Chronic toxicity of chromium -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[2]. To aid interpretation of the project results and improve ecological risk assessments, Ecofide was asked to determine the chronic toxicity of chromium-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 chromium concentration below an estimated threshold of 5 µg/l (see intermezzo). Chronic toxicity of chromium-spiked sediments (Cr^{3+}) 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 chromium (Cr^{3+}) to *Hyalella* in water-only or sediment exposures (data provided by Arche consulting)

* Chronic Cr^{3+}-toxicity water-only (survival; 42 days) – around 40% mortality at 29 (pH=8) and 48 µg/l (pH=7). Both treatments did not affect growth or reproduction. No toxic effects at pH 6 (Besser *et al.*, 2004).
* Chronic Cr^{3+}-toxicity sediment exposure (42 days). No effects on survival but minor effects on growth and inconsistent effects on reproduction. i) effects on growth and reproduction while exposed to 17680 mg Cr^{3+}/kg at an AVS of 9-13 mmol/kg); ii) slight effects on growth after 28 days but no effects on growth or reproduction after 42 days while exposed to 291 mg Cr^{3+}/kg at an AVS of <0.01 mmol/kg) (Besser *et al.*, 2004).
* $PNEC_{water}$ 4,7 µg Cr^{3+} /l (EU- RAR, 2005). Based on a NOEC for *Ceriodaphnia* and an assessment factor of 10.
* $PNEC_{sediment}$ 180 – 206 mg Cr/kg (ARCHE Consulting, 2017).

The data from Besser *et al* (2004) seems to indicate that *Hyalella* is less sensitive than *Ceriodaphnia*. For *Hyalella* an estimated threshold of 5 µg/l would probably be on the safe side to prevent intermingling effects in toxicity due to simultaneous exposure to chromium in sediment and overlying water.

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 rather sandy sediment (grain size between 31 and 1000 µm = 84%; $P_{10, 50, 90}$: 16, 248 and 516 µm), expected to resemble exposures under a high chromium availability as organic matter, Fe and AVS contents are low (Table 1[3]).

[1] Acronym for "Nature-based Remediation of Metal pollutants in Nature Areas to increase water storage capacity"
[2] https://webgate.ec.europa.eu/life/publicWebsite/project/LIFE18-ENV-BE-000286/nature-based-remediation-of-metal-pollutants-in-nature-areas-to-increase-water-storage-capacity-narmena;
[3] Preferably AVS <1mmol/kg; Total organic carbon <1-2% and Fe <7.5 g/kg DW.

Table 1. General characteristics of sediment from the Steenputbeek.

Parameter	Units	Value	Parameter	Units	Value
Org. matter	%	2,9	Cr	mg/kg dw	7.9
Moisture content	%	36	Cu	"	3.9
AVS	mmol/kg dw	6.5	Ni	"	4.8
AVS-SEM	"	6.2	Cd	"	0,04
Fe	g/kg dw	3.8	Zn	"	19.4

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, a chromium ($CrCl_3*6H_2O$; Cr^{3+}) solution was added to 5L sediment resulting in a final concentration of 3200 mg/kg dw. To compensate for possible effects of the increased chloride-concentration, the KCl amount of the EPA-medium was increased for the control treatment while additional NaCl was also added to keep the Na/K ratio of the EPA-medium constant. The possible chloride-effects were further reduced by increasing the sediment/water ratio from 1:1 up to 1:4 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.
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. In the second stage, this super-spike was diluted with varying amounts of reference sediment to produce a series of 5 chromium concentrations (nominal values: 0, 100, 320, 1000, 1800 and 3200 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 21-day pilot-experiment was started to determine the experimental set-up required to keep the Cr-concentrations in the overlying water below 5 µg/l. Sediment/water systems were prepared with the two highest chromium concentrations using a 1:8 and a 1:16 sediment/water ratio. 60% of the overlying water was refreshed twice daily. Weekly analyses of dissolved chromium concentrations (filtered over 0.45 µm; Rotilabo Cellulose-acetate filters) provided insight in diffusion rates and the possible flushing of excess unbound chromium from sediments. The overlying water was sampled near the sediment surface and approximately 30 min before a water renewal cycle to allow chromium concentrations to approach their maximum values.
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=0 (before the first refreshment cycle).

The results (table 2) illustrate that:
i) At the start of the experiment, chromium concentrations in overlying water of the 1800 mg/kg treatment were <5 µg/l, while in the 3200 mg/kg treatment increased but variable chromium concentrations were found (12 and 20 µg/l).
ii) After 7 days, chromium concentrations were increased in the 1800 mg/kg treatment (6-9 µg/l) and remained high in the 3200 mg/kg treatment.

iii) Continuation of the twice daily refreshments led for the 1800 mg/kg treatment to a gradual decrease of the chromium concentrations reaching values below 5 µg/l after 14 or 21 days. For the 3200 mg/kg treatment such a (slow) reduction was only observed when using a 1:16 sediment/water ratio. Concentrations in the 1:8 treatment remained high, indicating that the binding capacity of the sediment was surpassed. Such increased chromium concentrations in the overlying water will intermingle with toxicity of sediment bound chromium and should therefore be avoided.

The increase in chromium concentrations after the start of the pilot-experiment might be due to the sediment spiking procedure, performed in closed containers with only a limited amount of air. Equilibration between sediment and overlying water therefore resembled anaerobic conditions, while the pilot experiment was performed under aerobic conditions. In fact, visual observations confirmed that an aerobic sediment top layer took around two weeks to develop. It is therefore supposed that lowering chromium concentrations by a twice daily refreshment of the overlying water only took it's maximum effect after stable environmental conditions were reached.

Table 2. Dissolved chromium concentrations (µg/l) in the overlying water during a first 21-day pilot experiment to determine experimental set-up and refreshment rates.
☐ =Cr conc >15 µg/l; ☐ = 5<Cr<15 µg/l; ☐ = Cr <5 µg/l

Nominal Cr conc sediment (mg/kg)	Refreshment scheme	Sediment/water ratio	Sampling intervals from day 1 up to 21 days			
			0	7	14	21
1800	2*/day	1:8	2.5	8.9	7.6	4.2
"	2*/day	1:16	2.4	6.0	4.4	2.2
3200	2*/day	1:8	20	25	28	23
"	2*/day	1:16	12	20	11	9.8

In general, the results illustrate that a twice daily renewal might be sufficient to keep the chromium concentrations in the overlying water below the threshold of 5 µg/l, but at the same time demonstrate that the present set-up was not yet sufficient (especially for the 3200 mg/kg treatment). It was therefore decided to perform a second pilot experiment for which:

i) all spiked-sediment stocks were rinsed twice with EPA-medium to flush (possible present) unbound chromium from the porewater before the preparation of the new sediment-water testsystems.
ii) the duration of the experiment was prolonged to four weeks with a twice daily renewal cycle.
iii) a sediment/water ratio of 1:16 was used.
iv) the volume of daily refreshed overlying water was increased from 60 to 80% of the volume (a layer of 1.5 cm above the sediment was not refreshed)

The results of the second pilot experiment are summarized in table 3 and show that the average chromium concentrations in the overlying water after three and four weeks were all below 5 µg/l. The only exception was one replicate sample of the 3200 mg/kg treatment after 28 days (5.1 µg/l). Furthermore, the average concentration after 28 days was somewhat higher than the concentration after 21 days. With only two replicates it remained unclear whether this is normal variation. Based on these results it was decided to use the experimental set-up of this second pilot experiment also for the final toxicity test, with the exception that the sediment-water testsystems were prepared six weeks in advance. The first week was meant for sediment settlement, stabilization and reaching aerobic conditions (no refreshment of overlaying water), while all testsystems were twice daily refreshed during the other five weeks.

Table 3. Dissolved chromium concentrations (µg/l) in the overlying water during a second, 28-day pilot experiment to determine experimental set-up and refreshment rates.
☐ =Cr conc >15 µg/l; ☐ = 5<Cr<15 µg/l; ☐ = Cr <5 µg/l

Nominal Cr conc sediment (mg/kg)	Refreshment scheme	Sediment/water ratio	Time of sampling (days) 21	28
1000 (n=1)	2*/day	1:16	-	1,2
1800 (n=2)	2*/day	1:16	1,0 / 1,4 **Avg. 1.2**	1,0 / 2,0 **Avg. 1.5**
3200 (n=2)	2*/day	1:16	3,6 / 2,2 **Avg. 2.9**	5,1 / 3,9 **Avg. 4.5**

<u>Final toxicity test</u>
Chronic toxicity was assessed using 12 replicates for each chromium concentration. Sediment-water testsystems were based on a 1:16 v/v ratio between sediment and overlying water (bromide enriched EPA-medium). 80% of the overlying water was refreshed twice daily. Renewal started five weeks 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 chromium spiked sediment while previous studies using this set-up for cadmium and arsenic demonstrated suitable reproduction after 42 days (>50 juveniles per test beaker). Reducing the amount of sediment (see above: 1:16 v/v with overlying water) made the workload of finding these young neonates/juveniles acceptable.

Animals were fed daily with 1 ml YCT-suspension[4] (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 only), 28 and 42 days. The overlying water was sampled approximately 30 min before a water renewal cycle to allow chromium 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 100% after 28 days and 95% 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 7.2 mm) and a reproduction >2 juveniles/female after 42 days (present research 3.0 juveniles/female after 28 days and 21.7 juveniles/female after 42 days). 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 (21.7 juveniles/female after 42 days) corresponds well with these data.
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 278 mg/L the value fell within the range of 232-372 mg/l (mean 305 mg/l) from the

[4] YCT=Yeast, Cerophyl and Trout Chow

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. Oxygen saturation varied between 71 and 96%[5]. pH values in the overlying water varied between 7.6 and 7.9 with no differences between treatments. The same applied to conductivity (26 – 33 µS/mm), nitrite (<2 mg/l) and ammonia concentrations (<5 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 $T_{0,\ 14,\ 28\ \text{and}\ 42}$[6]. Besides chromium 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 chromium concentrations in the sediment were on average 2.8 times lower than the nominal intended values (Table 4) with low variation between treatments (range: 2.4-3.3). This low binding capacity of the sediment is a direct consequence of the experimental set-up resembling exposures under a high chromium availability as organic matter, Fe and AVS contents of the sediment were low. Average organic matter content of the sediment was 3.5% (organic carbon between 1-2%). AVS was significantly lower in the 3200 mg Cr/kg treatment (table 4; 1.7 mmol/kg dw) and increased during the experiment up to the 1000 mg Cr/kg treatment ($T_{0,\ 28,\ 42}$: control 3.8 – 8.6 – 11.3; 1000 mg Cr/kg 2.9 – 6.8 – 8.4; 1800 mg Cr/kg 4.8 – 5.8 – 5.7). Chromium concentrations in porewater increased from 0.56 µg/l in the control up to 6.0 µg/l in the 3200 mg Cr/kg treatment (Table 4). At T_0 chromium concentrations in the overlying water were on average twice as high as on $T_{14,\ 28,\ 42}$ even though the sediment-water systems were prepared one week in advance and the overlying water was daily refreshed (difference ranged from 2.9 in control down to 1.6 in 3200 mg Cr/kg treatment). With a maximum chromium concentration in the overlying water of 2.7 µg/l in the T_0 of the 3200 mg/kg treatment) all concentrations stayed well below the forementioned threshold of 5 µg/l. The porewater pH varied between 6.9 and 7.8 (average = 7.35) and did not differ significantly between treatments (average values 7.3-7.5). The DOC-concentrations in the porewater of the control and 100 mg Cr/kg treatment varied throughout the experiment between 4 and 11 mg/l (average 9.2 mg/l). A comparable range was observed for all higher treatments at $T_{0,28}$ (3-8 mg/l[7]), while DOC-concentrations in the 320 mg Cr/kg treatment and above were remarkably increased after 42 days (78 – 100 mg/l). Concentrations of other metals in sediment, porewater, SEM-AVS 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).

[5] Oxygen saturation directly after renewal: 91-96%; 30 min before the next renewal: 71-83%
[6] at T14 only overlying water
[7] One outlier of 16.8 mg/l in the 1800 mg/kg treatment at T_{28}.

Table 4. Physical chemical analyses during the toxicity tests. Average of three or four[8] replicates ($t_{0,(14),28,42}$ days) are presented together with the standard deviation between brackets.

Treatment (nominal Cr conc)	Sediment		Porewater			Overlying water		
	Cr	Org. matter	Cr [1)]	AVS	SEM	Cr [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	28.5 (3.0)	3.3 (0.14)	0.56 (0.31)	7.9 (3.80)	0.10 (0.02)	0.71 (0.46)	0.48 (0.21)	1.2 (0.38)
100	41.1 (3.3)	3.4 (0.07)	1.14 (0.88)	6.8 (2.15)	0.10 (0.01)	0.68 (0.38)	0.50 (0.21)	1.1 (0.37)
320	108 (14.9)	3.3 (0.03)	1.99 (1.82)	5.0 (1.80)	0.11 (0.03)	0.86 (0.47)	0.66 (0.28)	1.1 (0.44)
1000	336 (21.2)	3.5 (0.05)	3.31 (2.52)	6.0 (2.82)	0.11 (0.02)	1.11 (0.57)	0.90 (0.39)	1.0 (0.26)
1800	552 (30.2)	3.6 (0.21)	3.82 (1.07)	5.4 (0.54)	0.11 (0.03)	1.28 (0.63)	1.14 (0.65)	1.0 (0.23)
3200	1234 (123)	4.0 (0.40)	5.98 (2.42)	1.7 (1.02)	0.12 (0.03)	1.65 (0.74)	1.43 (0.70)	0.9 (0.17)

[1)] dissolved concentrations (filtered over 0.45 µm)

7. Toxicity

The lowest three treatments (100, 320 and 1000 mg Cr/kg dw) did not cause any effect on survival, growth, or reproduction (Table 5), while survival was still unaffected in the 1800 and 3200 mg Cr/kg treatments. These two highest treatments did however reduce the growth rate and/or reproduction of *Hyalella*. After 28 days a significant effect on the growth rate was observed in the 3200 mg/kg treatment, while significant effects on reproduction were observed in both the 1800 and 3200 mg/kg treatments. This effect on reproduction was also observed after 42 days. The effect percentages varied between 38 and 41% reduction and were quite comparable to the reduction percentages after 28 days (35%).

Table 5. 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 statistical significant effects. Estimated $L(E)C_{10}$ and $L(E)C_{50}$-values are presented with the 95% cl between brackets. Results are based on actual concentrations.

Treatment Cr conc (mg/kg dw)		Survival		Growth		Reproduction			
		T_{28}	T_{42}	T_{28}		T_{28}		T_{42}	
nom.	act.	%	%	mm [1)]	% reduction	Juveniles/ female	% reduction	Juveniles/ female	% reduction
0	**28**	100 (0)	95 (4.1)	5.25 (0.10)	-	3.0 (0.6)	-	21.7 (2.3)	-
100	**41**	98 (2.9)	99 (2.5)	5.28 (0.13)	+0.6	2.6 (0.3)	13.4	20.4 (2.7)	5.9
320	**108**	100 (0)	100 (0)	5.41 (0.17)	+3.1	3.1 (0.7)	+4.2	20.3 (1.9)	6.5
1000	**336**	100 (0)	95 (4.1)	5.13 (0.27)	2.3	2.5 (0.8)	16.8	22.2 (4.9)	+2.3
1800	**552**	98 (2.9)	99 (2.5)	5.08 (0.13)	3.2	2.0 (0.4)	34.5	13.5 (7.1)	37.6
3200	**1234**	96 (2.5)	96 (2.5)	4.78 (0.17)	9.0	1.9 (0.4)	35.4	12.8 (2.8)	41.1
Test parameters based on actual chromium concentrations in the sediment									
NOEC		1234		552		336		336	
$L(E)C_{10}$		>1234		>1234		155 (<1-381)		269 (<1-500)	
$L(E)C_{50}$		>1234		>1234		>1234		>1234	

[1)] Average length at T_0 was 1.95 mm

[8] Overlying water only

Calculated effect parameters (NOEC, EC_{10} and EC_{50} values; including 95% confidence intervals) are summarized in table 5. It should be noted that maximum effect percentages measured for growth and reproduction stayed below 50% and for growth even below 10% (Table 5). These effect ranges are sufficient for NOEC estimates, while statistical calculations of the EC_{10}- and especially EC_{50}-values could have benefited from higher exposure regimes.
EC_{10}-estimate for growth after 28 days was 1342 mg/kg dw.
EC_{50}-estimates for reproduction after 28 and 42 days were 2151 and 1474 mg/kg respectively.

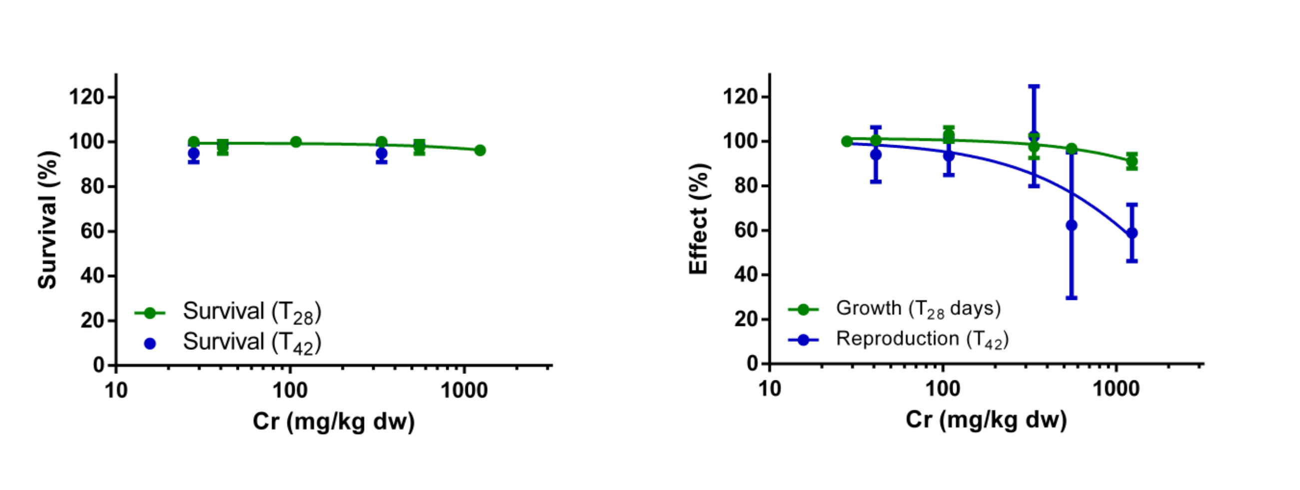

Figure 1. Dose-response curves for survival after 28 and 42 days (left) and growth (28 days) and reproduction (42 days) on the right-hand side. Effects on growth and reproduction are shown as effect percentages compared to the control sediment on the y-axis.

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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 Cr conc)	Sediment (mg/kg DW; except for Al and Fe; g/kg DW)									
(mg/kg dw)	Al	Fe	Mn	Co	Cu	Cd	Ni	Pb	Zn	As
0	4.4 (0.28)	4.8 (0.23)	95.7 (27.7)	0.88 (0.46)	1.8 (0.18)	0.18 (0.02)	3.3 (0.22)	8.3 (0.57)	11.3 (0.8)	4.1 (3.9)
100	3.9 (0.19)	4.2 (0.30)	85.8 (32.9)	0.57 (0.07)	1.7 (0.16)	0.17 (0.01)	3.0 (0.17)	7.2 (0.53)	10.7 (0.5)	6.4 (1.1)
320	4.0 (0.06)	4.3 (0.09)	85.4 (22.8)	0.59 (0.08)	1.7 (0.08)	0.17 (0.01)	3.3 (0.41)	7.4 (0.71)	10.6 (0.6)	2.2 (2.3)
1000	4.3 (0.21)	4.5 (0.28)	88.9 (26.3)	0.58 (0.03)	1.8 (0.16)	0.17 (0.01)	5.1 (1.99)	7.5 (0.88)	11.3 (1.0)	2.8 (3.4)
1800	4.1 (0.51)	4.4 (0.36)	84.8 (27.6)	0.51 (0.03)	1.6 (0.12)	0.17 (0.01)	3.2 (0.21)	7.7 (0.68)	10.3 (0.7)	4.7 (7.4)
3200	3.7 (0.25)	4.1 (0.37)	118 (51.6)	0.52 (0.02)	1.5 (0.17)	0.18 (0.02)	3.1 (0.40)	7.8 (0.89)	9.8 (0.8)	2.8 (1.9)

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 Cr)	Porewater									
(mg/kg dw)	Al (mg/l)	Fe (mg/l)	Mn (mg/l)	Co (µg/l)	As (µg/l)	Cu (µg/l)	Cd (µg/l)	Ni (µg/l)	Pb (µg/l)	Zn (µg/l)
0	*<0,01*	0.08 (0.05)	2.4 (0.87)	1.5 (1.16)	13.8 (9.2)	0.4 (0.26)	0.4 (0.09)	1.9 (1.06)	0.20 (0.26)	1.3 (0.79)
100	*<0,01*	0.13 (0.12)	2.3 (1.13)	3.2 (1.86)	17.8 (5.0)	0.5 (0.19)	0.6 (0.05)	2.2 (1.18)	0.13 (0.06)	1.4 (0.41)
320	*<0,01*	0.14 (0.13)	2.8 (0.70)	4.1 (1.63)	19.9 (1.8)	0.7 (0.16)	0.7 (0.09)	2.5 (0.44)	0.14 (0.05)	1.8 (0.56)
1000	*<0,01*	0.06 (0.05)	2.0 (0.74)	3.3 (1.19)	14.9 (11.2)	0.6 (0.35)	0.6 (0.14)	2.0 (0.57)	0.13 (0.06)	1.4 (0.71)
1800	*<0,01*	0.06 (0.05)	2.0 (0.28)	4.0 (1.97)	18.7 (8.7)	0.5 (0.10)	0.6 (0.03)	1.8 (0.28)	0.11 (0.03)	1.5 (0.25)
3200	*<0,01*	0.03 (0.02)	0.8 (0.54)	3.0 (1.44)	10.8 (3.4)	0.5 (0.09)	0.5 (0.12)	1.6 (0.31)	0.11 (0.03)	1.2 (0.16)

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 Cr conc)	Sediment (metal concentrations during AVS-SEM analyses in µmol/kg)							
(mg/kg dw)	Co	Cr	Cu	Cd	Ni	Pb	Zn	Ratio SEM/AVS
0	2.57 (0.46)	59.2 (30.2)	4.09 (2.33)	1.10 (0.25)	11.7 (2.08)	18.4 (5.6)	64.0 (14.1)	0.015 (0.007)
100	2.54 (0.41)	66.1 (5.4)	3.74 (1.01)	1.11 (0.24)	11.2 (1.50)	18.5 (4.5)	61.5 (8.8)	0.015 (0.003)
320	2.48 (0.42)	195 (54)	6.00 (1.13)	1.14 (0.21)	11.1 (2.04)	20.4 (4.9)	67.5 (19.7)	0.023 (0.004)
1000	2.47 (0.25)	767 (89)	5.36 (1.97)	1.09 (0.12)	11.4 (0.53)	19.2 (4.0)	71.0 (17.4)	0.021 (0.008)
1800	2.45 (0.17)	1473 (338)	7.57 (1.88)	1.07 (0.06)	11.0 (1.23)	18.8 (2.5)	66.5 (21.1)	0.020 (0.004)
3200	2.79 (0.56)	4525 (785)	13.4 (2.60)	1.09 (0.14)	11.4 (1.36)	19.2 (4.3)	71.8 (20.3)	0.086 (0.041)

Dissolved concentrations in overlying waters
Average concentration (n=4; $T_{0,14,28,42}$) 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 Cr)	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	1.1 (1.03)	15.9 (12.9)	9.0 (7.3)	15.0 (1.7)	2.6 (0.5)	6.7 (0.6)	32.2 (5.7)	65.1 (4.5)	7.81 (0.29)	28.4 (0.8)
100	1.3 (1.27)	8.1 (7.1)	7.2 (5.5)	14.7 (1.7)	2.6 (0.4)	6.6 (0.6)	31.7 (5.9)	63.8 (4.3)	7.83 (0.31)	27.6 (1.2)
320	1.1 (0.89)	5.3 (4.8)	7.5 (8.0)	14.6 (1.6)	2.5 (0.4)	6.4 (0.9)	30.5 (4.9)	62.8 (4.9)	7.85 (0.33)	28.0 (0.8)
1000	0.9 (1.45)	8.4 (13.5)	14.4 (19.4)	14.4 (1.2)	2.4 (0.4)	6.3 (1.0)	30.1 (4.9)	62.1 (5.7)	7.88 (0.30)	28.0 (1.2)
1800	0.6 (0.75)	2.5 (3.1)	5.1 (8.6)	14.5 (1.0)	2.5 (0.4)	6.6 (0.8)	30.6 (5.0)	63.1 (4.2)	7.85 (0.26)	28.1 (0.7)
3200	0.6 (0.97)	1.1 (0.5)	1.6 (2.1)	13.9 (1.3)	2.4 (0.4)	6.5 (0.9)	29.3 (4.9)	61.3 (5.9)	7.84 (0.24)	28.2 (0.9)

Treatment (nominal Cr conc)	Dissolved metal concentrations (µg/l)						
(mg/kg dw)	Co	As	Cu	Cd	Ni	Pb	Zn
0	0.20 (0.04)	3.9 (1.52)	0.41 (0.16)	0.14 (0.07)	0.45 (0.14)	0.13 (0.10)	0.92 (0.34)
100	0.17 (0.03)	5.2 (2.35)	0.39 (0.13)	0.12 (0.05)	0.42 (0.07)	0.10 (0.05)	0.96 (0.34)
320	0.17 (0.04)	4.5 (2.79)	0.38 (0.14)	0.11 (0.05)	0.43 (0.07)	0.08 (0.04)	0.81 (0.30)
1000	0.17 (0.02)	5.4 (2.11)	0.38 (0.14)	0.12 (0.06)	0.42 (0.05)	0.08 (0.04)	0.95 (0.25)
1800	0.18 (0.03)	3.9 (2.01)	0.36 (0.13)	0.11 (0.06)	0.44 (0.13)	0.07 (0.06)	0.89 (0.42)
3200	0.16 (0.03)	3.6 (1.40)	0.39 (0.18)	0.11 (0.06)	0.39 (0.04)	0.07 (0.04)	0.80 (0.35)