Chronic toxicity of cadmium-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 cadmium-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 refreshment frequency, required to keep the dissolved cadmium concentration below an estimated threshold of 1 µg/l (see intermezzo). Whole-sediment chronic toxicity of cadmium-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 cadmium to *Hyalella* species in water-only exposures (data provided by Arche consulting)

Hardness of the EPA-medium used in the present study is 90-100 mg/L $CaCO_3$

1) Borgmann et al (2005); *Hyallela azteca*
   7-day LC_{50} = 1.6 µg/l with a hardness of 124 mg/L $CaCO_3$
2) Ingersoll & Kemble (2000); *Hyallela azteca* (original article not recovered)
   42-day exposure; NOEC = 0,51 µg/l
3) Garcia et al., (2010); *Hyalella curvispina*; moderately hard water (80-100 mg/L $CaCO_3$)
   Neonates LC_{50} (4 days) = 1.7 µg/l
   Juveniles LC_{50} (14 days) = 10.2 µg/l (up to 8.1 µg/l less than 20% mortality after 14 days)

Annual average EQS-standard for dissolved cadmium within the European Water Framework Directive:
   <0.09 µg/l for class 3 (50 - <100 mg/L $CaCO_3$) and
   <0.15 µg/l for class 4 (100 - <200 mg/L $CaCO_3$)

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

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), expected to resemble exposures under a high cadmium availability as organic matter, Fe and AVS contents are low (Table 1).

Table 1. General characteristics of sediment from the Steenputbeek. All data refer to the sediment batch used in the present toxicity study. Average values (standard deviations between brackets) are shown based on triplicate measurements.

Parameter	Units	Value
Org. matter	%	0.71 (0.09)
Moisture content	%	28.1 (1.53)
AVS	mmol/kg dw	0.30 (0.06)
AVS-SEM	“	0.18 (0.05)
Fe	g/kg dw	2.16 (1.3)

Parameter	Units	Value
Cd	mg/kg dw	0.07 (0.002)
Cu	“	0.68 (0.08)
Ni	“	1.73 (0.30)
Pb	“	2.11 (0.16)
Zn	“	4.54 (0.49)

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 $CdCl_2$-solution was added directly to 5L sediment resulting in a final concentration of 1 g/kg dw. The pH of the overlying water was adjusted and kept on a constant value of 7.2-7.4 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 cadmium concentration of 442 mg/kg dw) was diluted with varying amounts of reference sediment to produce a series of 7 cadmium concentrations (nominal values: 0.9, 1.8, 5.6, 10, 56, 100 and 180 mg/kg dw). This series of spiked sediment was again equilibrated for 8-weeks during which pH-values were regularly checked and the sealed containers periodically mixed on a rolling mill.

4. Experimental set-up

After this equilibration period, 14-day pilot-experiments were started to determine the refreshment scheme required for the overlying water. 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 cadmium concentrations (filtered over 0.45 µm; Rotilabo Cellulose-acetate filters) provided insight in diffusion rates and the possible flushing of excess unbound cadmium from sediments (especially for the highest treatments). Based on the first series of experiments it was concluded that a daily refreshment of 50% of the overlying water seemed to be an appropriate balance between maintaining sufficiently low cadmium concentrations in the overlying water and keeping the workload acceptable. A second series of experiments was run to verify this for the range of test concentrations. The overlying water was typically sampled approximately 30 min before a water renewal cycle to allow cadmium concentrations to approach their maximum values. The results (table 2) illustrate that:

i) dissolved cadmium concentrations were the highest on the first day of sampling (A), while some increase was still observed in the second sample (B). It was therefore concluded that water renewal for the toxicity tests should be started one week ahead to remove the effect of flushing unbound cadmium from sediments and minimize the accumulation of toxic cadmium concentrations in the overlying water.

ii) the highest three cadmium treatments (56, 100 and 180 mg/kg) surpassed the binding capacity of the sediment and the increased cadmium concentrations in the overlying water will intermingle with toxicity of sediment bound cadmium. The toxicity experiment therefore focused on the lower cadmium treatments (0, 0.9, 1.8, 5.6 and 10 mg/kg) while the 56 mg/kg treatment was included as a positive control.

iii) dissolved cadmium concentrations in the 5.6 and 10 mg/kg treatment still exceeded the intended maximum concentration of 1 µg/l. It was therefore decided to increase

*the volume of daily refreshed overlying water (from 50 to 80% of the volume; a layer of 1.5 cm above the sediment was not refreshed).

*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.

Table 2. Dissolved cadmium concentrations (µg/l) in the overlying water during a pilot experiment to determine refreshment rates. The sediment concentration of 0.9 mg/kg was not tested in this pilot experiment.

Nominal Cd conc sediment (mg/kg)	Sampling intervals from day 0 (A) up to 14 days (E) A	B	C	D	E	Average (stdev) C – E
0	0.10	0.02	0.03	0.03	0.02	0.03 (0.01)
1.8	2.73	1.32	0.44	0.36	0.68	0.49 (0.17)
5.6	7.82	4.55	3.56	2.49	3.05	3.03 (0.54)
10	9.60	4.94	4.39	4.86	3.01	4.09 (0.96)
56	81.3	77.1	53.6	68.4	43.4	55.1 (12.6)
100	156.7	142.0	111.0	143.2	100.2	118.1 (22.3)
180	277.3	220.0	157.9	190.8	127.1	158.6 (31.9)

Chronic toxicity was assessed using 12 replicates for each cadmium 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 cadmium 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[2] (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). Due to this increased food amount, aeration of the test beakers started at T_{29} (slowly aerated six times a day). 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 28 and 42 days. The overlying water was sampled approximately 30 min before a water renewal cycle to allow cadmium concentrations to approach their maximum values. Separate test chambers were used for chemical analyses (2 replicates for each sampling and concentration) but they were stocked with test organisms and

[2] YCT=Yeast, Cerophyl and Trout Chow

maintained in the same manner as those used for assessing toxicity. Samples of the overlying water were taken near the sediment-water interface.

5. Test acceptability

The control treatment complied with acceptability criteria mentioned in EPA (2000) with a mean survival of 99% after 28 days and 98% 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 6.9 mm) and a reproduction >2juveniles/female after 42 days (present research 14 juveniles/female). Au et al (2015) mention a reproduction of 10-15 juveniles per female after 42 days, Ivey et al (2016) 5-15 juveniles/female while Besser et al. (2016) illustrate 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 (14 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 262 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. Overlying water was not aerated during the first four weeks and oxygen saturation varied between 61 and 96%. Due to the daily aeration in the fifth- and sixth-week oxygen saturation stayed above 90% during this second phase. pH values in the overlying water varied between 7.3 and 7.5 with no differences between treatments. The same applied to conductivity (25 – 29 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 $T_{0,\ 28\ and\ 42}$. Besides cadmium 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 cadmium concentrations in the sediment were on average 2.5 times higher than the nominal intended values but the rate of increase was comparable (Table 3). Average organic matter content of the sediment was 0,71%. Cadmium concentrations in porewater and AVS-SEM analyses increased with increasing cadmium treatments. Dissolved cadmium concentrations in the overlying water showed a slight increase in the first four treatments as compared to the control but a statistical significant increase was only noted in the highest treatment (56 mg/kg nominal). In addition, dissolved cadmium concentrations in the 0.9 and 1.8 mg/kg treatment were identical, while toxicity only occurred in the 1.8 mg/kg treatment (see below). These results suggest that toxicity up to the 10 mg/kg treatment was (primarily) caused by sediment exposure, while toxic effects in the 56 mg/kg treatment will also be influenced by exposure to dissolved cadmium in the overlying water. The strong increase in cadmium concentrations in the overlying water of the 56 mg/kg treatment was already noted in the pilot experiments (Table 2) and suggests that the binding capacity of the sediment was surpassed.

Concentrations of other metals in porewater, AVS-SEM and overlying water did not show treatment related differences (Appendix).

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

Treatment (nominal Cd conc)	Sediment		Porewater			Overlying water	
	Cd	Org. matter	Cd[1)]	AVS	SEM-Cd	Cd[1)]	DOC
(mg/kg dw)	mg/kg dw	%	µg/l	mmol/kg dw	mmol/kg dw	µg/l	mg/l
0	0.14 (0.04)	0.71 (0.09)	0.18 (0.09)	0.48 (0.25)	<0.01 (-)	0.92 (0,27)	2.0 (1.2)
0.9	3.2 (1.1)	0.91 (0.11)	1.33 (1.93)	1.47 (0.97)	0.03 (0.01)	1.5 (0.24)	1.9 (1.1)
1.8	4.3 (1.5)	0.71 (0.04)	2.08 (2.11)	1.04 (1.02)	0.03 (0.01)	1.4 (0.22)	1.8 (1.0)
5.6	11.4 (2.0)	0.59 (0.06)	3.39 (-)[2)]	0.55 (0.31)	0.11 (0.01)	1.8 (0.43)	1.8 (0.6)
10	22.9 (4.7)	0.67 (0.03)	7.60 (8.27)	0.65 (0.28)	0.21 (0.03)	2.0 (1.57)	1.7 (0.7)
56[3)]	131.4 (-)	0.63 (-)	14.0 (-)	1.19 (-)	1.07 (-)	29.7 (1.82)	2.0 (0.8)

1) dissolved concentrations (filtered over 0.45 µm)
2) duplicate; sample at t=0 was lost
3) duplicate; due to complete mortality after 28 days, no measurements after 42 days

7. Toxicity

The lowest treatment of 3.2 mg/kg dw (actual) did not cause any effect on survival, growth, or reproduction (Table 4). Survival was still unaffected in the 4.3 and 11.4 mg/kg treatments but significant effects occurred on both growth and reproduction. The growth was reduced with 13.5 and 22.4% respectively, while reduction percentages for reproduction were 32.5 and 52.6. First effects on survival were observed in the 22.9 mg/kg treatment (65% survival after 28 days and 43% after 42 days), while complete mortality occurred in the 131 mg/kg treatment.

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 Cd conc (mg/kg dw)		Survival		Growth		Reproduction	
		T_{28}	T_{42}	T_{28}		T_{42}	
nom.	act.	%	%	mm[1)]	% reduction	Juveniles/ female	% reduction
0	**<1**	99 (2.5)	98 (2.9)	5.19 (0.13)		13.5 (2.5)	
0.9	**3.2**	100 (-)	99 (2.5)	5.23 (0.17)	+0.7	11.8 (1.8)	-12.9
1.8	**4.3**	100 (-)	98 (2.9)	4.49 (0.03)	13.5	9.1 (0.9)	-32.5
5.6	**11.4**	85 (12.3)	91 (4.8)	4.03 (0.22)	22.4	6.4 (1.7)	-52.6
10	**22.9**	65 (5.8)	43 (11.9)	3.77 (0.21)	27.4	6.3 (2.4)	-53.5
56	**131.4**	0	0	-	-	-	-
Test parameters based on actual cadmium concentrations in the sediment							
NOEC		11.4	11.4	3.2		3.2	
L(E)C_{10}		11.1 (1.3-16.1)	12.6 (1.0-16.5)	8.9 (2.2-14.4)		1.6 (0.1-4.1)	
L(E)C_{50}		30.2 (22.0-116.1)	21.4 (15.9-28.5)	32.3 (21.6-70.1)		12.9 (6.0-43.5)	

1) Average length at T_0 was 1.68 mm

Calculated effect parameters (NOEC, L(E)C_{10} and L(E)C_{50} values; including 95% confidence intervals) are summarized in table 4 while the dose-response curves are illustrated in figure 4. It should be noted that the maximum effect percentages for growth and reproduction measured were 27.4 and 53.5 respectively (Table 4). Dose-response curves were forced to zero growth and reproduction in the highest treatment (131.4 mg/kg) due to the complete mortality in this treatment. The toxicity in this treatment is probably caused by a combined exposure to sediment and increased cadmium 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 L(E)C_{10}-values.

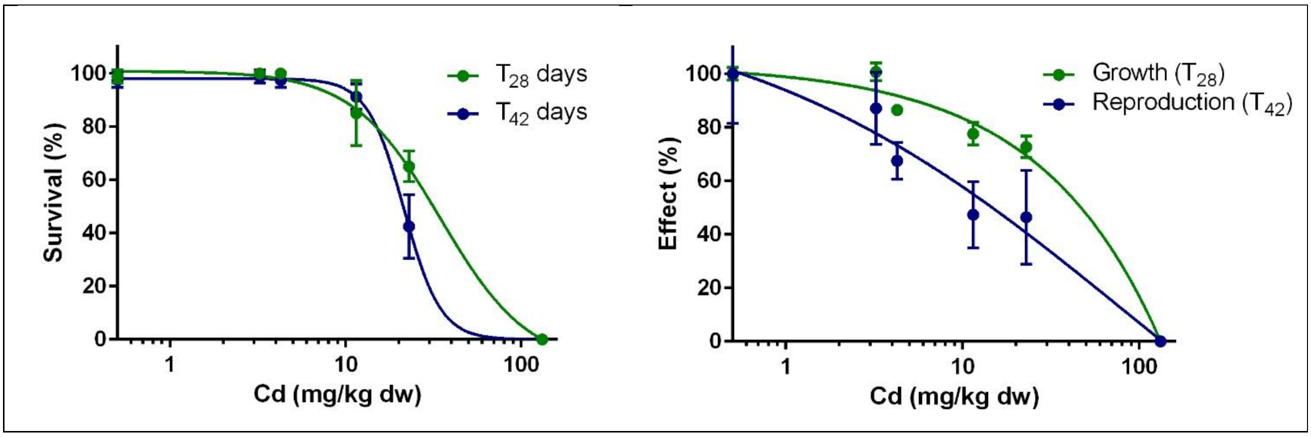

Figure 1. Dose-response curves for survival after 28 and 42 days (left) and growth (28 days) and reproduction (42 days) on the right-hand side. Effects on growth and reproduction are shown as effect percentages compared to the control sediment on the y-axis.

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Appendix chemical analyses

Concentrations in sediment
Average 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 Cd conc)	Sediment (mg/kg DW)									
(mg/kg dw)	Al	Fe	Mn	Co	Cr	Cu	Ni	Pb	V	Zn
0	746 (62.6)	1677 (161)	48 (11.9)	*0.7 (0.3)*	2.5 (0.4)	*0.8 (0.3)*	1.4 (0.4)	2.6 (0.7)	2.5 (0.4)	9.0 (1.5)
0.9	959 (195)	2123 (402)	70 (56.0)	*0.8 (0.3)*	2.8 (0.5)	1.1 (0.2)	2.5 (0.8)	3.2 (1.1)	3.1 (0.6)	11.9 (1.9)
1.8	710 (161)	1677 (369)	44 (31.4)	*0.5 (-)*	2.5 (0.5)	*0.9 (0.4)*	1.4 (0.5)	2.9 (0.3)	2.5 (0.5)	9.7 (1.1)
5.6	691 (160)	1556 (48)	48 (2.4)	*0.5 (-)*	1.9 (0.5)	*0.7 (0.3)*	1.5 (0.5)	3.6 (2.5)	2.7 (0.7)	7.6 (1.0)
10	639 (115)	1659 (298)	43 (25.9)	*0.7 (0.3)*	2.7 (0.2)	*1.0 (0.8)*	*1.0 (0.8)*	2.7 (0.2)	2.3 (0.9)	8.8 (1.6)
56[1)]	630 (-)	1528 (-)	50 (-)	*0.8 (-)*	2.3 (-)	*0.5 (-)*	*0.8 (-)*	2.3 (-)	2.3 (-)	7.0 (-)

[1)] duplicate; due to complete mortality after 28 days, no measurements after 42 days

Dissolved concentrations in porewater
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 Cd conc)	Porewater									
(mg/kg dw)	Al (mg/l)	Fe (mg/l)	Mn (mg/l)	Co (µg/l)	Cr (µg/l)	Cu (µg/l)	Ni (µg/l)	Pb (µg/l)	V (µg/l)	Zn (µg/l)
0	0.18 (0.21)	21.6 (17.9)	3.7 (2.9)	2.6 (1.2)	0.6 (0.6)	2.4 (0.6)	1.7 (1.2)	1.0 (0.6)	1.4 (0.8)	6.4 (5.7)
0.9	0.25 (0.39)	18.4 (15.2)	3.0 (2.1)	4.6 (5.0)	0.7 (1.0)	1.4 (0.5)	3.0 (2.8)	0.7 (0.7)	1.2 (1.1)	3.4 (2.8)
1.8	0.03 (0.05)	20.4 (24.6)	2.8 (2.4)	3.8 (3.2)	*0.2 (0.1)*	1.3 (0.6)	1.4 (0.2)	0.3 (0.4)	0.5 (0.3)	3.0 (1.3)
5.6[1)]	0.23 (-)	28.3 (-)	4.6 (-)	7.3 (-)	0.6 (-)	1.8 (-)	5.6 (-)	0.5 (-)	1.1 (-)	2.9 (-)
10	0.06 (0.07)	24.4 (21.7)	3.4 (2.9)	6.1 (7.3)	0.2 (0.2)	1.2 (0.7)	3.6 (3.1)	0.3 (0.1)	0.8 (0.2)	2.2 (0.1)
56[2)]	0.05 (-)	9.4 (-)	1.9 (-)	3.8 (-)	0.2 (-)	1.0 (-)	5.9 (-)	0.7 (-)	0.8 (-)	2.4 (-)

[1)] duplicate; sample at t=0 was lost
[2)] duplicate; due to complete mortality after 28 days, no measurements after 42 days

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 Cd conc)	Sediment (metal concentrations during AVS-SEM analyses in mmol/kg [Al, Fe, Mn] or µmol/kg [other metals])										
(mg/kg dw)	Al	Fe	Mn	Co	Cr	Cu	Ni	Pb	V	Zn	Ratio AVS/SEM
0	5,0 (0.5)	9,4 (1.0)	0,8 (0.3)	6,3 (0.6)	6,7 (0.1)	9,6 (0.8)	9,6 (1.4)	12,6 (1.7)	21,8 (2.3)	84 (7.8)	0.03 (0.02)
0.9	6,6 (0.3)	12,9 (1.7)	0,9 (0.7)	8,1 (1.1)	9,6 (0.7)	12,2 (2.5)	14,0 (0.9)	12,7 (2.1)	27,0 (4.7)	116 (4.9)	0.08 (0.05)
1.8	5,0 (0.5)	9,0 (0.6)	0,6 (0.4)	6,0 (1.5)	6,8 (1.0)	10,3 (0.4)	10,7 (1.7)	9,7 (1.7)	20,2 (1.9)	90 (6.4)	0.07 (0.07)
5.6	5,0 (0.4)	9,3 (1.0)	0,7 (0.2)	6,1 (0.3)	7,8 (1.0)	9,8 (1.9)	10,6 (2.3)	9,7 (0.4)	22,1 (1.4)	92 (7.1)	0.04 (0.02)
10	5,5 (0.3)	9,9 (1.1)	0,7 (0.4)	6,5 (1.4)	18,0 (18)	10,8 (0.6)	15,7 (8.8)	10,9 (2.0)	21,8 (2.7)	91 (4.9)	0.04 (0.02)
56[1)]	4,8 (-)	9,0 (-)	0,8 (-)	7,2 (-)	7,0 (-)	9,4 (-)	10,8 (-)	9,6 (-)	21,3 (-)	77 (-)	0.08 (-)

[1)] duplicate; due to complete mortality after 28 days, no measurements after 42 days

Dissolved concentrations in overlying waters
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 Cd conc)	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	<5	21.0 (16.5)	48 (65)	18,7 (1.9)	3,3 (0.2)	6,5 (0.2)	29,6 (1.2)	124 (12.7)	7.4 (0.20)	27 (1.4)
0.9	<5	9,7 (3.5)	35 (52)	19,3 (2.4)	3,4 (0.2)	6,5 (0.1)	29,6 (1.3)	128 (15.8)	7.4 (0.19)	27 (1.4)
1.8	<5	11,7 (12.4)	23 (32)	19,0 (2.1)	3,4 (0.1)	6,5 (0.2)	29,6 (1.4)	126 (14.0)	7.5 (0.23)	27 (1.2)
5.6	<5	27,3 (42.1)	11 (13)	18,0 (0.9)	3,4 (0.2)	6,4 (0.1)	29,6 (1.5)	119 (6.2)	7.5 (0.23)	27 (1.7)
10	<5	39,7 (60.9)	25 (22)	18,7 (2.3)	3,4 (0.2)	6,4 (0.2)	29,7 (1.4)	124 (15.2)	7.4 (0.19)	27 (1.5)
56[1]	<5	186 (-)	40 (-)	17,5 (-)	3,3 (-)	6,4 (-)	29,6 (-)	116 (-)	7.5 (0.25)	27 (1.3)

[1] duplicate; due to complete mortality after 28 days, no measurements after 42 days

Treatment (nominal Cd conc)	Dissolved metal concentrations (µg/l)						
(mg/kg dw)	Co	Cr	Cu	Ni	Pb	V	Zn
0	<1	<1	*1,2 (0.8)*	*1,0 (0.9)*	<1	13.0 (0.1)	4,0 (2.0)
0.9	<1	<1	*1,0 (0.9)*	*0,7 (0.3)*	<1	13.0 (0.1)	3,3 (1.2)
1.8	<1	<1	*1,5 (0.9)*	*0,7 (0.3)*	<1	13.0 (0.1)	4,7 (1.2)
5.6	<1	<1	*1,2 (0.8)*	*0,7 (0.3)*	<1	12.7 (0.6)	5,7 (3.8)
10	<1	<1	*1,8 (1.3)*	*1,5 (0.9)*	<1	12.7 (0.5)	5,3 (2.3)
56[1]	<1	<1	*1,3 (-)*	*0,8 (-)*	<1	13.0 (-)	4,0 (-)

[1] duplicate; due to complete mortality after 28 days, no measurements after 42 days