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Supplemental material

Observations and assessment of iron oxide and green rust nanoparticles in metal-polluted mine drainage within a steep redox gradient

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When taking water samples in the field, whether they are groundwater or surface water, it is well known that those water samples start to transform soon after removal from their native environment. In this study, because we were observing nanoscale features, it was especially critical that we understand the nature of the stability of our samples. Transmission electron microscopy (TEM) grids of water samples from the creek site (18 July 2011) were prepared in the field (~2 h old, with no air exposure until grid preparation which took less than 5 min), in the laboratory a few hours later, and the next day (with fixation with glutaraldehyde to preserve bacteria). Fig. S1 shows scanning electron microscopy images of these three grid types, and it is clear that the samples prepared on the same day look very similar (note the difference in magnifications when comparing particle sizes). However, the sample prepared one day later is different – characterised by larger primary particles and small needles growing out of the surface of those particles. Therefore, we only analysed water samples by TEM that were less than a day old. Sediment samples were prepared for TEM as soon as possible on the following day, and there was no obvious difference between fixed and unfixed samples.

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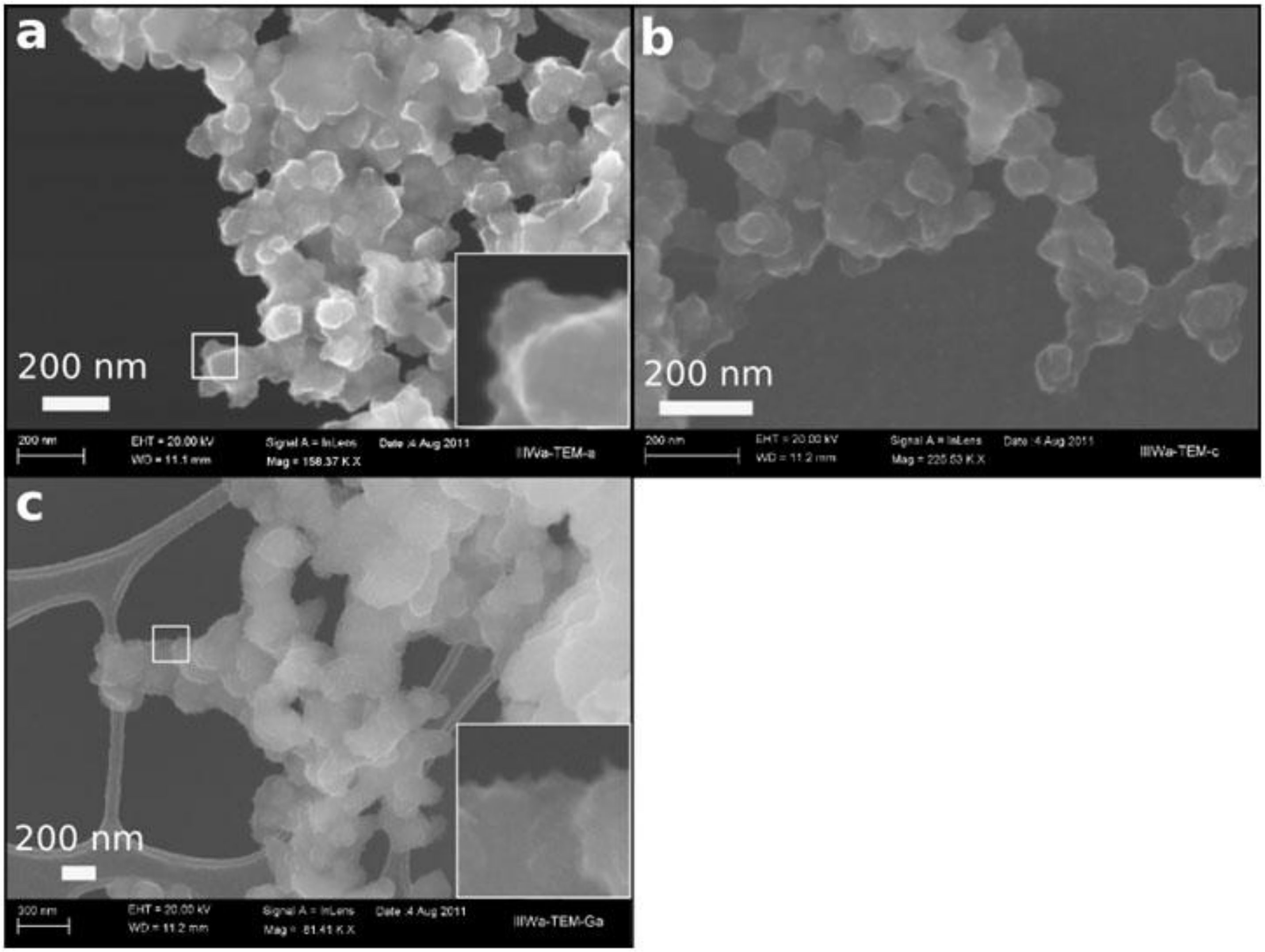

Fig. S1. Representative scanning electron microscopy images of grids showing the evolution of the water samples taken from the creek (18 July 2011) during storage. (a) Prepared grid in the field, (b) prepared grid in the laboratory on the same day as sampling, (c) fixed with glutaraldehyde and prepared the grid one day after sampling.

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Table S1. Water chemistry

Averages (standard deviations) of field triplicates. EC, electrical conductivity; DO, dissolved oxygen; Eh_{corr}, redox potential, corrected to the standard hydrogen electrode

	18 July 2011			25 October 2011		
	Outflow	Terraces	Creek	Outflow	Terraces	Creek
pH	n.d.	5.77	5.83	5.75	6.34	6.10
EC (mS cm^{-1})	n.d.	5.39	4.80	5.54	5.23	4.81
DO (mg L^{-1})	n.d.	n.d.	n.d.	0.6	8.5	5.0
Eh_{corr} (mV)	n.d.	n.d.	n.d.	260	280	307
pe	n.d.	n.d.	n.d.	4.39	4.74	5.20
Total Fe[C] (mM)	6.1 (1.1)[A]	7.2 (1.6)	4.8 (0.4)	5.9 (0.1)	4.9 (0.0)	4.4 (0.1)
<0.1 µm Fe(tot)[D] (mM)	5.9 (0.8)[A,B]	5.1 (0.1)	3.4 (0.2)	5.6 (0.1)	4.3 (0.3)	3.8 (0.0)
<0.2 µm Fe^{II} [E] (mM)	6.1 (0.1)[A]	5.8 (0.2)	3.8 (0.4)	5.64 (0.03)	4.6 (0.1)	3.9 (0.1)
<0.2 µm SO_4^{2-} (mM)	42 (1)[A]	45 (1)	39 (2)	40 (9)	33 (2)	33 (3)

[A]Sample triplicates.

[B]Filtered through a 0.2-µm filter.

[C]Determined by ICP-OES

[D]Determined by ICP-OES

[E]Determined spectrophotometrically

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Table S2. Element concentrations in filtered and unfiltered water

Averages (Avg) and standard deviations (s.d.) of field triplicates in concentrations of micromoles per litre. Maximum contaminant levels (MCL) are from the World Health Organization drinking water quality standards.[1] n.d., not determined; standard deviations with no replicates are marked by an en-dash

			18 July 2011						25 October 2011					
			Outflow		Terraces		Creek		Outflow		Terraces		Creek	
		MCL	Total	<0.1 µm	Total	<0.1 µm	Total	<0.1 µm	Total	<0.1 µm	Total	<0.1 µm	Total	<0.1 µm
Al (µM)	Avg		72.4	3.3	77.7	4.1	113.0	11.2	80.5	6.5	4.2	1.1	71.6	16.3
	s.d.		n.d.	n.d.	32.9	0.4	34.2	0.3	0.3	0.1	0.8	0.5	15.7	0.8
As (µM)	Avg	0.133	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.98	0.48	0.25	0.06	0.78	0.13
	s.d.		–	–	–	–	–	–	0.22	0.02	0.05	0.04	0.19	0.01
Ca (µM)	Avg		11371	10867	11970	10766	12503	11494	12374	11791	12072	11599	12793	12808
	s.d.		–	–	1099	374	1030	464	159	675	288	1664	172	377
Cd (µM)	Avg	0.0267	0.0396	0.0336	0.0260	0.0157	0.0842	0.0721	0.0416	0.0294	0.0031	0.0022	0.1039	0.0967
	s.d.		–	–	0.0037	0.0012	0.0051	0.0039	0.0080	0.0000	0.0004	0.0004	0.0012	0.0034
Co (µM)	Avg		13.9	13.5	11.4	10.3	12.1	11.0	12.8	12.7	9.8	9.3	13.4	13.3
	s.d.		–	–	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.8	0.2	0.1
Cr (µM)	Avg	0.962	0.082	0.005	0.087	0.008	0.037	0.007	0.118	<0.01	<0.01	<0.01	0.028	<0.01
	s.d.		–	–	0.030	0.003	0.013	0.001	0.1	<0.01	<0.01	<0.01	0.0	<0.01
Cs (µM)	Avg		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.055	0.046	0.074	0.069	0.046	0.043
	s.d.		–	–	–	–	–	–	0.004	0.002	0.008	0.010	0.001	0.001
Cu (µM)	Avg	31.5	0.216	0.057	0.328	0.133	2.277	0.475	0.203	0.023	0.023	0.016	1.587	0.662
	s.d.		–	–	0.10	0.06	0.58	0.02	0.11	0.00	0.01	0.00	0.16	0.24
Fe (µM)	Avg	5.37	6131	5900	7239	5050	4783	3385	5903	5626	4880	4309	4405	3809
	s.d.		–	–	1565	78	377	155	130	112	10	329	122	31
Mn (µM)	Avg	7.28	201	200	194	179	249	227	241	240	213	203	258	258
	s.d.		–	–	1	3	4	7	2	2	4	2	3	2
Na (µM)	Avg		2839	2769	3143	2843	2818	2639	3000	2927	3072	3017	2763	2808
	s.d.		–	–	324	119	209	136	87	169	78	430	45	122
Ni (µM)	Avg	1.19	50.0	48.5	48.5	43.1	37.7	33.6	40.3	40.3	34.7	32.7	32.9	33.1
	s.d.		–	–	1.2	1.1	0.5	1.1	0.5	0.3	1.0	3.1	0.2	0.1
Pb (µM)	Avg	0.0483	0.0123	<0.0005	0.0121	0.0012	0.0073	0.0022	0.0201	0.0011	<0.0005	<0.0005	0.0038	<0.0005
	s.d.		–	–	0.0039	0.0011	0.0028	0.0024	0.0127	0.0004	<0.0005	<0.0005	0.0017	<0.0005
Si (µM)	Avg		0.541	0.391	0.537	0.358	0.454	0.360	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	s.d.		–	–	0.097	0.004	0.022	0.014	–	–	–	–	–	–
U (µM)	Avg	0.126	0.51	0.47	1.74	0.54	0.44	0.14	0.51	0.45	0.51	0.38	0.41	0.20
	s.d.		–	–	1.40	0.03	0.10	0.01	0.03	0.01	0.12	0.07	0.05	0.01
Zn (µM)	Avg	76.4[A]	23.7	22.9	19.0	16.2	17.9	14.8	20.5	20.1	7.3	6.9	14.2	14.2
	s.d.		–	–	0.8	0.3	2.1	0.3	8.0	7.6	0.5	0.5	0.4	0.0

[A]Suggested maximum level for aesthetic drinking water quality[1]

Fig. S2. Gessen Creek field site photos. (a) Gessen Creek just a few metres upstream from the 'creek' site (October 2011), (b) 'terraces' site (June 2011, note the more yellow colour where water is flowing, and reddish-brown where it is not), (c) close-up of terrace sediments (October 2011), (d) 'creek' site (June 2011), (e) creek site sediments (October 2011).

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Table S3. Sediment and sediment pore-water characteristics

Averages (standard deviations) of field triplicates. EC, electrical conductivity

	18 July 2011		25 October 2011	
	Terraces	Creek	Terraces	Creek
pH	5.56	5.51	4.75	5.62
EC (mS cm^{-1})	5.33	4.62	4.18	4.1
Density (g WW cm^{-3})	1.32	1.11	1.40 (0.03)	1.14 (0.10)
Dry weight fraction (g g^{-1} WW)	0.29 (0.05)	0.12 (0.02)	0.38	0.22
Total Fe (mmol g^{-1} DW)	8.41 (0.57)	8.37 (0.02)	9.63 (0.05)	9.12 (0.06)
Pore-water < 0.2 µm Fe^{II} (mM)	5.4 (2.7)	1.5 (0.3)	1.5 (0.6)	1.8 (0.2)

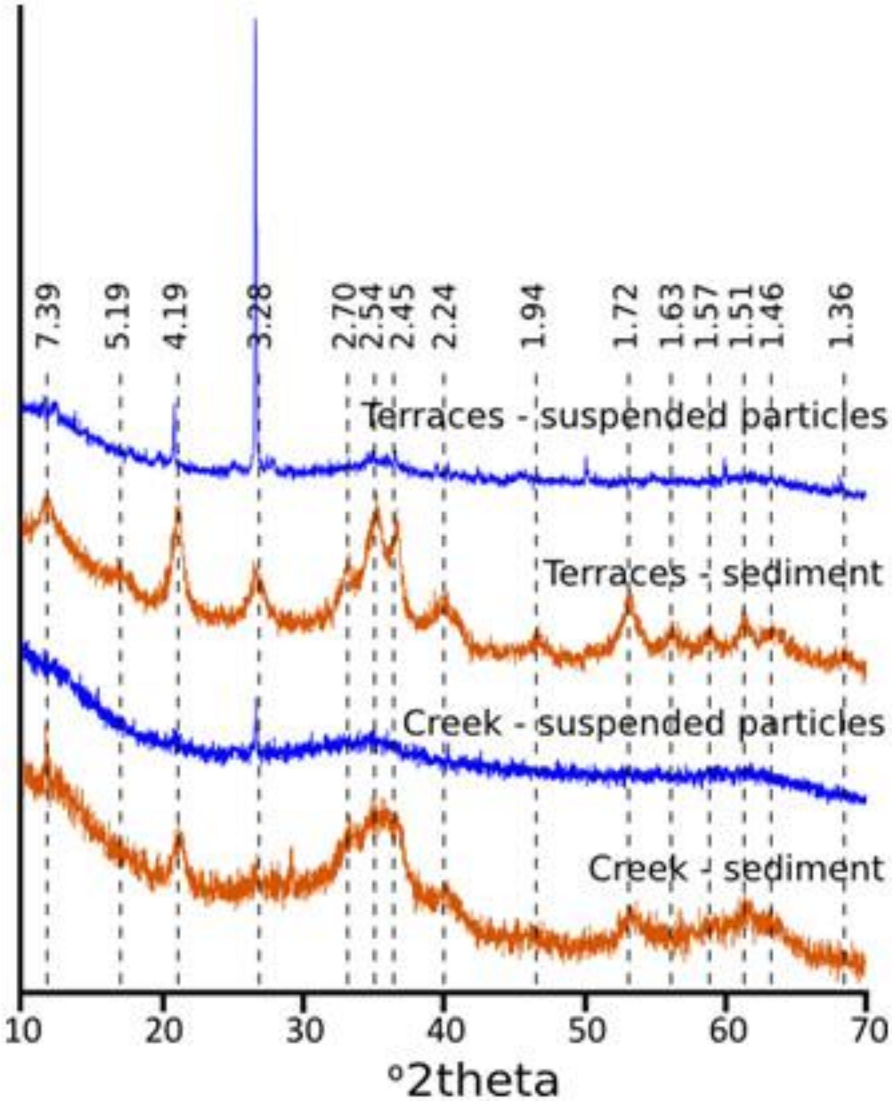

Fig. S3. X-Ray diffraction patterns of suspended particles and sediments in the terrace and creek sites (October 2011). Numbered lines are d-spacings in Angstroms. The *y*-axis represents the relative intensity.

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Table S4. ICP-MS data from the sequential extraction of sediments

All concentrations in micromoles per gram (dry weight sediment), sampled 18 July 2011 in triplicate. F6 averages are based on less than three replicates, so the standard deviation cannot be calculated

		Terraces							Creek						
		Whole	F1	F2	F3	F4	F5	F6	Whole	F1	F2	F3	F4	F5	F6
Al	Avg	109.13	1.51	0.40	7.13	17.70	0.64	37.47	145.99	1.48	5.68	79.21	17.62	0.48	13.17
	s.d.	3.30	0.03	0.04	0.21	0.56	0.11		3.59	0.35	0.07	0.14	1.77	0.05	
As	Avg	4.850	0.028	0.056	3.258	2.863	0.002	0.024	7.415	0.044	0.118	7.947	1.273	0.000	0.000
	s.d.	0.172	0.005	0.011	0.269	0.084	0.003		0.093	0.014	0.006	0.105	0.066	0.000	0.000
Ca	Avg	52.5	33.9	0.6	1.2	1.1	1.0	0.8	138.6	82.2	3.0	2.8	0.4	0.4	0.4
	s.d.	2.5	1.7	0.1	0.0	0.1	0.1		1.9	5.2	0.2	0.1	0.1	0.1	
Cu	Avg	0.166	0.010	0.008	0.018	0.047	0.000	0.032	4.546	0.036	0.584	2.044	0.748	0.022	0.036
	s.d.	0.011	0.007	0.010	0.006	0.004	0.000		0.165	0.010	0.006	0.009	0.044	0.003	
Fe	Avg	7076	68	71	2908	2645	0	43	5447	66	97	3636	697	0	10
	s.d.	87	7	8	9	79	0		20	20	4	33	67	0	
Mg	Avg	72.26	37.99	0.64	0.93	5.41	1.00	10.53	80.89	59.26	0.42	1.06	1.50	0.41	3.20
	s.d.	3.60	1.13	0.10	0.03	0.14	0.08		1.63	1.95	0.01	0.04	0.06	0.03	
Mn	Avg	1.55	0.37	0.00	0.08	0.53	0.00	0.15	2.40	0.67	0.05	0.49	0.36	0.00	0.04
	s.d.	0.06	0.01	0.00	0.00	0.01	0.00		0.05	0.07	0.00	0.01	0.02	0.00	
Na	Avg	7.81	4.15	0.29	0.00	0.00	0.73	1.12	9.66	5.90	0.00	0.00	0.14	0.19	0.63
	s.d.	0.69	0.17	0.50	0.00	0.00	0.85		2.71	0.34	0.00	0.00	0.24	0.05	
Ni	Avg	1.66	0.46	0.02	0.27	0.51	0.00	0.03	3.53	0.38	0.25	1.20	0.13	0.09	0.02
	s.d.	0.08	0.02	0.00	0.01	0.03	0.00		1.32	0.04	0.00	0.02	0.02	0.04	
P	Avg	32.3	0.3	0.0	9.6	16.2	1.4	1.1	48.1	0.3	0.0	22.8	7.7	2.2	0.4
	s.d.	1.7	0.0	0.0	0.3	0.8	0.4		1.0	0.1	0.0	0.1	0.5	0.1	
S	Avg	2660	633	5	137	904	8	258	4117	1140	89	845	611	2	74
	s.d.	95	9	8	4	23	1	0	85	113	3	15	30	2	
Si	Avg	7.4	8.4	17.9	48.6	68.8	1.2	2.4	11.1	9.2	32.3	133.6	27.9	0.3	1.2
	s.d.	2.7	0.1	3.4	0.9	2.4	0.3		4.6	0.9	0.6	2.4	1.4	0.1	
U	Avg	0.532	0.184	0.100	0.093	0.096	0.001	0.001	1.402	0.015	0.440	0.695	0.024	0.000	0.000
	s.d.	0.012	0.002	0.006	0.001	0.002	0.000		0.005	0.003	0.002	0.020	0.003	0.000	
Zn	Avg	3.75	0.28	0.00	0.56	2.00	0.00	0.09	3.74	0.15	0.13	1.39	0.83	0.00	0.03
	s.d.	0.17	0.02	0.00	0.02	0.05	0.00		0.66	0.02	0.01	0.09	0.03	0.00	

References

[1] World Health Organization, Chemical aspects, in *Guidelines for Drinking-Water Quality* **2011**, pp. 155–201 **(**WHO Press: Geneva, Switzerland).