Ecofide test report, 13-5-2023, final
Chronic toxicity of arsenic-spiked sediment to the amphipod *Hyalella azteca*

1. Goal of the present research

The EU funded project LIFE NARMENA[1] aims to increase available water storage capacity by removing metal pollutants from watercourses and floodplains through nature-based remediation. Its objectives are to demonstrate bacteria-assisted phytoremediation and constructed wetland techniques in Flemish watercourses polluted by chromium, arsenic, cadmium, and radium. The project also aims to develop an application framework to replicate these techniques and strengthen related nature and soil policies (https://webgate.ec.europa.eu/life/publicWebsite). To aid interpretation of the project results and improve ecological risk assessments, Ecofide was asked to determine the chronic toxicity of arsenic-spiked sediment to the amphipod *Hyalella azteca*.

2. General outline

A natural freshwater sediment was spiked in a 2-stage method with an equilibration period of at least 16 weeks. Semi-static pilot-experiments with varying refreshment schemes were performed to determine the minimum renewal frequency, required to keep the dissolved arsenic concentration below an estimated threshold of 100 µg/l (see intermezzo). Chronic toxicity of arsenic-spiked sediments was determined for the amphipod *Hyalella azteca* during a six-week exposure. Experimental set-up was based on EPA (2000) with some modifications to suit the characteristics and binding capacity of the natural sediment used in the present study. After four weeks survival and growth were assessed, while the reproductive output was determined after six weeks. Chemical analyses were performed to determine actual exposure conditions.

Intermezzo Toxicity of arsenic to *Hyalella* species in water-only exposures (data provided by Arche consulting)

* Acute toxicity water-only (mortality; 96 hrs) – NOEC 0.7 **m**g/l; LOEC 1.9 **m**g/l (Liber *et al*, 2011)
* Acute toxicity sediment-exposure (10 days) – NOEC <0.42 **m**g/l (based on porewater conc with high DOC)
* Chronic exposure (growth; 28 days) – EC_{25} 3920 nmol/L respectively 0.3 **m**g/L (Norwood *et al*., 2007)
* Most sensitive endpoint: Chronic exposure (growth; 28 days) – EC_{10} 200.8 **µ**g/L (ARCHE, 2022)

The median Hazardous Concentration (HC_{5-50}) of arsenic is recently set at 16.8 µg/l (Arche, 2020; Registration Dossier - ECHA (europa.eu)). In the same document an assessment factor of 3 is put forward, resulting in a $PNEC_{freshwater}$ of 5.6 µg/l.

3. Sediment spiking

The natural sediment was sampled from the Steenputbeek (50° 42’ 57,5” N; 4° 16’ 44,9” E), a small stream in the vicinity of the city Halle, 15 kilometers to the south of Brussels, Belgium. The sediment has been sampled for previous studies by the University of Antwerp and was found to contain low metal concentrations. It is a sandy sediment (grain size between 125 and 1000 µm; $P_{10, 50, 90}$: 224, 361 and 579 µm), expected to resemble exposures under a high arsenic availability as organic matter, Fe and AVS contents are low (Table 1[2]).

Note. The organic matter content of 0.32% in the freshly sampled batch was lower than the intended value of 1-2%. As this low value will influence the bioavailability of arsenic, the organic matter content was increased up to 1.3% by adding dried and grinded poplar leaves sampled from an unpolluted reference location. This procedure also led to a slight increase for several metals (Zn: 6.9; Cu: 0.9; Pb: 2.1; Fe: 1345 mg/kg; see also measurements of actual concentrations at the start of the experiment in the appendices).

[1] Acronym for “Nature-based Remediation of Metal pollutants in Nature Areas to increase water storage capacity”

[2] AVS <1mmol/kg; Total organic carbon <1-2% and Fe <7.5 g/kg DW

Table 1. General characteristics of sediment from the Steenputbeek. All data refer to the batch sampled for the present toxicity study (sampled December 2021) and were measured before addition of poplar leaves (see text). Average values (standard deviations between brackets) are shown based on triplicate measurements.

Parameter	Units	Value
Org. matter	%	0.32 (0.13)
Moisture content	%	18.8 (1.0)
AVS	mmol/kg dw	0.17 (0.01)
AVS-SEM	"	-0.11 (0.01)
Fe	g/kg dw	0.84 (0.06)

Parameter	Units	Value
As	mg/kg dw	0.59 (0.13)
Cu	"	0.45 (0.11)
Ni	"	1.37 (0.56)
Pb	"	1.30 (0.28)
Zn	"	3.87 (1.40)

Sediment spiking was based on the procedures described by Besser *et al*. (2013) and Brumbaugh *et al*. (2013) studying the chronic toxicity of nickel-spiked sediments. Twenty liter of fresh sediment was wet sieved over 2 mm, mechanically mixed and split into two separate batches.

In the first stage of spiking, an arsenate ($Na_2HAsO_4.7H_2O$) solution was added to 5L sediment resulting in a final concentration of 500 mg/kg dw. The pH of the overlying water was adjusted and kept on a constant value of 7.4-7.7 during an 8-week equilibration period. This equilibration took place in sealed containers at 20°C, which were periodically mixed on a rolling mill. The other batch of 15L was used as reference and for further dilutions of this 'super-spike'. Both batches followed the same procedures throughout all treatments. Spiking took place in a 1:1 v/v mixture of wet sediment and reconstituted medium as described in the EPA guidance 600/R-99/064 (EPA, 2000) to which bromide was added in a final concentration of 0.8 mg/L (Borgmann, 1996; Ivey & Ingersoll, 2016). This bromide enriched reconstituted medium was also used in the toxicity experiments. In the second stage, this super-spike (actual arsenic concentration of 258 mg/kg dw) was diluted with varying amounts of reference sediment to produce a series of 5 arsenic concentrations (nominal values: 0, 1, 5, 20, 50 and 200 mg/kg dw). This series of spiked sediment was again equilibrated for 8-weeks during which pH-values were regularly checked (and adjusted when necessary) while the sealed containers were periodically mixed on a rolling mill.

4. Experimental set-up

After this equilibration period, a 14-day pilot-experiment was started to determine the refreshment scheme required for the overlying water. Around 60% of the overlying water was intermittent refreshed with different time intervals varying from a control without renewal, twice and four times each week up to once, twice and four times per day. Regular analyses of dissolved arsenic concentrations (filtered over 0.45 µm; Rotilabo Cellulose-acetate filters) provided insight in diffusion rates and the possible flushing of excess unbound arsenic from sediments (especially for the highest treatments). The overlying water was typically sampled approximately 30 min before a water renewal cycle to allow arsenic concentrations to approach their maximum values. The results (table 2) illustrate that:

i) without renewal, arsenic concentrations in the overlying water increased during the experiment from 80 to 110-130 µg/l in the 50 mg/kg treatment and from 304 up to 520 µg/l in the 200 mg/kg treatment. This indicates that both treatments surpassed the binding capacity of the sediment. Such increased arsenic concentrations in the overlying water will intermingle with toxicity of sediment bound arsenic and should therefore be avoided.

ii) renewal of the overlying water on a daily base (or more frequently) led to a clear decrease of arsenic concentrations in the overlying water during the experiment. Remaining arsenic concentrations did no longer exceed forementioned threshold of 100 µg/l at the end of the experiment.
Note. The sediment-water systems were prepared one week ahead of starting the experiment to provide small sediment particles ample time to settle. The first samples for chemical analyses were taken at t=1.

iii) the difference between daily renewal and a renewal-frequency of 2 resp. 4 times per day was small for the 50 mg/kg treatment (except for the fact that the arsenic concentrations decreased faster at higher renewal frequencies). For the 200 mg/kg treatment this was not yet the case, again indicating the presence of a substantial amount of unbound arsenic in the porewater.

In general, the results illustrate that a daily renewal might be sufficient to keep the arsenic concentrations in the overlying water (far) below the threshold of 100 µg/l, but at the same time demonstrate that the present set-up was not yet sufficient (especially for the 200 mg/kg treatment). It was therefore decided:

i) to rinse all treatments twice with EPA-medium to flush the unbound arsenic from the porewater;
ii) to increase the overlying water/sediment ratio. Pilot experiments were run on a 4:1 v/v ratio, while a ratio of 8:1 v/v was used for the toxicity experiments;
iii) to increase the volume of daily refreshed overlying water from 60 to 80% of the volume (a layer of 1.5 cm above the sediment was not refreshed) and
iv) to start water renewal for the toxicity tests one week ahead to remove the effect of flushing unbound arsenic from sediments and minimize the accumulation of toxic arsenic concentrations in the overlying water

Table 2. Dissolved arsenic concentrations (µg/l) in the overlying water during a 14 day pilot experiment to determine refreshment rates.
☐ =As conc >30 µg/l; ☐ = 20<As<30 µg/l; ☐= 10<As<20 µg/l; ☐ = As <10 µg/l

Nominal As conc sediment (mg/kg)	Refreshment scheme	Sampling intervals from day 1 up to 14 days			
		1	6	9	14
50	no renewal	80.5	110.1	126.5	109.9
"	2*/week	61.5	46.3	42.5	28.4
"	daily	100.8	29.1	20.4	12.2
"	2*/day	180.3	20.3	16.8	9.9
"	4*/day	23.1	15.8	17.0	8.3
200	no renewal	303.9	570.4	565.9	519.7
"	2*/week	249.5	313.5	242.8	160.2
"	daily	583.6	137.2	119.5	102.3
"	2*/day	503.1	85.5	75.6	49.6
"	4*/day	277.0	55.0	36.1	33.5

Chronic toxicity was assessed using 12 replicates for each arsenic concentration. Glass beakers of 250 ml were filled with a 1 cm layer of sediment and 8 cm overlying water (bromide enriched EPA-medium). 80% of the overlying water was daily refreshed. Renewal started one week before adding the juvenile amphipods. Twenty juvenile *H. azteca* (7 days old) were added to each replicate at the start of the toxicity test. After 28 days 4 replicates were used to assess survival and growth, while survival and reproduction was determined in another 4 replicates after 42 days.

Note: The assessment of the reproduction within sediment-water test systems deviates from the water-only approach mentioned in the EPA (2000) guidelines. The present approach was chosen for its prolonged exposure to arsenic spiked sediment while pilot experiments demonstrated a suitable reproduction after 42 days (>50 juveniles per test beaker). Reducing the amount of sediment (see above: 1:8 v/v with overlying water) made the workload of finding these young neonates/juveniles acceptable.

Animals were daily fed with 1 ml YCT-suspension[3] (EPA, 2000). In addition, 1 ml of a Tetramin-suspension (6.3 g/L) was added three times a week during the first 28 days and daily during the 5th and 6th week to increase reproduction (Besser *et al.*, 2016). Temperature was maintained at 23 ± 1°C and the photoperiod was set at 16L:8D. Sediment and overlying water were sampled for chemical analyses at the start and after 14 (water

[3] YCT=Yeast, Cerophyl and Trout Chow

only), 28 and 42 days. The overlying water was sampled approximately 30 min before a water renewal cycle to allow arsenic concentrations to approach their maximum values. Separate test chambers were used for chemical analyses, but they were stocked with test organisms and maintained in the same manner as those used for assessing toxicity. Samples of the overlying water were taken 0.5 cm above the sediment-water interface with a low flowrate to prevent inclusion of small sediment particles.

5. Test acceptability

The control treatment complied with acceptability criteria mentioned in EPA (2000) with a mean survival of 96% after 28 days and 93% after 42 days. In addition, EPA (2000) also mentioned a few more indicative criteria based of a round-robin test: length after 28 days >3.2 mm (present research 4.5 mm) and a reproduction >2 juveniles/female after 42 days (present research 11.6 juveniles/female). In addition, Au *et al* (2015) mentioned a reproduction of 10-15 juveniles per female after 42 days, Ivey & Ingersoll (2016) 5-15 juveniles/female while Besser *et al.* (2016) illustrated the effect of different feeding regimes (1.6 juveniles/female on YCT and 9.1 juveniles/female on a Diatom+Tetramin diet). The control treatment of the present experiments (11.6 juveniles/female) corresponds well with these figures.
Sensitivity of the 7-day old juveniles was checked using the standard 96-h reference toxicity test with KCl. With a LC_{50}-value of 274 mg/L the value fell within the range of 232-372 mg/l (mean 305 mg/l) from the round-robin mentioned in EPA (2000). Oxygen saturation, pH, conductivity as well as nitrite and ammonia concentrations were checked weekly. All values stayed within criteria set for these possible confounding factors. Due to the daily renewal, overlying water was not aerated during the test and oxygen saturation varied between 60 and 93%[4]. pH values in the overlying water varied between 7.4 and 7.8 with no differences between treatments. The same applied to conductivity (26 – 28 µS/mm), nitrite (<2 mg/l) and ammonia concentrations (<10 mg/l). These physical-chemical parameters did therefore not affect the test results. Based on these criteria the toxicity test was deemed acceptable.

6. Results of physical-chemical analyses

Sediment, porewater and overlying water were sampled on $T_{0,\ 14,\ 28\ \text{and}\ 42}$[5]. Besides arsenic and several other metals, samples were used to determine organic matter and AVS-SEM in sediment as well as dissolved organic carbon (DOC), pH, $CaCO_3$ and conductivity in overlying water (average values and standard deviations are shown in the appendix).

Actual arsenic concentrations in the sediment were on average 4 times lower than the nominal intended values (Table 3). This low binding capacity of the sediment is a direct consequence of the experimental set-up resembling exposures under a high arsenic availability as organic matter, Fe and AVS contents of the sediment were low. Average organic matter content of the sediment was 1.1%. AVS (mmol/kg dw) increased during the experiment ($T_{0,\ 28,\ 42}$: control 0.6 – 1.2 – 1.4; treatment nominal 200 mg As/kg 0.6 – 1.1 – 11.7) Arsenic concentrations in porewater increased with increasing treatments (Table 3). At T_0 arsenic concentrations in the overlying water were on average six times higher compared to $T_{14,\ 28,\ 42}$ even though the sediment-water systems were prepared one week in advance and the overlying water was daily refreshed. Concentrations in the overlying water from the highest arsenic treatment (200 mg/kg) surpassed the 100 µg/l mentioned above, suggesting that toxicity up to the 50 mg/kg treatment was (primarily) caused by sediment exposure, while toxic effects in the 200 mg/kg treatment will also be influenced by exposure to dissolved arsenic in the overlying water. The increase in arsenic concentrations in the overlying water of the 200 mg/kg treatment was already noted in the pilot experiments (Table 2) and suggests that the binding capacity of the sediment was surpassed. The porewater pH varied between 7.3 and 7.6 (average = 7.46) and did not differ significantly between treatments. The average DOC-concentration in porewater was 21.1 mg/l

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[4] Oxygen saturation directly after renewal: 90-95%; 30 min before the next renewal: 60-72%
[5] at T14 only overlying water

(min-max: 16-32). Concentrations of other metals in porewater, AVS-SEM and overlying water did not show treatment related differences, except for a decrease in the Al and Fe concentrations in the overlying water of the two highest treatments (Appendix).

Table 3. Physical chemical analyses during the toxicity tests. Average of three or four replicates[6] ($t_{0,14,28,42}$ days) are presented together with the standard deviation between brackets.

Treatment (nominal As conc)	Sediment		Porewater			Overlying water		
	As	Org. matter	As[1)]	AVS	SEM	As[1)]		DOC
(mg/kg dw)	mg/kg dw	%	µg/l	mmol/ kg dw	mmol/ kg dw	µg/l t_{0-42}	µg/l t_{14-42}	mg/l
0	0.52 (0.21)	0.75 (0.24)[2)]	5.9 (2.8)	1.1 (0.4)	0.09 (0.02)	0.63 (0.74)	0.27 (0.15)	1.5 (0.40)
1	1.0 (0.21)	1.1 (0.20)	15.8 (4.7)	1.4 (0.7)	0.11 (0.01)	3.2 (4.0)	1.2 (0.65)	1.4 (0.03)
5	1.3 (0.43)	1.2 (0.38)	25.7 (7.7)	1.1 (0.5)	0.11 (0.01)	9.1 (12.0)	3.1 (1.8)	1.2 (0.39)
20	3.7 (0.87)	1.1 (0.38)	83.9 (9.7)	5.7 (7.9)	0.10 (0.01)	22.6 (28.1)	8.6 (3.4)	1.5 (0.48)
50	8.0 (1.5)	1.0 (0.25)	165 (31.8)	4.9 (6.8)	0.10 (0.01)	59.4 (58.5)	30.3 (6.9)	1.2 (0.47)
200	50.4 (8.6)	1.2 (0.53)	312 (167)	4.5 (6.3)	0.10 (0.02)	176 (40.5)	156 (1.5)	1.0 (0.47)

[1)] dissolved concentrations (filtered over 0.45 µm); [2)] duplicate; org matter at t=42 was not accurate (<0%)

7. Toxicity

The lowest three treatments (1, 1.3 and 3.7 mg As/kg dw; actual) did not cause any effect on survival, growth, or reproduction (Table 4), while survival was still unaffected in the 8.0 and 50.4 mg As/kg treatments. These two highest treatments did however reduce the growth rate and reproduction of *Hyalella*. Reduction percentages for the growth rate after 28 days were 12 and 16% respectively, while reduction percentages for reproduction after 42 days were 41 and 55%.
Indications for a reduced growth rate in these two treatments were already obtained after 14 days based on visual observations during the daily renewal of the overlying water. Furthermore, first reproduction in the controls up to 3.7 mg/kg was visually observed at T_{25}, while first reproduction in the 8.0 and 50.4 mg/kg was observed at T_{30}. These observations demonstrate a delay in the onset of reproduction in the two highest treatments, probably caused by a decreased growth rate.

[6] Overlying water only

Table 4. Survival, growth, and reproduction after 28 and 42 days. Average of four replicates are presented together with the standard deviation between brackets. Orange shading illustrate significant effects. Estimated NOEC, L(E)C_{10} and L(E)C_{50}-values are presented with the 95% cl between brackets.

Treatment		Survival		Growth		Reproduction	
As conc		T_{28}	T_{42}	T_{28}		T_{42}	
(mg/kg dw) nom.	act.	%	%	mm [1)]	% reduction	Juveniles/ female	% reduction
0	**0.5**	96 (2.5)	93 (2.9)	4.51 (0.18)	-	11.6 (0.6)	-
1	**1.0**	96 (2.5)	93 (2.9)	4.70 (0.25)	+4.1	12.0 (1.5)	+3.4
5	**1.3**	90 (7.1)	94 (6.3)	4.60 (0.31)	+2.0	12.6 (2.9)	+8.2
20	**3.7**	89 (11.1)	91 (4.8)	4.57 (0.28)	+1.4	12.4 (1.9)	+7.1
50	**8.0**	91 (4.8)	85 (4.1)	3.97 (0.29)	12.0	6.8 (2.8)	41.4
200	**50.4**	89 (4.8)	88 (6.5)	3.77 (0.13)	16.5	5.2 (1.1)	55.0
Test parameters based on actual arsenic concentrations in the sediment							
NOEC		50.4	50.4	3.7		3.7	
L(E)C_{10}		>50.4	>50.4	6.8 (4.0 – 23.7)		4.7 (2.0 – 5.7)	
L(E)C_{50}		>50.4	>50.4	>50.4		33.1 (22.2 – 55.7)	

[1)] Average length at T_0 was 1.81 mm

Calculated effect parameters (NOEC, L(E)C_{10} and L(E)C_{50} values; including 95% confidence intervals) are summarized in table 4. It should be noted that maximum effect percentages measured for growth and reproduction were 16.5 and 55 respectively (Table 4). This effect range is sufficient for NOEC and EC_{10}-estimations, while the statistical calculation of the EC_{50}-value for reproduction could have benefited from higher exposure regimes. However, the chosen sediment did not allow higher exposure regimes as the binding capacity already seemed to be surpassed in the 50.4 mg As/kg treatment. The toxicity in this treatment is probably caused by a combined exposure to sediment and increased arsenic concentrations in the overlying water. As the experiment intended to be a sediment-only exposure, interpretations should be focused on the chronic NOEC- and/or EC_{10}-values.

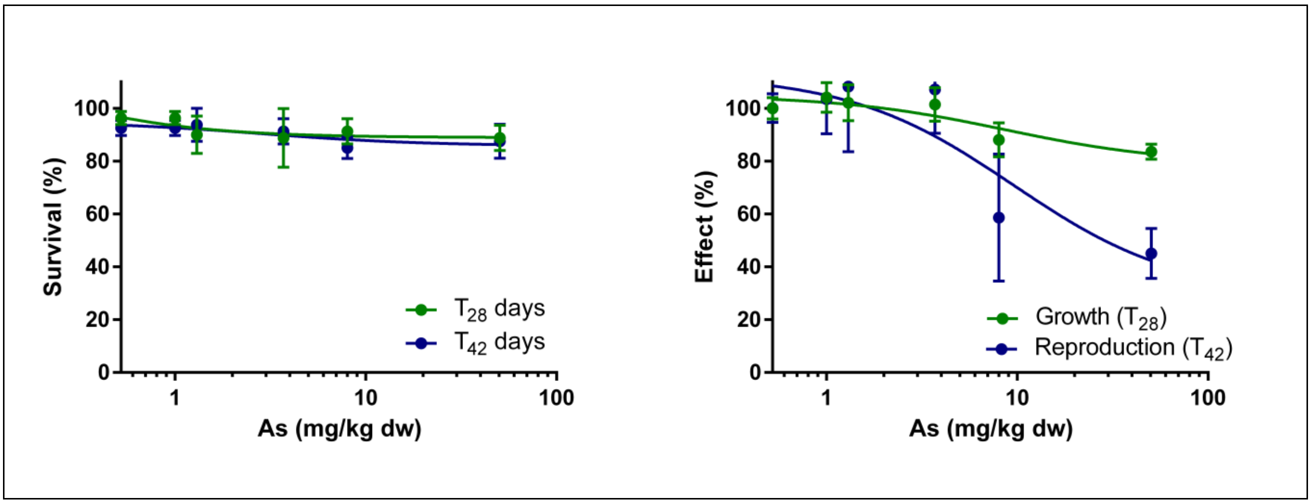

Figure 1. Dose-response curves for survival after 28 and 42 days (left) and growth (28 days) and reproduction (42 days) on the right-hand side. Effects on growth and reproduction are shown as effect percentages compared to the control sediment on the y-axis.

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Appendix chemical analyses

Concentrations in sediment
Average actual concentration (n=3) with the standard deviation between brackets.
When (some) concentrations were below the limit of quantification, average values are based on 0.5 * LOQ and shown in italic.

Treatment (nominal As conc)	Sediment (mg/kg DW)									
(mg/kg dw)	Al	Fe	Mn	Co	Cr	Cu	Cd	Ni	Pb	Zn
0	285 (70.8)	823 (77.4)	16 (4.3)	0.6 (0.1)	0.6 (0.3)	1.0 (0.6)	*0.02 (0.02)*	1.0 (0.4)	2.1 (0.5)	6.8 (0.6)
1	329 (66.8)	795 (101)	21 (12.5)	0.7 (0.2)	0.7 (0.1)	0.6 (<0.1)	*0.01 (0.01)*	0.8 (0.1)	1.9 (0.4)	9.4 (2.8)
5	320 (27.2)	825 (57.6)	16 (6.6)	0.5 (<0.1)	0.8 (0.1)	0.6 (0.1)	*0.01 (0.01)*	0.8 (0.1)	1.7 (0.1)	7.8 (0.3)
20	247 (23.2)	725 (62.2)	15 (6.1)	0.4 (0.1)	0.4 (0.1)	0.5 (0.1)	*<0.01 (-)*	0.6 (<0.1)	1.7 (0.1)	6.5 (1.2)
50	430 (145)	813 (236)	28 (7.1)	0.5 (0.1)	0.8 (0.2)	0.6 (0.2)	*0.01 (0.01)*	0.8 (0.2)	1.7 (0.4)	7.4 (2.1)
200	343 (30.1)	950 (99.2)	22 (11.3)	0.5 (<0.1)	0.8 (0.1)	1.3 (0.8)	*<0.01 (-)*	0.7 (0.1)	1.7 (0.1)	7.4 (1.5)

Dissolved concentrations in porewater (filtered over 0.45 µm)
Average concentration (n=3) with the standard deviation between brackets.
When (some) concentrations were below the limit of quantification, average values are based on 0.5 * LOQ and shown in italic.

Treatment (nominal As)	Porewater									
(mg/kg dw)	Al (mg/l)	Fe (mg/l)	Mn (mg/l)	Co (µg/l)	Cr (µg/l)	Cu (µg/l)	Cd (µg/l)	Ni (µg/l)	Pb (µg/l)	Zn (µg/l)
0	*<0,005*	0.1 (<0.1)	0.6 (0.4)	0.2 (0.2)	*<0.1 (0.06)*	1.4 (0.7)	*0.03 (0.02)*	1.3 (0.1)	0.2 (0.1)	1.7 (0.1)
1	*<0,005*	0.2 (0.1)	0.7 (0.5)	0.2 (0.1)	*<0.1 (0.01)*	1.0 (0.2)	*0.02 (0.02)*	1.6 (0.1)	0.1 (<0.1)	1.5 (0.3)
5	*<0,005*	0.2 (0.2)	0.7 (0.5)	0.3 (0.3)	*<0.1 (0.04)*	0.9 (0.1)	*<0.01 (-)*	1.8 (0.2)	0.2 (<0.1)	1.8 (0.5)
20	*<0,005*	0.1 (<0.1)	0.7 (0.4)	0.3 (0.2)	*<0.1 (-)*	0.6 (0.1)	*0.01 (0.02)*	2.1 (0.9)	0.1 (<0.1)	1.3 (0.1)
50	*<0,005*	0.1 (0.1)	0.7 (0.3)	0.4 (0.2)	*<0.1 (-)*	0.4 (0.2)	*0.01 (0.02)*	1.5 (0.5)	0.1 (<0.1)	1.0 (0.4)
200	*<0,005*	0.3 (0.1)	0.7 (0.5)	0.4 (0.3)	*<0.1 (-)*	0.5 (0.1)	*0.02 (0.03)*	2.2 (1.0)	0.1 (<0.1)	1.7 (0.1)

SEM-metals
Average concentration (n=3) with the standard deviation between brackets.
When (some) concentrations were below the limit of quantification, average values are based on 0.5 * LOQ and shown in italic.

Treatment (nominal As conc)	Sediment (metal concentrations during AVS-SEM analyses in µmol/kg)							
(mg/kg dw)	Co	Cr	Cu	Cd	Ni	Pb	Zn	Ratio AVS/SEM
0	1.6 (0.5)	29.1 (23.1)	3.8 (2.6)	0.49 (0.06)	14.0 (9.1)	8.1 (1.2)	66.7 (9.5)	11.9 (6.1)
1	1.8 (0.4)	26.2 (21.1)	2.3 (2.3)	0.55 (0.04)	12.7 (7.1)	9.1 (0.6)	80.0 (4.9)	13.0 (6.9)
5	2.0 (0.4)	44.7 (12.3)	2.1 (2.0)	0.55 (0.09)	20.2 (2.3)	9.4 (0.4)	78.6 (10.6)	9.6 (4.4)
20	1.7 (0.2)	13.7 (18.5)	1.5 (1.7)	0.53 (0.05)	9.1 (8.3)	8.0 (0.2)	75.2 (11.9)	54.5 (74.4)
50	1.9 (0.2)	34.4 (10.4)	1.5 (1.8)	0.50 (0.06)	16.0 (3.3)	9.0 (2.4)	67.2 (7.0)	46.7 (61.7)
200	2.4 (0.9)	33.8 (25.3)	1.5 (1.5)	0.45 (0.17)	16.3 (9.1)	6.8 (0.8)	70.2 (19.1)	47.6 (66.1)

Dissolved concentrations in overlying waters

Average concentration (n=4) with the standard deviation between brackets.
When (some) concentrations were below the limit of quantification, average values are based on 0.5 * LOQ and shown in italic.

Treatment (nominal As)	Dissolved metal concentrations (Ca, K, Mg, Na, $CaCO_3$ in mg/l; other metals in µg/l; Conductivity in µS/mm)									
(mg/kg dw)	Al	Fe	Mn	Ca	K	Mg	Na	$CaCO_3$	pH	Cond.
0	2.2 (2.5)	84.8 (47.2)	66.8 (41.4)	17.0 (1.2)	2.5 (0.1)	6.3 (0.2)	21.6 (1.2)	68.5 (2.8)	8.0 (0.15)	34 (11.7)
1	1.1 (1.7)	102.6 (72.4)	121.5 (134.3)	17.3 (1.1)	2.6 (0.1)	6.3 (0.2)	22.2 (2.6)	69.4 (2.6)	7.9 (0.13)	31 (6.4)
5	2.6 (3.9)	98.7 (79.8)	98.4 (70.8)	17.7 (1.5)	2.5 (0.1)	6.4 (0.1)	21.6 (1.3)	70.7 (3.3)	7.9 (0.13)	32 (7.3)
20	1.0 (1.2)	70.2 (30.1)	86.9 (66.6)	16.8 (1.3)	2.5 (0.1)	6.2 (0.2)	19.8 (1.7)	67.2 (3.1)	7.9 (0.16)	32 (8.5)
50	0.3 (0.4)	51.6 (12.6)	65.1 (43.0)	17.6 (2.1)	2.6 (0.1)	6.3 (0.2)	21.5 (2.7)	70.1 (5.6)	8.0 (0.18)	35 (8.5)
200	0.2 (0.3)	49.1 (19.5)	60.8 (30.5)	16.4 (0.9)	2.4 (0.1)	6.2 (0.2)	21.0 (2.5)	66.6 (1.8)	7.9 (0.09)	37 (10.4)

Treatment (nominal As conc)	Dissolved metal concentrations (µg/l)						
(mg/kg dw)	Co	Cr	Cu	Cd	Ni	Pb	Zn
0	0.10 (0.10)	0.19 (0.11)	0.18 (0.10)	0.03 (0.05)	0.29 (0.10)	0.07 (0.03)	1.9 (0.7)
1	0.14 (0.12)	0.14 (0.10)	0.19 (0.08)	0.03 (0.04)	0.30 (0.13)	0.08 (0.05)	1.6 (1.0)
5	0.15 (0.11)	0.17 (0.10)	0.20 (0.07)	0.01 (0.03)	0.32 (0.09)	0.09 (0.02)	1.6 (0.5)
20	0.12 (0.11)	0.12 (0.07)	0.21 (0.10)	0.02 (0.02)	0.31 (0.15)	0.07 (0.03)	1.2 (0.3)
50	0.09 (0.08)	0.08 (0.04)	0.18 (0.06)	0.01 (0.02)	0.28 (0.11)	0.08 (0.05)	1.1 (0.2)
200	0.11 (0.05)	0.11 (0.06)	0.19 (0.05)	0.01 (0.02)	0.32 (0.02)	0.08 (0.01)	2.6 (2.5)