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Supplementary Information

Heavy metal, organic matter, and disinfection byproduct release from drinking water pipe scales under stagnant conditions

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Parameter	WDS 1	WDS2	WDS3	MCL*
NO_3^- (mg/L)	6.1	2.2	3.7	50
$Cl^{-}(mg/L)$	21	1.7	101	250
$SO_4^{2-}(mg/L)$	4.5	6.7	22	250
Na (mg/L)	80	2.9	55	200
K (mg/L)	1.1	<1	1.4	/
Mg (mg/L)	24	2.1	8.2	/
Ca (mg/L)	78	5.9	66	/
pН	7.5	7.3	7.9	6.5-9.5
ORP (mV)	495	431.3	406	/
$HCO_3^-(mg/L)$	623	24.4	245	/
EC (µScm)	776	62.9	641	/
DCAA (µg/L)	1.0	16.2	<1.0	/
TCAA (µg/L)	2.9	14.6	< 0.4	/
DBAA (µg/L)	0.3	< 0.4	2.0	/
THAA (µg/L)	4.2	32.9	2.0	60
TCM (µg/L)	0.5	< 0.5	< 0.5	/
BCM (µg/L)	< 0.5	< 0.5	2.8	/
TTHM (µg/L)	0.5	< 0.5	2.8	100
DOC (mg/L)	3.3	1.5	/	

Table S1. Values of water quality parameters in tap water from the three water distribution systems of interest. Note that the samples for each WDS were sampled only once.

*MCL= maximum contaminant level according to current Croatian legislation

Table S2. Dissolved and particulate metal concentrations in tap water samples from the three water distribution systems under investigation. Blanks, triplicates, and controls were made during the analysis to ensure the quality of results. Analytical precision, evaluated from repeated measurements of standard solutions with known concentration, was better than 10% (relative standard deviation), whereas accuracy (better than 10%) was evaluated by analysing the reference sample. Quality assurance was conducted by participation in proficiency testing for all measured parameters.

Matal	dissolved	total	dissolved	total	dissolved	total	
Metal	WDS	1	WD	S 2	WDS	53	MCL*
Li (µg/L)	5.6	5.8	0.61	0.57	2.5	2.4	/
B (μ g/L)	35	48	8.3	7.9	309	294	1000
Al (µg/L)	< 0.25	0.61	65.5	100.5	29.8	33.4	200
V (μ g/L)	0.04	0.04	0.4	0.4	0.7	0.7	5.0
$Cr (\mu g/L)$	< 0.14	< 0.14	< 0.14	< 0.14	0.34	0.32	50
Mn (µg/L)	0.40	0.35	0.70	1.5	0.14	0.22	50
Fe (µg/L)	5.0	7.6	29	50	4.1	12.1	200
Ni (µg/L)	0.67	0.70	< 0.24	0.25	0.53	0.51	20
Cu (µg/L)	0.019	0.019	0.00096	0.00098	0.0017	0.0071	2000
$Zn \ (\mu g/L)$	107	107	27	32	16	23	3000
As $(\mu g/L)$	2.3	2.3	0.22	0.24	0.13	0.12	10
Se $(\mu g/L)$	< 0.06	< 0.06	< 0.06	< 0.06	1.17	1.17	10
Sr (µg/L)	397	403	19	19	674	659	/
Mo (µg/L)	0.73	0.73	0.30	0.46	1.1	1.2	/
Ba (µg/L)	84	82	4.1	4.7	13	12	700
Pb (µg/L)	0.65	0.89	0.07	0.15	0.11	0.22	10

*MCL= maximum contaminant level according to current Croatian legislation

Table S3. Excitation and emission wavelength for PARAFAC components for biofilm and every experiment (six models were generated in this research). Analyses were performed in a quartz cuvette with a path length of 1 cm. The blank solution (Milli-Q water) was subtracted from the EEM of samples. CCD gain was set at 'medium' and Saturation Mask Width was 10 nm. Data was corrected for inner filter effects and Rayleigh Masking (1st and 2nd order). After sample normalization, PARAFAC modeling was performed using Eigenvector Solo software (Eigenvector Research Inc., Manson, WA, USA).

	component	excitation	emission
	component	(nm)	(nm)
	humic-like	240	415
Pipe scale	tyrosine-like_1	240/275	314
	tyrosine-like_2	270	301
Release	humic-like_1	250	406
scales from pipe 1	humic-like_2	240/295	410
seales nom pipe i	humic-like_3	260	442
	humic-like_4	240/290	498
	humic-like 1	240/305	410
Release	humic-like 2	250	475
experiment with	tryptophan-like	240	342
scales nom pipe 2	tyrosine-like	275	305
	humic-like_1	240/330	419
Release	tryptophan-like	240/270	337
experiment with	humic-like_2	275	438
seales nom pipe 5	humic-like_3	295	406
Release	humic-like 1	240	438
experiment with	humic-like 2	240	373
scales from pipe 1	tryptophan-like	250	531
(addition of HAA)	humic-like 3	270	484
	humic-like 1	240	420
	humic-like 2	240	489
Release	humic-like 3	240	540
experiment with	humic-like 4	385	477
scales from pipe 2 (addition of $H \Delta \Delta$)	humic-like 5	355	438
	humic-like 6	280	442
	tryptophan-like	240	337

Table S4. Average values of measured anions, cations, pH value, ORP and electrical conductivity in the first three experiments (experiments with pipe 1, pipe 2 and pipe 3), average values od THAA and TTHM in the first three experiments. Note that THAA and TTHM were measure only at sampling points: 0, 0.08, 24 and 96 h, and average values of fluorescence intensities in the first three experiments (experiments with pipe 1, pipe 2 and pipe 3) pipe 3)

	sampling point	NO3 ²⁻ (mg/L)	Cl- (mg/L)	SO4 ²⁻ (mg/L)	Na ⁺ (mg/L)	K ₊ (mg/L)	Mg ²⁺ (mg/L)	Ca ²⁺ (mg/L)	HCO ₃ - (mg/L)	pH value	ORP (mV)	EV (µS/cm)
	0 h	6.1	21.0	4.5	80.0	1.1	24.0	78.0	622.5	7.5	495	776
	0.08 h	6.4	20.6	5.2	80.0	1.0	22.5	79.0	577.5	7.5	447	790
	2 h	6.9	22.1	7.0	78.0	1.1	23.5	75.5	-	7.6	430	801
ment 1	4 h	6.5	22.2	7.5	80.0	1.5	23.5	78.0	-	7.6	421	784
(pipe 1)	24 h	6.9	23.1	11.1	79.5	1.2	22.5	73.5	-	7.6	436	775
	72 h	6.1	22.1	14.5	80.0	1.1	21.5	72.5	-	7.9	399	807
	96 h	7.1	22.1	15.4	81.5	1.3	22.0	72.0	557.5	7.9	403	786
	0 h	2.2	1.7	6.7	2.9	<1	2.1	5.9	24.4	7.3	499	63
	0.08 h	2.0	2.9	8.3	3.0	1.0	2.1	5.9	25.0	7.2	543	69
ovnori	2 h	2.0	3.4	9.1	3.1	<1	2.0	5.8	-	7.3	511	70
ment 2	4 h	1.7	2.8	9.4	3.0	1.0	2.1	6.3	-	7.2	511	70
(pipe 2)	24 h	1.6	3.0	10.8	3.5	1.4	2.4	7.6	-	7.2	501	77
	72 h	3.2	4.0	12.7	3.5	1.6	2.2	7.6	-	7.4	507	82
	96 h	1.6	3.9	14.3	3.2	1.7	2.2	7.7	28.0	7.4	471	81
	0 h	3.7	101.0	22.0	55.0	1.4	8.2	66.0	244.9	7.9	406	641
	0.08 h	3.1	108.0	23.6	53.0	1.4	8.2	66.0	237.6	7.7	427	657
	2 h	2.8	109.0	23.7	53.7	1.4	8.3	75.3	-	7.7	419	662
ment 3	4 h	2.4	121.0	26.7	60.0	1.6	8.9	73.0	-	8.0	432	678
(pipe 3)	24 h	2.2	112.5	26.8	56.0	1.9	8.5	71.0	-	7.8	462	680
	72 h	2.4	112.0	26.8	56.5	1.5	8.7	73.5	-	7.9	405	691
	96 h	2.0	108.0	26.6	55.0	1.5	8.4	71.0	221.2	8.0	433	683

	sampling point	THAA (µg/L)	TTHM (µg/L)
	0 h	4.2	0.5
	0.08 h	3.2	< 0.5
experiment 1 (pipe 1)	24 h	4.1	0.7
	96 h	3.4	<0.5
	0 h	32.9	<0.5
avnarimant 2 (nina 2)	0.08 h	35.3	<0.5
experiment 2 (pipe 2)	24 h	36.2	<0.5
	96 h	35.9	<0.5
	0 h	2.0	2.8
experiment 3 (pipe 3)	0.08 h	2.0	1.9
experiment 5 (pipe 5)	24 h	2.1	1.8
	96 h	2.0	1.6

Experiment 1				
Exposure time h	humic-like1	humic-like 2	humic-like 3	humic-like 4
	(RU)	(RU)	(RU)	(RU)
0	386.0	205.5	185.0	138.8
0.08	396.7	209.6	189.3	142.3
2	395.4	211.1	188.0	141.1
4	407.2	219.4	191.7	141.8
24	394.6	211.7	188.6	139.7
72	374.4	202.2	186.0	134.5
96	367.0	202.3	179.0	131.9

Experiment 2				
Exposure time, h	humic-lke 1 (RU)	humic-like 2 (RU)	tryprophan-like (RU)	tyrosine-like (RU)
0	59.2	46.1	12.0	0.0
0.08	116.7	88.6	76.4	43.3
2	118.1	91.0	79.3	44.9
4	74.5	57.0	43.9	12.1
24	66.3	46.2	36.4	8.1
72	80.4	51.2	46.4	14.2
96	56.7	38.9	23.5	5.7

Experiment 3				
Exposure time h	humic-like 1	tryptophan-like	humic-like 2	humic-like 3
	(RU)	(RU)	(RU)	(RU)
0	0.0	0.0	0.0	0.0
0.08	61.4	43.4	7.9	104.8
2	154.2	114.3	10.8	56.9
4	60.0	36.7	146.2	16.7
24	88.8	84.2	7.6	43.2
72	7.0	6.5	2.2	18.1
96	4.5	7.5	2.1	22.5

Table S5. Spearman's correlation coefficients for measured parameters calculated from data from experiments with pipe 1, pipe 2 and pipe 3. Humic-like components were summed for

each experiments, as well as tyrosine-like and tryptophan-like components into protein-like component. Marked correlations are significant at p<0.05.

	NO ₃ -	Cŀ	SO42-	Na⁺	K⁺	Mg ²⁺	Ca ²⁺	pН	ORP	EC	As_{diss}	As _{tot}	Fe_{diss}	Fe _{tot}	AI_{diss}	Al _{tot}	Mn _{diss}	Mn _{tot}	Pb_{diss}	Pb _{tot}	Zn _{diss}	Zn _{tot}	humic-like	protein-like
NO ₃ -	1.0	0.3	-0.2	0.8	0.1	0.8	0.7	0.5	-0.5	0.8	0.5	0.5	-0.2	0.5	-0.4	0.4	-0.3	-0.2	0.8	0.8	-0.7	-0.7	0.7	-0.8
Cl-	0.3	1.0	0.7	0.5	0.8	0.5	0.5	0.8	-0.7	0.5	-0.5	-0.5	-0.7	0.0	-0.7	-0.4	0.6	0.6	0.0	0.0	-0.8	-0.8	0.1	-0.1
SO42-	-0.2	0.7	1.0	0.0	0.7	-0.1	0.0	0.6	-0.5	0.0	-0.8	-0.7	-0.5	-0.1	-0.3	-0.5	0.8	0.9	-0.5	-0.4	-0.4	-0.4	-0.5	0.3
Na⁺	0.8	0.5	0.0	1.0	0.3	0.9	0.8	0.7	-0.5	1.0	0.4	0.4	-0.4	0.5	-0.7	0.2	0.0	0.1	0.8	0.8	-0.8	-0.8	0.7	-0.8
K*	0.1	0.8	0.7	0.3	1.0	0.3	0.4	0.5	-0.5	0.3	-0.6	-0.5	-0.5	0.0	-0.6	-0.5	0.6	0.6	-0.2	-0.1	-0.6	-0.6	-0.1	0.0
Mg ²⁺	0.8	0.5	-0.1	0.9	0.3	1.0	0.9	0.6	-0.5	0.9	0.4	0.4	-0.5	0.4	-0.7	0.1	-0.1	0.0	0.8	0.8	-0.8	-0.8	0.7	-0.8
Ca ²⁺	0.7	0.5	0.0	0.8	0.4	0.9	1.0	0.5	-0.6	0.9	0.3	0.3	-0.5	0.4	-0.7	0.1	0.0	0.0	0.7	0.7	-0.8	-0.8	0.6	-0.6
рН	0.5	0.8	0.6	0.7	0.5	0.6	0.5	1.0	-0.8	0.7	-0.2	-0.1	-0.7	0.2	-0.6	-0.1	0.4	0.5	0.2	0.4	-0.8	-0.8	0.1	-0.5
ORP	-0.5	-0.7	-0.5	-0.5	-0.5	-0.5	-0.6	-0.8	1.0	-0.5	0.3	0.2	0.6	-0.2	0.5	0.0	-0.3	-0.3	-0.1	-0.3	0.8	0.8	-0.1	0.4
EC	0.8	0.5	0.0	1.0	0.3	0.9	0.9	0.7	-0.5	1.0	0.4	0.4	-0.5	0.5	-0.7	0.2	0.1	0.1	0.8	0.8	-0.8	-0.8	0.6	-0.8
As_{diss}	0.5	-0.5	-0.8	0.4	-0.6	0.4	0.3	-0.2	0.3	0.4	1.0	0.9	0.3	0.4	0.0	0.7	-0.7	-0.6	0.9	0.8	0.0	0.0	0.7	-0.6
As _{tot}	0.5	-0.5	-0.7	0.4	-0.5	0.4	0.3	-0.1	0.2	0.4	0.9	1.0	0.4	0.6	0.2	0.8	-0.6	-0.5	0.8	0.9	0.0	0.0	0.7	-0.6
Fe_{diss}	-0.2	-0.7	-0.5	-0.4	-0.5	-0.5	-0.5	-0.7	0.6	-0.5	0.3	0.4	1.0	0.3	0.8	0.5	-0.3	-0.3	-0.1	0.0	0.7	0.7	0.1	0.3
Fe _{tot}	0.5	0.0	-0.1	0.5	0.0	0.4	0.4	0.2	-0.2	0.5	0.4	0.6	0.3	1.0	0.0	0.8	0.0	0.1	0.5	0.7	-0.3	-0.3	0.7	-0.3
AI_{diss}	-0.4	-0.7	-0.3	-0.7	-0.6	-0.7	-0.7	-0.6	0.5	-0.7	0.0	0.2	0.8	0.0	1.0	0.4	-0.3	-0.3	-0.4	-0.3	0.8	0.8	-0.3	0.4
Al _{tot}	0.4	-0.4	-0.5	0.2	-0.5	0.1	0.1	-0.1	0.0	0.2	0.7	0.8	0.5	0.8	0.4	1.0	-0.4	-0.4	0.5	0.7	0.1	0.1	0.5	-0.4
Mn _{diss}	-0.3	0.6	0.8	0.0	0.6	-0.1	0.0	0.4	-0.3	0.1	-0.7	-0.6	-0.3	0.0	-0.3	-0.4	1.0	1.0	-0.5	-0.3	-0.3	-0.3	-0.3	0.3
Mn _{tot}	-0.2	0.6	0.9	0.1	0.6	0.0	0.0	0.5	-0.3	0.1	-0.6	-0.5	-0.3	0.1	-0.3	-0.4	1.0	1.0	-0.4	-0.3	-0.3	-0.3	-0.2	0.3
Pb_{diss}	0.8	0.0	-0.5	0.8	-0.2	0.8	0.7	0.2	-0.1	0.8	0.9	0.8	-0.1	0.5	-0.4	0.5	-0.5	-0.4	1.0	0.9	-0.5	-0.5	0.8	-0.8
Pb _{tot}	0.8	0.0	-0.4	0.8	-0.1	0.8	0.7	0.4	-0.3	0.8	0.8	0.9	0.0	0.7	-0.3	0.7	-0.3	-0.3	0.9	1.0	-0.5	-0.5	0.8	-0.8
Zn _{diss}	-0.7	-0.8	-0.4	-0.8	-0.6	-0.8	-0.8	-0.8	0.8	-0.8	0.0	0.0	0.7	-0.3	0.8	0.1	-0.3	-0.3	-0.5	-0.5	1.0	1.0	-0.4	0.6
Zn _{tot}	-0.7	-0.8	-0.4	-0.8	-0.6	-0.8	-0.8	-0.8	0.8	-0.8	0.0	0.0	0.7	-0.3	0.8	0.1	-0.3	-0.3	-0.5	-0.5	1.0	1.0	-0.4	0.6
humic-like	0.7	0.1	-0.5	0.7	-0.1	0.7	0.6	0.1	-0.1	0.6	0.7	0.7	0.1	0.7	-0.3	0.5	-0.3	-0.2	0.8	0.8	-0.4	-0.4	1.0	-0.4
protein-like	-0.8	-0.1	0.3	-0.8	0.0	-0.8	-0.6	-0.5	0.4	-0.8	-0.6	-0.6	0.3	-0.3	0.4	-0.4	0.3	0.3	-0.8	-0.8	0.6	0.6	-0.4	1.0

		nU voluo	DOC			pН	DOC
		pri value	(mg/L)			value	(mg/L)
	0 h	8.3	-		0 h	7.6	1.6
	0.08 h	7.8	-		0.08 h	7.3	1.8
	4 h	7.7	-		4 h	6.6	-
pipe I (HA=2 mg/I)	24 h	7.8	-	pipe 2 (HA=2 mg/I)	24 h	6.6	-
l ling/L)	48 h	8.0	-	ing/L)	72 h	6.7	-
	72 h	8.2	-		96 h	6.7	-
	96 h	8.4	-		240 h	6.8	1.3
	0 h	8.3	-		0 h	7.6	1.6
	0.08 h	7.9	-		0.08 h	7.1	2.1
· 1 /11 / 5	4 h	7.8	-	· • • • • • •	4 h	4.7	-
pipe I (HA=5	24 h	7.8	-	pipe 2 (HA=5 mg/I)	24 h	6.7	-
l ling/L)	48 h	7.9	-	ing/L)	72 h	6.9	-
	72 h	8.0	-		96 h	7.0	-
	96 h	8.2	-		240 h	6.9	1.5
	0 h	8.3	-		0 h	7.6	1.6
	0.08 h	7.8	-		0.08 h	7.2	3.5
	4 h	7.8	-		4 h	6.9	-
pipe I (HA=10 m_{α}/I)	24 h	7.8	-	pipe 2 (HA=10 m_{α}/L)	24 h	6.8	-
mg/L)	48 h	7.9	-	IIIg/L)	72 h	6.9	-
	72 h	8.1	-		96 h	7.0	-
	96 h	8.2	-		240 h	6.9	1.6

Table S6. pH value and DOC in the experiments with added humic acid.

Table S7. Percentage of released metals from the deposits during the experiments with tap water.

Metal	pipe 1 % released	pipe 2 % released	pipe 3 % released		
Fe (mg/kg)	0.19	0.05	0.06		
As (mg/kg)	1.2	1.1	0.4		
Mn (mg/kg)	3.9	4.6	12.7		
Al (mg/kg)	6.2	1.0	3.6		
Zn (mg/kg)	1.3	12.9	2.8		
V (mg/kg)	2.2	0.5	0.8		
Cr (mg/kg)	1.8	0.2	0.6		
Co (mg/kg)	6.2	2.1	5.5		
Ni (mg/kg)	19.4	5.5	10.6		
Cu (mg/kg)	20.5	3.0	0.7		
Se (mg/kg)	9.4	10.8	44.4		
Mo (mg/kg)	2.0	1.2	1.7		
Cd (mg/kg)	4.0	13.1	11.9		
Sn (mg/kg)	/	/	/		
Sb (mg/kg)	3.1	3.8	1.0		
Ba (mg/kg)	48.3	8.3	4.2		
Pb (mg/kg)	2.1	1.4	0.4		
U (mg/kg)	2.1	/	3.1		

Table S8. Percentage of released metal under different HA concentrations in the experiment with pipe 2.

Metal	% released (without HA)	% released (HA=2 mg/L)	% released (HA=5 mg/L)	% released (HA=10 mg/L)
Fe (mg/kg)	0.05	0.4	0.5	0.8
As (mg/kg)	1.1	2.1	3.8	3.4
Mn (mg/kg)	4.6	7.3	7.1	7.9
Al (mg/kg)	1.0	1.7	1.4	1.8
Zn (mg/kg)	12.9	23.8	21.4	22.8
V (mg/kg)	0.5	1.3	3.0	4.1
Cr (mg/kg)	0.2	2.6	3.2	6.4
Co (mg/kg)	2.1	3.5	4.3	5.6
Ni (mg/kg)	5.5	17.0	12.9	18.1
Cu (mg/kg)	3.0	7.2	6.3	12.7
Se (mg/kg)	10.8	19.9	29.1	29.6
Mo (mg/kg)	1.2	10.3	15.9	8.3
Cd (mg/kg)	13.1	37.7	28.2	33.6
Sn (mg/kg)	/	/	/	/
Sb (mg/kg)	3.8	9.6	9.4	11.6
Ba (mg/kg)	8.3	19.4	19.4	88.4
Pb (mg/kg)	1.4	8.2	5.8	6.6
U (mg/kg)	/	5.9	7.0	12.6







Figure S1. Additional SEM images of pipe scale 1 (a), pipe scale 2 (b) and pipe scale 3(c). Note the different magnifications used.



Figure S2. EDS spectra of particles from (a) pipe 1, (b) pipe 2 and (c) pipe 3.



Figure S3. Diffractograms of samples from (a) pipe 1, (b) pipe 2 and (c) pipe 3. Figure S3d shows diffractogram of sample from pipe 1 after the experiment. Data base ICDD PDF-2 (The International Centre for Diffraction Data Powder Diffraction, 2003) was used to determine phase composition of samples.



Figure S4. Time profiles of the OM components in experiments with scale from (a) pipe 1, (b) pipe 2 and (c) pipe 3.



pipe 2





Figure S5. The linear co-release of Mn and sulfate in (a) pipe 1, (b) pipe 2 and (c) pipe 3 and (d) Mn and calcium in pipe 2.









Figure S6. The linear co-release of (a) Fe and As, (b) Fe and Al and (c) Pb and As in pipe 1.



Figure S7. PARAFAC components intensities during the experiment with pipe scale 1 (after addition of HA).



Figure S8. PARAFAC components intensities during the experiment with pipe scale 2 (after addition of HA).

Experiment under stagnation water conditions

- Fe, Al, Mn release, Pb and As precipitation
- Predominance of humic substances
- Stable and low THAA concentrations
- PCCA analysis group 1: Fe, Pb, As – co-existance and co-release of these metals

Pipe 1 – groundwater with higher As concentrations



Experiment under stagnation water conditions (addition of HA) • The greatest influence on the release of Fe, Al and Pb

HA concentrations were independent of the quantity of As, however they induced its release

Experiment under stagnation water

The greatest influence on the

The differences in HA

concentrations least affected Mn

The addition of HA caused the

release of all monitored metals

conditions (addition of HA)

release of Fe

concentrations

Experiment under stagnation water conditions

- Fe, Mn release, Al precipitation
- Presence of humic and protein substances
- Higher THAA concentrations which increased marginally
- PCCA analysis group 2: Fe, Al, Zn

Experiment under stagnation water conditions

- Fe, Mn, Pb release, Al precipitation
- Only Mn reached quasiequilibrium over the experimental time course
- Mn in this pipe has the highest tendency to be released potential problem at the end of the network
- Similar increasing concentration trend between Mn and SO₄ ions
- Presence of humic and protein substances
- Stable and low TTHM and THAA concentrations
- PCCA analysis group 3: Mn, SO₄ ions

Pipe 2 - surface water -

higher content of protein-

like OM

10 cm

- Pipe 3 brackish water – higher Mn
 - concentration



Figure S9. Summary of the major findings under stagnant conditions with and without addition of HA. Note the conditions for each pipe were as follows: pipe 1 was in contact with groundwater with higher As concentrations, pipe 2 was in contact with surface water with higher contents of protein-like OM, and pipe 3 was in contact with brackish water with higher Mn concentrations.