.78 ± 0.50	3.51 ± 0.38	3.65 ± 0.69	A−E
SUVA ($L\ mg^{-1}\ m^{-1}$)	1.97 ± 1.22	1.18 ± 0.64	1.89 ± 0.45	2.06 ± 0.48	D, E
FI	1.69 ± 0.24	1.47 ± 0.10	1.38 ± 0.04	1.40 ± 0.03	A−E
RI	0.35 ± 0.07	0.50 ± 0.04	0.51 ± 0.02	0.48 ± 0.02	A, B, C, F
AA-like (%)	0.084 ± 0.034	0.10 ± 0.07	0.05 ± 0.01	0.04 ± 0.01	B, C, E

[a]Means, standard deviations, and T-tests comparing young (< 5 yr), old (5−30 yr), and very old (> 30 yr) groundwater collected from site K are shown. [b]Total dissolved arsenic (As_T), % arsenic as As(V), total dissolved Fe (Fe_T), and dissolved organic carbon (DOC); n = sample size; dash = not measured or performed. [c]FI and RI are dimensionless; AA-like = amino acid-like fluorescence. [d]Sample size is six for As, %As(V), Fe, SO_4, Ca, and Mn values (Table S1). [e]t test results show significant difference between (A) surface water and <5 year old groundwater, (B) surface water and 5−30 year old groundwater, (C) surface water and >30 year old groundwater, (D) <5 year old and 5−30 year old groundwater, (E) <5 year old groundwater and >30 year old groundwater, and (F) 5−30 year old and >30 year old groundwater. G represents no statistical difference in any sample sets.

water samples representing the Old Brahmaputra River and local streams and ponds in Araihazar in March 2008 (Table S1). These "small volume" whole water samples were analyzed for anions and cations, dissolved As and Fe concentrations and speciation, DOC concentration, and UV−visible absorbance and fluorescence spectroscopy (see Supporting Information for details).

For both FA isolates and small volume water samples, key metrics from UV−vis absorbance and fluorescence spectroscopy were evaluated. Specific UV absorbance (SUVA)[47] was utilized to provide information about the aromaticity of whole waters, whereas for FAs the aromatic C content was measured directly with solid state ^{13}C NMR. Three dimensional fluorescence excitation emission matrices (EEMs) were acquired and instrument specific corrections, inner-filter correction, Raman normalization, and blank subtraction were applied. EEMs were fit to the parallel factor analysis (PARAFAC) model of Cory and McKnight,[48] and individual component loadings are presented in Raman units (RU) and as percentages of total fluorescence. The relative amount of amino acid-like fluorescence, taken as the sum of tyrosine-like and tryptophan-like PARAFAC components (C8 + C13 in Cory and McKnight[48]), which are described as the more biologically labile components in the fluorescence literature, was tracked in all samples. The fluorescence source index (FI) was also calculated[48] to provide information about the DOM sources in whole waters and FAs. The FI has provided consistent and reliable information on the sources of DOM and humic substances in natural waters that were consistent with characterization using other techniques. For example, Mladenov et al. isolated FAs from wetland surface water and found that spatial changes in the FA content, C:N ratios, C functional groups, and FI reflected an influx of plant-derived organic compounds during flooding.[49] The redox index (RI) has been used to track the presence of reduced quinone-like compounds in surface water and groundwater samples[49] and is the ratio of

reduced quinone-like PARAFAC components to total quinone-like PARAFAC components.[50]

A multivariate analysis of variance (MANOVA; vegan package, R project) was employed to test whether groundwater chemistry (As, Fe, sulfate, DOC, total dissolved nitrogen (TDN), SUVA, FI, and percent amino acid-like fluorescence) was significantly different among surface water ($n = 10$) and groundwater samples in the age groups of younger than 5 years ($n = 10$), 5−30 years ($n = 6$), and greater than 30 years old ($n = 8$). Additionally, an analysis of variance (ANOVA) was also used to determine significance in As concentration difference between data sets using a two-tailed t test (R project).

3. RESULTS AND DISCUSSION

Surface water DOM and FA Isolate. Surface water samples (Table S1), consisting of the old Brahmaputra River (OBR) channel and local streams and ponds near Site K, displayed high average DOC concentrations, greater than groundwater of any age (Table 1). Because small volume water samples were obtained in March when the groundwater tables are substantially higher than the surface water tables,[41] the river and stream waters are primarily groundwater fed, with the water chemistry subject to "redox trapping" during groundwater discharge.[51,52] "Redox trapping" means that redox-sensitive elements such as As, Fe, and Mn have a tendency to be removed from solution and "trapped" in sediments under oxidizing conditions, and therefore, their concentrations are lower than when reducing conditions prevail. The low surface water concentrations of As, Fe, and Mn compared to those in old groundwater and similar concentrations of Ca, which is not redox sensitive, in surface water and groundwater are consistent with this discharge process (Table 1). Additionally, because the surface water environment is oxic, sulfate concentrations in surface water are higher than in old groundwater. Similarly, young groundwater, which is influenced by oxic surface water inputs, is known to have substantial variability in concentrations of these redox-sensitive elements (As, Fe, and Mn; Table 1).[53]
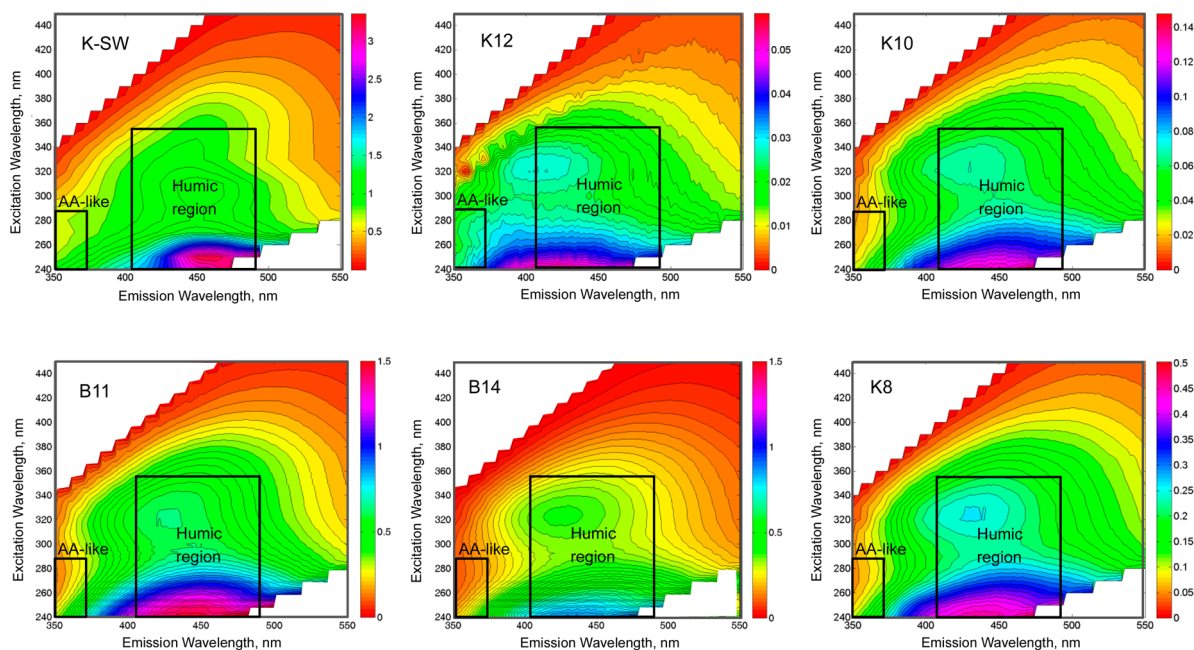
Figure 1. EEMs of surface water (K-SW) and Site K and Site B groundwater whole waters collected for fulvic acid isolation (samples described in Table 2). Fluorescence intensities (z-axis) are in Raman units (RU). Regions of amino acid-like fluorescence (proxy for labile DOM) and humic-like fluorescence are marked.

The low RI of surface water DOM (Table 1) also reflects this oxic state of surface water and the presence of more oxidized quinone-like moieties[50] than in groundwater. Because surface water DOC concentrations are two to three times higher than those in old groundwater, additional allochthonous inputs from the surrounding landscape[33] and autochthonous biological inputs (e.g., refs 53 and 54) contribute to the high DOC concentrations. The FI value is highest in surface water samples (Table 1), indicating a microbial source, which would support autochthonous inputs such as bacteria and algae. This is not surprising considering the surface water quality of rural Bangladesh, where wastewater is frequently disposed of directly into ponds and other surface water bodies.[54] The EEMs of the small volume surface water samples showed a peak in the region of amino acid-like fluorescence near excitation/emission of 275/350 nm (Figure 1) and provide further evidence of a microbial DOM source in this water type. However, there is also substantial humic fluorescence in the Peaks A and C regions (Figure 1 K-SW), which reflects contributions from soil and terrestrial plant sources to the DOM pool in surface water. Thus, the DOM of surface water is characterized by protein-like as well as humic DOM.

Although we have only one fulvic acid isolate of a local streamwater (K-SW) sample, the characteristics of this surface water FA isolate are illustrative. The DOC of this surface water sample comprised greater than 40%, fulvi acid, which was greater than the FA fraction of all other large volume groundwater samples (Table 2). Further, the combination of high SUVA, reflecting terrestrial DOM contributions, and high FI and amino acid-like fluorescence, reflecting autochthonous microbial contributions, would also suggest that, like the small volume water sample, this fulvic acid was derived from a combination of microbial constituents as well as soil and vascular plant sources in the area surrounding the river.

Groundwater DOM and FA Isolates. The extensive spatial survey of groundwater from different wells and depths showed that there were significant differences in DOC concentration as well as in the quality of DOM among groundwater of different ages. Differences in DOC concentration, SUVA, FI, and %AA-like fluorescence of small volume samples were significant between young, younger than 5 year old groundwater, and older groundwater (Table 1). All groundwater samples have comparable RI values consistent with reducing conditions indicated by elevated levels of Fe and Mn concentrations in groundwater compared to surface water (Table 1). Further, the results showing significantly ($p <$ 0.00001) higher concentrations of As, Fe, Ca, and TDN, mostly as ammonia (Table S2) and lower sulfate concentrations in older groundwater than in young groundwater (Table 1) are consistent with progressively greater reducing conditions as groundwater ages and subsequent occurrence of mineral weathering (calcite dissolution) and redox reactions.[39,44] Additionally, significant differences, determined with MANOVA ($R^2 = 0.38$; $p < 0.0001$), were also observed for DOC, SUVA, FI, and percent amino acid-like fluorescence between groups of groundwater. The lower FI, lower AA-like fluorescence, and higher SUVA in the two groups of older groundwater samples suggest inputs of "terrestrial" sources, such as lignaceous and aromatic C compounds, common in dispersed sedimentary organic matter. One explanation for the more terrestrial DOM signatures in the older groundwater is that DOM is mobilized from sediments as a consequence of microbial Fe reduction and Fe mineral dissolution. This scenario would be consistent with other studies showing that sedimentary organic matter contributes substantially to the groundwater DOM pool.[55,56] DOM mobilization from sediments is supported by the results of sediment incubations from our earlier study conducted also at Araihazar, which showed an increase in DOC and Fe concentrations and DOM terrestrial fluorescence signatures after incubation with native groundwater.[33]

Table 2. Characteristics of Six Large Volume Whole Waters, Including Site Description, Solute Chemistry (total dissolved arsenic (As_T), % Arsenic as As(III), Total Dissolved Fe (Fe_T), Dissolved Organic Carbon (DOC), and Fulvic Acid (FA) content and concentration), and DOM Optical Spectroscopic Properties (specific UV absorbance (SUVA), Fluorescence index (FI), and Amino Acid-like (AA-like) fluorescence), Collected from Site K and Site B Groundwater and Site K Surface Water (K-SW) in October 2009

	site description				solute chemistry						optical spectroscopic properties		
sample	latitude (degrees)	longitude (degrees)	depth (m)	Age[a] (yrs)	As_T (μg L^{-1})	%As (III)	Fe_T (mg L^{-1})	DOC[b] (mg C L^{-1})	FA content (% of DOC)	FA conc. (mg FA L^{-1})	SUVA[c] (L mg^{-1} m^{-1})	FI	AA-like (% of fluorescence)
K-SW	23.7916	90.6101	0	N.A.	23.3	40	0.01	4.80	42.7	4.63	2.66	1.65	5.7
K12.1	23.7948	90.6284	7.50	<5	2.0	90	0.5	0.59	2.80	0.04	1.60	1.57	24
K10.2	23.7941	90.6281	11.0	<5	69	100	3.5	0.92	8.30	0.26	1.72	1.46	8.0
K8.3	23.7932	90.6280	14.8	>30	363	94	11.0	0.93	25.0	1.28	2.24	1.49	4.0
B11	23.780	90.640	11.4	5–30	213	95	9.6	2.08	13.3	0.54	2.52	1.57	3.3
B14	23.780	90.640	14.0	5–30	340	95	10.2	2.51	34.4	1.70	2.73	1.57	4.1

[a]Water age category based on 3H/3He dating reported in Stute et al.[39] and Radloff.[45] [b]DOC concentrations are lower in large volume whole waters collected in October 2009 post-monsoon than in small volume samples collected in March 2008 during dry season (Table S1). [c]High SUVA values (>5.0) may indicate additional Fe absorbance in the UV-range (Weishaar et al.).[47]

Similarly, the higher fulvic acid content of large volume isolates B11, B14, and K8, representative of older groundwater (>5 years old), compared to the younger K10 and K12 groundwater (Table 2) indicates that the older groundwater has higher amounts of dissolved humic substances, most likely originating from organic material in the sediments. Also, the FAs isolated from large volume isolates of older groundwater had higher aromatic C concentration, lower FI values, and lower amino acid-like fluorescence than FA isolates of young groundwater (Table 3), consistent with the trends observed in the small volume samples that point to a terrestrial, sedimentary DOM source. The lower amino acid-like fluorescence was also evident in EEM spectra of the large volume isolates of old groundwater (Figure 1), which were instead dominated by fulvic acid-like fluorescence.

By contrast, several lines of evidence suggest that DOM in younger groundwater (<5 years old) is influenced by microbial sources. The average FI value of small volume samples of young groundwater is similar to surface water, and the average value of %AA-like fluorescence is the highest among all water types (Table 1), likely reflecting the influence of sewage-polluted surface water on young groundwater. This influence was identified at the same study site[57] in young groundwater, which has been shown to contain high counts of the fecal indicator bacterium *E. coli*.[58] The lowest average SUVA value in young groundwater suggests that the young groundwater is the least influenced by terrestrially derived DOM among all water types (Table 1). Similarly the large volume isolates of young groundwater from sites K10 and K12 had low fulvic acid content (Table 2), and the FA isolated from those sites had higher FI and amino acid-like fluorescence and lower %C (Table 3) than older groundwater.

Nonhumic DOM. In addition to humic DOM in Araihazar groundwater, nonhumic DOM is present in both young and old groundwater. Although nonhumic fractions were not measured directly, the influence of nonhumic DOM can be observed in the optical spectroscopic properties of whole water samples in this study. For the five large volume isolates described above, fluorescence properties were measured of the initial whole water, prior to isolation. The whole water samples comprise multiple fractions of organic matter, including fulvic acids as well as nonhumic and potentially more labile compounds, such as amino acids, carbohydrates, low molecular weight organic acids, bases, and neutrals. The FI and percent amino acid-like fluorescence of whole water samples prior to FA isolation (Table S1) were higher than for the FA isolates (Figure 2) because the nonhumic fraction was still present in those whole water samples. The nonhumic fraction, therefore, reflects contributions to the DOM pool from microbial and other nonhumic DOM sources. FI values of whole waters are known to be higher when compared to FA isolates, in part, because of the presence of other fluorescent microbial compounds, such as extracellular quinone-containing exudates, that would act to raise the FI.[59] These microbial sources may include microbial exudates or microbial products from lysed microbial cells, which may derive from sewage-influenced surface water drawn laterally[60] or to depth or from the sediments themselves. Other microbial consortia, including bacterial taxa capable of Fe, As, and humics reduction, which are key members of the community in Araihazar sediments,[43] may also contribute to the amino acid-like fluorescence, which is known to represent biodegradable DOM,[61] that was observed in the whole water DOM. Therefore, this nonhumic fraction is also potentially

Table 3. Elemental Content, ^{13}C-NMR Distribution of C Functional Groups of Fulvic Acid Isolates, and Optical Spectroscopic Characteristics of Fulvic Acids Isolated from Site K and Site B Groundwater and Site K Surface Water (K-SW)

sample	elemental content			^{13}C NMR distribution (%) and C functional group distribution[a]							optical spectroscopic characteristics		
	C (%)	N (%)	C:N ratio	aliphatic (60–0)	heteroaliphatic (90–60)	acetal (110–90)	aromatic (165–110)	carboxyl (190–165)	carbonyl (220–190)	Ar:Al ratio	SUVA (L mg^{-1} m^{-1})	FI	AA-like fluorescence (%)
K-SW	44.3	2.9	15.28	48	15.62	0.01	19.43	13.61	3.27	0.40	4.36	1.69	11.0
K12.1	45.2	1.41	32.1	46.1	8.57	1.92	24.7	16.1	2.58	0.54	3.86	1.48	4.9
K10.2	28.8	1.30	22.2	63.2	11.4	0.12	14.1	10.6	0.59	0.22	5.16	1.45	4.1
K8.3	15.4	0.77	19.9	47.1	5.76	3.34	30.2	11.9	1.75	0.64	4.71	1.40	3.7
B11	51.3	1.57	32.7	53.1	9.30	0.56	19.6	16.0	1.47	0.37	3.96	1.43	3.5
B14	50.6	1.50	33.7	45.3	10.4	2.98	25.8	14.1	1.50	0.57	4.15	1.41	3.3

[a]Parentheses list range of wavelengths for each functional group (nm).

labile and important for setting in motion reductive dissolution of Fe minerals.

Humic DOM and As Mobility. Having illustrated the differences between two pools of groundwater DOM, humic and nonhumic, we now attempt to shed light on the variable relationship, either positive, negative or a lack thereof, between groundwater As and DOC concentrations observed in several field studies.[26−28] There was no significant correlation between whole water DOC concentration and As in this set of shallow groundwater samples from Bangladesh (Figure S2) and only a weak correlation for the five large volume isolates (Figure 2). Instead, the FA concentration of groundwater had significant positive relationships with As and Fe (Figure 2). We acknowledge that the sample size is small given the extensive sampling and preparation time needed for FA isolation (see Supporting Information); however, FA isolation is a highly representative technique.[46] Moreover, the change in DOM quality supports the transition of DOM source from nonhumic, microbially derived material in young water to humic, terrestrially derived material as the groundwater ages. Although FA content would be expected to increase as a result of microbial degradation of labile fractions that may leave behind more recalcitrant components, the FI of such microbially derived FAs is typically higher (>1.50).[48,62] Instead the isolates have a lower FI (~1.40 to 1.43 (Figure 2), which suggests that the original source of fulvic acids in older groundwater is probably not labile microbial organic matter transported from other zones but rather sedimentary material from the local aquifer.

We interpret the correlation between the chemical characteristics (aromaticity, FI, % amino acid-like fluorescence) of FAs and both As and Fe (Figure 2) as indicating the involvement of humic DOM in reactions that maintain As in solution. Prior laboratory and field studies suggest a likely mechanism through formation of As−Fe−DOM complexes.[22−24] Although humic DOM, As, and Fe may all be present as free molecules as a consequence of reductive dissolution, the stoichiometric relationship showing proportionately greater humic DOM content in water with greater dissolved As or Fe concentrations (Figure 2) implies potential complex formation among these species. Indeed the aromatic carbon content of humic substances has been shown to have a strong positive correlation with Fe(III).[63] Multiple laboratory studies have also shown that As is able to form ternary complexes with Fe and DOM.[25] Using Pahokee Peat Humic Acid as their DOM source, Sharma et al.[23] demonstrated that at neutral pH As(V) formed colloids and complexes with Fe-DOM but not with DOM in the absence of Fe. Formation of complexes between As and DOM in the presence of metals but not in their absence was also demonstrated for DOM extract from compost.[64] Fulvic acids readily complex Fe at neutral pH, and As can bind strongly to such complexes via bridging mechanisms[24] to stay in solution. Therefore, it is not unexpected that humic DOM correlates well with As in the presence of Fe in the neutral pH groundwater of our study. Using a molecular weight of 470 Da for groundwater FA from a similar environment,[65] we calculated that molar Fe:As:FA ratios were about 47:1.3:1 (see slopes of linear regressions in Figure 2). The average molar ratios of Fe to As for large volume isolates from groundwater younger than 5 years old ranged from 40 to 68 in our study, which is slightly higher than the 20 to 55 range reported in laboratory studies of As−Fe−DOM complexes under controlled conditions.[23,24]
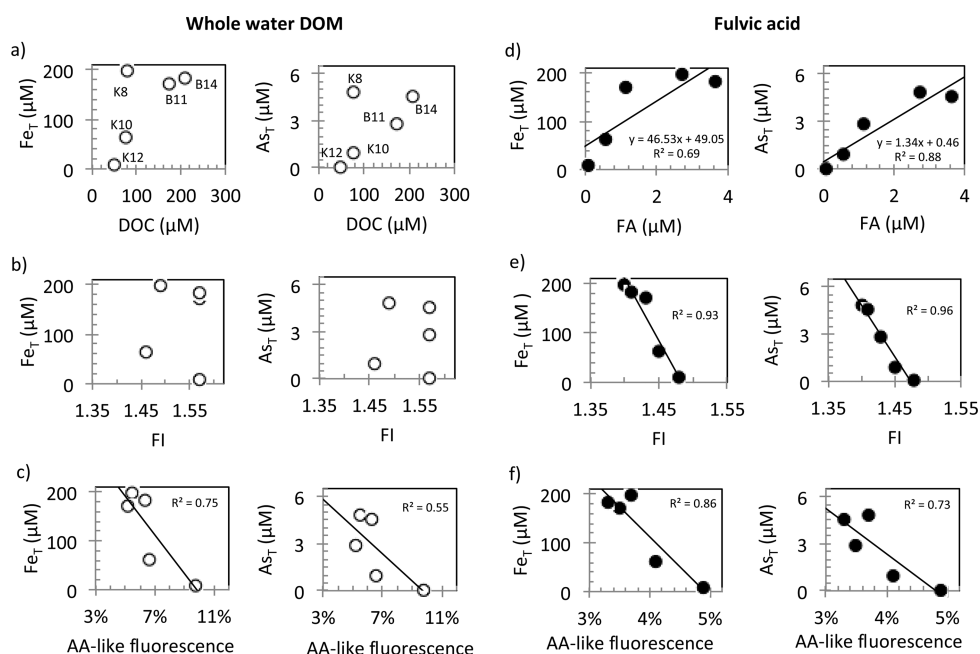
Figure 2. Scatterplots of total dissolved As and Fe vs (a) DOC concentration, (b) fluorescence index (FI), and (c) amino acid-like (AA-like) fluorescence of large volume whole water samples prior to isolation at Site B and Site K. (d) Fulvic acid concentration of large volume samples. (e) Fluorescence index and (f) amino acid-like fluorescence of fulvic acids isolated from the large volume samples at Site B and Site K (sample labels shown in panel a). Regression lines shown only for significant relationships ($p < 0.05$).

Organic matter that passed through the approximately 0.9 μm Ballston filter, which may consist of colloids and small particles, also appears to have undergone associations with As and Fe. Prior to isolation of the FAs in this study, we recovered orange-colored organic matter on the filters after filtering the large volume water samples that were acidified to pH 2 to prevent Fe precipitation. This orange-colored retentate (material retained on filters) for three samples, K12, K8, and B11, was redissolved in 0.1 N HCl, and its FA fraction was also isolated.[46] The FA content of the filter retentates was substantially higher (FA of 18%, 28%, and 37% for samples K12, K8 and B11, respectively) than in the corresponding large volume isolates (Table 2), suggesting that organic matter in larger molecules was more humic than DOM in groundwater. The FAs of filter retentates also displayed the most terrestrially derived signatures with the much lower mean FI values (FI values of 1.24, 1.23, and 1.29 for samples K12, K8 and B11, respectively) than dissolved FA isolates of groundwater from the same three sites (Table 3; mean FI = 1.54).

Therefore, the influence of colloidal and particulate organic matter and Fe on As mobility also merits further study. Ultrafiltration of As- and DOM-rich groundwater from Hetao Plain, Inner Mongolia has demonstrated stronger association between As and smaller organic colloids than with larger Fe colloids.[66] Additionally, SEM images, EDS analysis, and synchrotron XRF analyses in their study confirmed the association of As with natural organic matter (NOM) with molecular weights of 5−10 kDa.

Implications for Groundwater As Enrichment. Deltaic regions with elevated As concentrations in reducing groundwater tend to be rich in sedimentary organic compounds derived from the deposition of plant and animal biomass detrital compounds over time. In Bengal Deltaic sediments, Meharg et al. postulated that As and organic matter were co-deposited during the Holocene,[67] especially in productive coastal wetland ecosystems that developed at the time. Similar co-deposition of As and organic matter may be true for other deltaic or depositional environments with elevated As in groundwater, such as the Red River Delta, Mississippi River Delta, Okavango Delta, and Pearl River Delta; however, previous studies have focused more on relationships with total organic matter content of sediments or DOC of whole water. Our results suggest that the relationships between As, Fe, and humic DOM that may influence As mobility in reducing groundwater environments deserve further exploration. With respect to the linear relationships between As, DOM, and Fe observed in our study, it is possible that As—DOM−Fe complexes were sequestered in the sediments simultaneously during deposition, thousands of years ago. Co-deposition of organic matter and As with Fe-containing minerals, as proposed by Meharg et al.,[67] and later ternary complex formation after reductive dissolution is a plausible scenario to explain why groundwater with elevated As contains more terrestrially derived, humic DOM than groundwater with low As concentrations. Regardless of whether the association with As is via dissolved complex or colloidal complex, our results suggest that the humic fraction of DOM, in particular, undergoes reactions with As. Differentiating the humic, chemically reactive, from the nonhumic, biologically reactive, fraction of DOM in reducing groundwater of deltaic aquifers is a first step to better elucidating that role.

It is also important to note that the labile, biologically reactive nature of DOM in young groundwater at our study site should stimulate microorganisms capable of Fe reductive dissolution leading to As mobilization. However, instead of finding high As concentration in young groundwater with labile DOM, our results showed that As concentration was significantly higher in old groundwater, which contained higher fulvic acid content, DOC concentrations, and terrestrially or sediment-derived DOM sources, compared to young ground-

water. These results indicate that the nature of the As—Fe—DOM relationship is more complex than the simple picture of labile DOM as a driver for As mobilization. The involvement of humic DOM in reactions with As and/or Fe, such as complexation, competitive sorption, or electron shuttling, may help explain why As concentrations are elevated in old groundwater. Field research and experimental studies directly investigating the role of humic DOM for As mobilization under environmentally relevant conditions are needed.

In addition to its potential influence on As mobility in reducing aquifers, the chemical reactivity of humic DOM influences treatment and remediation strategies used for dealing with high As concentrations in drinking water. For example, the presence of colloidal As—Fe—DOM associations may hamper filtration efforts,[68] and competition with humic or fulvic acids inhibits several treatment processes, such as As sorption on Fe minerals,[69] As(V) adsorption during coagulation with ferric chloride,[70] and As sorption on Fe nanoparticles.[71]

Despite the important role of organic matter in As cycling and the clear value of elucidating its chemical structure to understand and predict biogeochemical reactions, much still remains to be understood regarding the quality of DOM found in groundwater of different age and As concentrations. This study provides new information on the role of DOM fractions as being both biologically and chemically reactive, with the humic DOM acting to maintain As in solution in reducing groundwater. In particular, we have shown that DOM in older groundwater (>5 years old) is characterized by higher FA content, more terrestrial, sedimentary sources, and less microbial spectroscopic signatures than DOM in younger groundwater or surface water. The highly significant relationships we observed between As and Fe concentrations and FA content, the FI, and amino acid-like fluorescence of FA isolates further suggest that humic As—Fe—DOM interactions may have occurred long ago during the precipitation of As-containing Fe minerals or recently as complexation reactions after free As was mobilized from sediments. The presence of humic DOM in groundwater and the heterogeneous distribution of dispersed organic matter in sediments may, therefore, be additional factors contributing to the large spatial variability in As concentrations over short distances. In addition to improving our understanding of the heterogeneity of elevated groundwater As across the landscape, accounting for the quality of DOM in groundwater of different ages is important to the development remediation and treatment strategies.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

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Detailed methods, supporting tables, and supporting figures (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: nmladenov@sdsu.edu. Phone: (619) 594-0725. Fax: (619) 594-6005.

Present Addresses

Natalie Mladenov: Department of Civil, Construction, and Environmental Engineering, San Diego State University, San Diego, California 92182, United States.

Diana Nemergut: Department of Biology, Duke University, Durham, North Carolina 27708, United States.
Kathleen A. Radloff: Gradient, Cambridge, Massachusetts 02130, United States.

Notes

The authors declare no competing financial interest.

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