Supporting Information for:

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

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The authors declare no conflict of interest.

The supporting material contains 24 pages, 11 figures and 3 tables.

Summary of SI contents

S1 Supplementary Methods

Investigation Strategy and Sampling

Preliminary maps were created based on inverse distance weighting (IDW) to estimate areas of unknown As concentrations based on a linearly weighted combination of a set of points with a known concentration. The weight is a function of inverse distance, i.e. the influence of a known point decreases with distance to the unknown cell value. [1] With the help of IDW interpolation, the As contamination level could be estimated for unknown areas based on punctual, known tube-well measurements. Based on this overview of the As distribution, additional As measurements were taken in the field with a portable field device (Palintest Arsenator, Wagtech) that delivers an immediate estimation of the level of As contamination. [2] The purpose of taking new measurements was to extend the dataset and to verify and modify the initial distribution maps. Based on the modified distribution maps, 50 locations were chosen from which samples for laboratory analysis in Denmark were collected (S2 Fig. S2).

IDW maps were created for several depth levels for the shallow aquifer, including [10; 17], [19 ; 25] and [29 ; 33] mbgs, respectively (S2 Fig. S2). These depth levels are expected to represent local sub-aquifers, separated by thin clay layers. Due to uncertain depth information and due to the fact that the sub-aquifers are assumed to be hydrologically interconnected, the study will address the shallow aquifer as one unit.

Batch experiments

To investigate a possible interference from the high As concentrations (i.e. quenching effects) on the fluorescence measurements, the As concentration was stepwise raised to 800 µg/L after

the initial measurement for one sample and the change of fluorescence signal with changing As concentration was analysed. In a second experiment, the As concentration of the same sample was immediately raised to 800 µg/L and the change of fluorescence signal was analysed over three weeks.

HPSEC-EEM spectroscopy

For each of the seven samples analysed with HPSEC-EEM spectroscopy, a sequence of 35 chromatographic runs was performed, whereby instrument parameters where systematically changed for each of the 35 injections to allow the determination of absorbance properties (one run) and fluorescence properties at each excitation wavelength (34 runs). For each sample, this resulted in 1534 individual absorbance spectra and 1534 fluorescence emission scans at each excitation wavelength. The 1534 fluorescence emission scans for each excitation wavelength were then combined to to form EEMs, resulting in 1534 chromatographically separated EEMs per sample.

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S2 Supplementary Figures

Mapping plan (ArcGIS maps)

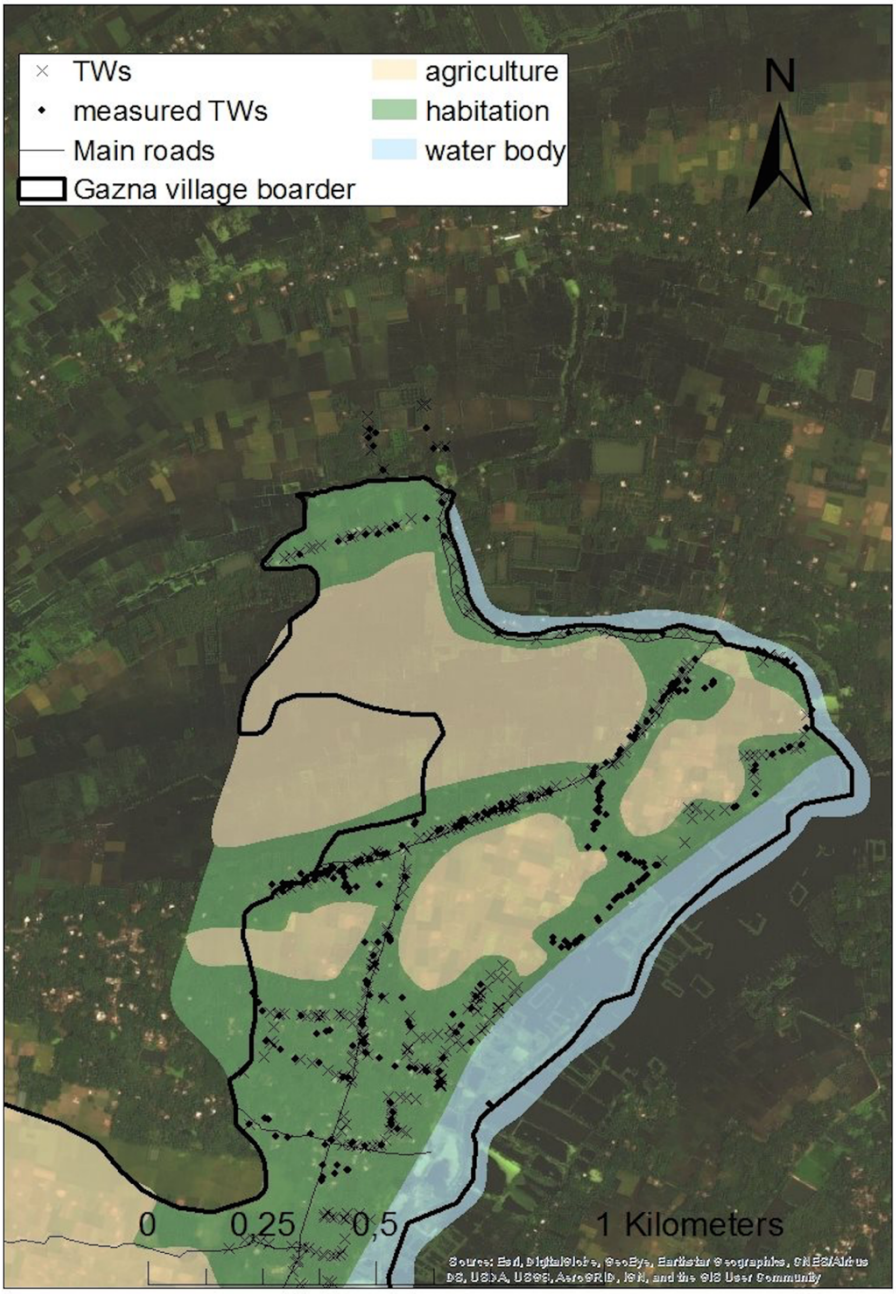

Figure S1: Overview for the registered household tube-wells in Gazna village before the field campaign in April 2017. (No registration of irrigation tube wells)

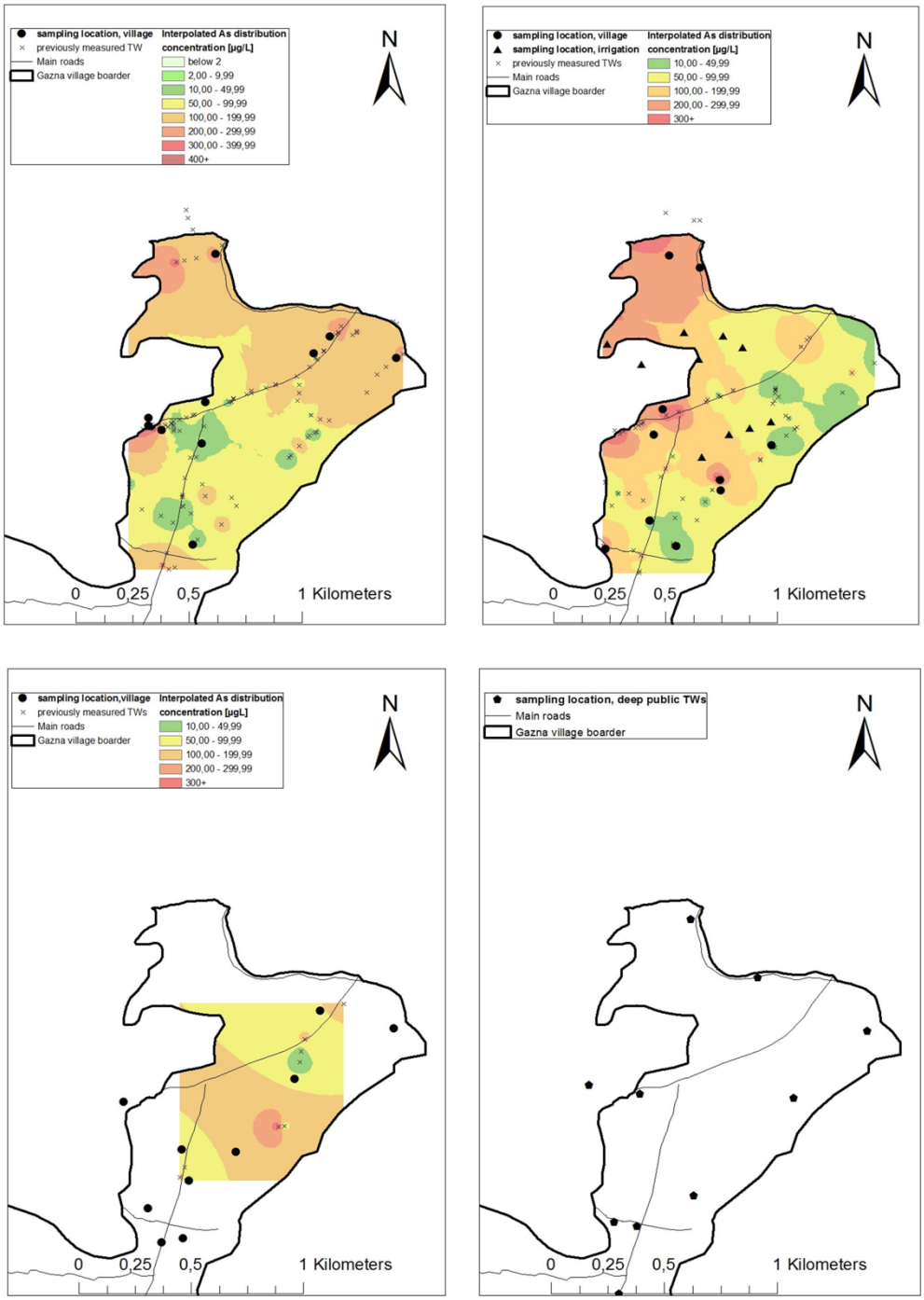

Figure S2: Preliminary maps and sampling locations for the shallow aquifer at 10-17 mbgs (top, left), 19-25 mbgs (top, right), 29-33 mbgs (bottom, left) and for the deep aquifer (bottom, right). **Circles** indicate sampling locations for household tube-wells. **Triangles** indicate sampling locations for irrigation tube-wells. **Pentagons** indicate sampling location for deep public tube-wells.

One-Sample Approach, sum of squared error

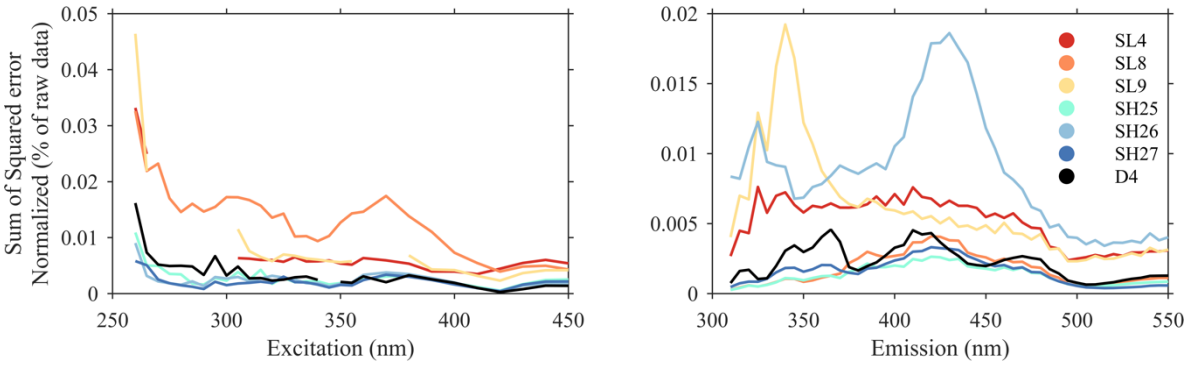

Figure S3: Sum of squared error between the final 'one-sample' model and the seven individual models for excitation (left) and emission curves (right).

Bulk PARAFAC Model

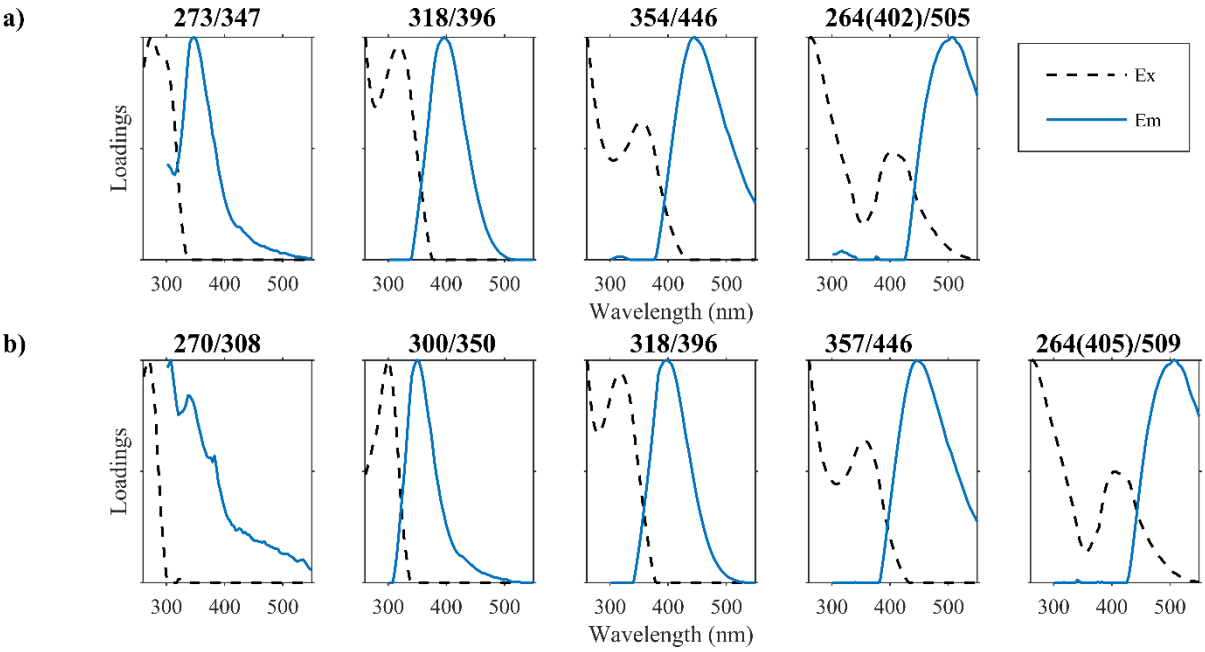

Figure S4: Four (a) and five (b) component bulk PARAFAC model. Excitation curves shown as dashed lines and emission curves shown as solid lines. Location of excitation (Ex) and emission (Em) peaks are indicated as Ex/Em in [nm].

Bulk EEMs

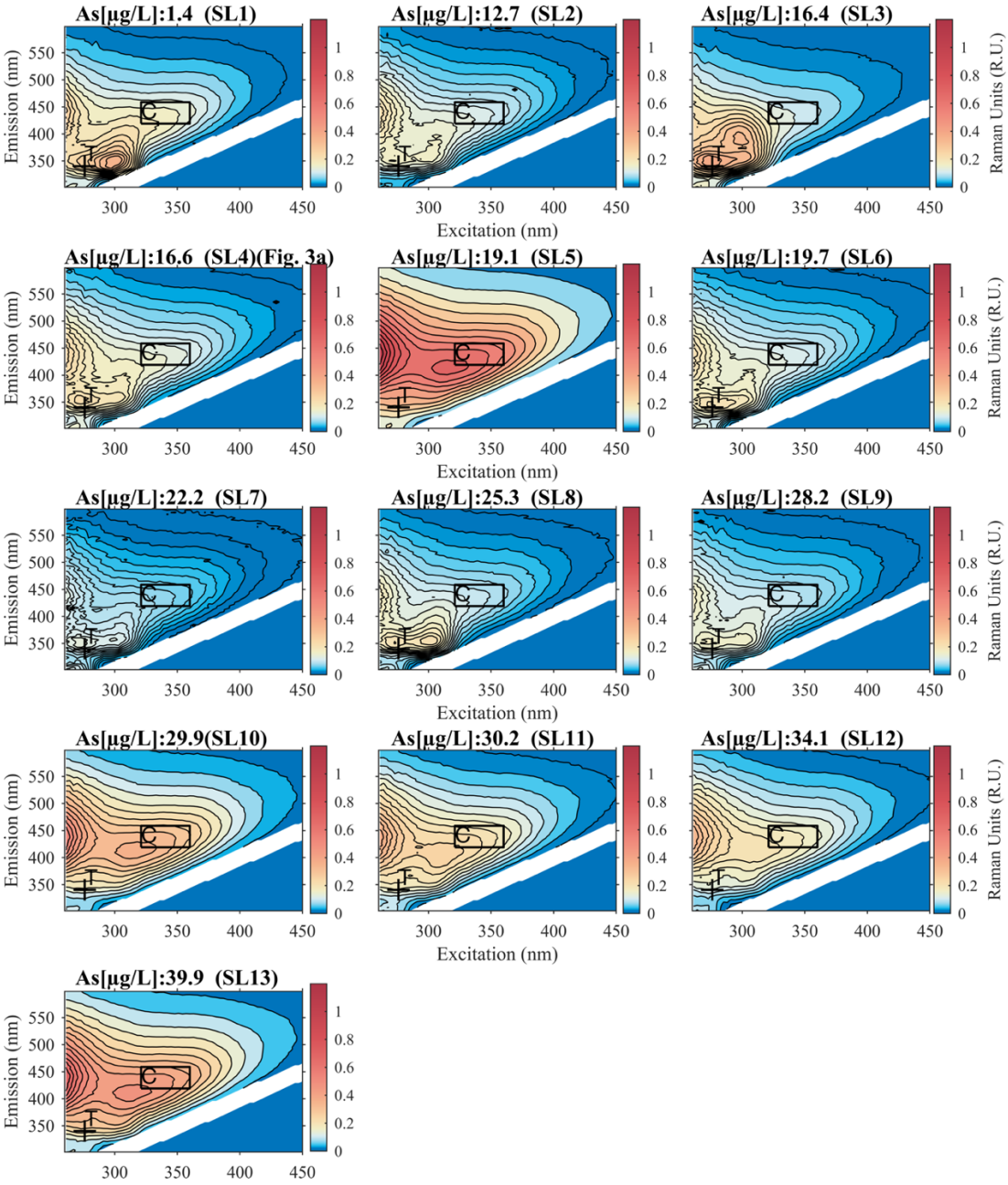

Figure S5: Bulk EEMs for all 13 SL samples, sorted by increasing As concentration.

The samples SL4, SL8 and SL9 were additionally analyzed by HPSEC; see chromatograms in S2 Fig. S8a-c. The bulk EEM of SL4 is also shown in the main manuscript in Fig. 3a.

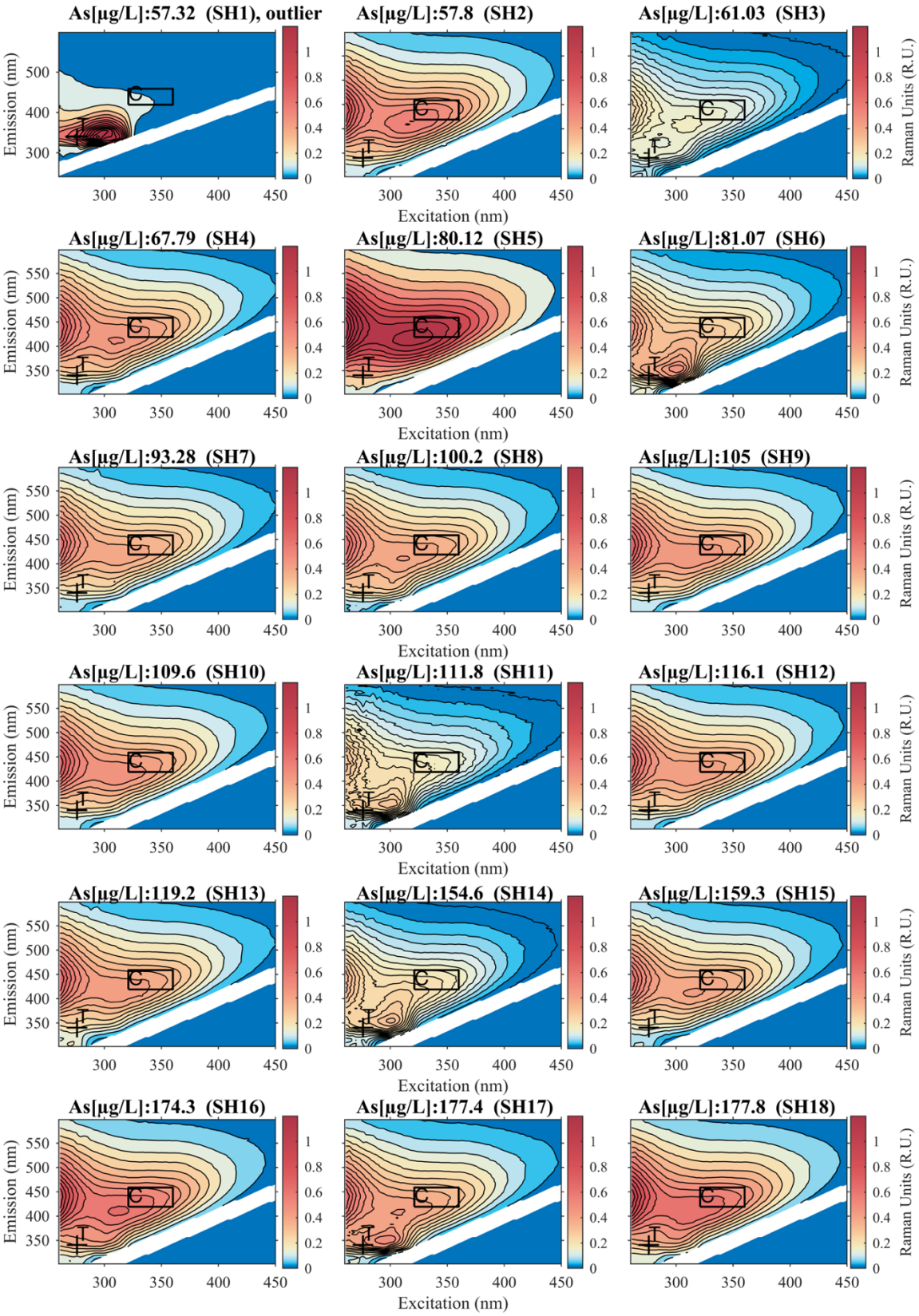

Figure S6a: Bulk EEMs of SH samples (SH1-SH18), sorted by increasing As concentration.

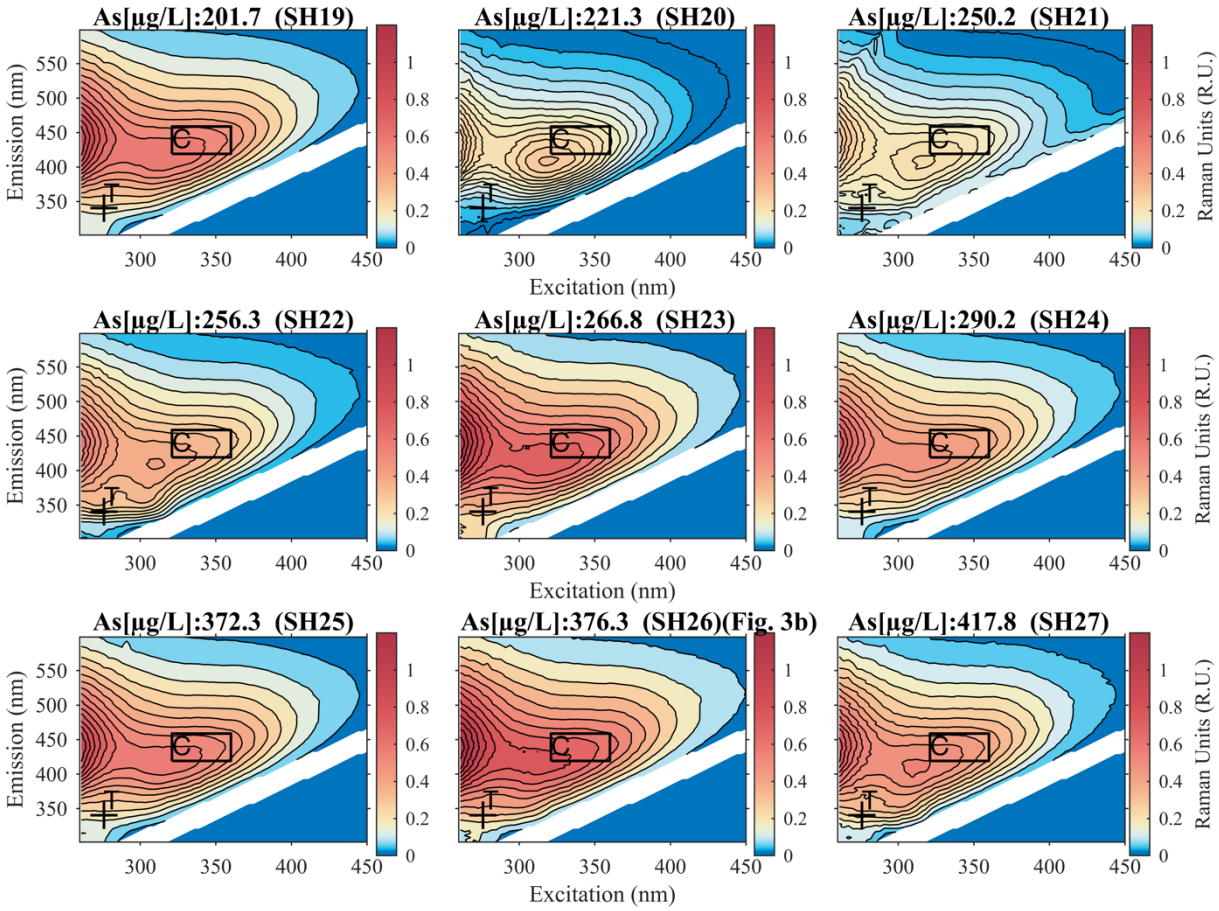

Figure S6b: Bulk EEMs of SH samples (SH19-SH27), sorted by increasing As concentration.

The samples SH25, SH26 and SH27 were additionally analyzed by HPSEC; see chromatograms in S2 Fig. S8d-f. The bulk EEM of SH26 is also shown in the main manuscript in Fig. 3b.

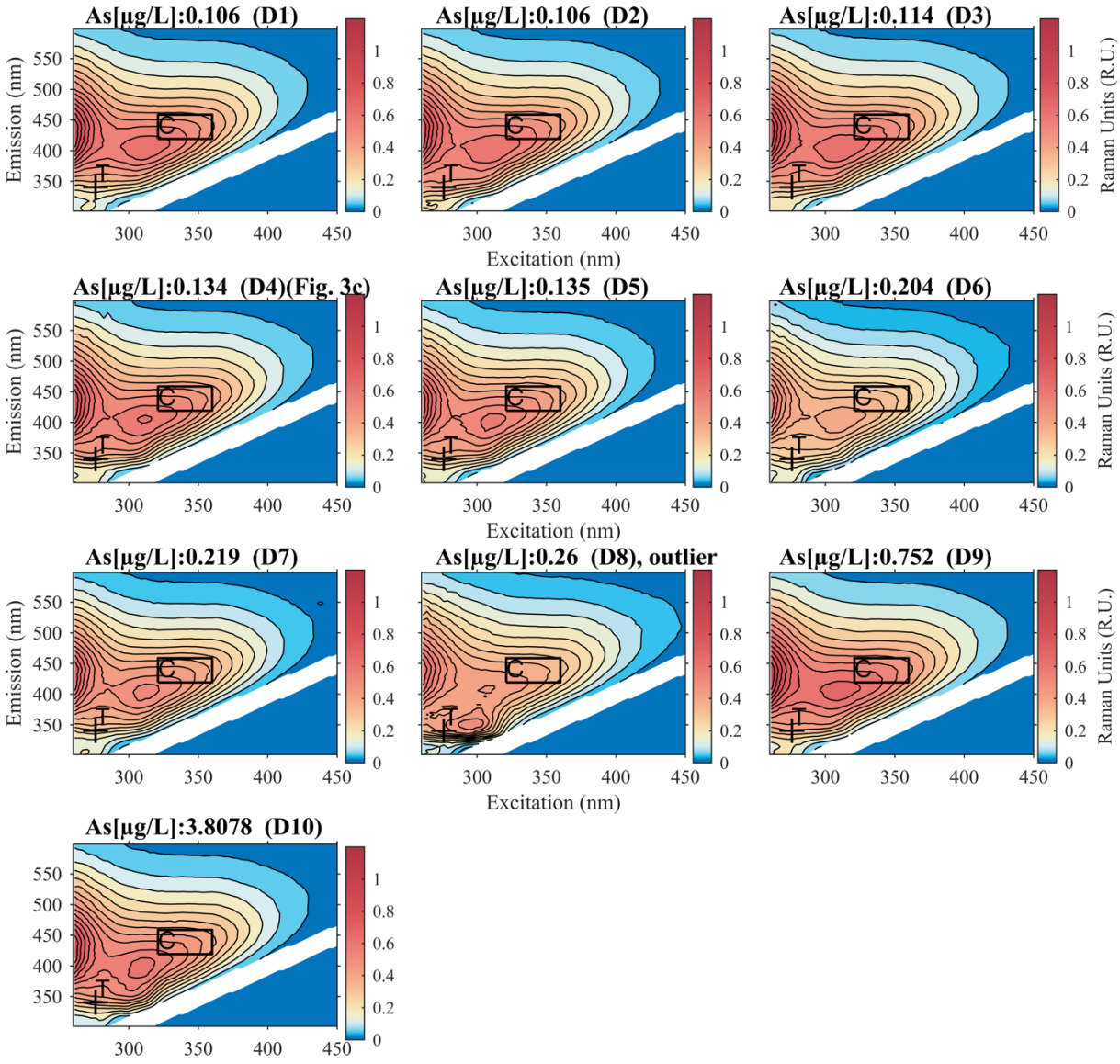

Figure S7: Bulk EEMs of all 10 D samples. (Note, detection limit of As is 1µg/L).

The sample D4 was additionally analyzed by HPSEC; see chromatograms in S2 Fig. S8g. The bulk EEM of D4 is also shown in the main manuscript in Fig. 3c.

Chromatograms

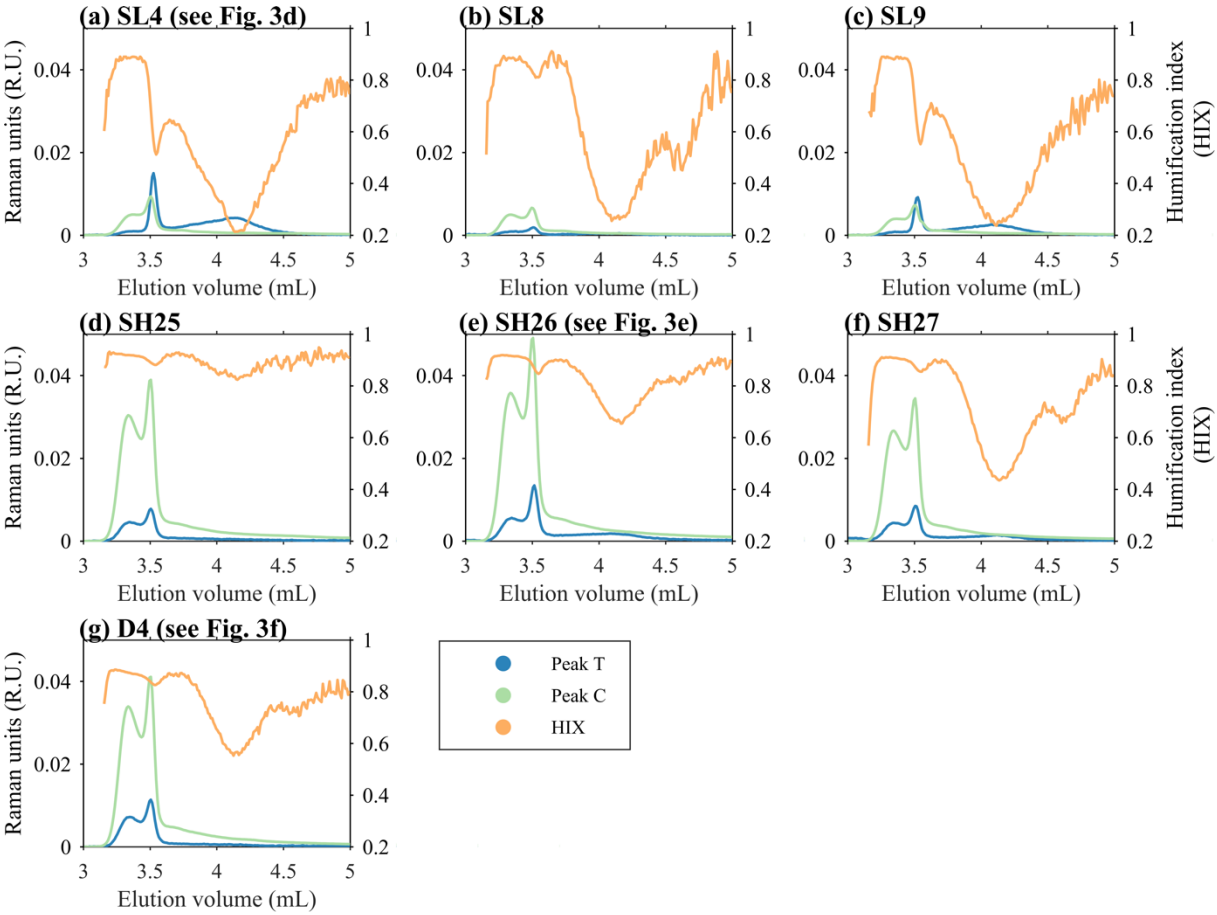

Figure S8: Chromatograms of peak T, peak C and HIX for the seven samples which were analyzed with HPSEC.

The chromatograms of SL4, SH26 and D4 are also shown in the main manuscript in Fig. 3d-f.

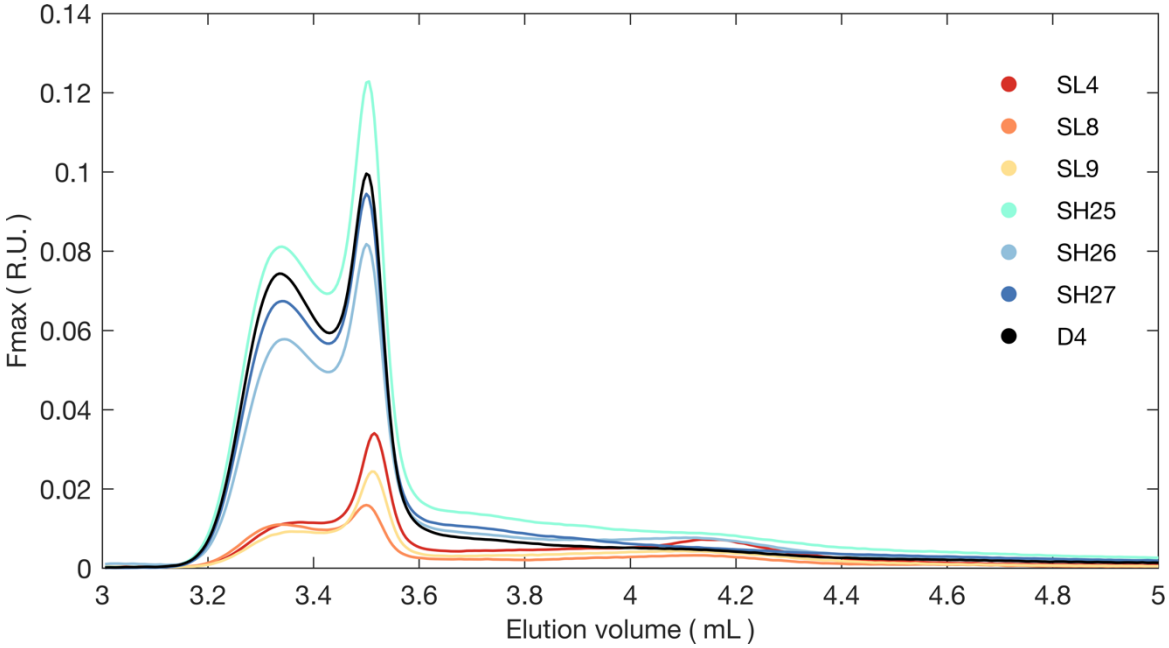

Figure S9: Chromatograms showing bulk fluorescence for the seven samples analyzed with HPSEC.

Batch experiments

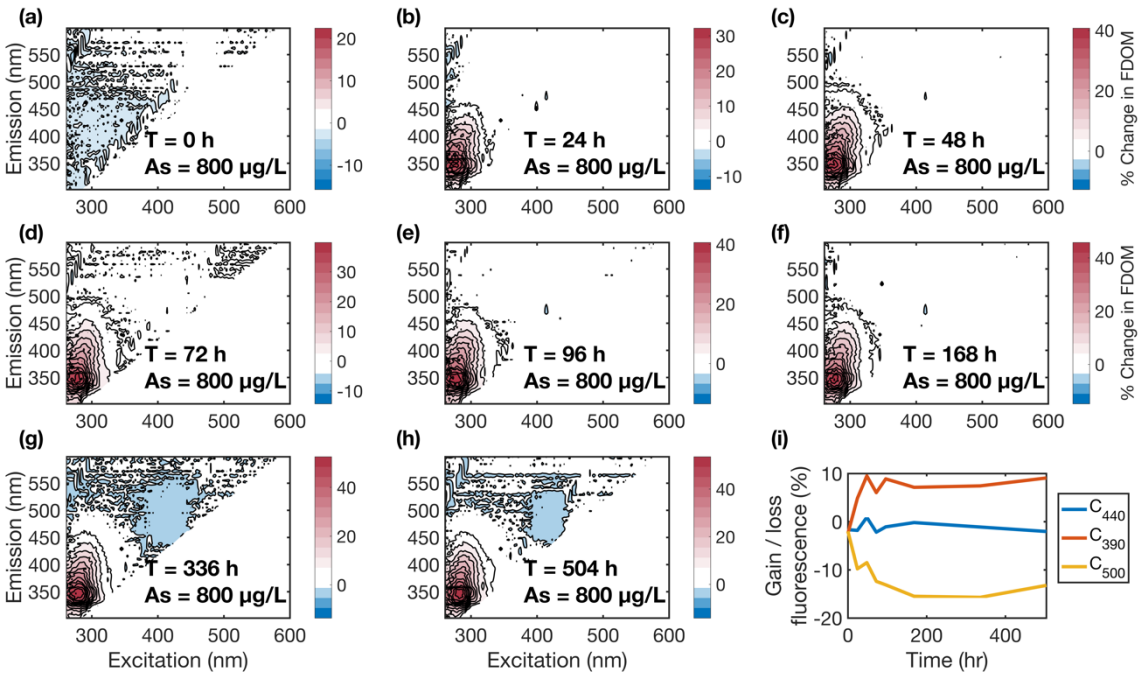

Figure S10: Change of FDOM (%) of sample SL6 over time at an As concentration of 800 µg/L (normalized subtraction EEMs) (a-h) and corresponding change of fluorescence (%) of PARAFAC components C_{390}, C_{440}, C_{500} (i). The increase in peak T, when measuring the fluorescence signal over time, most likely indicates impurities in the prepared As solution. A loss of fluorescence signal, as would be expected from quenching effects, could not be observed.

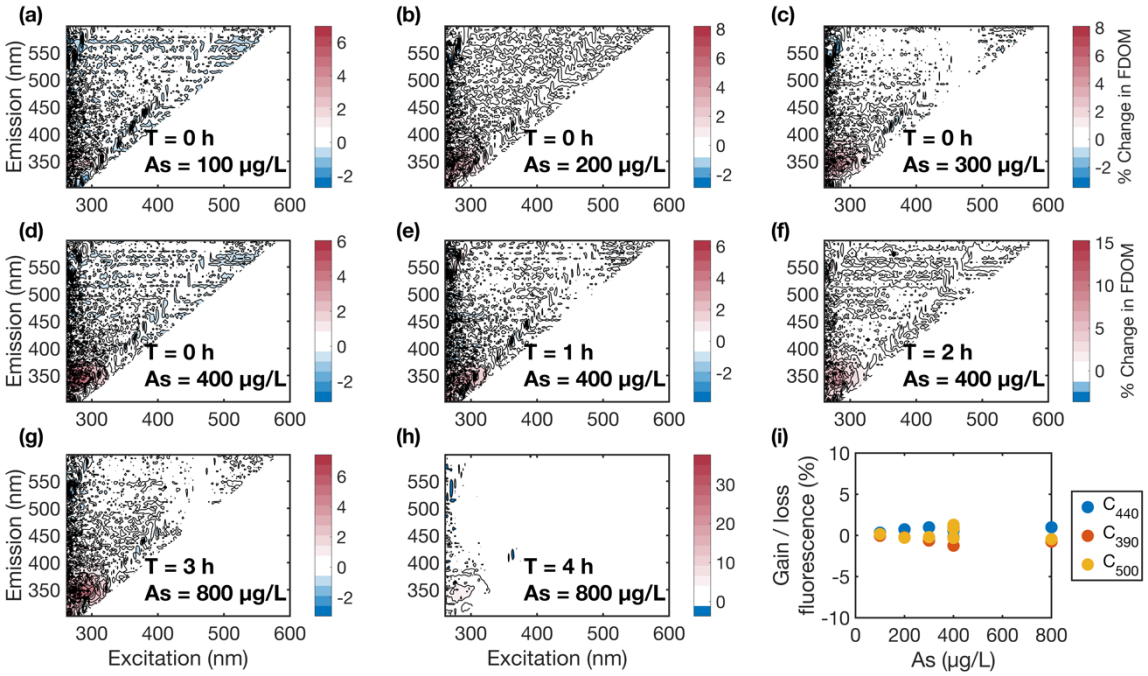

Figure S11: Change of FDOM (%) of sample SL6 for increasing As concentration (normalized subtraction EEMs) (a-h) and corresponding change of fluorescence (%) of PARAFAC components C_{390}, C_{440}, C_{500} (i).

S3 Supplementary Tables

Groundwater and fluorescence characteristics

Table S1: Groundwater and fluorescence characteristics of the shallow (SL; SH) and the deep aquifer (D). All numbers are expressed as mean value ± standard deviation, with minimum - maximum range in parentheses

	Shallow Aquifer		[a] **Deep Aquifer**
	(SL)	**(SH)**	**(D)**
Depth [mbgs]	[10-33]	[10-33]	[170-200]
As conc. level	<50 [µg/L]	>50 [µg/L]	<10 [µg/L]
n samples	13	27	10
Water Chemistry			
As [µg/L]	23 ± 10	172 ± 101	<1
	(1- 40)	(57 - 418)	(<1 - 4)
Fe [mg/L]	2.62 ± 2.56	4.68 ± 3.44	0.58 ± 0.20
	(0.09 - 9.14)	(0.06 - 13.51)	(0.21 - 0.96)
Mn [mg/L]	0.24 ± 0.07	0.43 ± 0.20	0.08 ± 0.01
	(0.14 - 0.34)	(0.09- 0.88)	(0.06 - 0.097)
P [mg/L]	0.40±0.41	0.62±0.29	0.03±0.001

	(0.02-1.25)	(0.07-1.16)	(0.02-0.04)
DOC[mg/L]	1.43 ± 0.51	1.76 ± 0.58	1.88 ± 0.72
	(0.64 - 2.28)	(0.80 - 2.89)	(1.05 - 3.39)
Spectroscopic Properties			
(based on bulk analysis)			
[b] $SUVA_{254}$[L/mg m]	1.85±1.52	2.53±1.45	2.31±0.70
	(0.82-6.84)	(0.82-5.44)	(0.92-3.36)
[b, c] HIX	0.72 ± 0.09	0.83 ± 0.05	0.79 ± 0.06
	(0.56 – 0.84)	(0.70 – 0.89)	(0.64 – 0.84)

[a] Most samples from the deep aquifer show dissolved As concentrations below the detection limit of 1 µg/L.

[b] Values given for $SUVA_{254}$ and HIX do not include sample SH1 due to sample contamination, see Fig. S6a.

[c] Note, that the HIX shown in this table is calculated based on Ohno et al.; [3] the conventional HIX based on Zsolnay et al. [4] resulted in values of 1.27-5.40, 2.37-7.92 and 1.78-5.11 for SL, SH and D samples, respectively, which might be relevant for comparison with other studies.

Table S2: Groundwater and fluorescence characteristics of all 50 samples.

Index	Depth [mbgs]	As [µg/L]	Fe [mg/L]	Mn [mg/L]	P [mg/L]	[a] DOC [mg/L]	$SUVA_{254}$ [L/mg m]	[b] HIX
D1	183	<1	0.96	0.09	0.02	1.96	1.98	0.84
D2	183	<1	0.70	0.10	0.03	2.64	1.84	0.73
D3	198	<1	0.44	0.07	0.04	2.00	2.04	0.77
D4	174	<1	0.67	0.07	0.03	1.91	2.25	0.82
D5	183	<1	0.64	0.07	0.04	1.08	3.33	0.81
D6	183	<1	0.46	0.06	0.03	3.39	0.92	0.80
D7	183	<1	0.42	0.07	0.02	1.05	3.36	0.81
D8	183	<1	0.59	0.06	0.04	1.65	2.14	0.64
D9	183	<1	0.77	0.09	0.04	–	2.30	0.82
D10	183	4	0.21	0.09	0.02	1.25	2.89	0.82
SL1	15	<1	0.22	0.14	0.02	1.73	1.21	0.68
SL2	21	13	0.72	0.17	0.08	1.77	0.90	0.75
SL3	21	16	2.80	0.29	0.03	–	2.58	0.56
SL4	30	17	1.99	0.28	0.15	1.77	1.13	0.69
SL5	27	19	4.70	0.27	0.62	0.92	6.84	0.84
SL6	21	20	1.15	0.19	0.14	0.64	2.07	0.64

SL7	21	22	0.23	0.20	0.10	1.16	0.82	0.61
SL8	16	25	0.09	0.21	0.12	1.19	1.07	0.67
SL9	24	28	0.95	0.22	0.13	1.06	1.14	0.65
SL10	23	30	2.30	0.38	0.89	2.17	1.54	0.83
SL11	30	30	9.14	0.28	0.89	1.62	1.37	0.78
SL12	26	34	5.58	0.19	0.79	0.88	1.85	0.80
SL13	17	40	4.23	0.35	1.25	2.28	1.58	0.84
SH1	10	57	10.99	0.74	0.74	1.03	1.79	0.35
SH2	33	58	6.62	0.32	0.98	2.89	1.85	0.86
SH3	24	61	1.72	0.22	0.15	1.46	0.82	0.73
SH4	18	68	0.06	0.34	0.39	2.32	1.83	0.85
SH5	21	80	10.42	0.53	0.43	2.35	4.88	0.89
SH6	15	81	2.51	0.39	0.19	1.85	1.32	0.74
SH7	29	93	2.55	0.09	0.61	0.81	4.74	0.86
SH8	28	100	4.48	0.31	0.76	2.08	1.59	0.85
SH9	30	105	5.43	0.24	1.16	1.64	2.12	0.84
SH10	21	110	2.21	0.38	0.69	2.35	1.59	0.85
SH11	30	112	2.61	0.43	0.74	1.64	1.07	0.70
SH12	15	116	5.37	0.18	0.69	1.65	2.06	0.83

SH13	30	119	4.79	0.37	1.04	1.93	1.65	0.85
SH14	15	155	0.22	0.57	0.12	1.66	1.42	0.74
SH15	24	159	4.23	0.59	0.67	1.83	1.78	0.85
SH16	16	174	2.10	0.38	0.29	2.67	2.36	0.84
SH17	24	177	7.08	0.58	0.96	1.24	2.33	0.77
SH18	15	178	10.04	0.28	0.89	0.87	5.44	0.84
SH19	26	202	3.45	0.46	0.61	2.63	1.65	0.87
SH20	18	221	0.25	0.15	0.07	1.05	2.33	0.89
SH21	22	250	6.74	0.80	0.54	2.15	1.60	0.81
SH22	26	256	1.89	0.83	0.70	1.63	1.80	0.83
SH23	18	267	13.51	0.51	0.72	1.86	5.01	0.81
SH24	24	290	3.37	0.33	0.78	2.13	1.70	0.87
SH25	18	372	7.63	0.44	0.74	0.80	5.25	0.87
SH26	33	376	4.20	0.25	1.02	1.01	5.20	0.88
SH27	15	418	1.80	0.88	0.54	1.99	2.48	0.84

[a] For two samples DOC could not be measured due to damage of the sampling bottles, indicated by –.

[b] Calculated according to Ohno et al. [3]

Position of peak maxima

Table S3: Chromatographic positions of peak maxima (peak T, peak C)

		Peak T			Peak C	
		Max. 1	**Max. 2**	**Max. 3**	**Max. 1**	**Max. 2**
	Elution Vol. [ml]	3.38	3.53	4.13	3.37	3.50
SL4	*Mol. Size [Da]*	1647.00	545.96	6.12	1772.80	632.56
	Intensity [R.U.]	0.001	0.015	0.004	0.005	0.009
	Elution Vol. [ml]	3.38	3.50	4.16	3.34	3.50
SL8	*Mol. Size [Da]*	1647.00	632.56	5.29	2210.89	680.88
	Intensity [R.U.]	0.001	0.002	0.000	0.005	0.007
	Elution Vol. [ml]	3.35	3.53	4.07	3.37	3.50
SL9	*Mol. Size [Da]*	1908.25	545.96	9.53	1772.80	632.56
	Intensity [R.U.]	0.001	0.009	0.003	0.004	0.007
	Elution Vol. [ml]	3.35	3.50	3.80	3.34	3.50
SH25	*Mol. Size [Da]*	2054.00	632.56	69.51	2210.89	632.56
	Intensity [R.U.]	0.005	0.008	0.001	0.030	0.039

	Elution Vol. [ml]	3.35	3.52	4.09	3.34	3.50
SH26	*Mol. Size [Da]*	2054.00	587.66	8.85	2210.89	632.56
	Intensity [R.U.]	0.006	0.013	0.002	0.036	0.049
	Elution Vol. [ml]	3.35	3.50	4.12	3.35	3.50
SH27	*Mol. Size [Da]*	2054.00	632.56	7.10	2054.00	632.56
	Intensity [R.U.]	0.004	0.008	0.001	0.027	0.034
	Elution Vol. [ml]	3.35	3.50	3.91	3.34	3.50
D4	*Mol. Size [Da]*	2054.00	632.56	33.29	2210.89	632.56
	Intensity [R.U.]	0.007	0.011	0.001	0.034	0.041
	mean E. Vol [ml]	**3.4**	**3.5**	**4.0**	**3.3**	**3.5**
	mean Mol. Size [Da]	**2E+03**	**6E+02**	**2E+01**	**2E+03**	**6E+02**

Supplementary References

(1) esri. How IDW works https://pro.arcgis.com/en/pro-app/tool-reference/3d-analyst/how-idw-works.htm (accessed Jul 19, 2018).

(2) Palintest. Digital Arsenic Test Kit https://www.palintest.com/en/products/digital-arsenic-test-kit (accessed Jul 19, 2018).

(3) Ohno, T. Fluorescence Inner-Filtering Correction for Determining the Humification Index of Dissolved Organic Matter. *Environ. Sci. Technol.* **2002**, *36* (4), 742–746.

(4) Zsolnay, A.; Baigar, E.; Jimenez, M. Differentiating with Fluorescence Spectroscopy the Sources of Dissolved Organic Matter in Soils Subjected to Drying. *Chemosphere* **1999**, *38* (1), 45–50.