Roles of different molecular weights of dissolved organic matter in arsenic enrichment in groundwater: Evidences from ultrafiltration and EEM-PARAFAC

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\textbf{A B S T R A C T}

Molecular weight (MW) is a fundamental property of dissolved organic matter (DOM) that may affect the interaction between DOM and metals/metalloids. However, understanding of MW property of groundwater DOM and its roles in arsenic (As) mobilization needs to be improved. In this study, two surface water samples and 12 groundwater samples with different concentrations of As and dissolved organic carbon (DOC) were taken to evaluate the effects of DOM MW on As mobilization by using sequential ultrafiltration technique under a N\textsubscript{2} atmosphere. Concentrations of As, Fe, DOC, and spectroscopic properties of OM in each ultrafiltered sample were analyzed. Variations of As, Fe and DOC concentrations in different ultrafiltration fractions show that Fe colloids mainly exist in large size particles (10 kDa-0.45 \textmu m fractions), while organic colloids are mainly present in middle size particles (5-10 kDa fractions). The positive correlations between As and Fe in 10 kDa-0.45 \textmu m fractions ($R^2 = 0.76, p < 0.01$) and between As and DOC in 5-10 kDa fractions ($R^2 = 0.61, p < 0.05$) indicate that the complexation of As with large-size Fe colloids and middle-size DOM promotes As mobilization. Parallel factor analysis (PARAFAC) of the three-dimensional fluorescence spectra reveals that DOM in all size fractions comprises three major components, namely C1 (terrestrial humic-like component), C2 (microbial humic-like component), and C3 (protein-like component). The positive correlation between C1 (%) and As concentrations ($R^2 = 0.73, p < 0.05$) possibly confirms that terrestrial-derived humic-like substance (HS) should be conducive to As enrichment. The spectroscopic indices of various fractions show that low MW DOM is characterized by more microbe-associated OM. Some samples have more than 90\% of As in the ‘truly dissolved’ fractions, suggesting that microbial utilization of labile low MW DOM should lead to the enrichment of As, in addition to complexation reactions. Therefore, both As-Fe-HS complexation and microbial degradation of low MW DOM being related to the reductive dissolution of As-bearing Fe oxide minerals enhance As mobilization and enrichment in groundwater.

\textbf{1. Introduction}

High arsenic (As) groundwater is widespread in many parts of the world, including Argentina, Bangladesh, China, India, and Pakistan (Smedley and Kinniburgh, 2002), which is considered as a serious environmental problem. High As groundwater in China is mainly distributed in Guizhou, Inner Mongolia, Shanxi, Taiwan, Yunnan, and Xinjiang (Guo et al., 2014). Long-term ingestion of food and/or water with high As contents can result in As chronic poisoning, which endangers the health of residents (Smith et al., 1992; Chen et al., 2011).

Dissolved organic matter (DOM), as a carbon source for the metabolism of microorganisms, promotes and accelerates As biogeochemical processes in groundwater systems (Mladenov et al., 2010; Guo et al., 2019), which plays an important role in As mobilization. Moreover, complexation of refractory organic matter with As (Kulkarni et al., 2017), competitive adsorption with humic substances (Bauer and Bledau, 2006), and DOM acting as an electron shuttle (Klüpfel et al., 2014) also affect the mobilization and transformation of As. Competitive adsorption of humic substances on mineral surfaces may promote As desorption from the solid phase (Davis et al., 2001), and complexation of DOM with As and Fe can also enhance As solubility (Sharma et al., 2010; Liu et al., 2011). In recent years, the commonly...
accepted mechanism of As enrichment is that the synergism of microorganisms and DOM catalyzes reductive dissolution of As-adsorbing Fe/Mn oxide minerals and reduction of adsorbed As(V) in reducing conditions (Mearthur et al., 2001; Charlet and Polya, 2006; Guo et al., 2008, 2019). The introduction of bioreactive OM enhances microbial activity and reduces the redox potential of groundwater, thereby promoting the reductive dissolution of Fe-oxide minerals (Oremland and Stolz, 2005; Campbell et al., 2006; Stuckey et al., 2015). Molecular weight (MW) is a fundamental property of DOM and an important factor affecting the bioavailability of DOM (Gao and Güéguen, 2017) and the association between organic matter and As (Chen et al., 2016). Different MW organic colloids have different capacities for As binding (Bauer and Blodau, 2009; Chen et al., 2016). Our previous investigation on size distributions of OM, As and Fe colloids showed that As was related to middle-size organic colloids with MWs of 5–10 kDa (Guo et al., 2011). In contrast, amino acids with low molecular weight are bioactive and readily utilized by microorganisms (Keil and Kirchman, 1993; Meckler et al., 2004; Guo et al., 2019). Laboratory experiments showed that low-MW-DOM would be labile for microorganism metabolisms (Wu et al., 2016; Brailsford et al., 2017). Therefore, exploring the source, characteristics, biological reactivity and geochemical reactivity of DOM with different MWs is of significance in revealing the mechanism of As enrichment in groundwater.

Spectroscopic properties well reflect the source and reactivity of DOM in groundwater and surface water (Mladenov et al., 2010). Of the spectroscopic properties, the fluorescence emission-emission matrix (EEM) is the simple and fast way to identify DOM sources and compositions (Coble, 1996). Parallel factor analysis (PARAFAC) is a statistical tool that divides a large number of EEM datasets into several independent fluorescence components (Stedmon et al., 2003). EEM coupled with PARAFAC (EEM-PARAFAC) can separate the overlapping peaks to objectively identify characteristics and relative content of each fluorescent component in the DOM samples (Stedmon and Bro, 2008; Cuss and Güéguen, 2012). Regarding the associations of fluorescence characteristics with MW of DOM, Gontijo et al. (2017) found that high-MW-DOM fractions are more likely of terrestrial origin with the higher humification and less fresh components, whereas low-MW-DOM fractions are related to microbial/biogenic origin and fresh products. However, spectroscopic properties of different MW DOM and their relevance to As mobilization are still unclear in real high-As groundwater, which would improve our understanding of As enrichment mechanisms in groundwater systems.

The Hetao Basin is typical sediment-filled basin in the northwest of China, with groundwater As concentrations between 0.33 and 857 μg/L (Guo et al., 2011) and dissolved organic carbon (DOC) concentrations from 0.73 to 35.7 mg/L (Deng et al., 2009). A thick layer of aquifers entraps clay lenses enriched in OM, which contain large amounts of humus, forming reducing environments. The reductive dissolution of iron oxides is considered to be the main process for the formation of high-As groundwater in the region (Guo et al., 2013, 2016). Although sequential ultrafiltration showed that As is related to middle-size organic colloids with MWs of 5–10 kDa (Guo et al., 2011), the source and characteristics of different MW DOM and their roles on As enrichment are unclear. Therefore, taking the Hetao basin as the study area, this study mainly aims at (1) characterizing differences in MW distribution of DOM in groundwater with different As concentrations, (2) investigating spectroscopic properties, source and reactivity of DOM with different MWs, and (3) evaluating the roles of MW specific DOM on As mobilization in groundwater systems.

2. Materials and methods

2.1. Study area

The Hetao Basin is located to the north of the Yellow River and the south of Langshan Mountains in the west of Inner Mongolia (Fig. 1a).

The basin is a flat terrain with the slight tilt from SW to NE, which belongs to a typical semiarid-arid climate with low precipitation (the annual precipitation 130–220 mm), strong evaporation (the annual evaporation 2000–2500 mm) and long sunshine periods (annually 3000–3200 h). Average annual temperatures range from 5.6 to 7.8 °C (Guo et al., 2013).

The study area is located in the northwest of the Hetao basin (Fig. 1), which is rich in groundwater sources. But high-As and/or high-fluoride groundwaters are widely distributed. The aquifer in the study area is multi-layer staggered, with complexed morphology and lithologic types. Groundwater in the northern part is mainly recharged by precipitation, surface runoff and lateral fracture water from the bedrock of the Langshan Mountain, while in the southern part mainly by farmland irrigation backflow and the seepage from irrigation channels which are used to divert the Yellow River for agricultural irrigation. Groundwater is mainly discharged by evapotranspiration and manual extraction (Deng et al., 2009).

2.2. Sampling of groundwater and surface water

According to the previous hydrogeological and geochemical investigation, we collected groundwater samples with different As concentrations and relatively high DOC from twelve representative shallow wells (depths between 10 and 30 m) (Fig. 1b). Two surface water samples were taken as well for comparison.

Groundwater was sampled from shallow wells after pumping for around 20 min until temperature, pH, oxidation-reduction potential (ORP), and electrical conductivity (EC) were stable. Under dark conditions, all water samples were filtered through a 0.45 μm glass fiber membrane which had been burned at 450 °C for 5 h. Dissolved organic matter in the filtered (< 0.45 μm) samples is called by “bulk DOM”. Immediately after filtrated through a 0.45 μm glass fiber membrane, samples were subject to sequential ultrafiltration through nominal MW membranes of 100 kDa (< 100 kDa fraction), 30 kDa (< 30 kDa fraction), 10 kDa (< 10 kDa fraction) and 5 kDa (< 5 kDa fraction) (Polyethersulfone membrane, Sartorius Stedim Biotech) in the field. The ultrafiltration membranes were cleaned with ultrapure water for three times before use and then stored moistly. During (ultra)filtration, the first 30–50 mL of solution was discarded. Ultrafiltration was carried out with a 200-μL polysulfone cell (Millipore 8200, USA) equipped
with a magnet stirring bar to prevent the membranes from clogging. Ultrapure N₂ gas (> 99.999%) was used to stabilize the redox conditions of the samples and to push the sample through the membranes (0.35 MPa) during both filtration and ultrafiltration (Guo et al., 2011).

Samples were collected in HNO₃-washed polyethylene bottles and acidified with 1:1 ultrapure HNO₃ to pH < 2.0 for subsequent laboratory analysis of major and trace elements. Aliquots for As species analysis were preserved with 0.25 M EDTA. Those for dissolved organic carbon (DOC) and spectroscopic analysis were stored in 40 mL amber glass bottles having been burned at 450 °C for 5 h and acidified with H₂PO₄ to pH < 2. Although hydrochloric acid and nitric acid acidification may affect the optical properties of groundwater (Kulkarni et al., 2019), it is advisable for the overall trend of optical properties during ultrafiltration process due to the same degree of phosphoric acid acidification (Table S2). All water samples were stored in the dark at 4 °C.

2.3. Water analysis

During groundwater sampling, water temperature, EC, pH, and ORP were measured using a multiparameter portable meter (HANNA, HI 9828). Concentrations of S²⁻, Fe²⁺ and NH₄⁺ were determined using a portable spectrophotometer (HACH, DR2800). Alkalinity was analyzed using a Model 16900 digital titrator (HACH) with bromocresol green-methyl red indicator. The Eh readings reported in this study are the results of the ORP values corrected to the standard hydrogen electrode (SHE).

Concentrations of major cations and trace elements were analyzed by Inductively Coupled Plasma Atomic Emission Spectroscopy (iCAP6300, Thermo) and Inductively Coupled Plasma Mass Spectroscopy (7500C, Agilent), with a relative standard deviation (RSD) of ± 5% and an analytical precision of 2%. Concentrations of S²⁻, Fe²⁺ and NH₄⁺ were estimated by a Shimadzu TOC-VCPH analyzer.

2.4. Optical spectroscopic measurements

UV-visible absorbance and fluorescence spectroscopy measurements were made for DOM by a UV-Visible spectrophotometer with a 10 mm path length (UV1010, LabTech Ltd.) and a Fluoromax-4 Spectrofluorometer (Jobin Yvon Horiba, respectively). The specific UV absorbance (SUVA₂₅₄), routinely as an indicator of aromaticity, was determined by the exponential equation (Eq. (1)) (Twardowski et al., 2004). Absorbance (SUVA 254), routinely as an indicator of aromaticity, was determined by the exponential equation (Eq. (1)) (Twardowski et al., 2004).

\[ a_\lambda = a_{1 \lambda} e^{-(\lambda - \lambda_{ref})} \]  
(1)

where \( a_\lambda \) is absorption coefficient at the specified wavelength, which is calculated as 2.303Aₐ/I (A is the absorbance, and I is the path length), \( a_{1 \lambda} \), is the absorption coefficient at the reference wavelength, and \( \lambda \) is the slope fitting parameter.

The scanning wavelengths for all excitation–emission matrix (EEM) spectra were set between 250 and 400 nm for excitation and between 300 and 550 nm for emission, with the integration time of 0.1 s. The measurements were conducted at 4 nm steps for excitation, and 2 nm for emission. The slits were set to 3 nm for both excitation and emission monochromators. Although high Fe concentrations may cause quenching effects (Poulin et al., 2014), adding H₃PO₄ to pH < 2.0 for the studied samples would minimize the fluorescence quenching caused by metal complexes due to the dissociation of most metal complexes at low pH (McKnight et al., 2001). The biological index (BIX) is calculated as a ratio of the intensity at emission of 380 nm and 430 nm both at 310 nm excitation to illustrate protozoa activity and OM sources (Toosi et al., 2012). The humification index (HIX) is obtained as a ratio of the areas under the emission spectra over 434–480 nm to 300–345 nm with an excitation wavelength of 254 nm to estimate the degree of humification and sources of OM (Zsolnay et al., 1999). The fluorescence index (FI) is calculated as a ratio of the intensity at emission of 450 nm and 500 nm both at 370 nm excitation to characterize the source of humic substances (HS) (McKnight et al., 2001).

2.5. PARAFAC analysis

Prior to PARAFAC analysis, the raw EEM fluorescence spectra were subjected to an internal filter correction to remove the inner filtration effect of samples (Huguet et al., 2009), and subsequently deducted by the ultra-pure water background fluorescence signal to eliminate the effects of Raman scattering (Huang et al., 2015). PARAFAC model was established for our sample dataset (a total of 70 EEMs of bulk DOM and different MW DOM) using DOMFlour toolbox (Stedmon and Bro, 2008). A three-component model was verified by split half analysis and residual analysis (Stedmon et al., 2003). Residual intensities, subtracting the PARAFAC-modeled EEM from the measured EEM, were within 10% of measured EEM intensities for all samples. Referring to previous studies, three components were recognized, including terrestrial humic-like component (C1), microbial humic-like component (C2), and tryptophan-like component (C3) (See Section 3.4 for details). The relative concentrations of these components were estimated by the Fmax in Raman units (R.U.) output from DOMFlour (Saadi et al., 2006; Yang et al., 2015). The relative abundance of each component (C%) was calculated as the percentage of its fluorescence intensity divided by the sum of the fluorescence intensities of all derived components.

Based on the PARAFAC components, two indices were used to further characterize the nature of DOM. The protein to humic index (PHI) was calculated as the ratio of the protein-like DOM components (C3) to the sum of humic-like DOM components (C1 + C2) obtained from the PARAFAC model, according to Eq. (2). The microbial to terrestrial index (MTI) was calculated as the ratio of the sum of microbially-derived (C2 + C3) to the terrestrially-derived DOM components (C1) (Eq. (3)).

\[ \text{PHI} = \frac{F_{\text{max},C3}}{F_{\text{max},C1} + F_{\text{max},C2}} \]  
(2)

\[ \text{MTI} = \frac{F_{\text{max},C2} + F_{\text{max},C3}}{F_{\text{max},C1}} \]  
(3)

where \( F_{\text{max},C1} \), \( F_{\text{max},C2} \) and \( F_{\text{max},C3} \) (R.U.) are the maximum fluorescence intensities of the three components output from DOMFlour, respectively.

3. Results

3.1. Chemical characteristics of groundwater and surface water

Physicochemical parameters of water samples are given in Table 1. Groundwaters have pH from 7.35 to 8.80, indicating a weakly alkaline environment. The Na⁺ ion is the major cation (mostly > 50% meq%), while HCO₃⁻ and Cl⁻ are the major anions. Groundwater samples generally have high salinity, with total dissolved solid (TDS) between 676 and 2280 mg/L, which are higher than those of two surface water samples (436 and 661 mg/L).
Table 2

Correlations of Fe and DOC concentrations with As concentrations in each corresponding fraction.

<table>
<thead>
<tr>
<th>MW fractions</th>
<th>Fe</th>
<th>DOC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>R²</strong></td>
<td><strong>p</strong></td>
<td><strong>R²</strong></td>
</tr>
<tr>
<td>&lt; 0.45 μm</td>
<td>0.305</td>
<td>0.288</td>
</tr>
<tr>
<td>100kDa-0.45μm</td>
<td>0.749**</td>
<td>0.002</td>
</tr>
<tr>
<td>30–100 kDa</td>
<td>0.543*</td>
<td>0.045</td>
</tr>
<tr>
<td>10–30 kDa</td>
<td>0.565*</td>
<td>0.035</td>
</tr>
<tr>
<td>10kDa-0.45μm</td>
<td>0.758**</td>
<td>0.002</td>
</tr>
<tr>
<td>5–10 kDa</td>
<td>–0.169</td>
<td>0.563</td>
</tr>
<tr>
<td>&lt; 0.5 kDa</td>
<td>0.376</td>
<td>0.185</td>
</tr>
</tbody>
</table>

* Denotes statistically significant at < 0.01.
** Denotes statistically significant at < 0.05.

Relatively low Eh is observed in groundwater (Table 1), indicating a medium-strong reducing environment. Sulfate concentrations show big variations from < 0.1 to 651 mg/L, with three samples having SO$_4^{2-}$ concentrations exceeding 250 mg/L (the Chinese drinking water guideline). The presence of S$^{2-}$ and the relatively low SO$_4^{2-}$ concentrations indicate that SO$_4^{2-}$ reduction has occurred in the reducing environment. Nitrate concentrations are mostly below the detection limit (< 0.01 mg/L). The NO$_3^-$ concentrations of GW-01-07 and SW-05 are 4.02 and 10.3 mg/L (Table 1), respectively, which may be due to the impact of pollution from agricultural activities.

Groundwater DOC concentrations range from 4.70 to 12.8 mg/L (Table 1), which are slightly higher than those of surface water. Among 12 groundwater samples, eight contain > 0.3 mg/L of Fe and four contain > 0.1 mg/L of Mn (Chinese drinking water guideline) (Table 1). Arsenic concentrations range from 13.2 to 758 μg/L, which are mostly present as As(III) species. The As concentrations and DOC show a positive correlation ($R^2 = 0.727$, $p < 0.01$; Table 2, Fig. S1), indicating that under weakly alkaline conditions, As release from the aquifer to the groundwater increases with increasing DOC concentrations. In surface water samples, As, Fe and Mn concentrations are relatively low.

3.2. Concentrations of As, Fe and DOC in different ultrafiltrated fractions

Arsenic concentrations range from 13.2 to 758 μg/L and As(III) is the major species in the 0.45 μm filtrates, accounting for 61%–100% of total As (Table 1). Groundwater samples are divided into three groups according to the change of As concentrations during sequential ultrafiltration (Fig. 2). In samples GW-01, GW-08, GW-09, GW-13, GW-01-07, and GW-4-5 (Group I), no significant change was found, and > 90% of As is concentrated in the < 5 kDa fraction, indicating that As is mainly present as a truly dissolved form. For other samples, a large drop in As concentration was observed between 100 kDa fraction and 5 kDa fraction, while there is a gentle decrease between 0.45 μm and 100 kDa. In samples GW-06, GW-07, and GW-2-4 (Group II), the biggest difference was found between < 100 kDa fraction and < 10 kDa fraction in comparison with those between the neighboring fractions, while in samples GW-03, GW-04 and GW-12 (Group III), the biggest difference is between < 10 kDa fraction and < 5 kDa fraction. It shows that in the colloidal particles, the 10–100 kDa fraction contains the most As in Group II samples, while the 5–10 kDa fraction retains the largest proportion of As in Group III samples.

During the sequential ultrafiltration, Fe concentration decreases with ultrafilter pore size (Fig. 3). For most of studied samples, there is a large drop in Fe concentration between 0.45 μm and 100 kDa, and some of these samples also have evident decreases between 100 and 10 kDa (Samples GW-06, GW-07, GW-08, GW-2-4 and GW-4-5). It indicates that Fe is mostly present as large size colloids (10 kDa–0.45 μm).

However, the distribution of DOC in ultrafiltrates with different pore sizes is completely different from that of Fe (Fig. 3). For all samples, DOC concentrations decrease systematically through continuous
Fig. 2. Relative change of As concentrations in different (ultra)filtrates during sequential ultrafiltration of groundwater samples.

Fig. 3. Variations of Fe and DOC concentrations in different (ultra)filtrates during ultrafiltration of groundwater samples.
Box-whisker plots of BIX (a), HIX (b), FI (c), and SUVA\textsubscript{254} (d) for different size fractions of groundwater DOM. Black solid points represent the average. Box range indicates lower quartile, median (middle line) and upper quartile; two whisker ends indicate maximum and minimum, respectively.

(ultra)filtration. Most samples (GW-04, GW-08, GW-09, GW-12, GW-01-07 and GW-4-5) show a large drop between 10 and 5 kDa, indicating that the OM mainly exists as the organic colloids between 5 and 10 kDa.

Although the variations of Fe, DOC and As are different on the whole, they have the similar trends in specific ultrafiltration ranges. In Group II samples, the 10 kDa-0.45 μm fraction contains the most As, similar to the variation of Fe. However, in Group III samples, the 5–10 kDa fraction retains the largest proportion of As, similar to the distribution of DOC.

For the two surface water samples, As concentrations are low and decrease progressively during ultrafiltration (Fig. S2). Concentrations of DOC and Fe also decrease gradually during the sequential ultrafiltration (Fig. S3), indicating that As may exist as the inorganic-organic colloids.

### 3.3. Fluorescent characteristics of different MW organic matter

BIX, HIX, FI and SUVA\textsubscript{254} of DOM with different MWs are shown in Fig. 4. During the sequential ultrafiltration, BIX slightly increases and HIX obviously decreases (Fig. 4a and b). High BIX values (> 1) indicate a predominantly autochthonous origin of DOM, while low values (0.6–0.7) show a lower DOM production in natural waters (Huguet et al., 2009). High HIX values (between 10 and 16) correspond to the humified DOM being mainly of terrestrial source, while low values (< 4) connect to autochthonous DOM (Huguet et al., 2009). HIX values decrease from 13.06 for <0.45 μm fraction to 8.82 for < 5 kDa fraction. It indicates that lower MW DOM has stronger biological activity and higher MW DOM has higher degree of humification.

The FI values around 1.9 indicate that OM is the source of microorganisms, mainly from the process of microbial metabolism, while FI values around 1.4 reflect that the terrestrial sources make up the major contribution (McKnight et al., 2001). The FI values range from 1.63 to 2.22 for groundwater samples, with an average of 1.95, indicating that most of OM is primarily of microorganism origin. During the sequential ultrafiltration, FI value slightly increases (Fig. 4c), illustrating a greater contribution from microbial sources in low MW DOM. The value of SUVA\textsubscript{254} decreases during the sequential ultrafiltration (Fig. 4d), suggesting more aromaticity and more complex structure in high MW DOM. Normally, lower MW DOM exhibits higher BIX and FI values, and lower HIX and SUVA\textsubscript{254} values than higher MW DOM. It indicates that higher MW DOM has higher humification and aromaticity levels, whereas lower MW DOM are characterized by more microbe-associated OM. Furthermore, BIX values are negatively correlated with HIX values, and groundwater samples with higher MWs generally have higher HIX values and lower BIX values (Fig. 5).

Concerning surface water, although BIX is higher and HIX is lower than groundwater (Fig. 5), the trends of BIX and HIX after sequential ultrafiltration are similar to those of groundwater (Table S1). During ultrafiltration, the BIX values of DOM do not change significantly, while the HIX values slightly decrease. It also shows that the higher MW DOM has a higher degree of humification.

### 3.4. 3D-EEM fluorescence spectral characteristics of DOM in ultrafiltered samples

Three fluorescent components are successfully obtained for all the samples by PARAFAC modeling (Fig. 6), which are similar to components identified in other studies (Table 3). C1 and C2 have excitation and emission wavelength maxima consistent with humic-like components, whereas C3 is identified as a protein-like component, with a peak similar to that of tryptophan. Fluorescence peaks at long wavelengths (i.e., red-shifting) are related to the structural condensation and polymerization of organic compounds (Chen et al., 2003; Lee et al., 2015). Fluorescent characteristics at longer emission wavelengths are more pronounced in EEMs of larger size HS (Hur and Kim, 2009). The components at the longer wavelength contain more conjugated fluorescent molecules, which have the higher aromaticity and more complicated structure. Thus, the humic-like C1 fluorophore is probably associated with the more condensed structure and larger molecular size, indicating a relatively more refractory nature, whereas the tryptophan-like C3 fluorophore is less structurally condensed and more labile.

The percentage of C1 is positively correlated with C2, while the relative percentage of C3 is negatively correlated with C1 and C2 (Fig. S4), indicating that the tryptophan-like component may be the degradation product of other components (Chen et al., 2010). As shown in Fig. S5, the fluorescence intensities of the C1 and C2 components in groundwater are relatively higher, while the intensities of the C3 component in surface water are higher. It shows that DOM in groundwater may be more humified than in surface water.
During the sequential ultrafiltration, the relative abundances of C1 and C2 in groundwater are slightly reduced, while the relative abundance of C3 significantly increased (Fig. 7). The results indicate that the protein-like component of the microbial origin is mainly present in low MW DOM. Based on the three PARAFAC components, PHI and MTI are developed to provide further insights into the nature of DOM. With step-by-step ultrafiltration, the average groundwater PHI value increases from 0.288 in the “bulk DOM” to 0.443 in the < 5 kDa fraction, and the mean value of the MTI rises from 1.86 to 2.32 (Figs. S6b and c). Overall, the < 5 kDa fraction has higher contents of protein-like

![Fig. 6. Spectral properties of the three fluorophores identified by the parallel factor analysis (PARAFAC). The high degree of similarity validates the three-component model (dashed lines). The solid lines represent the loadings from the three-component model using the whole data set.](image)

<table>
<thead>
<tr>
<th>Component</th>
<th>Ex/Em (nm)</th>
<th>DOM groups and descriptions by previous studies</th>
<th>Description in this study</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>&lt; 250 (334)/442</td>
<td>C1: &lt; 250/452, terrestrial humic-like substances (Kowalczuk et al., 2009)</td>
<td>Terrestrial humic-like component</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C1: 250 (330–340)/440, fulvic-acid like component (Huang et al., 2016)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C1: &lt; 240/436, UV humic-like material (Stedmon et al., 2003)</td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>318 (&lt; 250)/400</td>
<td>C3: &lt; 250 (310)/400, marine humic substances and also as DOM that has been altered by microbial reprocessing (Kowalczuk et al., 2009) Microbial humic-like components, also being regarded as the blue shift peak position of C1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C2: &lt; 250 (320)/390, humic-like materials (Huang et al., 2016)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C1: 240 (320)/404, humic-like materials produced from organic matter as a result of microbial activities (Wei et al., 2016)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>C6: &lt; 250 (325)/410, microbially-derived components (Chen et al., 2010)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C2: 240 (320)/400, humic-like materials, impacted by agriculture, marine humic (Kalkarni et al., 2017)</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>&lt; 250/364</td>
<td>C5: &lt; 250 (270)/370, protein- and tryptophan-like, microbial-produced (Williams et al., 2010) Tryptophan-like component</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C4: &lt; 250 (290)/360, amino acids, free or protein bound (Baghoth et al., 2011)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C3: 220 (280)/352, protein-like peaks (Murphy et al., 2008)</td>
<td></td>
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</table>
components and more labile components of microbial origin, in relative to other fractions. It is in line with the relationship between BIX and OM fractions, confirming that lower MW DOM is more favorable for degradation by microorganisms. However, surface water has significantly higher PHI and MTI than groundwater (Fig. S6a).

4. Discussion

4.1. Arsenic association in Fe colloids

Iron mainly exists as large-size colloids in groundwater. As shown in Fig. 3, Fe concentrations change greatly in the >100 kDa and 10–100 kDa fractions. Pourret et al. (2007) found that in the shallow groundwater, Fe mostly occurred as inorganic Fe colloids with grain sizes between 0.22 μm and 30 kDa. Gontijo et al. (2017) also observed that most of Fe was distributed in the fraction of larger sizes (> 10 kDa) in the freshwater.

Groundwater As is associated with large-size Fe colloids. It is found that there is a positive correlation between As and Fe in the 10 kDa-0.45 μm fraction (Pourret et al., 2007) found that in the shallow groundwater, Fe mostly occurred as inorganic Fe colloids with grain sizes between 0.22 μm and 30 kDa. Gontijo et al. (2017) also observed that most of Fe was distributed in the fraction of larger sizes (> 10 kDa) in the freshwater.

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4.2. Relationship between As and organic matter

Organic matter has a significant role in As accumulation. On the one side, the formation of As-DOM complexes through positively charged amino groups in the DOM or through metal cation bridges can contribute to the high mobility of As (Redman et al., 2002; Saada et al., 2003). On the other hand, the decomposition of bioactive OM, which can be utilized by microorganisms, may result in microbiologically-mediated reduction of Fe(III) oxides to Fe^{2+} and reduction of As(V) to As(III) (Fendorf et al., 2010; Guo et al., 2019), which lead to As release from solid surfaces.

The formation of As–Fe–DOM complexes enhances As mobility in the studied groundwater systems. The humic-like components in groundwater are higher than those in surface water (Fig. S5), which is consistent with the observation of Chen et al. (2010). Among the relative abundances of three PARAFAC components, only C1 (%) is positively correlated with As concentrations (Pourret et al., 2007) found that in the shallow groundwater, Fe mostly occurred as inorganic Fe colloids with grain sizes between 0.22 μm and 30 kDa. Gontijo et al. (2017) also observed that most of Fe was distributed in the fraction of larger sizes (> 10 kDa) in the freshwater.

4.3. Bioreactivity of low MW OM and its role in As mobilization

The low MW DOM, having high bioreactivity, also promotes As mobility by providing carbon source and electrons for microbiologically-mediated reduction of Fe(III) oxides. During the sequential ultrafiltration of groundwater, lower HIX and higher BIX, FI (Fig. 4), PHI and MTI
values (Fig. S6) were observed in < 5 kDa fractions, which are indicative of more-processed microbial and more biologically labile DOM in low MW fractions. Schittich et al. (2018) also found that in shallow groundwater with high dissolved As, protein-like components were associated with small molecular size fractions (∼ 2 kDa). Normally, low MW DOM is more susceptible to microbial degradation, and therefore has higher BIX values (Huang et al., 2015). Under reducing conditions, the biologically labile DOM can theoretically drive the microbial reduction of Fe oxides and/or adsorbed As, and therefore mobilize As (Kulkarni et al., 2017; Guo et al., 2019). Furthermore, more than 50% of dissolved As is in the ‘truly dissolved’ phase in most groundwater samples (Huang et al., 2015). Under reducing conditions, the biologically labile DOM can theoretically drive the microbial reduction of Fe oxides and/or adsorbed As, and therefore mobilize As (Kulkarni et al., 2017; Guo et al., 2019). Furthermore, more than 50% of dissolved As is in the ‘truly dissolved’ phase in most groundwater samples (Fig. 2). In Group I samples, although the variations of As concentrations are not obvious during ultrafiltration, more than 90% of As is present in the < 5 kDa fraction (Fig. 2), and four samples have As concentration greater than 600 μg/L (Table 1). This result indicates that, in addition to the complexation reaction with Fe and/or OM, more As would be mobilized via reductive dissolution of Fe oxides with low MW DOM as carbon sources for microorganisms.

In summary, it is speculated that organic matter in the 5–10 kDa fractions promotes the accumulation of As in groundwater through As complexation, whereas the low MW DOM in the "truly dissolved" phase of < 5 kDa fraction mainly acts as carbon sources for microorganisms leading to the reduction of Fe oxide/hydroxides, which promotes the release of As.

5. Conclusions

Two major types of colloids, namely large-sized Fe colloids and middle-sized organic colloids, were observed in the studied groundwater. Iron concentrations are positively correlated with As in large-sized (10 kDa-0.45 μm) fractions, while DOC and As have a positive correlation in middle-sized (5–10 kDa) fractions. The results indicate that both the large-sized Fe colloids and the middle-sized organic colloids have the ability to bind to As. The positive correlation between C1 (%) and As in < 0.45 μm fractions further evidences that the OM involved in the complexation reaction would be mainly terrestrial-derived HS. High MW DOM with the higher HIX and SUVA254 values, showing a high degree of humification and aromaticity, is more conducive to the complexation between As and DOM. In the middle-sized organic colloids, the phenolic hydroxyl functional groups of humic acid may readily bind to As and enhance As mobility. In Group I samples, more than 90% of dissolved As is present in the ‘truly dissolved’ phase (< 5 kDa), indicating that As enrichment in these groundwaters is not only the result of As-Fe-HS complexation. The low MW DOM with the higher BIX, FI, PHI and MTI values shows the greater biological activity and more microbe-associated OM, which can be better utilized by microorganisms. The presence of low MW OM, promoting microbial respiration, leads to As enrichment in groundwater via microbially-mediated reductive dissolution of As-containing Fe oxides/hydroxides. Therefore, both As-Fe-HS complexation as large-size and middle-size colloids and microbially mediated reductive dissolution of As-bearing minerals triggered by low MW OM result in As mobilization and enrichment in groundwater. The large-size and middle-size colloids with chemical activity and the low MW DOM with biological activity have substantial contributions to As mobility in high As groundwater. However, due to the limitations of EEM method, future investigation should be focused on molecular characteristics of DOM and their roles in As behavior in groundwater systems in combination with field analysis.

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Appendix A. Supplementary data

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References


