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Induced Metal Tolerance in Microbenthic Communities from Three Lowland Rivers with Different Metal Loads

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Abstract. The response of microbenthic communities to sustained metal stress was studied in three lowland rivers with different levels of pollution. Tolerance against zinc and cadmium was determined in short-term toxicity tests with microbenthic assemblages colonizing glass discs. Photosynthetic activity served as an endpoint in tests for algae, whereas for bacteria thymidine incorporation was determined. For bacterial assemblages from unpolluted locations, EC_{50} values in short-term tests ranged between 6.7 and 56.2 µM zinc, and 8.7 and 25.5 µM cadmium, respectively. Bacterial assemblages from the two most polluted sites were significantly more tolerant for zinc (EC_{50}: 994 µM and >1,000 µM) and cadmium (EC_{50}: 218 µM and 154 µM). Results indicated a shift in community composition toward pollution-adapted organisms when a threshold concentration of 1 µM zinc is exceeded. Although an increasing community tolerance was also indicated for algae, EC_{50} values for microbenthic algae from all sites exceeded in most cases the highest metal concentrations tested (Zn: 1,000 µM; Cd: 320 µM). Since species composition of algal assemblages was found to change at much lower metal levels, it is concluded that short-term toxicity tests measuring photosynthesis inhibition do not reflect well the long-term effects of these metals. Toxic effects of metals on both algal and bacterial assemblages are attenuated by precipitation and complexing capacities of the biofilm.

Algal and bacterial toxicity tests are extensively applied to assess the effects of hazardous substances in water. However, many of these tests are performed with single species in the laboratory under ecologically unrealistic circumstances (Lewis 1990). Furthermore, the tested species is often not a representative of the field communities of concern. Since response to toxicants can differ among algal species by two to three orders of magnitude (Blanck *et al.* 1984; Kasai *et al.* 1993), toxicity levels cannot simply be extrapolated from one species to others or to natural assemblages. Yet, these assemblages reflect the taxonomic and physiological diversity within communities more properly (Joern and Hoagland 1996). Microbenthic communities play an important role in the energy and nutrient cycles of running waters (Allan 1995) and their suitability for toxicity testing has been indicated earlier (Cairns *et al.* 1986). An ecologically relevant impact of a chemical triggers succession of tolerant life forms, which outcompete more vulnerable members of the community. As a result, the restructured assemblage becomes less sensitive against this substance as a whole (Blanck and Wängberg 1988b; Molander and Blanck 1992). So far, investigations on zinc exposure of communities have employed structural parameters such as species composition rather than short-term toxicity tests with a physiological endpoint (Genter *et al.* 1987; Niederlehner and Cairns 1992). Therefore, these studies do not reveal if the restructuring of the communities was due to selection for zinc tolerance. The present study investigated if long-term metal stress leads to increased tolerance of microbenthic communities. It was questioned if assemblages of microalgae and bacteria from rivers with different levels of zinc and cadmium show adaptation to these metals. To detect differences in tolerance, short-term toxicity tests were performed, measuring algal photosynthesis and bacterial thymidine incorporation. Special attention was given to the biofilm composition and its role in mediating metal impact.

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Materials and Methods

Sampling Sites

Experiments on the rivers Dommel, Keersop, and Donge were done between April 6, 1995, and January 16, 1996 (Table 1). At the sampling locations, these three lowland rivers are of similar size (second- or third-order stream). They all belong to a part of the watershed of the River Meuse (Figure 1) and are characterized by an area of similar geomorphological features like sandy, poor soils with intense agricultural use. At the village of Neerpelt, the Dommel shows a steep increase in metal concentrations downstream from the tributary Eindergatloop (Table 2). This stream receives high loads of zinc and cadmium due to the discharge of waste water from a metallurgic factory. At the Dommel, samples were taken from upstream, downstream, and in the Eindergatloop. Zinc concentrations upstream from the polluting tributary were also elevated because of atmospheric deposition of metals in the past. Sampling sites at the rivers Keersop and Donge show low background zinc and cadmium levels and were therefore chosen as control sites.

Collection of Microbenthos

Polyethylene racks with etched glass-discs (surface 1.5 cm^2) were kept at the stream sites, floating parallel to the water current as described by

Table 1. List of sampling sites and dates for bacterial and algal short-term toxicity tests with zinc and cadmium

	Bacterial Cell Replication		Algal Photosynthesis	
Sampling Site	Zn	Cd	Zn	Cd
Keersop	6/8/95	6/8/95	6/22/95	6/22/95
	6/29/95	6/29/95	7/13/95	7/13/95
	7/20/95	7/20/95	8/4/95	8/4/95
Donge	1/16/96	1/16/96	11/28/95	11/28/95
			12/6/95	12/6/95
			12/20/95	12/20/95
Dommel				
upstream	4/7/95	8/9/95	4/7/95	8/9/95
downstream	4/6/95	8/11/95	4/6/95	8/11/95
Eindergatloop	4/8/95	8/14/95	4/8/95	8/14/95

Blanck and Wängberg (1988a). Depending on growth conditions, it took 2 to 4 weeks to establish periphytic communities on this artificial substrate. Racks with glass discs were transported to the laboratory in insulated boxes with site water and subjected to toxicity testing within 4 h. Short-term toxicity tests with zinc and cadmium were carried out for both bacterial cell duplication and algal photosynthetic activity. Table 1 summarizes the sampling schedule.

Water Chemistry

Conductivity, pH, and temperature were measured *in situ.* For further water analysis, two 250-ml polyethylene bottles, prerinsed with HNO_3, were taken from the respective location on the day of the toxicity experiment. Dissolved inorganic and organic carbon (DIC and DOC, respectively) were determined with a total carbon analyzer (model 700 C, OI Analytical, USA). Total phosphate was assessed according to Murphy and Riley (1962). Alkalinity of the waters was computed after titration with 0.002 N H_2SO_4. Concentrations of zinc and iron were measured by flame atomic absorption spectrometry (Perkin Elmer 1100B). Samples for cadmium concentrations were analyzed using graphite furnace atomic absorption spectrometry (Perkin Elmer 5100) with Zeeman background correction.

Composition of Biofilm

Chlorophyll *a*, dry weight, and ash-free dry weight were determined per sampling date at least in triplicate. Chlorophyll *a* content of microbenthic communities was determined after sonicating the glass discs (47 kHz for 5 min) in 5 ml distilled water; the suspension was filtered through a Whatman GF/C filter and filters were extracted with 80% (v/v) ethanol according to the Dutch standard procedures (Anonymous 1981). Freeze-dried microbenthic matter was weighed before and after ignition at 500°C to assess dry weight (DW) and ash-free dry weight (AFDW). Samples for metal contents of the biofilms were digested with Ultrex HNO_3 70% in a microwave (model MDS-200, CEM laboratories, USA), using Buffalo River sediment (NIST 2704, USA) as reference material. Measured values normally deviated less than 10% from the certified concentrations. Subsamples of the solutions were subjected to measurements of metals by atomic absorption spectrometry.

Short-Term Toxicity Tests with Microbenthic Algae

The rate of photosynthesis was used to determine the response to metals. Photosynthetic activity of periphytic algae was measured as ^{14}C-bicarbonate uptake. To avoid interaction of added and background metals and to improve the comparison, samples from all three Dommel locations were incubated in water from the upstream location. The other incubations were carried out using site water. The test water was filtered with Whatman GF/F filters. Geometric concentration series for both zinc and cadmium were prepared with Titrisol® (Merck, Germany) ranging from 1 to 1,000 µM Zn and 0.32 to 320 µM Cd. Actual concentrations were checked by atomic absorption spectrometry, showing less than 10% deviation from the nominal values. Each glass disc was incubated in 2 ml test solution. Five replicate glass discs per metal concentration as well as five control samples without toxicants were measured. Two samples killed by a final concentration of 2% (v/v) formaldehyde were included to determine abiotic sorption of radiolabeled material. A laboratory incubator was used to provide standard light conditions (85 $\mu mol \cdot m^{-2} \cdot s^{-1}$), gentle shaking, and maintaining of the average site temperature of river waters. After preexposure to the metals for 2 h, 2 µCi of Sodium [^{14}C]bicarbonate (5 mCi, Amersham, England) were added to each vial. Photosynthesis was stopped after 1 h by adding 200 µl of 37% (v/v) formaldehyde. To remove inorganic bicarbonate, acetic acid was added, and the samples were dried on a heating plate. Subsequently, 1 ml DMSO was added to dissolve the organic material, followed by 7 ml of scintillation liquid (Instagel, Packard, USA). Incorporated ^{14}C was measured in a liquid scintillation counter (Tri-Carb 1600 TR, Packard). According to a counting quench curve, activities were calculated as disintegrations per min (dpm).

Short-Term Toxicity Tests with Microbenthic Bacteria

The incorporation of [*methyl*-^{3}H]thymidine into alkaline and TCA-insoluble material, presumably bacterial DNA, was measured as an indication for bacterial cell multiplication. The test setup followed the procedures for photosynthesis measurement, using sterile, filtered (Schleicher and Schüll 0.22 µm pore size, cellulose acetate) water instead. [*methyl*-^{3}H]Thymidine (43 $Ci.mmol^{-1}$, Amersham) was added in a concentration of 20 nM. In a pre-experiment, 20 nM was found to be saturating for most locations. Only for the Dommel sites, 40 nM were added to avoid that higher bacterial activity might lead to a depletion of radiolabeled material. Cell replication was stopped with formaline after 0.5 h, and bacterial DNA was extracted as described by Tubbing and Admiraal (1991). The amount of incorporated ^{3}H was corrected for quenching and abiotic sorption.

Determination of EC_{50} Values

Dose-effect curves for metals and algal or bacterial thymidine uptake were computed as follows: the average activity of the controls was set to 100% and activities under metal exposure were related to this value. Means and standard deviation for each metal concentration were calculated from the five replicates. EC_{50} values and confidence intervals were quantified from each dose-effect curve using the logistic response model by Haanstra *et al.* (1985). Differences in effect concentrations were regarded as significant when 95% confidence limits did not overlap. For sites tested repeatedly, the pooled data set was also fitted to the model.

Results

Water Chemistry and Composition of the Biofilm

River waters differed strongly in the concentrations of metals. The most polluted water from Eindergatloop contained over 100 times more zinc and over 1,000 times more cadmium than water from the clean reference: the River Donge (Table 2). A high level of

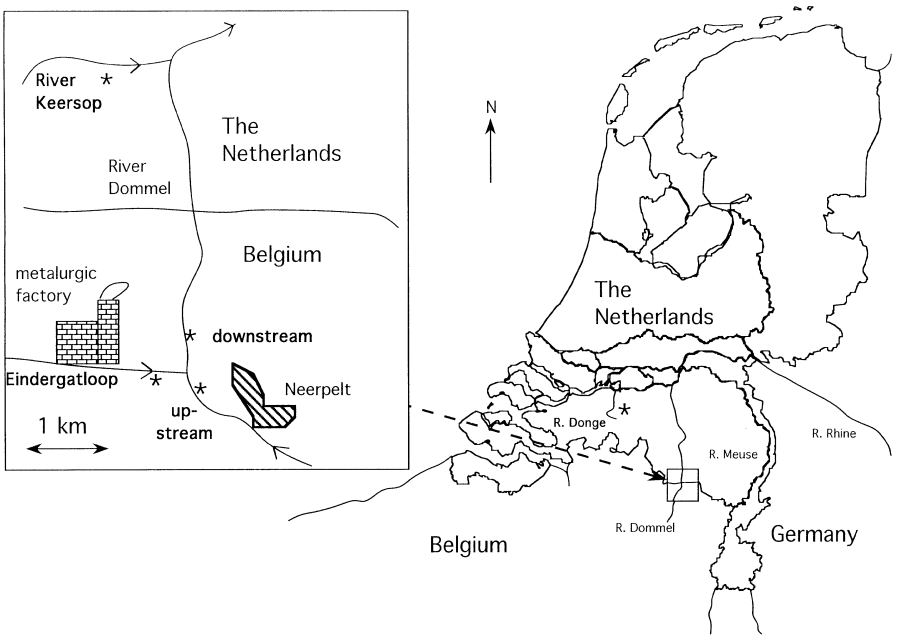

Fig. 1. Map of the watershed of the River Meuse showing the sampling sites at the rivers Dommel, Keersop, and Donge. ✱ sampling site

Table 2. Physical and chemical data of the three lowland rivers at the sampling sites (measurements were done parallel to toxicity tests)

Sampling Site	Temp. (°C)	pH	Conductivity (µS/cm)	Alkalinity (mEq/L)	Total P (µM P)	DIC (mg/L)	DOC (mg/L)	Zn (µM)	Cd (µM)	Fe (µM)
Keersop										
Mean	16.8	6.89	408	0.94	2.13	15.40	7.72	0.96	0.003	3.55
SD	2.1	0.18	131	0.23	0.73	2.74	0.62	0.60	0.004	2.48
n	6	6	5	3	3	6	6	6	6	6
Donge										
Mean	8.1	7.12	1,720	2.57	15.89	30.46	10.50	0.18	0.004	9.49
SD	1.9	0.04	254	—	9.09	7.60	2.00	0.04	0.005	0.67
n	3	3	3	1	3	3	3	3	3	3
Dommel										
Upstream										
Mean	11.8	6.95	393	0.54	9.32	9.48	8.86	1.93	0.007	4.36
SD	2.7	0.64	—	—	3.12	1.72	0.91	1.45	0.004	4.68
n	3	3	1	1	2	3	3	3	3	3
Downstream										
Mean	12.6	7.08	983	1.05	17.74	14.46	8.85	21.84	1.291	4.50
SD	2.2	0.45	74	0.14	5.17	1.02	0.59	13.75	0.935	5.02
n	3	3	2	2	2	3	3	3	3	3
Eindergatloop										
Mean	13.1	7.23	2,777	1.57	20.10	23.08	8.41	68.00	4.181	3.50
SD	3	0.60	2,112	0.17	9.04	0.45	3.13	27.36	1.674	5.53
n	3	3	2	2	2	3	3	3	3	3

eutrophication (2.13–20.1 µM total phosphorus) was found in all rivers. High concentrations of dissolved inorganic carbon indicated the hardness of the water and the levels of organic carbon confirmed some input of organic waste water. Values of pH and dissolved iron were in the same ranges and confirmed the similarity of the catchments. Microbenthic communities from all locations contained very little chlorophyll *a*, ranging from 0.20 µg/cm^2 for assemblages from Donge and Eindergatloop to 0.51 µg/cm^2 for Keersop samples (Figure 2). Dry weight was highest for samples from upstream and downstream the Eindergatloop (1.85 and 2.31 mg/cm^2, respectively), and lowest for Donge and Keersop communities (0.54 and 0.73 mg/cm^2, respectively). Organic matter made up 51.9% of the DW for Dommel upstream samples, whereas all other biofilms contained more inorganic than organic material. The Autotrophic Index (chl*a* content per organic matter) was highest for assemblages from the Keersop ($2.3 \cdot 10^{-3}$) and lowest for samples from upstream and downstream the Eindergatloop ($0.32 \cdot 10^{-3}$ and $0.37 \cdot 10^{-3}$, respectively). Metal concentrations in the biofilm increased according to the differences in metal concentrations in the water (Figure 3): Microbenthos from the Eindergatloop contained much zinc (538 µmol/g DW) and cadmium (167 µmol/g DW) equivalent to a heavy metal content of 5.4% of the whole DW. For assemblages downstream the Eindergatloop, metal concentrations in the biofilm accounted for 2.6% of the DW (350 µmol Zn and 27.7 µmol Cd/g

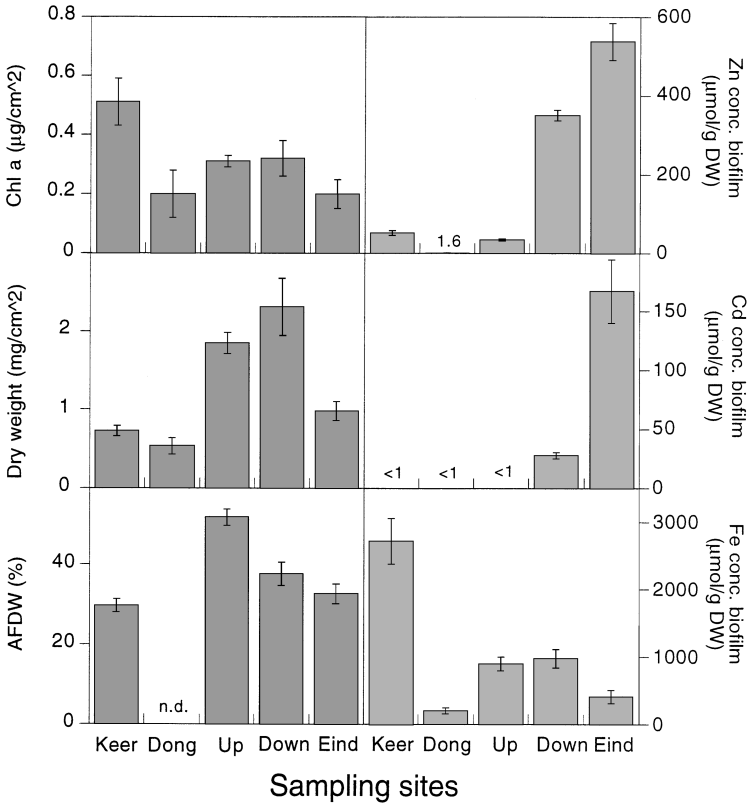

Fig. 2. Composition of the microbenthic biofilm. AFDW: Ash-free dry weight; DW, Dry weight; n.d., not detectable; Error bars, 1 SE

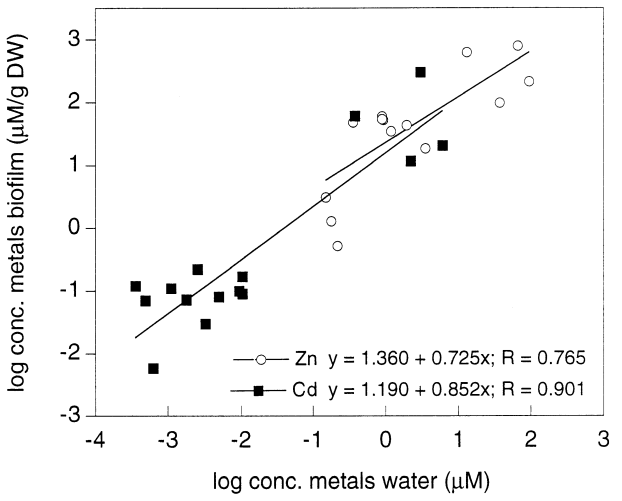

Fig. 3. Relation between metal concentrations in the biofilm and in the water. ○, zinc; ■, cadmium

DW). Zinc and cadmium concentrations in samples from other locations were, respectively, one or more than two orders of magnitude lower. Iron contents were highest in Keersop communities (2,716 µmol/g DW), mounting up to 15.2% of the DW.

Bacterial Thymidine Uptake

Toxic effects of zinc and cadmium on microbenthic bacteria were indicated in short-term tests (Figure 4). Zinc EC_{50} values for samples from the unpolluted rivers Keersop and Donge ranged between 6.7 and 56.2 µM (Table 3), thereby indicating a significantly higher sensitivity than samples from the Dommel upstream location (EC_{50}: 601 µM). Assemblages from this location were still less resistant than microbenthic bacteria from inside and downstream from the Eindergatloop (EC_{50}: >1,000 µM and 994 µM, respectively). However, this difference in zinc toxicity was not significant (Table 3). Short-term toxicity of cadmium was also highest for assemblages from Donge and Keersop (EC_{50}: 8.7–25.5 µM). Except for the first Keersop measurement (6/8/95), all EC_{50} values for bacteria from these sites were significantly lower than for those from the Dommel upstream location (Table 3). The EC_{50} for cadmium toxicity to bacterial assemblages at this site was 65.4 µM, significantly lower than for communities taken from downstream and inside the Eindergatloop (EC_{50}: 218 and 154 µM, respectively). EC_{50} values were related to the concentrations of both metals at test sites (Figure 5), indicating an increase in effective concentrations at elevated background levels.

Algal Photosynthesis

Generally, the metals tested showed little effect on microbenthic algal primary production (Figure 6). Short-term zinc exposure even stimulated the photosynthesis of algal assemblages from the polluted sites. EC_{50} values could only be determined for two Keersop samples (EC_{50}: 478 and 440 µM), all others were beyond the highest test concentration of 1,000 µM (Table 4). Values of EC_{50} for cadmium short-term toxicity were measurable for only two samples from Donge and Keersop (EC_{50}: 49.6 and 177 µM, respectively). All other assemblages showed no inhibition of photosynthetisis rate or

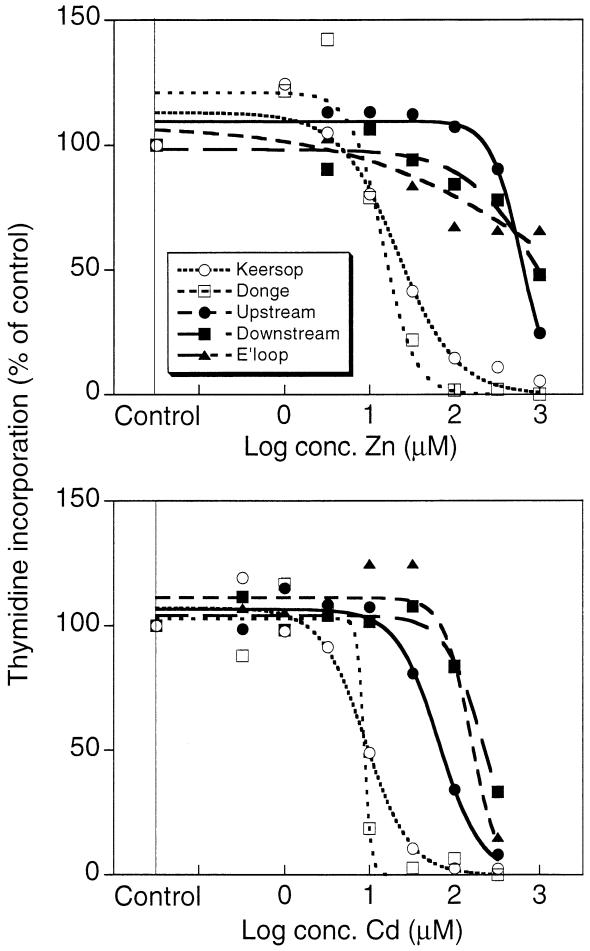

Fig. 4. Dose–effect curves in short-term toxicity tests using bacterial thymidine incorporation. (Upper panel) zinc, (lower panel) cadmium. Keersop: measurement at 6/29/95

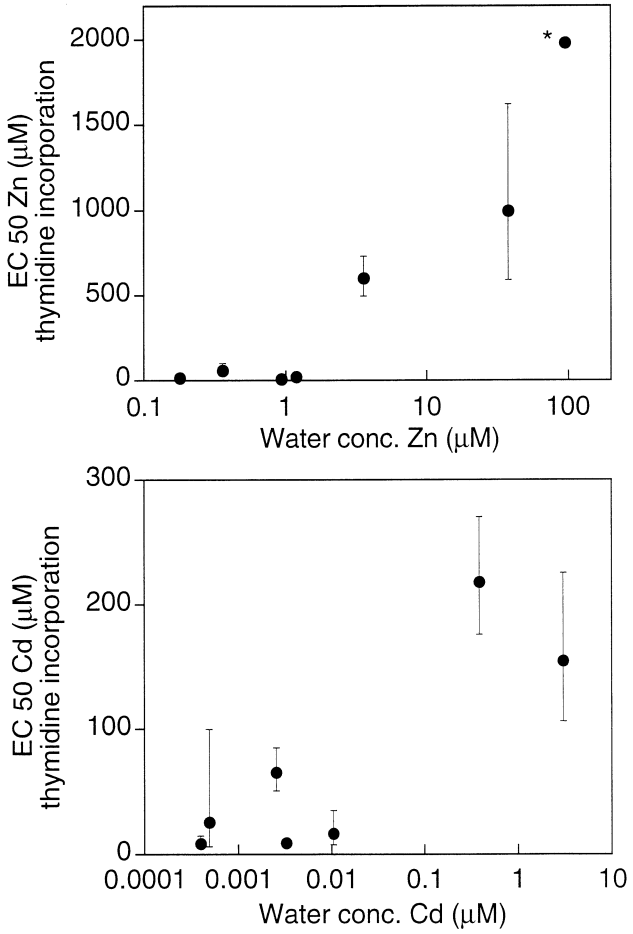

Fig. 5. EC_{50} values from short-term bacterial toxicity tests with concentrations of zinc (upper panel) and cadmium (lower panel) in site water. Error bars: 95% confidence limits; ✱: extrapolated value

Table 3. Effect concentrations zinc and cadmium in short-term toxicity tests with bacterial cell replication (EC_{50} values and 95% confidence limits)

	Zinc (µmol/L)		Cadmium (µmol/L)	
Sampling Site	EC_{50}	95% Confidence Limits	EC_{50}	95% Confidence Limits
Keersop				
1	6.7	(2.4–18.5)	25.5	(6.0–99.7)
2	21.5	(13.4–34.6)	9.0	(6.6–12.2)
3	56.2	(31.7–99.3)	16.4	(7.7–35.0)
all data	22.9	(11.4–46.3)	15.3	(11.0–21.4)
Donge	14.5	(8.9–23.2)	8.7	(6.5–14.8)
Dommel				
upstream	601	(494–731)	65.4	(50.4–84.9)
downstream	994	(588–>1,000)	218	(176–270)
Eindergatloop	>1,000	ND	154	(106–225)

ND = not detectable

effective concentrations exceeded the highest cadmium concentration tested (320 µM). Photosynthetic activity under cadmium exposure was even found to be enhanced for microbenthic algae downstream from the Eindergatloop (Figure 6).

Discussion

Chemical Composition of Water and Biofilm

Our study focused on rivers of the same stream order belonging to the same watershed. Water chemistry was generally comparable, but differed prominently in metal loads. Microbial biofilms are known to immobilize metals, not only because of the binding capacity of the extracellular polymer matrix but also by serving as a template for the precipitation of insoluble mineral phases (Ferris *et al.* 1989). Newman *et al.* (1983) suggested that iron and manganese oxides accumulating on the substrate surfaces may contribute more to the metal concentrations of the biofilm than the microfloral community. These precipitation processes were most prominent for Keersop assemblages (Figure 2), in which iron accounted for 15.2% of the total DW. However, at the most polluted site in the Eindergatloop, zinc and cadmium composed, respectively, 3.5 and 1.9% of the DW, while iron content was only 2.3%. Microfloral assemblages also stimulate metal accumulation in their own micro-environment because algal photosynthesis creates a pH inside the biofilm several units higher than the pH of the surrounding water (Liehr *et al.* 1994). By this, metal

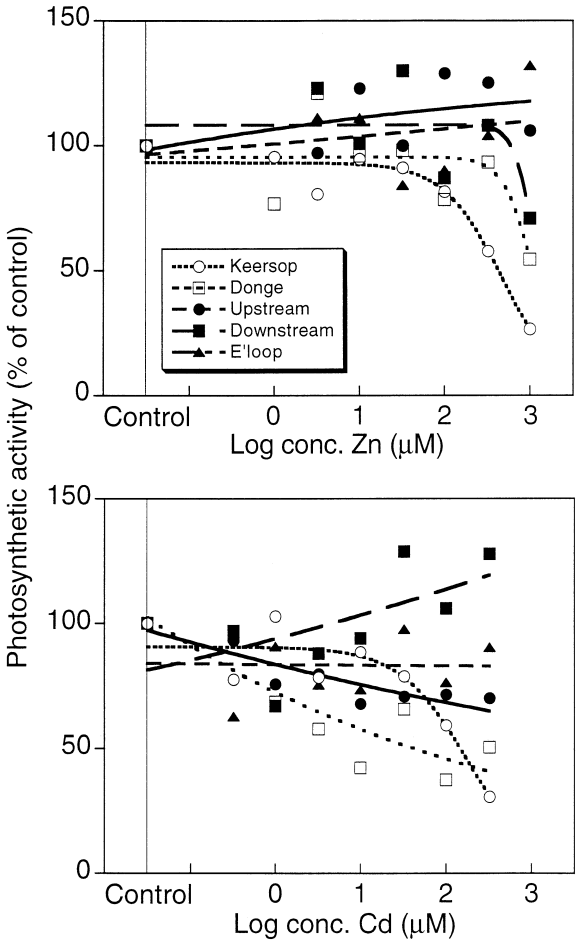

Fig. 6. Dose-effect curves in short-term toxicity tests using algal photosynthesis. (Upper panel) zinc; (lower panel) cadmium. Keersop: measurement at 7/13/95; Donge: measurement at 11/28/95

sorption and precipitation are favored and bioavailable metal hydroxide ions are removed. Despite these different mechanisms involved in metal precipitation, zinc and cadmium concentrations in the microbenthic assemblages displayed the gradient of metal concentrations in the water phase as indicated by the regressions in Figure 3. Although samples from polluted Dommel sites exceeded metal contents normally regarded as toxic chemical waste, the combination of precipitation mechanisms may act as a protective barrier, complicating the assessment of toxic effects on living organisms embedded in the biofilm.

Bacterial Thymidine Incorporation

Tolerance for both metals clearly distinguish Keersop and Donge assemblages on the one hand from Dommel communities on the other (Figure 4). For zinc, tolerance was already elevated in samples upstream from the Eindergatloop, whereas cadmium sensitivity was significantly reduced only at extremely high levels of this metal in the two most polluted Dommel sites (Figure 5). Our observations corroborate earlier ones on the increased incidence of zinc tolerant bacteria in

Table 4. Effect concentrations zinc and cadmium in short-term toxicity tests with algal photosynthesis (EC_{50} values and 95% confidence limits)

	Zinc (µmol/L)		Cadmium (µmol/L)	
Sampling Site	EC_{50}	95% Confidence Limits	EC_{50}	95% Confidence Limits
Keersop				
1	>1000	ND	>320	ND
2	478	(333–686)	177	(90.2–>320)
3	440	(286–677)	>320	ND
all data	799	(514–>1,000)	>320	ND
Donge				
1	>1000	ND	49.6	(7.0–>320)
2	>1000	ND	>320	ND
3	>1000	ND	>320	ND
all data	>1000	ND	>320	ND
Dommel				
upstream	>1000	ND	>320	ND
downstream	>1000	ND	>320	ND
Eindergatloop	>1000	ND	>320	ND

ND = not detectable

metal polluted stream sediments (Hornor and Hilt 1985; Chappel and Goulder 1994). The precipitation mechanisms mentioned above are likely to protect intact microbenthic assemblages. However, when used for short-term toxicity tests, effect levels were not only in the same range as for bacterial single species toxicity tests (Gosh *et al.* 1996) but were also suitable to detect a shift in community tolerance at much lower levels of long-term zinc exposure. It can be concluded that an *in situ* concentration of about 1 µM zinc represents a threshold level. When it is exceeded, succession toward a more tolerant assemblage is triggered. This concentration supports the environmental standards set by the Dutch authorities, which adopted 0.5 µM zinc as NOEC for aquatic ecosystems (Cleven *et al.* 1993). Our study could not distinguish between adaptation to zinc and to cadmium stress. Also, metal-tolerance in bacteria seems to be based on a general level of cotolerance for metals. For instance, zinc-tolerance is often linked with multiple metal and antibiotic resistance (Houba and Remacle 1980) and appears to be more prevalent in Gram-negative bacteria (Duxbury and Bicknell 1983). Therefore, it is concluded that the metal pollution in the River Dommel restructured microbial communities, favoring bacteria possessing these resistance mechanisms.

Algal Photosynthesis

Although EC_{50} values for photosynthetic activity were mostly beyond the test range, dose-effect curves for samples from different locations showed an increase in tolerance according to the degree of metal pollution (Figure 6). Stimulation of photosynthesis in assemblages from downstream and inside the Eindergatloop might be caused by the use of upstream water in the short-term test and could be interpreted as a requirement for high metal concentrations. However, due to the general insensitivity of the measurements, this trend was not significant (Table 4). Microbenthic algal photosynthesis was several orders of magnitude less susceptible to metal stress than algal growth in

single-species tests. The latter displayed harmful effects at concentrations as low as 0.075 µM zinc (Kuwabara 1985) and 0.035 µM cadmium (Chiaudini and Vighi 1978). This difference in sensitivity is partly due to the protective micro-environment inside the biofilm described above. Furthermore, it is known that diffusion of zinc through the biofilm is a process that takes longer than the 3 h of incubation we applied in our experiments (Rose and Cushing 1970). A second reason for the insensitivity of microbenthic algal primary productivity to zinc and cadmium is based on their unspecific mode of action on photosynthetic activity. An additional experiment confirmed this: when pre-incubation time was prolonged up to 22 h, doses of respectively 320 µM Zn and 100 µM Cd still had no significant effect on algal photosynthesis (Lehmann, unpublished results). Takamura *et al.* (1989) found growth inhibition of eucaryotic algae at far lower Cd or Zn concentrations than applied in our photosynthesis measurement, indicating that both metals did not damage this physiological process directly. In contrast to the PICT-concept (Blanck and Wängberg 1988a,b), microbenthic algal assemblages from sites with a different degree of pollution showed little differences in metabolic sensitivity to the tested substances, but appeared responsive to the deteriorating conditions on a structural level. Recent taxonomic studies of Eindergatloop and downstream samples indicated that only low numbers of algae were present, confirming their low autotrophic index. On the contrary, a high heterotrophic activity caused by ciliates (*Carchesium* sp.) was evident. This is in agreement with findings of Williams and Mount (1965), who reported a decrease in the diversity of primary producers and an increase of decomposers and consumers at elevated zinc levels. Additionally, diatom communities from downstream and inside the Eindergatloop showed a significantly elevated abundance of *Fragilaria capucina* and *Achnanthes minutissima*, species that are known to be tolerant to metal stress (Ivorra *et al.* 1997). On the species level, metal resistance of algae from rivers polluted by metals were reported earlier (Foster 1982b; Takamura *et al.* 1989). Genter *et al.* (1987) and Colwell *et al.* (1989) applied artificial streams to demonstrate that long-term zinc treatments as low as 0.76 µM changed the structure of microbenthic algal communities. Furthermore, field surveys proved the shift of algal assemblages provoked by metal pollution in rivers (Foster 1982a; Deniseger *et al.* 1986). None of these changes toward metal-adapted organisms could be detected by measuring algal photosynthesis in the present short-term test. Substances like zinc and cadmium that have an unspecific mode of action on photosynthesis probably damage other physiological processes at much lower levels. Such processes should be reexamined for further toxicity studies on microbenthic algal assemblages.

Conclusions

Zinc acts as a structuring agent on microbenthic bacterial assemblages when its long-term concentration exceeds a threshold level of about 1 µM.

The toxic effects of metals on living organisms embedded in biofilms are complicated by protective precipitation mechanisms.

For microalgae embedded in biofilms, photosynthesis is not an appropriate toxicological endpoint to measure the tolerance to zinc and cadmium.

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