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Schittich, Anna-Ricarda; Wünsch, Urban; Kulkarni, Harshad; Battistel, Maria; Bregnhøj, Henrik; Stedmon, Colin ; McKnight, Ursula S.

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Characterization of Natural and Affected Environments

Investigating fluorescent organic matter composition as a key predictor for arsenic mobility in groundwater aquifers

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1 Investigating fluorescent organic matter composition as a key predictor for

2 arsenic mobility in groundwater aquifers

- Anna-Ricarda Schittich ^{a,*}, Urban J. Wünsch ^{b,c}, Harshad V. Kulkarni ^d, Maria Battistel ^a, Henrik
 Bregnhøj ^e, Colin A. Stedmon ^c, Ursula S. McKnight ^a
- 5
- 6 ^a Department of Environmental Engineering, Technical University of Denmark, Bygningstorvet,
- 7 Building 115, 2800 Kgs. Lyngby, Denmark
- 8 ^b Water Environment Technology, Chalmers University of Technology, Architecture and Civil
- 9 Engineering, Sven Hultins Gata 6, 41296 Gothenburg, Sweden
- ^c National Institute of Aquatic Resources, Technical University of Denmark, Kemitorvet, Building 202,
- 11 2800 Kgs. Lyngby, Denmark
- ^d Department of Geology, Kansas State University, Manhattan, KS, USA, 66502
- ¹³ ^e School of Global Health, University of Copenhagen, 1353 Copenhagen K, Denmark
- ^{*} To whom the correspondence should be addressed: Email: annsc@env.dtu.dk
- 15
- 16
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- 18
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21 Graphical abstract



24 Abstract

25 Dissolved organic matter (DOM) is linked to the heterogeneous distribution of elevated arsenic (As) in 26 groundwater used for drinking and irrigation purposes, but the relationship between DOM 27 characteristics and arsenic mobility has yet to be fully understood. Here, DOM from groundwater 28 sampled in the Bengal Basin region was characterized using both conventional bulk emission-excitation 29 (EEM) spectroscopy and high-performance size exclusion chromatography coupled to spectroscopy 30 (HPSEC-EEM). Notably, application of the novel HPSEC-EEM approach permitted the total 31 fluorescence of individual samples to be independently resolved into its underlying components. This 32 allowed the external validation of the bulk-sample fluorescence decomposition and offered insight into 33 the molecular size distribution of fluorescent DOM. Molecular size distributions were similar for the 34 UVA fluorescent (C_{310} , C_{340}) as well as the three visible fluorescent (C_{390} , C_{440} , C_{500}) components. There 35 was a greater visible fluorescence in shallow aquifer samples (10-33 m) with high As (SH, up to 36 418 μ g/L) than in samples from the same depth with lower As (up to 40 μ g/L). This indicated a link 37 between DOM quality and As mobility within the shallow aquifer. The deep aquifer samples (170–200 m) revealed DOM characteristics similar to SH samples but had low As concentrations (<4µg/L), 38 signifying that the deep aquifer is potentially vulnerable to As contamination. These findings pave the 39 40 way for a more comprehensive assessment of the susceptibility of drinking water aquifers, thereby 41 supporting the management of groundwater resources.

43 1 Introduction

44 Many groundwater aquifers serving as the primary drinking water resource in Asia are severely polluted 45 with arsenic (As). Some of the most problematic areas are located in the geological formations of the 46 Bengal Basin, which cover large parts of West Bengal (India) and Bangladesh. Total As concentrations 47 considerably exceeding the Indian drinking water standard of 10 μ g/L (50 μ g/L in the absence of alternative water sources) are a widespread concern for public health. ^{1–8} The degradation of labile 48 49 dissolved organic matter (DOM) is considered to be the main driver for As mobilization in this area. ^{2,3,9,10} Specifically, the microbial utilization of DOM causes reducing conditions in the aquifer which 50 subsequently leads to the dissolution of As-bearing oxide minerals, as well as the release of As sorbed 51 onto iron hydr(oxides).^{2,3,7,11-13} While sedimentary DOM from peat layers, ^{9,14-16} as well as surface-52 derived organic carbon, ^{17–19} have been suggested, the source of labile DOM is still a matter of much 53 debate.^{20,21} Beyond labile DOM, recent studies also highlight the potential influence of humic and fulvic 54 acids (refractory organic polymers) in As mobilization through electron shuttling, ^{22–25} or competition 55 for sorption, ²⁶ and complexation processes. ^{27,28} Since the chemical composition of DOM defines its 56 reactivity in the environment, ^{29,30} the ability to track the different chemical fractions of DOM is essential 57 58 to better understand its role on arsenic mobilization in groundwater.

Over the last decades, absorbance and fluorescence spectroscopy have increasingly been applied to 59 characterize groundwater DOM in relation to As contamination.^{8,24,31,32} The fluorescing fraction of 60 DOM, fluorescent dissolved organic matter (FDOM), can be characterized by recording fluorescence 61 62 emission as a function of excitation wavelength. The resulting emission-excitation matrices (EEMs) can further be mathematically decomposed into their underlying individual fluorescent phenomena by 63 methods such as parallel factor analysis (PARAFAC). ^{33,34} Recent studies used fluorescence ratios, such 64 65 as freshness and the humification index, as well as PARAFAC modelling to analyze shallow groundwater samples from West Bengal and have observed a link between humic-like DOM and high 66 As concentrations.^{8,32,35} However, a common caveat in PARAFAC modeling is that datasets must 67 68 contain sufficient variability in fluorescent properties; therefore large datasets (> 100 samples) are

69 preferable. ³⁶ This represents a barrier preventing the widespread implementation of this method in 70 practice, i.e. in field studies, as it is often not possible to collect such large datasets due both to financial 71 and practical constraints.

72 Recently, a new analytical framework has been developed that produces >1000 EEMs originating from 73 a single water sample, which allows the mathematical decomposition of fluorescence in single samples. ³⁷ This is achieved through the online-coupling of high-performance size exclusive chromatography 74 75 (HPSEC) with fluorescence EEM spectroscopy. Application of the HPSEC approach also links 76 fluorescence phenomena to their molecular size distributions, an important indicator that may reveal information about the susceptibility to degradation of different DOM compounds. ^{38,39} HPSEC coupled 77 to spectroscopic measurements additionally offers the possibility to investigate interactions between 78 79 DOM and other elements such as As in contaminated aquifers.²⁸

80 The overall goal of this study was to characterize groundwater DOM taken from a village in West Bengal 81 by applying fluorescence spectroscopy and to investigate links to As mobilization. We aimed to 82 characterize FDOM of bulk samples and link fluorescence to molecular size by applying HPSEC-EEM-83 PARAFAC for groundwater samples. Based on this information, we aimed to group samples taken from 84 different aquifers with respect to the hydrochemistry. The final goal was to establish links between 85 FDOM characteristics and the abundance of As, i.e. to investigate whether distinct fluorescent 86 characteristics can be observed for samples from specific aquifers with differing arsenic content (below 87 and above the local As drinking water standard of 50 μ g/L). This could provide information about the 88 mechanisms driving As distributions and ultimately support the management of groundwater resources.

89 2 Material and Methods

90 2.1 Study site

91 The field study site is located in the village of Gazna (22° 54' 15.9" N, 88° 48' 28.7" E), in the western 92 part of the Bengal Basin. The geology of the site shows relatively shallow, organic rich, Holocene

93 sediments (<11 thousand years before present [bp]) separated from deeper Pleistocene sand and gravel deposits (2600–12 thousand years bp) by a low permeable clay layer, typical of the Bengal Basin.^{40–44} 94 95 The vertical extension of Holocene sediments varies locally, and while it can reach depth levels up to 100 meters below ground surface (mbgs), ^{45,46} it may also be limited to much shallower depths. ^{47,48}A 96 97 study located in the Jamuna sub-basin (roughly 30 km west of this study site) described the interface between Holocene and Pleistocene sediments at approximately 30 mbgs.⁴⁹ The Holocene aquifer is 98 99 henceforth referred to as the shallow aquifer and is locally used for domestic and irrigation purposes. 100 Several authors suggest this aquifer system displays a very complex, heterogeneous structure, comprised 101 of a series of sub-aquifers separated by thin clay layers that may extend for up to a few kilometers horizontally. ^{50–52} Precise geochemical and hydrologic information about this aquifer at the study site is 102 very limited, even though the lithology in neighboring areas is known.⁴⁹ Shallow aquifer samples have 103 been documented throughout this region by As concentrations greatly exceeding the Indian drinking 104 water standard, reaching 1000 μ g/L, ⁵ where the sediment is most commonly described as grey-colored 105 and anoxic. ⁴⁶ Holocene sediment cores from a neighbouring district (ca. 75 km south-west of our study 106 site) revealed As concentrations up to 12.6 mg/kg sediment, ⁵³ where the Holocene sediments are 107 overlain by a silty clay layer of overbank deposits forming the surface aquitard. ⁵⁴ 108

109 The primary aquifer, henceforth referred to as the *deep aquifer*, flows in the Pleistocene sediments and 110 is located ca. 170 mbgs at this study site. In contrast to the high lateral variability found in the shallow 111 aquifers, groundwater extracted from depths >150 mbgs rarely contain As concentrations above 50 112 μ g/L. ^{44,55} Low dissolved As concentrations in deep aquifers with reducing conditions were previously 113 reported due either to a low As source in the sediment, ^{46,56,57} or the presence of pyrite acting as an As 114 sink, ^{58,59} while oxidized Pleistocene sediments showed a large capacity for adsorbing dissolved As 115 consequently leading to low As concentrations in the water. ⁶⁰

116 **2.2 Investigation strategy and sampling**

117 The local Indian NGO (non-governmental organization) Kishalaya Tarun Tirtha (KTT) and the Danish

118 NGO Ulandsforeningen for Bæredygtig Udvikling (UBU) have worked together on awareness and

supported a number of measurement campaigns in the village of Gazna since 2004 (Supporting Information (SI), Fig. S1). For this study, additional fieldwork was carried out in April 2017 in cooperation with the two NGOs. To obtain an overview of the level of As contamination in the surrounding study area and to guide the additional collection of samples, existing groundwater borehole (i.e. tube well) data was used to create preliminary maps in ArcGIS (v.10.3) applying inverse distance weighting (IDW) interpolation (for more details, see SI Methods, SI Figs. S1-S2).

125 For the laboratory analysis, a total of 50 samples were collected. Forty were sampled from the shallow 126 aquifer system containing both low (< 50 μ g/L) and high (> 50 μ g/L) total dissolved As concentrations 127 (SI Fig. S2). For the deep aquifer, no initial data was available and thus groundwater was collected from 128 nine deep tube wells distributed throughout the different parts of the village (SI Fig. S2) and one deep 129 tube well of a school, just outside the village borders. Note that Gazna is one of the villages where the 130 exceptional standard of 50 µg/L applies for arsenic. All samples were collected in April 2017 during a very dry period, just before the start of the rainy season. All samples for the chemical analysis (see 131 section 2.3) were immediately filtered through a 0.45 µm nylon filter for particulates removal and 132 acidified with nitric acid (HNO₃) to pH < 2. Samples for absorbance and fluorescence spectroscopy 133 (section 2.4) were not acidified to avoid fluorescence quenching, ⁶¹ and filtered through a 0.2 µm nylon 134 filter. ⁶² After collection, the samples were stored at 4-8 °C for a maximum of 2-7 days and refrigerated 135 during transportation to the Technical University of Denmark (DTU) for analysis. Upon arrival, samples 136 137 were stored at 4 °C in the dark.

138 **2.3 Chemical analysis**

Concentrations of As, phosphorus (P), iron (Fe) and manganese (Mn), as well as dissolved organic carbon (DOC) were measured for all sampling sites. Dissolved As and P were measured using inductively coupled plasma mass spectrometry (ICP-MS) ca. 15 days after collection using an Agilent Technologies 7700 Series ICP-MS. Detection limits were 1.0 µg/L and 13.0 µg/L for As and P, respectively. Fe and Mn were measured with inductively coupled plasma optical emission spectrometry 144 (ICP-OES) within five weeks of collection. The measurements were carried out with a Perkin Elmer 145 AvioTM 200 duel view ICP-OES. Detection limits were 0.3 μ g/L and 0.1 μ g/L for Fe and Mn, 146 respectively. DOC was measured within two weeks of collection using high-temperature combustion 147 (TOC-VWP, Shimadzu) in the form of non-volatile organic carbon (NVOC) with a detection limit of 148 0.07 mg/L.

149 **2.4 Spectroscopic data**

Absorbance and fluorescence spectroscopy was carried out with two approaches described in detailbelow.

152 **2.4.1 Bulk EEM spectroscopy**

153 Spectroscopic data for all 50 bulk samples was obtained within three weeks after sample collection using a Horiba Aqualog fluorometer with a quartz cuvette of 0.01 m path length. Absorbance data was 154 collected between 240-600 nm (increment 3 nm). Specific UV absorbance at 254 nm (SUVA₂₅₄) was 155 used to obtain information about the aromatic carbon content of the samples and calculated as the ratio 156 of absorbance intensity at 254 nm, divided by the product of path length and DOC concentration.⁶³ 157 Fluorescence emission was recorded at excitation wavelengths between 240-600 nm at 3 nm increments 158 159 and then measured at emission wavelengths between 240-600 nm at increments of \sim 3.28 nm (instrument default). The integration time varied between 1 s and 3 s, depending on the carbon concentration of the 160 161 samples.

To investigate a possible interference from the high As concentrations (i.e. quenching effects) on the
fluorescence measurements, two batch experiments were carried out as described in the SI (SI Methods).

164 **2.4.2 HPSEC-EEM spectroscopy**

Seven samples were additionally analyzed with HPSEC coupled to absorbance and fluorescence detection within seven months of sample collection, including samples from the shallow aquifer with low dissolved As (< 50 μ g/L, SL); samples from the shallow aquifer with high dissolved As (> 50 μ g/L,

SH) and samples from the deep aquifer (D). HPSEC was performed using a Shimadzu Nexera X2UFLC system equipped with a TSKgel SuperAWM-H column and two sequential detectors, following the methodology described elsewhere. ³⁷ Absorbance was measured between 240-700 nm at 1 nm intervals using a Shimadzu SPD-M30 detector. Fluorescence emission was detected between 300-600 nm with an interval of 5 nm across an excitation of 240-450 nm (increment 5 nm between 240-360 nm, then 10 nm between 360-450 nm) using a Shimadzu RF-20Axs detector (for more details, see SI Methods and Wünsch et al.³⁷).

175 **2.4.3 Data processing**

For both datasets (obtained from bulk EEM and HPSEC-EEM measurements), EEMs were processed using the drEEM toolbox (v.0.3.0) in MATLAB 9.3. ⁶⁴ For bulk EEMs, inner filter effects were corrected with the absorbance-based approach, which was appropriate for the absorbance intensities of the dataset. ⁶⁵ After the subtraction of a blank EEM, first and 2nd order physical scatter was removed and interpolated only for the first order Rayleigh scatter. HPSEC-EEMs were corrected following the protocol described elsewhere. ³⁷ The fluorescence intensity in all EEMs was normalized by division of fluorescence counts with the Raman peak area at 350 nm.

For both datasets, the humification index (HIX) was calculated from the emission intensities at the peak area 435-480 nm divided by the sum of the peak areas 300-345 nm and 435-480 nm at an excitation wavelength of 254 nm. ⁶⁶ Fluorescence index and freshness index were calculated as described elsewhere. ^{67,68} UV fluorescence at peak T, which represents the signal at 275 nm/340 nm (Ex/Em), was extracted from the datasets. ^{69,70} Peak C was extracted at 320-360 nm/420-460 nm (Ex/Em) as a measure for the visible (Ex > 300 nm) fluorescence emission region. ^{69,70}

189 **2.5 Chemometric analyses**

190 PARAFAC was used to identify the fluorescence components that comprise bulk and HPSEC EEMs.

191 Independent PARAFAC models were fit to the 50 bulk EEMs, as well as the EEMs resulting from

HPSEC-EEMs. Models were constrained to non-negativity; a relative change of 10⁻⁷ from one iteration to the next was used as a convergence criterion. For the classical PARAFAC model, a dataset of 48 samples was used after removing two outliers. With only 48 samples, we were able to split-validate a four-component model. The spectral loadings of models with up to six components appeared reasonable, but could not be validated.

197 For HPSEC-EEMs, one PARAFAC model was fit to a dataset consisting of a total of 1330 EEMs. This 198 dataset was comprised of 190 equally spaced EEMs from the seven samples (190 x 7) measured by 199 HPSEC-EEM between elution volumes of 3.1 and 5 mL. This is equal to one EEM every 10 µL or 2s 200 in the separation. The best fit was obtained with an overall model with five components, which was 201 subsequently fit to the full datasets of all seven samples. To evaluate the appropriateness of this 202 approach, sum-of-squared-errors for the global model in all seven samples were subsequently 203 investigated. Fitting the global solution to individual samples resulted in less than 0.05 % unexplained 204 variance in all cases (SI Fig. S3). Furthermore, we used the spectral comparison between the un-205 validated bulk FDOM five-component model fit to the 48 bulk EEMs and the five-component model fit to the 1330 HPSEC EEMs to provide a means to externally validate both models. The Tucker 206 congruence coefficient (TCC) was used to assess spectral congruence between components.^{71,72} 207

208 2.6 Statistical data treatment

209 Principal Component Analysis (PCA) was used to group and characterize the groundwater samples according to their geochemical (As, Fe, Mn, P) and spectral composition (SUVA254, PARAFAC 210 components). Ordination techniques, such as PCA, can be combined with cluster analyses (here, 211 212 hierarchical cluster analysis (HCA) was used, applying a SIMilarity PROFile analysis (SIMPROF) p < 0.05) to obtain a better interpretation of the ordination diagrams and help identify wells belonging to 213 the same clusters. ⁷³ Data were 4th root transformed across the sample dimension to equalize sample 214 leverage, and centered and scaled across variables to equalize variable leverage (zero mean, unit 215 216 variance) before the PCA and cluster analyses were performed.

217 **3 Results and Discussion**

218 **3.1** Fluorescence composition and molecular size distributions of DOM

The optical properties of bulk groundwater samples were typical for DOM samples from this region. Fluorescence intensities at peak T and peak C varied from 0.06-0.42 R.U. and 0.09-1.32 R.U., respectively. HIX and SUVA₂₅₄ showed values from 0.56-0.89 and 0.82-6.84, respectively (SI Tables S1-S2). The fluorescence index was relatively constant throughout all samples (1.64-1.89) while the freshness index varied between 0.75-1.26, where smaller values are representative for more decomposed DOM. ⁷⁴

225 PARAFAC conducted on the 50 bulk samples revealed that four to six components described 99.83-99.92% of the variability. The successfully validated four-component PARAFAC model featured one 226 UVA fluorescent (protein-like) component, as well as three visible wavelength (humic-like) components 227 (Ex. > 300 nm) (SI Fig. S4a). Despite spectral loadings that resembled pure fluorophores, a five-228 229 component model could not be validated (SI Fig. S4b). Therefore, HPSEC-EEM-PARAFAC was used 230 to externally cross-validate the five-component bulk PARAFAC model based on the seven-sample 231 subset (Fig. 1a-e). The results show that the fluorescence signal can be decomposed into similar components (Fig. 1a-e), named C₃₁₀, C₃₄₀, C₃₉₀, C₄₄₀ and C₅₀₀ according to their fluorescence emission 232 233 maxima with respective excitation maxima at 265 nm, 280 nm, 305 nm, 350 nm and 390 nm. However, 234 the external cross-validation revealed some degree of dissimilarity for C_{310} and C_{340} , (Fig. 1a-b). Considering that the comparison of models fit to data obtained on separate instruments under different 235 conditions is challenging, ⁷⁵ the failure to obtain highly similar fluorescence components at low 236 237 wavelengths most likely reflects the varying degree of influence of scatter peaks at low wavelengths between detectors. For C₃₉₀, C₄₄₀ and C₅₀₀ (Fig. 1c-e), conventional validation thresholds were met for 238 congruence between components (TCC > 0.95). ⁷⁶ Thus, the results offer compelling evidence that bulk 239 240 EEM fluorescence can be described by means of PARAFAC models obtained on a single sample. The 241 external cross-validation approach offers a unique opportunity to validate otherwise unstable PARAFAC models from a small dataset. The UVA fluorescence components C_{310} and C_{340} have previously been defined as protein-like components, ^{75,77,78} and show a mean contribution of 11% and 19% to the total fluorescence, respectively. The visible fluorescence components C_{390} , C_{440} and C_{500} show mean contributions of 26%, 34% and 10%, respectively and are often defined as humic-like. ^{8,78–} ⁸⁰ C_{390} and C_{440} match with two components (C2, C1) found in shallow groundwater at a nearby site (ca. 75 km distance), ⁸ reaching similarity scores of 0.99 and 0.97, respectively.

248 The utilization of HPSEC-EEM analysis permitted the in-depth analysis of apparent molecular size 249 distributions of FDOM for the seven-sample subset (see definition in section 2.4.2). In general, HPSEC 250 yields apparent molecular size distributions of DOM after calibration with a standard. However, 251 apparent size distributions may be influenced by the occurrence of secondary interactions with the 252 column, ⁸¹ i.e. adsorption of hydrophobic compounds resulting in artificially low molecular weight. ⁸² 253 Distributions are therefore shown as a function of elution volume (Fig. 1f-j), which is generally inversely 254 correlated to molecular size. Fractions eluting at a volume larger than 4 mL are outside the calibration 255 range (i.e. appear smaller than 20 Da), but the separation still offers insight into DOM composition 256 unrelated to molecular size.

UVA fluorescence (C₃₁₀, C₃₄₀) revealed two elution peaks in the larger molecular size fraction at ca. 3.3 257 258 mL and 3.5 mL (~2 kDa and ~0.6 kDa, respectively) and a third elution peak in the smaller molecular 259 size fraction at ca. 4.0 mL (\sim 20 Da), where the contribution of secondary interactions probably led to the artificially large elution volume (Fig. 1f-g). For the SL samples, fluorescence of component C₃₁₀ is 260 261 clearly associated with smaller molecular size fractions (the third peak is dominant in SL samples) while 262 a shift towards the larger molecular size fraction tends to occur for SH and D samples (Fig. 1f). A similar pattern was observed for C₃₄₀, which is mainly associated with the larger molecular size fraction for SH 263 264 and D samples, while for SL samples, the third peak also contributes considerably to the fluorescence 265 signal (Fig. 1g). Visible fluorescence (C₃₉₀, C₄₄₀, C₅₀₀) is consistently associated with the large size 266 fraction (two peaks at ca. 3.3 mL and 3.5 mL) independent of the sample group (Fig. 1h-j), but is 267 considerably higher for the SH and D samples compared to SL samples. Overall, the HPSEC-EEM-12 PARAFAC analysis indicated similar fluorescence distributions over the chromatograms between the two protein-like, as well as the three humic-like components. Small shifts in molecular size distribution and different fluorescence intensities for different sample groups point towards a qualitative difference in DOM among the samples.



Figure 1: Cross-validation (a-e) of the five-component bulk PARAFAC model (blue) by using an independent HPSEC-EEMPARAFAC model (black) and TCC values indicating similarity scores between the two models for the excitation (first number,
dashed line) and emission (second number, solid line) curves. For each PARAFAC component (a-e), the distribution of the
maximum fluorescence intensity (F_{max}) over the chromatogram is shown for all 7 samples analyzed by HPSEC (f-j).

277 **3.2 Hydro-chemical classification of aquifers**

278 The characterization of the shallow and deep aquifers was based on dissolved As, Fe, Mn, P and DOC 279 concentrations, as well as absorbance and fluorescence characteristics. The reliability of the preliminary 280 As distribution maps (section 2.2) was tested during the fieldwork by checking the predicted 281 concentrations for several areas. As a result, the maps served as a satisfying tool to distinguish 282 contamination levels (SI Fig. S2), which was especially helpful regarding very limited information and 283 equipment at the study site. The shallow aquifer showed very heterogeneous, and much higher, dissolved As concentrations than the deep aquifer. Dissolved As concentrations ranged from 1-40 µg/L for SL 284 samples, from 57-418 μ g/L for SH samples, and from < 1-4 μ g/L for D samples (SI Tables S1-S2). DOC 285

concentrations did not differ much between the three groups, for all samples DOC varied from 0.64–
3.39 mg/L (SI Tables S1-S2), and were lower than reported in related studies. ^{8,23} However, shallow
aquifer samples (SL; SH) showed considerably higher dissolved Fe, Mn and P concentrations compared
to D samples (SI Tables S1-S2).

290 The PCA based on As, Fe, Mn and P could explain 90.58% of the variance between the samples with 291 two principal components (PC1 + PC2) and the HCA split the samples into four significantly different clusters (SIMPROF p < 0.05, Fig. 2a). With one exception (D sample of 4 µg AsL⁻¹ clustering with SL 292 sample of 1 µg AsL⁻¹), all D samples clustered in one group, indicative of a hydrologically distinct 293 294 (deep) aquifer. A low arsenic concentration may reflect the lack of As-containing iron/manganese 295 minerals in the deep aquifer sediments, which also aligns with the low dissolved Fe and Mn 296 concentrations observed in the D-samples. The shallow aquifer samples split into two clusters where SH 297 samples tended to gather in one cluster. However, a clear separation between SL and SH samples is not given (Fig. 2a), indicating that, although the shallow aquifer has been (arbitrarily) divided into different 298 299 sub-aquifers, the interaction between them is widespread and probably enhanced by the local use of the tube-wells. 300

301 Generally, high concentrations of dissolved Fe and Mn in the shallow aquifer are consistent with other 302 studies in the Bengal Basin and are indicative for the reductive dissolution processes of Fe and Mn minerals, possibly leading to arsenic mobilization.^{11,83} However, this does not explain the very 303 304 heterogeneous distribution of As within the shallow aquifer. As mobilization due to competition with phosphate (PO₄³⁻), ⁸⁴ or the enhancement of OM degradation through phosphate, ⁷ might be relevant 305 306 processes to analyze further. Moreover, groundwater age linked to hydrogeological conditions in the flow system may also play a role in determining the spatial As distribution, for example through flushing 307 of As.⁸⁵ Hydrogeological conditions, likely influenced by pumping activities, can additionally cause 308 the cycling of labile organic carbon, ^{44,86} possibly leading to a spatial variability in DOM source and 309 guality along with varying redox potential.³² For example, Harvey et al.,⁴⁴ found that the mobilization 310 311 of As may be driven by young carbon transported to depth through irrigation pumping.

312 Even though DOC concentrations were similar for all samples in this study, the degree of colored 313 material differed between samples. A PCA based on As, C310, C340, C390, C440, C500 and SUVA254 (Fig. 314 2b) explained 81.38% of the variance with PC1 and PC2 and indicated a positive correlation between 315 most of the SH samples and the visible fluorescent components (C_{390} , C_{440} , C_{500}) as well as SUVA₂₅₄, 316 indicating increasing aromaticity. SL samples were mostly located diagonally opposite to C_{390} , C_{440} , C_{500} 317 and SUVA₂₅₄ (negative correlation). This points towards a qualitative difference in DOM between the 318 samples of the shallow aquifer possibly linked to different DOC sources. The two UVA components 319 (C_{310}, C_{340}) were negatively correlated to As. SH and D samples mainly differed in PC2 (y-axis), driven by the difference in As concentration (Fig. 2b). 320



Figure 2: (a) Principal component analysis (PCA) based on As, Fe, Mn and P, and (b) PCA based on As, SUVA₂₅₄ and the five PARAFAC components. Triangles indicate the seven samples additionally analyzed with HPSEC. Black circles indicate significantly different cluster groups based on HCA.

325 **3.3 Links between DOM composition and As mobility**

General fluorescence parameters (peak T, peak C, HIX) and their distribution over the chromatogram were used to investigate whether distinct fluorescent characteristics can be observed for the three different sample groups (SL, SH and D). The general fluorescence characteristics of one representative sample for each group are shown in Fig. 3 (characteristics for all samples are given in SI Figs. S5-S9).
DOM fluorescence intensity was lower for SL samples (0-0.2 R.U.) than for SH (0-1.2 R.U.) and D (00.8 R.U.) samples (Fig. 3a-c, z-axis), whereby the high fluorescence intensities in the SH and D samples
align with a high fluorescence in the C peak region. The distribution of peak T (UV fluorescence) and
peak C (visible fluorescence) over the chromatogram (Fig. 3d-e) is comparable with the
chromatographic distribution of the UV and visible fluorescence PARAFAC-components, respectively
(Fig. 1f-j) (for detailed chromatographic peak positions of peak T and peak C, see SI Table S3).

336 HIX, which is roughly comparable to the ratio of peak T to peak C, displays clear differences along the 337 chromatogram and a consistent pattern can be seen across all three sample groups (Fig. 3d-f). Our results 338 suggest that, along the chromatogram (changing molecular size), an increase in HIX is related to a 339 decrease in protein-like fluorescence. The late minimum (4.0 mL) in HIX in the chromatograms results 340 from a relative increase in the T peak fluorescence (Fig. 3d-e) even though fluorescence is low (Fig. 3f). 341 The greatest variability in the chromatograms across the three sample groups was due to the increase of 342 peak C for SH and D samples (Fig. 3d-f), whereas peak T was relatively comparable. This drove the 343 slight increase in the measured HIX in the bulk samples (SI Tables S1-S2) and is linked to the process 344 of humification where labile substrate is transformed into material with visible wavelength fluorescence 345 (peak C). Peak T and C are often referred to as protein-like and humic-like fluorescence, respectively. 70 346

From this perspective, our results for the shallow aquifer fit with the findings of Kulkarni et al.,^{8,35} who 347 348 characterized bulk sample DOM from shallow groundwater with high arsenic concentrations in a nearby 349 study area as more humic-like, compared to samples with low arsenic concentrations. To test if our 350 findings reflected other effects such as fluorescence quenching, rather than differences in DOM 351 characteristics for SL and SH samples, two batch experiments were carried out (SI Methods) where a loss of fluorescence signal, as would be expected from quenching effects, could not be observed (SI Fig. 352 S10-S11). Quenching effects caused by high iron concentrations might also play a role, ⁸⁷ however, they 353 354 appear to not be driving the variability observed in our samples. Sample SH26 has similar fluorescence 16 355 characteristics to sample D4 (compare Fig. 3e and Fig. 3f), despite an order of magnitude difference in iron concentrations (4.20 mg/L and 0.67 mg/L, respectively). Similarly, the SH samples have relatively 356 357 higher Fe concentrations and fluorescence compared SL samples. If quenching was important in driving the trends seen one would expect an inverse correlation. The different EEM characteristics therefore 358 likely arise from qualitatively different DOM, which promotes or prevents As mobility in groundwater. 359 360 Remarkably, the deep aquifer (low in As) shows DOM characteristics similar to SH, not SL samples, 361 having a high fluorescence in the humic-peak region (compare Fig. 3b-c, 3e-f). The similarity between 362 SH and D samples is also reflected in the PCA in Fig. 2b, where SH and D samples show similar characteristics along PC1 (x-axis). The combination of low As and dominant humic peak may indicate 363 364 an aquifer with reducing conditions where, however, the source of As is missing in the sediment. Even though low concentrations of sedimentary As (1.8 mg/kg, average crustal abundance) are sufficient to 365 mobilize As with dissolved concentrations greater than 10 µg/L, ⁸⁸ it has been documented that sorbed 366 As has been removed from the Pleistocene sediments in the Bengal Basin delta, ^{2,3,57} and Zheng et al.⁸⁹ 367 368 suggested that the concentration of mobilizable As in the sediment is an important variable regarding low As concentrations in the deep aquifer. Such conditions could potentially make the deep aquifer 369 370 vulnerable to arsenic contamination, through drawdown of As from the shallow aquifer by deep tube wells, considering that humic-like DOM in the deep aquifer might promote As mobility to groundwater. 371 ^{8,90} The risk of As drawdown to deep aquifers has been addressed by various studies discussing 372 sustainable and As-safe water supply in the Bengal Basin delta and has to be evaluated carefully 373 depending on individual hydrogeological conditions. ^{46,55,91} Moreover, other chemical properties of 374 DOM, which are not directly visible from our DOM characterization might play a role regarding As 375 376 mobility and should additionally be assessed to obtain a more detailed classification. For example, a previous study reported higher C:N ratios for deep, Pleistocene aquifer samples with low As compared 377 to shallow, Holocene aquifer samples with high As concentration.³⁵ 378

Our results present qualitative DOM characteristics for different aquifers and sample groups. It was
 possible to clearly differentiate fluorescence characteristics between SL and SH samples, supporting the

theory that humic-like DOM is linked to As mobility under reducing conditions e.g. through electron shuttling or competition processes. Considering the high similarity between SH and D samples and the likely absence of an As source in the deep aquifer, these DOM characteristics may serve as a useful tool when assessing potential aquifer vulnerability towards As mobility, especially in relation to the management of sustainable groundwater extraction.



Figure 3: Bulk EEM characteristics of the samples SL4 (a), SH26 (b) and D4 (c), as representatives for the three analyzed
 groups (SL, SH, D), and their corresponding chromatograms (d-f) showing peak T, peak C and HIX.

389 4. Perspectives

The successful cross-validation between conventional PARAFAC and HPSEC-EEM-PARAFAC models offers the opportunity to carry out stable data analysis on relatively small datasets, effectually eliminating the barrier typically associated with field sampling (related to number of samples taken), and potentially opening new areas for research related to contaminated groundwater and DOM composition. Additional studies similar to Liu et al., ²⁸ are now necessary, where HPSEC-fluorescence can be coupled to ICP-MS to investigate direct interactions between DOM and metals such as As, Fe, and Mn. If such direct interactions exist, this would reveal which metals associate with which organic

matter size fractions. In addition, respective UV spectroscopic properties of the relevant organic matter
 size fractions could be characterized.

To better understand the relationships between fluorescence, DOM characteristics and arsenic mobility, batch experiments should be conducted based on aquifer sediments collected preferably from areas with well-characterized hydrology and geology. Besides the As content of the sediment, specific redox kinetics and microbial processes, as well as stoichiometry and chemical composition should be further investigated. ³⁵ This is of special interest for assessing the potential vulnerability of the deep, Pleistocene aquifer to As mobilization, which is an important clean source of drinking water in the region.

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414 Associated Content

415 Supporting Information

Supplementary Methods regarding, investigation strategy and sampling, batch experiments and HPSECEEM spectroscopy. Supplementary Figures: mapping plan (ArcGIS maps) (Fig. S1-S2), sum of squared
errors for the one-sample approach (Fig. S3), bulk PARAFAC model (Fig. S4), bulk EEMS for all
samples (Fig. S5-S7), chromatograms (Fig. S8-S9), batch experiments (Fig. S10-S11). Supplementary

- 420 Tables: groundwater and fluorescence characteristics (Table S1-S2), chromatographic positions of peak
- 421 maxima for peak T and peak C (Table S3). The supporting information is available free of charge at
- 422 https://pubs.acs.org/.

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