Contrasting dissolved organic matter quality in groundwater in Holocene and Pleistocene aquifers and implications for influencing arsenic mobility

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Abstract

The discontinuous nature of elevated arsenic (As) in drinking water wells of West Bengal and other regions in the Bengal Basin has led to increased interest in the role that sediment-derived organic matter may play in enhancing reductive dissolution and AS mobilization. Higher As concentrations have been observed in groundwater in reduced Holocene (grey) aquifers when compared to oxidized Pleistocene (orange) aquifers. In order to evaluate if differences in the chemical character of dissolved organic matter (DOM) are present in groundwater in the Holocene and the Pleistocene aquifers that may influence dissolved As concentrations, shallow groundwater and surface water samples were collected from four study sites in Murshidabad district, West Bengal, India, and analyzed for water chemistry parameters and characteristics of DOM. For wells known to typically contain high As concentrations (in Holocene sediments) in Beldanga (10 – 4622 µg/L, at 35-45 m depth) and Hariharpara (5 – 695 µg/L, at 6-37, depth) sites, as well as wells characterized by low As concentrations (Pleistocene sediments) in Nabagram (0 – 16 µg/L, at 20-45 m depth) and Kandi (5 – 50 µg/L, at 20-55 m depth), detailed

DOM characterization was carried out using fluorescence spectroscopy and parallel factor analysis (PARAFAC). Results from statistical analysis of a variety of optical (absorbance and fluorescence) DOM properties revealed that the DOM in groundwater in the Holocene aquifer had high humification index (HIX) and low freshness index ($\beta:\alpha$) values, whereas groundwater in the Pleistocene aquifer comprised more labile and microbial DOM sources. Consistent with the more labile nature of DOM in groundwater in the Pleistocene aquifer, two ratios 1) humic-like to protein-like components (humic:protein) and 2) terrestrially-derived to microbially-derived components (terr:microb) obtained from a four-component PARAFAC model were 1.9 and 2.9 times greater, respectively, in groundwater in the Holocene aquifer than that in Pleistocene aquifer, which suggests that the absence of humic-like DOM may be an important limitation to As mobility.

Keywords

Dissolved organic matter characterization; Fluorescence spectroscopy; Parallel Factor Analysis (PARAFAC); Groundwater arsenic.

1. Introduction

Approximately 43 million people in West Bengal and 22 million in Bangladesh have been exposed to arsenic (As) concentrations greater than the World Health Organization guideline for drinking water (10 $\mu\text{g/L}$) (*Sankar et al., 2014; Datta et al., 2011; Bhattacharya et al., 1997; Nickson et al., 1998; Smith et al., 2000; McArthur et al., 2001; Dowling et al., 2002; Roychowdhury et al., 2002; Ravenscroft et al., 2005; Acharyya and Shah, 2006; Datta et al., 2009*). Microbially-mediated reductive dissolution of iron oxyhydroxide minerals has been

invoked as one of the prominent mechanisms for the mobilization of sediment-bound As into reducing groundwater (McArthur *et al.*, 2001; Dowling *et al.*, 2002; Hasan *et al.*, 2007; Sankar *et al.*, 2014). The driver of this mechanism is labile dissolved organic matter (DOM). It has been hypothesized that labile DOM is drawn to depth from ponds and other surface waters laden with sewage (Harvey *et al.*, 2006; Neumann *et al.*, 2010), however, dispersed sedimentary organic matter has also been implicated as a source (McArthur *et al.*, 2004; Sengupta *et al.*, 2008; Datta *et al.*, 2011, Neumann *et al.*, 2014).

Recently, additional roles have been proposed for DOM in aquifers in the Bengal Basin that may have important consequences for iron and As mobilization (Wang *et al.*, 2006; Mladenov *et al.*, 2010). For instance, humic substances, a biologically refractory class of DOM, are involved in complexation reactions with Fe and As (Liu *et al.*, 2011; Sharma, 2010), competition with As for sorption sites (Bauer and Blodau, 2006), and electron shuttling reactions (Lovley *et al.*, 1996, 1998 and 1999; Scott *et al.*, 1998; Klapper *et al.*, 2002; Kappler *et al.*, 2004; Jiang *et al.*, 2008; Wolf *et al.*, 2009; Mladenov *et al.*, 2010, 2015). Therefore, DOM of both labile and recalcitrant quality may enhance the process of As release by several mechanisms, including by serving as an electron donor (i.e. as direct source of energy) and by more chemically reactive mechanisms involving humic substances (Mladenov *et al.*, 2015).

Characterization of biologically labile as well as humic and chemically reactive fractions of DOM provides insights into the sources of DOM and, therefore, its potential influence on As mobility. Absorbance and fluorescence spectroscopy techniques have opened a new window for the characterization of DOM (Coble, 1996) and have proven to be sensitive, reliable and rapid techniques for identifying DOM sources and transformations. For example, absorbance over the ultraviolet range of wavelengths provides a quantitative measure of source and molecular weight

1 of DOM (*Helms et al., 2008*), while indices obtained from three dimensional fluorescence
2 analysis provide insight into DOM source, age, freshness, and degree of processing in the
3 environment. In addition, PARAFAC modeling of excitation-emission matrix (EEM) data allows
4 quantification of humic-like, protein-like, terrestrially-derived and microbially-derived
5 fluorescent components of DOM (*McKnight et al., 2001; Cory and McKnight, 2005; Mladenov*
6 *et al., 2011; Mladenov et al., 2015*).

7 Shallow groundwater (< 40 m deep), the main source of drinking water for most
8 communities within the Bengal Basin, is largely free of pathogenic bacteria. However, it has a
9 wide range of As concentrations and its DOM sources may comprise both labile microbially-
10 derived DOM transported to depth and terrestrially-derived DOM originating from dispersed
11 sedimentary materials, subsurface peat deposits, or other lignaceous organic matter sources. In
12 an investigation of the characteristics of the DOM in groundwater in the Holocene aquifer near
13 Araihasar, Bangladesh, *Mladenov et al. 2010* found through incubation experiments that, over
14 time, sediments released DOM with terrestrially-derived fluorescence properties. Results
15 obtained from fluorescence spectroscopy of groundwater samples in the same aquifer found
16 higher amino acid-like fluorescence in samples from young (~1.6 years), low As wells, whereas
17 samples from 11 – 14 m depth with As concentration ~550 µg/l and age of ~ 19 years had lower
18 amino acid-like fluorescence and more lignaceous dissolved organic matter (*Mladenov et al.,*
19 *2010*). In groundwater samples in 12,300 – 48,500 years old (*Acharyya et al., 2000*) Pleistocene
20 aquifers, more oxidized organic matter and microbial signatures were reported (*Sutton et al.,*
21 *2009*).

22 The geochemistry of water and sediments in the Pleistocene aquifer in the region has also
23 been studied, and the Pleistocene aquifer is known to contain much lower dissolved As

1 concentrations than the Holocene aquifer as a result of more strongly oxidizing conditions that
2 occurred when the sediments were deposited (*Fendorf et al., 2010*). Anaerobic incubation
3 experiments with Pleistocene sediments, lactate as carbon source and metal reducing bacteria
4 *Shewanella*, showed that phosphorous-extractable As increased even without addition of lactate
5 (*Dhar et al., 2011*). Therefore, arsenic mobilization from the Pleistocene sediments may not have
6 been caused by labile DOM. Much still remains to be understood about the similarities or
7 differences in groundwater chemistry, particularly of the organic geochemistry that may
8 influence As mobilization in Pleistocene and Holocene aquifers.

9 To our knowledge, the chemical and optical properties of DOM in groundwater in the
10 Pleistocene aquifer have not yet been extensively studied. Therefore, this study evaluates the
11 optical spectroscopic characteristics of DOM in groundwater in both the Pleistocene and
12 Holocene aquifers in wells that are high and low in As. A unique feature of this study is that the
13 sampling sites in Holocene and Pleistocene aquifers are located at similar depths, in close
14 proximity to each other, on either side of a hydrologic divide that separates the two geologic
15 formations, and are thus subject to similar environmental and population pressures. Dissolved
16 organic carbon (DOC) concentrations and UV-visible absorbance and fluorescence spectroscopy
17 were employed to characterize the freshness and humification of groundwater DOM. This study
18 further employs parallel factor analysis (PARAFAC) multivariate analysis modeling of
19 fluorescence spectra from samples collected exclusively from the Bengal Basin. This first
20 PARAFAC model of Bengal Basin groundwater fluorescence quantifies the relative amounts of
21 protein-like and humic-like DOM components in groundwater in the Pleistocene and Holocene
22 aquifers with different As concentrations.

2. Materials and Methods

2.1 Study site

To characterize DOM in groundwater in the Holocene aquifer, 32 wells were sampled covering a wide spatial extent ($\sim 623 \text{ km}^2$) in the Beldanga and Hariharpara blocks located to the east of River Bhagirathi (Figure S1) in which the geology is characterized by ~ 7000 years before present younger Holocene sediments (*Acharyya et al., 2000, Biwas and Roy, Mukherjee et al., 2007*). These sites to the east of the river are known to have a large proportion of high dissolved As wells (*Datta et al., 2011; Sankar et al., 2014*). To characterize DOM in groundwater in the Pleistocene areas, 23 wells covering a wide spatial extent ($\sim 544 \text{ km}^2$) in the Nabagram and Kandi blocks, located to the West of River Bhagirathi and characterized by 12,300 – 48,600 years before present (*Acharyya et al., 2000*) Pleistocene sediments, were sampled. These sites are known to have wells generally low in dissolved As (*Datta et al., 2011; Moran et al., 2000*). Lithological evidence suggests the presence of a clay layer at 100 m depth below the study area on both sides of the river (*Mukherjee et al., 2007*). Description of sediment type, total As, total iron and organic matter in sediments at all study sites is given in Table 1 (*Sankar, 2013*). In Beldanga, the maximum As concentration in older (Holocene) silty clay was 18.09 mg/kg, the total Fe concentration varied between 14.72 – 32.97 g/kg, and organic matter content was between 57.7 – 171.4 mg/kg at 15 m depth. In Hariharpara, the maximum As concentration was 15.54 mg/kg in older (Holocene) dark grey sticky clay while the concentration of total Fe was 26.22 – 31.73 g/kg and organic matter was 55.85 – 61.15 mg/kg at 6 m depth. Characterization of sedimentary organic matter in similar geological setting in Nadia district, West Bengal (*Rowland et al., 2006*) revealed the abundance of apolar compounds dominated by high molecular weight n-alkanes with small amounts of hopanes and steranes. Components of less abundant polar compounds were primarily the n-alkanols and small amounts of sterols. Rowland

et al. (2006) also suggested that a significant fraction of organic matter was presumably recalcitrant macromolecular material, which would be less available as a labile carbon source (electron donor) for microbial activities. In Nabagram, the highest sediment As concentration was 10.42 mg/kg with total Fe of 12.9 – 25.61 g/kg and organic matter content of 62.78 – 74.64 mg/kg at 3 m depth in reddish (Pleistocene) grey colored clay. In Kandi, the maximum sediment As was 15.67 mg/kg with total Fe of 25.39 – 36.12 g/kg and organic matter of 118.9 – 122.4 mg/kg at 12.2 m depth in reddish (Pleistocene) grey colored clay (*Sankar, 2013*).

The characteristics of sedimentary organic matter in Pleistocene age sediments have been reported in the literature. Ghosh et al. (2015) (a study conducted in Pleistocene brown sand aquifer of Nadia, West Bengal, a site just to the south of our study area) reported that the organic matter deposited during the Pleistocene was mainly from terrestrial, higher plant origin indicated by high molecular weight (HMW) n-alkanes and unsaturated alkanolic acids. However, low molecular weight (LMW) n-alkanes and n-alkanoic acids were also present and believed to be derived from microbial cells (*Ghosh et al., 2015*). Presence of terrestrially derived as well as microbially derived organic matter in Pleistocene sediments at other locations has also been reported by several other studies such as in aquifers of Red River Delta, Vietnam (*Lawati et al., 2012; Eiche et al., 2016*), in eastern Netherlands (*Hartog et al., 2004*) and Bangladesh (*Hossain et al. 2009*).

Mukherjee et al. (2007) reported the transmissivity of 3300 – 7000 m²/day, permeability of 42 m/day for sand and 0.01 m/day for clay and porosity of 0.2 for the Holocene aquifer. Generally the older (Pleistocene, typically yellow-reddish) sediments have a lower permeability than recent (Holocene, typically grey) sediments (*Mukherjee et al., 2007*). Ravenscroft et al. (2005) had reported the permeability of Pleistocene sands to be 20-30 m/day while that for

Holocene sands to be 40-60 m/day for Pleistocene and Holocene sands with the same medium grain size. The lower permeability of Pleistocene sands was thus attributed to the presence of secondary clays and iron oxides that partially clog the pore throats (*Ravenscroft et al., 2005*). The same study also reported that Pleistocene clays were thicker and more consolidated than Holocene aquitards, resulting in lower vertical permeability and lower specific yield in Pleistocene aquifer systems (*Ravenscroft et al., 2005*). Groundwater pumping for domestic and irrigation purposes was estimated as $3.32\text{E-}04$, $8.84\text{E-}05$ and $1.33\text{E-}04$ $\text{m}^3/\text{m}^2/\text{day}$ during pre-monsoon, monsoon and post-monsoon seasons respectively. (*Mukherjee et al., 2007*).

2.2 Sample collection and storage

For representative sampling of surface water and groundwater, samples were collected from 11 surface ponds (PW), 37 shallow depth tube wells (TW) (10 - 40 m), 3 deep tube wells (TW) (>40 m) and 11 irrigation wells (IW) (10-46 m). A total of 43 samples from the Holocene area (27 TW, 5 IW and 11 PW) and 29 from the Pleistocene area (20 TW, 3 IW and 6 PW) were analyzed. Samples were filtered through a $0.7\text{ }\mu\text{m}$ nominal pore size pre-combusted glass fiber filter in the field and stored at 4°C until analysis. To avoid quenching effects from lowering pH, samples were not acidified either in field or in lab prior to fluorescence analysis.

2.3 Spectroscopic Analyses

UV-visible absorbance and fluorescence spectroscopic techniques have been used to study DOM in a wide range of environments from marine waters (*Kalle, 1949; Duursma and Marchand, 1974*) to groundwater (*Mladenov et al, 2010, 2013, 2015*). Spectroscopic data was acquired using Jobin Yvon Aqualog Fluorometer. A filtered water sample was excited with range of wavelengths from 240 nm to 450 nm with 3 nm increments and integration time of 0.25 seconds. From absorbance data, absorption coefficient “a” was calculated as, $a = 2.303 \cdot A/L$,

1 where A is the absorbance and L is the path length (0.01 m). Absorbance at 254 nm (abs_{254}) has
2 been used to understand characteristics of organic molecules as the molecules with specific
3 binding arrangements such as the conjugated systems in aromatic compounds exhibit greatest
4 absorptivity at 254 nm (*Weishaar et al., 2013*). Log-transformed absorbance coefficients were
5 used to calculate spectral slope between 275 nm to 295 nm ($S_{275-295}$) and between 350 nm to 400
6 nm ($S_{350-400}$). The former has been shown to be an indicator of molecular weight and degree of
7 photobleaching while the latter represents chromophoric DOM (CDOM) and contributions from
8 terrestrially-derived DOM (*Helms et al., 2008*). The spectral slope ratio (S_R) was calculated by
9 taking the ratio of $S_{275-295}$ and $S_{350-400}$. A $S_R > 1$ indicates marine-like DOM and $S_R < 1$ is
10 characteristics of terrestrially dominated high CDOM samples (*Helms et al., 2008*). An increase
11 in S_R values has been attributed to reduced molecular weight of DOM and photobleaching while
12 microbial activities such as microbial production or preservation of long wavelength absorbing
13 substances have been observed to decrease S_R (*Helms et al., 2008; Moran et al., 2000; Vahatalo*
14 *and Wetzel, 2004*).

15 Emission spectra were collected in signal to reference (S:R) mode from 212 nm to 619
16 nm with 3.28 nm increment (instrument default). EEMs were corrected for the inner filter effect
17 (*Ohno, 2002*), Raman normalized (using 18.3 M Ω cm Milli-Q ultra-pure water at ~350 nm), and
18 blank subtracted. First and second order Rayleigh scattering bands were excised (*Stedmon and*
19 *Bro, 2008*). A quinine sulfate standard was also run, giving a value of 1 R.U. = 2.495 quinine
20 sulfate units (QSU). The fluorescence Index (FI) was calculated as the ratio of fluorescence
21 intensities at 470 nm and 520 nm emission and 370 nm excitation. Lower values of FI (~1.3-1.4)
22 indicate terrestrial sources of DOM while higher values (~1.7-1.9) indicate microbially-derived
23 DOM (*McKnight et al., 2001; Cory and McKnight, 2005*). The freshness index ($\beta:\alpha$) was

calculated as the ratio of emission intensity at 380 nm to the maximum intensity between 420 nm to 435 nm at an excitation wavelength of 310 nm (*Parlanti et al., 2000*). More recently derived DOM is represented by β , while α represents highly decomposed DOM (*Wilson and Xenopoulos, 2008; Fellman et al., 2010*). The humification index (HIX) was calculated as the ratio of peak area under the emission spectra at 435 nm to 480 nm to peak area from 300 nm to 345 nm at an excitation wavelength of 254 nm (*Zsolnay, 2003*). Higher values of the HIX indicate a higher degree of DOM humification (*Ohno, 2002*). All the corrections, data processing and calculations of indices were performed using MATLAB R2014a (Figure S3 and Figure S4). Corrected three dimensional fluorescence excitation emission matrix (EEM) data was fitted to a four component PARAFAC model (*Harshman, 1970; Stedmon et al., 2003; Stedmon and Bro, 2008*). Statistical analyses were performed for descriptive statistics parameters. Since the As concentrations were not normally distributed, a non-parametric Mann – Whitney – Wilcoxon (MWW) U test was used to compare the statistically significant variability among samples.

2.4 PARAFAC modeling

The DOM Fluor toolbox (*Stedmon and Bro, 2008*) was used to build a PARAFAC model for this sample dataset. The sample set included corrected EEMs from surface and groundwater with varying fluorescence intensities. In order to avoid false identification of extremely high or low concentration samples as outliers by PARAFAC, these EEMs were normalized using a modified method of Murphy et al. (2013). In brief, each intensity value in the matrix was divided by the maximum intensity of that sample instead of dividing by the sum of squares of all intensities in that sample. This presets all the EEMs to a maximum intensity of 1, according to:

$$I(i,j)_{corrected} = \frac{I(i,j)_{initial}}{I_{max}}.$$

Outliers were removed from the PARAFAC model dataset if they met one of these conditions: negative spectral slope ratios, very high FI (> 2) that corresponded to a FI peak emission location > 470 nm, and, $\beta:\alpha$ values >1 indicating shift of peak in the 310 nm emission spectra. The dataset was then fitted to a non-negativity constrained PARAFAC model according to Stedmon et al. (2003). Additional outliers were identified by calculating the leverage of each sample and wavelength. Samples with leverages approaching 1 were examined to ensure that there was no analytical error before being considered as outliers (Stedmon and Bro, 2008). After removing outliers, a total of 60 samples were used. A four-component model was validated by split half analysis (Figure 3) and random initialization techniques by running ten, four component models. For all the samples, the residual (difference between actual and modeled EEM) did not exceed 10% of the intensity in the original EEM (Cawley et al., 2012), which indicates an acceptable model fit (Stedmon and Bro, 2008). After complete validation, the actual intensities of each component were obtained by multiplying the intensity of each component by the maximum intensity (I_{max}) of the original EEM used in the normalization. Two indices based on PARAFAC components were developed to provide further insights into the nature of DOM. The humic:protein index was calculated as the ratio of the sum of humic-like DOM components to the sum of protein-like DOM components obtained from the PARAFAC model, according to:

$$humic:protein = \frac{\sum Humic - like DOM Components}{\sum Protein - like DOM Components}.$$

The terrestrial:microbial (terr:microb) index was calculated as the ratio of the sum of terrestrially-derived to microbially-derived DOM components obtained from the PARAFAC model, according to:

$$\text{terr: microb} = \frac{\sum \text{Terrestrially Derived DOM Components}}{\sum \text{Microbially Derived DOM Components}}.$$

3. Results

3.1 Comparison of chemical and DOM properties in groundwater in the Holocene and the Pleistocene aquifers

Significantly higher dissolved As concentrations in the groundwater in Holocene aquifer than that in Pleistocene ($p < 0.05$, Table 3) were observed, where concentrations in the former ranged from 5 µg/L to 1264 µg/L (average = 261 µg/L), and those in the latter ranged from 0.4 µg/L to 50 µg/L (average = 14.5 µg/L) (Table 2). More than 70% of the groundwater samples in the Holocene aquifer contained As concentrations > 50 µg/L, which is the Indian drinking water standard (*Datta et al., 2011; IS 10500, 2012*). All of the groundwater samples in the Pleistocene aquifer contained As concentrations < 50 µg/L. Groundwater collected from the Holocene aquifer had higher average dissolved Fe concentrations (average = 3.19 mg/L), and was more reducing (average = -44.3 mV) than in Pleistocene sites. DOC concentrations were also significantly higher ($p < 0.05$) in groundwater in the Holocene aquifer (average = 2.94 mg/L) than in the Pleistocene aquifer (average = 1.79 mg/L) (Table 2).

Similar to the trends noted for DOC concentration, a_{254} values (Table 2) were significantly higher ($p < 0.05$; Table 3) in groundwater in the Holocene aquifer (average = 0.047 a.u.) compared to that in the Pleistocene aquifer (average = 0.034 a.u.). However, $SUVA_{254}$ values did not show any statistically significant difference ($p > 0.05$; Table 3) between groundwater in the Holocene (average = 2.26 L.mg⁻¹.m⁻¹) and Pleistocene (average = 2.35 L.mg⁻¹.m⁻¹) aquifer. S_R values (Table 2) were significantly lower ($p < 0.05$; Table 3) in groundwater in

the Holocene aquifer (average = 1.33) than that in the Pleistocene aquifer (average = 1.78). Groundwater in the Pleistocene aquifer had notably higher intensities 1.65 Raman Units (R.U.) in the protein-like region (PARAFAC component C3), at 1.65 Raman Units (R.U.), compared to that in the Holocene aquifer, at, 0.38 R.U. (Figure 1a and 1b). The FI (Table 2) was similar for groundwater in both the Pleistocene (mean of 1.73) and Holocene (mean of 1.72) aquifers, and at the high end of the typical FI range (1.2 – 1.8). The $\beta:\alpha$ was statistically lower ($p < 0.05$; Table 3) in groundwater in the Holocene aquifer (average = 0.77) than that in the Pleistocene aquifer (average = 1.0; Table 2). The HIX was significantly higher ($p < 0.05$; Table 3) in groundwater in the Holocene aquifer (average = 6.79) than that in the Pleistocene (average = 3.34).

3.2 Comparison of groundwater and surface water DOM properties

In both the high As (Holocene sediments) and low As (Pleistocene sediments) locations in our study area, DOC concentrations and optical spectroscopic properties related to DOC concentration (abs_{254}) were significantly different between groundwater and surface water samples (Tables 2 and 3). Overall, the EEMs of surface water samples had distinct fluorescence signatures when compared to groundwater samples, with very pronounced peaks in the protein-like region and elevated total fluorescence intensity (Figure 1c). Indeed, all fluorescence and PARAFAC parameters were significantly different in surface water and groundwater samples (Table 3). However, in the low As (Pleistocene sediments) areas, the optical properties of DOM in groundwater samples were statistically similar to surface water samples in most properties other than Abs_{254} , S_R , $\beta:\alpha$, HIX (Table 3).

3.3 PARAFAC model components

The PARAFAC model identified four components (Figure 2), which were similar to components identified in other studies (Table 4). C1, C2 and C4 had excitation and emission

wavelength maxima consistent with humic-like components, whereas C3 was identified as a protein-like component, with a peak similar to that of tryptophan. C4 has been identified in other studies as a microbial humic-like peak, and C1 and C2 were found to be more commonly derived from terrestrial organic material (Table 3). Therefore C1 and C2 are considered to be the terrestrial components, and C3 and C4 are taken as the microbial components in this study. The humic:protein ratio showed a good correlation ($R^2 = 0.72$, Figure S2) with the ratio of intensities of traditional fluorescence peaks Peak B and Peak T to the intensities of Peak A, C and M described in Coble et al., 1998.

The HIX, humic:protein ratio, and terr:microb ratio showed distinct differences between the fluorescent components in groundwater in the Holocene and Pleistocene aquifers (Tables 1 and 2). The average percentages of two of the microbial components, C3 and C4 were statistically lower ($p < 0.05$; Table 3) in groundwater in the Holocene aquifer (at 11.8% and 8.53%, respectively) than in groundwater in the Pleistocene aquifer (at 18.2% and 28.3%, respectively; Table 3). As a result, the terr:microb ratio (average = 4.27) of groundwater in the Holocene aquifer was significantly higher than in groundwater in the Pleistocene aquifer (average = 1.44; Table 2). Similarly, the humic:protein ratio was found to be 1.8 times higher in groundwater in the Holocene aquifer (average = 8.53) than in the Pleistocene aquifer (average = 4.71). Surface waters from the Hariharpara, Beldanga (underlying Holocene aquifer) and Nabagram, Kandi (underlying Pleistocene aquifer) were not statistically different with respect to all the parameters analyzed except for the terr:microb ratio, which was significantly lower (average = 2.23) in Hariharpara and Beldanga areas compared to the Nabagram and Kandi areas (average = 3.15) and $\beta:\alpha$, which was significantly higher (average = 0.85) in Hariharpara and Beldanga areas compared to the Nabagram and Kandi areas (average = 0.76) (Table 2). Groundwater samples

were statistically different from surface water samples with respect to all parameters except S_R for the Holocene sites (Table 3). In Pleistocene areas, groundwater samples were statistically different from surface water samples in S_R , $\beta:\alpha$ (average 0.99 and 0.77), HIX (average 3.34 and 6.79), humic:protein (average 4.71 and 8.53) and terr:microb (average 1.44 and 4.27) respectively (Table 3).

4. Discussion

The difference in As concentration in groundwater in Holocene and Pleistocene aquifers is well documented, with Holocene aquifers generally having higher dissolved As concentrations than Pleistocene aquifers (*Fendorf et al., 2010*). Even though Pleistocene sediments can contain substantial amounts of As, dissolved As concentrations were reported as insignificant in groundwater of the aquifer (*Eiche et al., 2008*). It has been suggested that the lower As concentrations result from Pleistocene aquifers experiencing more flushing than Holocene aquifers due to their older age of deposition (*Morgan and McIntire, 1959*). However, Ravenscroft et al. (2005) instead showed that flushing of Pleistocene sediments is limited because of their lower permeability than Holocene sands due to clogging by secondary clays and iron oxides. Even for sediments with low As content, Hering and Kneebone (2002) found that generally very low concentrations of sedimentary As (1.8 mg/kg) are sufficient to produce dissolved As concentration exceeding $10 \mu\text{gL}^{-1}$. Sedimentary As found in all the sites in this study including those in the Pleistocene aquifer is $> 1.8 \text{ mg/kg}$ (Table 1), and sediment Fe_T and organic matter content are also present in both Holocene as well as Pleistocene aquifers in significant amounts (Table 1). Therefore, the limitation for As mobilization is more complicated, and consideration of microbial processing, redox state, and DOM chemical quality merit further investigation.

4.1 DOM characteristics in groundwaters in the Holocene and Pleistocene aquifer

Previous studies have shown that DOC concentrations tend to be substantially higher in groundwater of the Holocene aquifer than in groundwater of the Pleistocene aquifer (*Sutton et al., 2009; Datta et al., 2011; Sankar et al., 2014*). The lower DOC concentrations in groundwater of Pleistocene than Holocene aquifers may limit the release of As from those sediments (*Fendorf et al., 2010; Berg et al., 2008; Postma et al., 2007; Polizzotto et al., 2008; Harvey et al., 2002*). Similarly, our study found lower DOC concentrations in groundwater in Pleistocene sediments. Harvey et al. (2002) and Saunders et al. (2008) both showed that an increase in dissolved As concentration occurs upon injection of labile carbon into the aquifer. However, even without labile carbon addition, DOC concentrations can increase under reducing conditions. Mladenov et al. (2010) found that DOC increased from 4.84 to 6.10 mgL⁻¹ in sediment incubations with native groundwater in Bangladesh due to the release of DOM from the sediment. Neumann et al. (2014) also performed incubations with sediments in Vietnam high in As and found those sediments to release DOM that was easily biodegradable.

Our analyses shed light on the spectroscopic properties of CDOM and suggest that differences in the chemical character of CDOM in these two geologically distinct settings may have an influence on As release. Despite high FI values in both settings that reflect high amounts of microbial activity, several independent measures of DOM character indicate that DOM in groundwater in the Pleistocene aquifer was fresher, less biologically processed and less humified, than DOM in groundwater in the Holocene aquifer. First, the high S_R (~1.78) in groundwater in the Pleistocene aquifer was similar to values in systems with low-molecular-weight microbial contributions to the DOM pool (*Helms et al., 2008*). Second, PARAFAC modeling identified components that been previously reported in a variety of aquatic

environments (Table 4). The ratios of terr:microb and humic:protein PARAFAC components in groundwater in the Pleistocene aquifer were $\sim 1/2$ and $1/3$, respectively, of those in groundwater in the Holocene aquifer, indicating that CDOM in groundwater in the Pleistocene aquifer was more microbially-derived and less humic than in the Holocene aquifer.

The burial history and lack of microbial communities may help explain why these older sediments contain such seemingly young DOM. Paleo-sedimentary studies indicate that the Pleistocene sediments in the study area are floodplain deposits of the early Ganges-Brahmaputra river system (*Hasan et al., 2007; Stollenwerk et al., 2007; Acharyya et al., 2000*). In other river systems as well, such as in the Gulf coast Pleistocene sediments in the United States, the Pleistocene sediments have been reported to be early river flood plain deposits (*Morgan and McIntire, 1959; Robert, 1997; Blum and Roberts, 2012*). The quantity of glacial ice on the continents caused fluctuation in the sea level during the Pleistocene time. Sea level dropped abruptly with each glacial advance causing an increase in the discharge of rivers into the sea. This caused rapid erosion, weathering and oxidation of the river sediments. At the end of the interglacial period, sea level rose with the waning of the glaciers, which lowered the flow gradient of rivers and filled the valleys with alluvial sediments (*Morgan and McIntire, 1959*). The highly weathered and oxidized Pleistocene sediments were then buried under recent alluvial deposition (*Morgan and McIntire, 1959*). The buried Pleistocene sediments have been reported to contain 0.13-0.17% TOC by weight in sand and 0.2-0.67% by weight in clay and comprise organic matter mainly derived from both terrestrial vegetation and microbial constituents (*Ghosh et al., 2015*). These chemical characteristics are consistent with our findings of low HIX, terr:microb and humic:protein ratios, and high FI, S_R and $\beta:\alpha$ values, which are indicative of less-processed microbial DOM, in groundwater that is in contact with these Pleistocene sediments.

Dissolved organic matter with such characteristics should be biologically labile and theoretically be able to drive microbial reduction of Fe and As in the sediments if reducing conditions predominate, Fe-reducing microorganisms are present, and other terminal electron acceptors (oxygen and nitrate) are depleted. Although reducing conditions have not explicitly been investigated at our sites, Sankar et al., (2014) did measure dissolved oxygen, nitrate, nitrite and sulfate, and did not find significant differences between the concentrations of these electron acceptors in the groundwater of the Holocene (high As) and Pleistocene (low As) aquifers. Along the same lines, an emerging area of research has shown that biologically-labile soil organic matter can persist over long periods of time if conditions, such as physical disconnection between organic matter and microbial communities and energy or nutrient limitation, prevail (*Schmidt et al., 2012*). At least one study has investigated microbial communities in the Pleistocene sediments. Lawati et al. (2012) demonstrated that the Pleistocene sediments contain a very low abundance of microbial population, which they concluded could be responsible for lower As concentrations in the groundwater. This microbial limitation may explain why the organic matter in the groundwater seems, from our absorbance and fluorescence analyses, to be relatively fresh and unprocessed by microorganisms. However, the high FI values and low terr:microb ratios we measured in groundwater of the Pleistocene aquifer suggest that microbial constituents should be elevated rather than lacking in this setting. To gain more clarity on the presence and role of microbes in DOM processing and to better understand why the cascade of reductive dissolution is not underway in the Pleistocene aquifer, the composition of the microbial consortia and other factors that influence Fe reductive dissolution must be further explored.

In contrast to the labile character of CDOM in groundwater of the Pleistocene aquifer, groundwater of the Holocene aquifer was found to have CDOM with optical properties similar to

humic substances. Based on previous studies showing release of Fe, humic DOM (*Mladenov et al., 2010*), and biodegradable DOM (*Neumann et al., 2015*) from sediments during incubations with native groundwater, we expect that sediments are a likely source of DOM in groundwater of the Holocene aquifer. A potential scenario, proposed by Mladenov et al. (2015), is that humic DOM, containing quinone-like moieties known to shuttle electrons to accelerate Fe reduction (*Lovley et al. 1996, 1998 and 1999*), could be enhancing the same reaction in groundwater leading to associated mobilization of As from sediments into groundwater.

The lower $\beta:\alpha$ and S_R values (Table 2), similar to those found in wetlands and swamps with large amounts of high molecular weight DOM (*Helms et al., 2008*), for groundwater in the Holocene aquifer further indicate more biologically-processed DOM (*Fellman et al., 2010*). Higher HIX values, representative of greater humification of DOM (*Zsolnay, 2003; Ohno, 2002*), also point to a less labile DOM source that may instead participate in humic DOM-stimulated reductive dissolution. In addition, DOM with this more chemically-reactive character participates in other reactions that may lead to As mobilization, such as complexation and competitive sorption, which have been described in other studies (*Wang and Mulligan, 2006; Mikutta and Kretzschmar, 2011*). The observation that CDOM in groundwater of the Pleistocene aquifer lacks the humic character found in CDOM in groundwater of the Holocene aquifer may reflect both the unprocessed nature of the DOM and an absence of humic moieties that could serve important functions related to As mobilization, as described above.

4.2 Comparisons of DOM chemical character in surface water and groundwater

Surface water samples from Hariharpara and Beldanga contained younger (higher $\beta:\alpha$) and more microbial (lower terr:microb ratio) DOM than those in Nabagram and Kandi. Characteristics of DOM differed significantly between groundwater and surface water.

Spectroscopic properties suggested that humic and more biologically-processed DOM was found in this order: groundwater in Holocene > surface water > groundwater in Pleistocene. The higher values of $\beta:\alpha$ and lower HIX in surface water in Hariharpara and Beldanga, than underlying groundwater in the Holocene aquifer may be attributed to mixing of groundwater and surface water, which has been known to contain more fresh, microbially-derived and less humified organic matter from sewage and other wastewater inputs (*Knappett et al., 2011; Mladenov et al., 2015*) and algae (*Parlanti et al., 2000*). In contrast, the surface water samples from Nabagram and Kandi were less fresh (lower $\beta:\alpha$) and more humified (higher HIX) than the underlying groundwater in the Pleistocene aquifer, thus eliminating the possibility of surface water mixing.

5. Conclusions

The factors affecting the variability in groundwater As concentration in Holocene as well as Pleistocene aquifers are still in question and are relevant for an improved understanding of As mobility throughout the Bengal Basin. The quality of DOM in these two geological settings in the context of As mobility has not been studied. In this study, for Pleistocene sediments located in close proximity to Holocene sediments, multiple lines of evidence, including S_R , HIX and $\beta:\alpha$ values and humic:protein and terr:microb ratios of PARAFAC components, indicate that the CDOM in groundwater in the Pleistocene aquifer contains a higher proportion of protein-like and microbially-derived constituents and may be less humified and less biologically-processed than DOM in groundwater in the Holocene aquifer, which had elevated As concentrations. The absence of humic DOM may indicate that microbial degradation of DOM did not proceed in the Pleistocene aquifer as it had in the Holocene. The more proteinaceous and less humic DOM in groundwater of the Pleistocene aquifer also would be less likely to participate in reactions, such as complexation, electron shuttling, and competition with As for sorption sites. The absence of

humic-like DOM from groundwater in Pleistocene aquifer may, therefore, be an important limitation to As mobility.

On the other hand, our spectroscopic analyses indicate that the predominance of humic-like DOM in groundwater in the Holocene aquifer may result from mobilization of humic substances from the sediments as well as greater microbial processing of DOM. This more humic DOM may promote chemical reactions that mobilize or maintain As in solution, such as DOM-As binary or DOM-Fe-As ternary complex formation and humic DOM serving as an electron shuttle to accelerate the process of reductive dissolution. Therefore, the results of this study are also relevant to the understanding of As mobilization in Holocene aquifers.

6. Acknowledgements

We acknowledge field assistance by several students from the Department of Geology at Kansas State University. Funding was provided to Natalie Mladenov by the National Science Foundation Engineering Directorate CBET Division Award #1449247, and NSF Hydrologic Sciences grants EAR-1014971 and EAR-1014946 to Datta and Johannesson, respectively.

8. References

- Acharyya, S.K., Lahiri, S., Raymahashay, B.C., Bhowmik, A., 2000. Arsenic toxicity of groundwater in parts of the Bengal basin in India and Bangladesh: The role of Quaternary stratigraphy and Holocene sea-level fluctuation. *Environ. Geol.* 39, 1127–1137.
doi:10.1007/s002540000107
- Acharyya, S.K., Shah, B. a., 2007. Arsenic-contaminated groundwater from parts of Damodar fan-delta and west of Bhagirathi River, West Bengal, India: Influence of fluvial geomorphology and Quaternary morphostratigraphy. *Environ. Geol.* 52, 489–501.
doi:10.1007/s00254-006-0482-z
- Al Lawati, W.M., Jean, J.S., Kulp, T.R., Lee, M.K., Polya, D.A., Liu, C.C., Van Dongen, B.E., 2013. Characterisation of organic matter associated with groundwater arsenic in reducing aquifers of southwestern Taiwan. *J. Hazard. Mater.* 262, 970–979.
doi:10.1016/j.jhazmat.2012.08.007
- Al Lawati, W.M., Rizoulis, A., Eiche, E., Boothman, C., Polya, D. a., Lloyd, J.R., Berg, M., Vasquez-Aguilar, P., van Dongen, B.E., 2012. Characterisation of organic matter and microbial communities in contrasting arsenic-rich Holocene and arsenic-poor Pleistocene aquifers, Red River Delta, Vietnam. *Appl. Geochemistry* 27, 315–325.
doi:10.1016/j.apgeochem.2011.09.030
- Bauer, M., Blodau, C., 2006. Mobilization of arsenic by dissolved organic matter from iron oxides, soils and sediments. *Sci. Total Environ.* 354, 179–90.
doi:10.1016/j.scitotenv.2005.01.027
- Berg, M., Trang, P.T.K., Stengel, C., Buschmann, J., Viet, P.H., Van Dan, N., Giger, W., Stüben, D., 2008. Hydrological and sedimentary controls leading to arsenic contamination of groundwater in the Hanoi area, Vietnam: The impact of iron-arsenic ratios, peat, river bank deposits, and excessive groundwater abstraction. *Chem. Geol.* 249, 91–112.
doi:10.1016/j.chemgeo.2007.12.007
- Bhattacharya, P., Chatterjee, D., Jacks, G., 1997. Occurrence of Arsenic-contaminated Groundwater in Alluvial Aquifers from Delta Plains, Eastern India: Options for Safe Drinking Water Supply. *Int. J. Water Resour. Dev.* 13, 79–92.
doi:10.1080/07900629749944

- 1 Blum, M.D., Roberts, H.H., 2012. The Mississippi Delta Region: Past, Present, and Future.
2 Annu. Rev. Earth Planet. Sci. 40, 655–683. doi:10.1146/annurev-earth-042711-105248
- 3 Cawley, K.M., Wolski, P., Mladenov, N., Jaffé, R., 2012. Dissolved organic matter
4 biogeochemistry along a transect of the okavango delta, botswana. Wetlands 32, 475–486.
5 doi:10.1007/s13157-012-0281-0
- 6 Coble, P.G., 1996. Characterization of marine and terrestrial DOM in seawater using excitation-
7 emission matrix spectroscopy. Mar. Chem. 51, 325–346. doi:10.1016/0304-4203(95)00062-
8 3
- 9 Coble, P.G., Del Castillo, C.E., Avril, B., 1998. Distribution and optical properties of CDOM in
10 the Arabian Sea during the 1995 Southwest Monsoon. Deep. Res. Part II Top. Stud.
11 Oceanogr. 45, 2195–2223. doi:10.1016/S0967-0645(98)00068-X
- 12 Coble, P.G., Green, S.A., Blough, N. V, Gagosian, R.B., 1990. Characterization of dissolved
13 organic matter in the Black Sea by fluorescence spectroscopy. Nature 348, 432–435.
- 14 Cory, R.M., McKnight, D.M., 2005. Fluorescence spectroscopy reveals ubiquitous presence of
15 oxidized and reduced quinones in dissolved organic matter. Environ. Sci. Technol. 39,
16 8142–9.
- 17 Datta, S., Mailloux, B., Jung, H.-B., Hoque, M. a, Stute, M., Ahmed, K.M., Zheng, Y., 2009.
18 Redox trapping of arsenic during groundwater discharge in sediments from the Meghna
19 riverbank in Bangladesh. Proc. Natl. Acad. Sci. U. S. A. 106, 16930–5.
20 doi:10.1073/pnas.0908168106
- 21 Datta, S., Neal, A.W., Mohajerin, T.J., Ocheltree, T., Rosenheim, B.E., White, C.D.,
22 Johannesson, K.H., 2011. Perennial ponds are not an important source of water or dissolved
23 organic matter to groundwaters with high arsenic concentrations in West Bengal, India.
24 Geophys. Res. Lett. 38, 1–5. doi:10.1029/2011GL049301
- 25 Dhar, R.K., Zheng, Y., Saltikov, C.W., Radloff, K. a., Mailloux, B.J., Ahmed, K.M., Van Geen,
26 A., 2011. Microbes enhance mobility of arsenic in pleistocene aquifer sand from
27 Bangladesh. Environ. Sci. Technol. 45, 2648–2654. doi:10.1021/es1022015
- 28 Dowling, C.B., 2002. Geochemical study of arsenic release mechanisms in the Bengal Basin
29 groundwater. Water Resour. Res. 38, 1–18. doi:10.1029/2001WR000968
- 30 Duursma, Egbert K., and M.M., Duursma, E.K., Marchand., M., 1974. Aspects of organic marine
31 pollution. Oceanogr. Mar. Biol. An Annu. Rev. 12, 315–431.
- 32 Eiche, E., Berg, M., Hönig, S.-M., Neumann, T., Lan, V.M., Pham, T.K.T., Pham, H.V., 2016.
33 Origin and availability of organic matter leading to arsenic mobilisation in aquifers of the
34 Red River Delta, Vietnam. Appl. Geochemistry 1–10.

- 1 Eiche, E., Neumann, T., Berg, M., Weinman, B., van Geen, A., Norra, S., Berner, Z., Trang,
2 P.T.K., Viet, P.H., Stüben, D., 2008. Geochemical processes underlying a sharp contrast in
3 groundwater arsenic concentrations in a village on the Red River delta, Vietnam. *Appl.*
4 *Geochemistry* 23, 3143–3154. doi:10.1016/j.apgeochem.2008.06.023
- 5 Fellman, J.B., Hood, E., Spencer, R.G.M., 2010. Fluorescence spectroscopy opens new windows
6 into dissolved organic matter dynamics in freshwater ecosystems: A review. *Limnol.*
7 *Oceanogr.* 55, 2452–2462. doi:10.4319/lo.2010.55.6.2452
- 8 Fendorf, S., Michael, H. a, van Geen, A., 2010. Spatial and temporal variations of groundwater
9 arsenic in South and Southeast Asia. *Science* 328, 1123–1127. doi:10.1126/science.1172974
- 10 Ghosh, D., Routh, J., Dario, M., Bhadury, P., 2015. Elemental and biomarker characteristics in a
11 Pleistocene aquifer vulnerable to arsenic contamination in the Bengal Delta Plain, India.
12 *Appl. Geochemistry* 61, 87–98. doi:10.1016/j.apgeochem.2015.05.007
- 13 Harshman, R. a, 1970. Foundations of the PARAFAC procedure: Models and conditions for an
14 “explanatory” multimodal factor analysis. *UCLA Work. Pap. Phonetics* 16, 1– 84.
- 15 Hartog, N., van Bergen, P.F., de Leeuw, J.W., Griffioen, J., 2004. Reactivity of organic matter in
16 aquifer sediments: Geological and geochemical controls. *Geochim. Cosmochim. Acta* 68,
17 1281–1292. doi:10.1016/j.gca.2003.09.004
- 18 Harvey, C.F., Ashfaq, K.N., Yu, W., Badruzzaman, a. B.M., Ali, M.A., Oates, P.M., Michael,
19 H. a., Neumann, R.B., Beckie, R., Islam, S., Ahmed, M.F., 2006. Groundwater dynamics
20 and arsenic contamination in Bangladesh. *Chem. Geol.* 228, 112–136.
21 doi:10.1016/j.chemgeo.2005.11.025
- 22 Hasan, M.A., Ahmed, K.M., Sracek, O., Bhattacharya, P., Brömssen, M., Broms, S., Fogelström,
23 J., Mazumder, M.L., Jacks, G., 2007. Arsenic in shallow groundwater of Bangladesh:
24 Investigations from three different physiographic settings. *Hydrogeol. J.* 15, 1507–1522.
25 doi:10.1007/s10040-007-0203-z
- 26 Helms, J.R., Stubbins, A., Ritchie, J.D., Minor, E.C., Kieber, D.J., Mopper, K., 2008. Absorption
27 spectral slopes and slope ratios as indicators of molecular weight, source, and
28 photobleaching of chromophoric dissolved organic matter. *Limnol. Oceanogr.* 53, 955–969.
29 doi:10.4319/lo.2008.53.3.0955
- 30 Hering, J.G., Kneebone, P.E., 2002. Biogeochemical controls on arsenic occurrence and mobility
31 in water supplies. *Environ. Chem. Arsen.* 2001, 183–215.
- 32 Hood, E., Fellman, J., Spencer, R.G.M., Hernes, P.J., Edwards, R., D’Amore, D., Scott, D.,
33 2009. Glaciers as a source of ancient and labile organic matter to the marine environment.
34 *Nature* 462, 1044–1047.

- 1 Hoque, M.A., Burgess, W.G., Shamsudduha, M., Ahmed, K.M., 2011. Delineating low-arsenic
2 groundwater environments in the Bengal Aquifer System, Bangladesh. *Appl. Geochemistry*
3 26, 614–623. doi:10.1016/j.apgeochem.2011.01.018
- 4 Horneman, A., van Geen, A., Kent, D. V., Mathe, P.E., Zheng, Y., Dhar, R.K., O’Connell, S.,
5 Hoque, M.A., Aziz, Z., Shamsudduha, M., Seddique, A.A., Ahmed, K.M., 2004.
6 Decoupling of As and Fe release to Bangladesh groundwater under reducing conditions.
7 Part I: Evidence from sediment profiles. *Geochim. Cosmochim. Acta* 68, 3459–3473.
8 doi:10.1016/j.gca.2004.01.026
- 9 Jin, H., Wen, H., Yang, H., Liu, Z., Ren, Z., Che, G., Zhao, Z., 2003. Arsenic poisoning of
10 Bangladesh groundwater. *Sci. Technol.* 395, 16–18.
- 11 Kalle, K., 1949. Fluoreszenz und Gelbstoff im Bottnischen und Finnischen Meerbusen. *Dtsch.*
12 *Hydrogr. Zeitschrift* 2, 117–124. doi:10.1007/BF02225972
- 13 Kalbitz, K., Schmerwitz, J., Schwesig, D., Matzner, E., 2003. Biodegradation of soil-derived
14 dissolved organic matter as related to its properties. *Geoderma* 113, 273–291.
15 doi:10.1016/S0016-7061(02)00365-8
- 16 Kappler, A., Benz, M., Schink, B., Brune, A., 2004. Electron shuttling via humic acids in
17 microbial iron(III) reduction in a freshwater sediment. *FEMS Microbiol. Ecol.* 47, 85–92.
18 doi:10.1016/S0168-6496(03)00245-9
- 19 Knappett, P.S.K., Escamilla, V., Layton, A., McKay, L.D., Emch, M., Williams, D.E., Huq, R.,
20 Alam, J., Farhana, L., Mailloux, B.J., Ferguson, A., Sayler, G.S., Ahmed, K.M., van Geen,
21 A., 2011. Impact of population and latrines on fecal contamination of ponds in rural
22 Bangladesh. *Sci. Total Environ.* 409, 3174–3182. doi:10.1016/j.scitotenv.2011.04.043
- 23 Korak, J.A., Dotson, A.D., Summers, R.S., Rosario-ortiz, F.L., 2013. Critical analysis of
24 commonly used fluorescence metrics to characterize dissolved organic matter 9.
- 25 Liu, G., Fernandez, A., Cai, Y., 2011. Complexation of arsenite with humic acid in the presence
26 of ferric iron. *Environ. Sci. Technol.* 45, 3210–6. doi:10.1021/es102931p
- 27 Lovley, D. R., Coates, J. D., Blunt-Harris, E. L., Phillips, E. J., & Woodward, J. C. (1996).
28 Humic substances as electron acceptors for microbial respiration. *Nature*, 382(6590), 445-
29 448.
- 30 Lovley, D.R., Fraga, J.L., Blunt-Harris, E.L., Hayes, L. a., Phillips, E.J.P., Coates, J.D., 1998.
31 Humic Substances as a Mediator for Microbially Catalyzed Metal Reduction. *Acta*
32 *Hydrochim. Hydrobiol.* 26, 152–157. doi:10.1002/(SICI)1521-
33 401X(199805)26:3<152::AID-AHEH152>3.0.CO;2-D
- 34 Lovley, D.R., Fraga, J.L., Coates, J.D., Blunt-Harris, E.L., 1999. Humics as an electron donor for
35 anaerobic respiration. *Environ. Microbiol.* 1, 89–98.

- 1 Mann, H.B., Whitney, D.R., 1947. On a Test of Whether one of Two Random Variables is
2 Stochastically Larger than the Other. *Ann. Math. Stat.* 18, 50–60.
3 doi:10.1214/aoms/1177730491
- 4 McArthur, J.M., Banerjee, D.M., Hudson-Edwards, K. a., Mishra, R., Purohit, R., Ravenscroft,
5 P., Cronin, a., Howarth, R.J., Chatterjee, a., Talukder, T., Lowry, D., Houghton, S., Chadha,
6 D.K., 2004. Natural organic matter in sedimentary basins and its relation to arsenic in
7 anoxic ground water: The example of West Bengal and its worldwide implications. *Appl.*
8 *Geochemistry* 19, 1255–1293. doi:10.1016/j.apgeochem.2004.02.001
- 9 McArthur, J.M., Ravenscroft, P., Safiulla, S., Thirlwall, M.F., 2001. Arsenic in groundwater:
10 Testing pollution mechanisms for sedimentary aquifers in Bangladesh. *Water Resour. Res.*
11 37, 109–117. doi:10.1029/2000WR900270
- 12 McArthur P. AU3 - Safiullah, S. AU4 - Thirlwall, M.F., J.M.A.-R., 2001. Arsenic in
13 groundwater: testing pollution mechanisms for sedimentary aquifer in Bangladesh RN -
14 *Water. Resour. Res.*, vol. 37, pp. 109-117. *Water Resour. Res.* 37, 109–117.
- 15 McKnight, D.M., Boyer, E.W., Westerhoff, P.K., Doran, P.T., Kulbe, T., Andersen, D.T., 2001.
16 Spectrofluorometric characterization of dissolved organic matter for indication of precursor
17 organic material and aromaticity. *Limnol. Oceanogr.* 46, 38–48.
18 doi:10.4319/lo.2001.46.1.0038
- 19 Mikutta, C., Kretzschmar, R., 2011. Spectroscopic evidence for ternary complex formation
20 between arsenate and ferric iron complexes of humic substances. *Environ. Sci. Technol.*
- 21 Mladenov, N., Zheng, Y., Miller, M.P., Nemergut, D.R., Legg, T., Simone, B., Hageman, C.,
22 Rahman, M.M., Ahmed, K.M., McKnight, D.M., 2010. Dissolved organic matter sources
23 and consequences for iron and arsenic mobilization in Bangladesh aquifers. *Environ. Sci.*
24 *Technol.* 44, 123–8. doi:10.1021/es901472g
- 25 Mladenov, N., Zheng, Y., Simone, B., Bilinski, T.M., McKnight, D.M., Nemergut, D., Radloff,
26 K. a., Rahman, M.M., Ahmed, K.M., 2015. Dissolved Organic Matter Quality in a Shallow
27 Aquifer of Bangladesh: Implications for Arsenic Mobility. *Environ. Sci. Technol.*
28 150827163840003. doi:10.1021/acs.est.5b01962
- 29 Moran, M.A., Sheldon, W.M., Zepp, R.G., 2000. Carbon loss and optical property changes
30 during long-term photochemical and biological degradation of estuarine dissolved organic
31 matter. *Limnol. Oceanogr.* 45, 1254–1264. doi:10.4319/lo.2000.45.6.1254
- 32 Morgan, J.P., G., M.W., 1959. Quaternary geology of the Bengal Basin, East Pakistan and India.
33 *Bull. Geol. Soc. Am.* 70, 319–342.
- 34 Mukherjee, A., Fryar, A.E., Howell, P.D., 2007a. Regional hydrostratigraphy and groundwater
35 flow modeling in the arsenic-affected areas of the western Bengal basin, West Bengal,
36 India. *Hydrogeol. J.* 15, 1397–1418. doi:10.1007/s10040-007-0208-7

- 1 Mukherjee, A., Fryar, A.E., Rowe, H.D., 2007b. Regional-scale stable isotopic signatures of
2 recharge and deep groundwater in the arsenic affected areas of West Bengal, India. *J.*
3 *Hydrol.* 334, 151–161. doi:10.1016/j.jhydrol.2006.10.004
- 4 Murphy, K.R., Stedmon, C. a., Graeber, D., Bro, R., 2013. Fluorescence spectroscopy and multi-
5 way techniques. *PARAFAC. Anal. Methods* 5, 6557. doi:10.1039/c3ay41160e
- 6 Neidhardt, H., Biswas, A., Freikowski, D., Majumder, S., Chatterjee, D., Berner, Z.A., 2013.
7 Reconstructing the sedimentation history of the Bengal Delta Plain by means of
8 geochemical and stable isotopic data. *Appl. Geochemistry* 36, 70–82.
9 doi:http://dx.doi.org/10.1016/j.apgeochem.2013.06.017
- 10 Neumann, R.B., Ashfaq, K.N., Badruzzaman, a. B.M., Ashraf Ali, M., Shoemaker, J.K.,
11 Harvey, C.F., 2010. Anthropogenic influences on groundwater arsenic concentrations in
12 Bangladesh. *Nat. Geosci.* 3, 46–52. doi:10.1038/ngeo685
- 13 Neumann, R.B., Pracht, L.E., Polizzotto, M.L., Badruzzaman, A.B.M., Ali, M.A., 2014.
14 Biodegradable Organic Carbon in Sediments of an Arsenic-Contaminated Aquifer in
15 Bangladesh. *Environ. Sci. Technol. Lett.* 1, 221–225. doi:10.1021/ez5000644
- 16 Nevin, K.P., Lovley, D.R., 2000. Lack of production of electron-shuttling compounds or
17 solubilization of Fe(III) during reduction of insoluble Fe(III) oxide by *Geobacter*
18 *metallireducens*. *Appl. Environ. Microbiol.* 66, 2248–2251. doi:10.1128/AEM.66.5.2248-
19 2251.2000
- 20 Ohno, T., 2002. Response to Comment on “Fluorescence Inner-Filtering Correction for
21 Determining the Humification Index of Dissolved Organic Matter” †. *Environ. Sci.*
22 *Technol.* 36, 4196–4196. doi:10.1021/es020113d
- 23 Parlanti, E., Wörz, K., Geoffroy, L., Lamotte, M., Wo, K., Geo, L., Lamotte, M., 2000.
24 Dissolved organic matter fluorescence spectroscopy as a tool to estimate biological activity
25 in a coastal zone submitted to anthropogenic inputs. *Org. Geochem.* 31, 1765–1781.
26 doi:10.1016/S0146-6380(00)00124-8
- 27 Polizzotto, M.L., Kocar, B.D., Benner, S.G., Sampson, M., Fendorf, S., 2008. Near-surface
28 wetland sediments as a source of arsenic release to ground water in Asia. *Nature* 454, 505–
29 508. doi:10.1038/nature07093
- 30 Postma, D., Larsen, F., Minh Hue, N.T., Duc, M.T., Viet, P.H., Nhan, P.Q., Jessen, S., 2007.
31 Arsenic in groundwater of the Red River floodplain, Vietnam: Controlling geochemical
32 processes and reactive transport modeling. *Geochim. Cosmochim. Acta* 71, 5054–5071.
33 doi:10.1016/j.gca.2007.08.020
- 34 Ravenscroft, P., Burgess, W.G., Ahmed, K.M., Burren, M., Perrin, J., 2005. Arsenic in
35 groundwater of the Bengal Basin, Bangladesh: Distribution, field relations, and
36 hydrogeological setting. *Hydrogeol. J.* 13, 727–751. doi:10.1007/s10040-003-0314-0

1 Roberts, H., 1997. Dynamic changes of the Holocene Mississippi River delta plain: the delta
2 cycle. *J. Coast. Res.* 13, 605–627.

3 Rowland, H.A.L., Polya, D.A., Lloyd, J.R., Pancost, R.D., 2006. Characterisation of organic
4 matter in a shallow, reducing, arsenic-rich aquifer, West Bengal. *Org. Geochem.* 37, 1101–
5 1114. doi:10.1016/j.orggeochem.2006.04.011

6 Roychowdhury, T., Uchino, T., Tokunaga, H., Ando, M., 2002. Arsenic and other heavy metals
7 in soils from an arsenic-affected area of West Bengal, India. *Chemosphere* 49, 605–618.
8 doi:10.1016/S0045-6535(02)00309-0

9 Sankar, M.S., Vega, M. a., Defoe, P.P., Kibria, M.G., Ford, S., Telfeyan, K., Neal, a., Mohajerin,
10 T.J., Hettiarachchi, G.M., Barua, S., Hobson, C., Johannesson, K., Datta, S., 2014. Elevated
11 arsenic and manganese in groundwaters of Murshidabad, West Bengal, India. *Sci. Total*
12 *Environ.* 488-489, 570–579. doi:10.1016/j.scitotenv.2014.02.077

13 Sankar, M.S., 2013. Geochemical significance of Arsenic and Manganese toxicity in
14 groundwaters from Murshidabad district, West Bengal, India.

15 Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. a., Kleber,
16 M., Kögel-Knabner, I., Lehmann, J., Manning, D. a. C., Nannipieri, P., Rasse, D.P., Weiner,
17 S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property.
18 *Nature* 478, 49–56. doi:10.1038/nature10386

19 Sengupta, S., Mcarthur, J.M., Sarkar, a., Leng, M.J., Ravenscroft, P., Howarth, R.J., Banerjee,
20 D.M., 2008. Do ponds cause arsenic-pollution of groundwater in the Bengal Basin? An
21 answer from West Bengal. *Environ. Sci. Technol.* 42, 5156–5164. doi:10.1021/es702988m

22 Smith, A., Lingas, E., Rahman, M., 2000. Contamination of drinking-water by arsenic in
23 Bangladesh: a public health emergency: RN - Bull. W.H.O., v. 78, p. 1093-1103. Bull.
24 World Health Organ. 78, 1093–1103.

25 Stedmon, C. a, Markager, S., Bro, R., 2003. Tracing dissolved organic matter in aquatic
26 environments using a new approach to fluorescence spectroscopy. *Mar. Chem.* 82, 239–254.
27 doi:10.1016/S0304-4203(03)00072-0

28 Stedmon, C.A., Bro, R., 2008. Characterizing dissolved organic matter fluorescence with parallel
29 factor analysis: a tutorial. *Limnol. Oceanogr. Methods* 6, 572–579.
30 doi:10.4319/lom.2008.6.572

31 Stollenwerk, K.G., Breit, G.N., Welch, A.H., Yount, J.C., Whitney, J.W., Foster, A.L., Uddin,
32 M.N., Majumder, R.K., Ahmed, N., 2007. Arsenic attenuation by oxidized aquifer
33 sediments in Bangladesh. *Sci. Total Environ.* 379, 133–50.
34 doi:10.1016/j.scitotenv.2006.11.029

- Sutton, N.B., van der Kraan, G.M., van Loosdrecht, M.C.M., Muyzer, G., Bruining, J., Schotting, R.J., 2009. Characterization of geochemical constituents and bacterial populations associated with As mobilization in deep and shallow tube wells in Bangladesh. *Water Res.* 43, 1720–1730. doi:10.1016/j.watres.2009.01.006
- Vähätalo, A. V., Wetzel, R.G., 2004. Photochemical and microbial decomposition of chromophoric dissolved organic matter during long (months-years) exposures. *Mar. Chem.* 89, 313–326. doi:10.1016/j.marchem.2004.03.010
- Walker, M., Kristensen, T.B., Huuse, M., Piotrowski, J. a, Clausen, O.R., 2009. A morphometric analysis of tunnel valleys in the eastern North Sea based on 3D seismic data. *J. Quat. Sci.* 22, 801–815. doi:10.1002/jqs
- Wang, S., Mulligan, C.N.C., 2006. Effect of natural organic matter on arsenic release from soils and sediments into groundwater. *Environ. Geochem. Health* 28, 197–214. doi:10.1007/s10653-005-9032-y
- Weishaar, J.L., Aiken, G.R., Bergamaschi, B. a., Fram, M.S., Fujii, R., Mopper, K., 2003. Evaluation of Specific Ultraviolet Absorbance as an Indicator of the Chemical Composition and Reactivity of Dissolved Organic Carbon. *Environ. Sci. Technol.* 37, 4702–4708. doi:10.1021/es030360x
- Wilson, H.F., Xenopoulos, M. a., 2008. Effects of agricultural land use on the composition of fluvial dissolved organic matter. *Nat. Geosci.* 2, 37–41. doi:10.1038/ngeo391
- Zakir Hossain, H.M., Sampei, Y., Roser, B.P., 2009. Characterization of organic matter and depositional environment of Tertiary mudstones from the Sylhet Basin, Bangladesh. *Org. Geochem.* 40, 743–754. doi:10.1016/j.orggeochem.2009.04.009
- Zheng, Y., van Geen, A., Stute, M., Dhar, R., Mo, Z., Cheng, Z., Horneman, A., Gavrieli, I., Simpson, H.J., Versteeg, R., Steckler, M., Grazioli-Venier, A., Goodbred, S., Shahnewaz, M., Shamsudduha, M., Hoque, M.A., Ahmed, K.M., 2005. Geochemical and hydrogeological contrasts between shallow and deeper aquifers in two villages of Arai-hazar, Bangladesh: Implications for deeper aquifers as drinking water sources. *Geochim. Cosmochim. Acta* 69, 5203–5218. doi:10.1016/j.gca.2005.06.001
- Zsolnay, Á., 2003. Dissolved organic matter: artefacts, definitions, and functions. *Geoderma* 113, 187–209. doi:10.1016/S0016-7061(02)00361-0

9. List of Tables and Figures

9.1 Table Captions

Table 1: Description of sediment characteristics based on analyses of four sediment cores collected from study area (*Sankar, 2013 and Sankar et al., 2014*) and the characteristics of sedimentary organic matter obtained from sites in similar geologic settings (*Rowland et al., 2006 and Ghosh et al., 2015*).

Table 2: Properties of groundwater (GW) in Holocene and Pleistocene aquifers and overlying surface water (SW).

Table 3: P statistics (non-parametric Mann-Whitney-Wilcoxon Test)* showing statistical variation among DOM properties in samples.

Table 4: Description of PARAFAC components from this study and similar components identified in other studies (not an exhaustive list).

9.2 Figure Captions

Figure 1: Representative EEM spectra of (a) Groundwater in Holocene aquifer, (b) Groundwater in Pleistocene aquifer, (c) Surface water. Note different intensity scale on (c).

Figure 2: EEM spectra (left) and emission-excitation curves (right) showing loadings of four PARAFAC components identified in the model.

Figure 3: Split half analysis of four component PARAFAC model showing validation of the model.

Table 1

Location	Depth (m)	As _T (mg/kg)	Fe _T (g/kg)	Organic Matter (mg/kg)	Sediment Description [#]	Age	Organic Matter Characteristics
Beldanga	3	18.06	32.97	57.7	1	Holocene, ~7000 years before present	Abundant high molecular weight n-alkanes, hopanes and steranes; Less abundant n-alkanols and sterols (Rowland <i>et al.</i> , 2006 [*])
N 23 56.392	15	18.09	21.56	171.4	1		
E 88 16.206	27	16.52	24.6	55.3	1		
	30.5	9.71	11.91	53.2	2		
Hariharpara	3	12.78	26.22	59.2	3		
N 24 03.651	6	15.54	31.73	56.5	3		
E 88 21.395	15	8.3	6.8	55.85	4		
	27.4	6.03	31.73	61.15	4		
Nabagram	9.1	10.42	19.55	74.64	5	Pleistocene, 12,300 – 48,600 years before present	High molecular weight (HMW) n-alkanes; Low molecular weight (LMW) n- alkanes; Mono-unsaturated n-alkanoic acids; LMW n- alkanoic acids; Sterol and stanol monomers; Lipids (Ghosh <i>et al.</i> , 2015 ⁺)
N 23 12.156	43	6.36	8.75	62.78	6		
	3	7.4	6.46	-	7		
Kandi	12.2	15.67	25.39	118.9	5		
N 23 58.570	21	11.04	36.12	-	5		
E 88 06.814	27	5.6	5.29	121.8	8		
	30.5	9.51	31.74	-	3		
	33.5	9.92	35.5	122.4	3		

[#]Sediment Description from Sankar (2013): ¹Silty clay; ²Sand; ³Dark grey sticky clay; ⁴Medium grained sand; ⁵Reddish grey colored clay; ⁶Grey colored medium grained sand; ⁷Reddish color medium grained sand; ⁸Dark grey colored fine-medium sand.

^{*}Data obtained from analyses of Holocene sediments from Nadia district, West Bengal, India.

⁺Data obtained from Pleistocene sediments from Nadia district, West Bengal, India.

1 Table 2

Analysis type	Holocene SW	Pleistocene SW	Holocene GW	Pleistocene GW
² Water Chemistry				
Total Dissolved	35.2 ± 38.1	3.13 ± 2.71	261 ± 285	14.5 ± 10
Arsenic (µg/L)	(0 – 110.3)	(0 – 4.69)	(5 – 1263)	(0.4 – 50)
Total Dissolved	6.57 ± 21.3	0.06 ± 0.08	3.19 ± 3.86	0.52 ± 0.39
Iron (mg/L)	(0 – 70.8)	(0 – 0.15)	(0 – 13.6)	(0.07 – 1.03)
Dissolved Organic	7.28 ± 2.49	9.84 ± 1.90	2.94 ± 1.97	1.79 ± 1.48
Carbon (mg/L)	(2.64 – 9.62)	(8.67 – 12.03)	(1.25 – 6.76)	(0.85 – 4.91)
ORP	NA	NA	-44.25 ± 49.08 (-108 – 69)	+74.48 ± 71.58 (-55 – 156.5)
Absorbance				
³ Abs ₂₅₄ (a.u.)	0.23 ± 0.08 (0.08 – 0.38)	0.22 ± 0.11 (0.11 – 0.35)	0.047 ± 0.01 (0.02 – 0.08)	0.034 ± 0.01 (0.02 – 0.05)
⁴ SUVA (L.mg ⁻¹ .m ⁻¹)	2.65 ± 0.22 2.48 – 3.16	3.03 ± 0.17 2.92 – 3.23	2.26 ± 1.13 0.60 – 4.19	2.35 ± 1.20 0.93 – 4.65
⁵ S _{275_295}	-0.02 ± 0 (-0.02 – -0.01)	-0.02 ± 0 (-0.02 – -0.01)	-0.02 ± 0.00 (-0.02 – -0.01)	-0.02 ± 0.00 (-0.02 – -0.01)
⁶ S _{350_400}	-0.01 ± 0 (-0.02 – -0.01)	-0.01 ± 0 (-0.01 – -0.01)	-0.01 ± 0.01 (-0.03 – 0)	-0.01 ± 0.00 (-0.02 – -0.01)
⁷ S _R	1.26 ± 0.22 (0.84 – 1.73)	1.27 ± 0.14 (1.08 – 1.42)	1.33 ± 0.47 (0.69 – 2.34)	1.78 ± 0.43 (1.10 – 2.54)
⁸ Fluorescence Indices				
FI	1.60 ± 0.04 (1.55 – 1.70)	1.63 ± 0.03 (1.60 – 1.67)	1.73 ± 0.06 (1.59 – 1.83)	1.72 ± 0.10 (1.54 – 1.88)
β:α	0.85 ± 0.10 (0.77 – 1.12)	0.76 ± 0.04 (0.72 – 0.81)	0.77 ± 0.06 (0.68 – 0.96)	0.99 ± 0.14 (0.66 – 1.09)
HIX	5.39 ± 0.89 (3.38 – 6.31)	6.38 ± 2.21 (4.06 – 9.06)	6.79 ± 1.98 (3.48 – 10.45)	3.34 ± 1.38 (1.67 – 7.25)
⁹ PARAFAC (R.U.)				

Analysis type	Holocene SW	Pleistocene SW	Holocene GW	Pleistocene GW
C1	3.57 ± 2.99 (1 – 11.48)	3.73 ± 2.54 (0.89 – 6.07)	1.87 ± 3.21 (0.23 – 17.13)	2.47 ± 3.37 (0.17 – 10.08)
C2	2.28 ± 1.41 (0.79 – 5.30)	2.56 ± 1.69 (0.66 – 4.07)	1.18 ± 1.56 (0.19 – 8.14)	2.18 ± 2.82 (0.19 – 8.15)
C3	1.24 ± 0.87 (0.38 – 3.21)	0.94 ± 0.47 (0.42 – 1.55)	0.38 ± 0.5 (0.05 – 2.59)	1.65 ± 2.26 (0.17 – 6.67)
C4	1.73 ± 2.60 (0.38 – 9.51)	1.27 ± 1.16 (0 – 2.30)	0.6 ± 1.76 (0 – 9.9)	3.93 ± 6.4 (0.03 – 20.02)
Calculated Parameters				
Total Fluorescence (R.U.)	8.84 ± 7.60 (2.74 – 29.50)	8.50 ± 5.84 (1.96 – 13.83)	4.03 ± 6.88 (0.53 – 37.02)	10.23 ± 14.77 (0.63 – 44.93)
% C1	40.45 ± 1.97 (36.56 – 43.77)	44.27 ± 0.81 (43.29 – 45.22)	46.25 ± 3.19 (40.25 – 51.43)	27.76 ± 8.59 (22.37 – 52.25)
% C2	28.14 ± 3.42 (17.96 – 30.33)	31.29 ± 2.35 (28.98 – 34.18)	33.40 ± 3.63 (22 – 39.06)	25.79 ± 4.77 (18.14 – 34.72)
% C3	14.96 ± 3.47 (10.87 – 20.67)	14.43 ± 6.19 (8.28 – 21.40)	11.81 ± 4.35 (4.98 – 23.19)	18.17 ± 4.08 (13.90 – 29.05)
% C4	16.46 ± 5.81 (11.01 – 32.24)	10.01 ± 9.17 (0 – 17.33)	8.53 ± 6.66 (0 – 26.75)	26.28 ± 13.10 (2.45 – 44.57)
¹⁰ Humic:protein	5.98 ± 1.43 (3.84 – 8.20)	7.07 ± 3.21 (3.67 – 11.08)	8.53 ± 3.42 (3.31 – 19.10)	4.71 ± 1.03 (2.44 – 6.19)
¹¹ terr:microb	2.23 ± 0.38 (1.32 – 2.78)	3.15 ± 0.55 (2.61 – 3.80)	4.27 ± 1.52 (2.15 – 7.71)	1.44 ± 1.24 (0.68 – 5.11)

¹All numbers are expressed as average value ± standard deviation, minimum – maximum range in parenthesis.

²Data source: Sankar et al., 2014, except oxidation reduction potential (ORP) data from 2014 field season.

³Absorbance at 254 nm.

⁴Specific ultra-violet absorbance.

⁵Spectral Slope between 275 nm and 295 nm.

⁶Spectral Slope between 400 nm and 350 nm

⁷Spectral Slope Ratio ($S_{275-295} / S_{350-400}$)

⁸Fluorescence Index (FI), Freshness Index ($\beta:\alpha$), Humification Index (HIX).

⁹C1 to C4 = Four components obtained by PARAFAC model.

1 ¹⁰Ratio of humic like to protein like component obtained by PARAFAC model = $(C1+C2+C4) / (C3)$.

2 ¹¹Ratio of terrestrially produced DOM to microbially produced DOM = $(C1+C2) / (C3+C4)$.

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1 Table 3

Analysis type	Holocene – Pleistocene SW	Holocene – Pleistocene GW	GW – SW Holocene	GW – SW Pleistocene
Total Dissolved Arsenic (µg/l)	0.048	<0.001	0.012	0.014
DOC (mg/l)	0.121	0.003	0.00014	0.00275
Absorbance				
Abs 254 (a.u.)	0.5	0.01	<0.001	<0.001
SUVA (L.mg ⁻¹ .m ⁻¹)	0.024	0.303	0.197	0.146
S275-295	0.01	0.49	0.46	0.44
S350-400	0.19	0.02	0.47	0.02
S _R	0.33	<0.001	0.30	0.01
Fluorescence Indices				
FI	0.07	0.40	<0.001	0.04
β:α	<0.001	<0.001	<0.001	<0.001
HIX	0.22	<0.001	0.01	<0.001
PARAFAC (R.U.)				
C1	0.50	0.18	<0.001	0.12
C2	0.50	0.45	<0.001	0.19
C3	0.37	0.03	<0.001	0.25
C4	0.40	<0.001	<0.001	0.39
Calculated Parameters				
Humic: protein	0.33	<0.001	0.01	0.22
terr:microb	<0.001	<0.001	<0.001	<0.001

2 *Values in shaded cells: $P < 0.05$ indicate statistically different parameter

Reference	Component 1 240 (360)/460	Component 2 240 (320) / 400	Component 3 240 (280) / 344	Component 4 240 (280) / 388
This Study	Terrestrial humic-like	humic-like, impacted by agriculture, marine humic	protein-like, tyrosine and tryptophan	microbial humic-like
Parlanti et al., (2000)	α' - humic like, 250-260/380-480 α - humic like, 330-350/420-480 Terrestrial source, HMW* and aromatic humic-like	α' - humic like, 250-260/380-480 β -marine humic like, 310-320/380-420 Terrestrial source associated with microbial, wastewater, agricultural activity, LMW* humic-like	δ - tryptophan like, 270-280/320-350 γ - tyrosine like, 270-280/300-320 amino acids, free or bound in proteins, free tryptophan and tyrosine, intact proteins, more or less degraded peptide material	-
Stedmon et al., (2003)	C1 – humic like, 240-320/428	C2 – marine humic like, impacted by agriculture, 315/384	C4 – tryptophan like, 278/348	-
Fellman et al., (2009)	UVC humic like 1, 320-360/420-460 UVC humic like 2, <260/448-480	UVA humic like, 290-325(<250)/370-430	tyrosine-like, 270-275/304-312 tryptophan like, 270-280/330-368	U – Unknown, 250(320)/370 HMW humic, widespread, but highest in wetlands and forested environments, very labile, associated with freshly produced DOM
Williams et al., (2013)	C1 – terrestrial humic, <250/440(468)	C2 – terrestrial humic, <250(310)/420(388)	C7 – protein like, 280/342 (318)	C6 – microbial humic, <250(285)/386
Coble, (1996)	A = UV humic, 260/400-460; C = humic like, 350/420-480	A = UV humic, 260/400-460; M = marine humic, 290-310/370-410	T = tryptophan like, 275/340 B = tyrosine like, 270/305	-
Coble et al., (1990)	A – UV humic like, 260/400-460 C – visible humic like, 320-360/420-460	A – UV humic like, 260/400-460, M – visible marine humic, 290-310/370-410	T – tryptophan like, 275/340, B – tyrosine like, 275/305	N – unknown, 280/370

1 Table 4

2 *HMW = High Molecular Weight, LMW = Low Molecular Weight

Figure 1

1 Figure 2

1 Figure 3

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