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KINETICS OF CADMIUM ACCUMULATION IN PERIPHYTON UNDER FRESHWATER CONDITIONS

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Abstract—The aim of the present study was to investigate the kinetics of cadmium (Cd) accumulation (total and intracellular) in periphyton under freshwater conditions in a short-term microcosm experiment. Periphyton was precolonized in artificial flow-through channels supplied with natural freshwater and then exposed for 26.4 h to nominal Cd concentrations of 5 and 20 nM added to natural freshwater. Labile Cd in water determined with diffusion gradient in thin films was 60 to 69% of total dissolved Cd in the exposure channels and 11% in the control channel. Intracellular Cd concentrations in periphyton increased rapidly and linearly during the first 71 min. Initial intracellular uptake rates were 0.05 and 0.18 nmol of Cd/g of dry weight × min in the 5 nM and 20 nM exposures, respectively. The subsequent intracellular uptake was slower, approaching steady state at the end of Cd exposure. Uptake kinetics of Cd was slower when compared to experiments with planktonic algal cultures, probably due to diffusion limitations. Intracellular Cd uptake during the entire exposure was modeled with a nonlinear, one-compartment model from which uptake and clearance rate constants, as well as bioconcentration factors, were obtained. The release of Cd from periphyton after the end of Cd exposure was slow when compared to the initial uptake rates.

Keywords—Cadmium uptake Periphyton Freshwater Microcosms Uptake kinetics

INTRODUCTION

Cadmium (Cd) is a toxic, nonessential trace metal that has been designated a priority pollutant. It is introduced into the environment by several anthropogenic sources, e.g., phosphate fertilizers, mining, or fossil fuel combustion [1]. Concentrations of dissolved Cd in pristine river water are between 0.03 and 0.2 nM [2,3] but can be much higher near sources of metal pollution, e.g., 438 nM [4] downstream of coal mining and Zn ore treatment and between 4.2 μM [5] and 6.8 μM [6] downstream of metallurgic factories.

Bioavailability and hence uptake and effects of trace metals on aquatic organisms strongly depend on chemical speciation [7]. The free ion activity model and the biotic ligand model can be used to predict this bioavailability, especially for algae. Their applicability has been demonstrated for algae in defined media exposed to Cd [8–10], although some exceptions have been found [11–13]. Algae are at the basis of the food chain; therefore, metal uptake by algae strongly influences metal accumulation by invertebrates, which may take up metals both over water and over their diet [14,15].

Periphyton is the most important primary producer in running waters and responsible for the uptake and retention of organic carbon and inorganic nutrients [16]. It is a complex, three-dimensional community of heterotrophic and autotrophic organisms (algae, bacteria, and fungi) and nonliving components that grows on various substrates in rivers, lakes, and wetlands [16]. The community is embedded in a heterogeneous matrix, composed of different macromolecules such as polysaccharides, proteins, nucleic acids, (phospho)lipids, and other polymeric compounds [17]. It has been shown in some laboratory studies [18–20] and field studies [6,21] that periphyton is effective in accumulating metals from water. As periphyton can be used as diet by invertebrates, accumulated metals in periphyton may then be transferred into the food chain. Elevated Cd concentrations in water can have effects on biomass-related parameters (chlorophyll *a*, dry weight) [4,22,23] and on settlement, development, and species composition of periphyton [24]. Evidence also shows Cd biomagnification among trophic levels [25]. Periphyton can accumulate metals by three processes, namely, adsorption to components of the extracellular matrix, adsorption to cell surface molecules, and intracellular uptake [16]. Kinetic studies with planktonic algae cultures under laboratory conditions showed a rapid increase of Cd uptake and a slow release when cells were transferred to Cd-free medium [8,12]. It is expected that accumulation of Cd in periphyton under freshwater conditions will differ from Cd uptake by planktonic algae in defined media, since periphyton includes different algal species and microbial and fungal species, which are embedded in an organic matrix. Only few studies are available investigating the uptake and release of Cd in periphyton [20,26,27], which in particular do not give information about the intracellular Cd content in combination with exposure to natural freshwater. Uptake kinetics of Cd in periphyton is of interest to evaluate the effects of Cd concentration variations in natural waters.

The aim of the present study was to examine the kinetics of Cd accumulation (total and intracellular) in periphyton under freshwater conditions exposed to environmentally relevant Cd concentrations in a short-term microcosm experiment. Periphyton was precolonized in artificial flow-through channels supplied with natural freshwater and then exposed to nominal Cd concentrations of 5 and 20 nM for 26.4 h. Accumulation of Cd was related to either dissolved or labile Cd concentrations in water. The latter was measured in situ with the technique of diffusion gradient in thin films (DGT) [28,29], allowing the simultaneous measurement of average concentrations of free,

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inorganic metal complexes and some part of the organic metal complexes over the deployment time. Intracellular Cd uptake in periphyton during Cd exposure was modeled with a first-order one-compartment model. The release of Cd from periphyton upon cessation of Cd exposure was also investigated. Uptake and release kinetics were compared with data from algae and periphyton experiments, and factors affecting the accumulation of Cd in periphyton are discussed.

MATERIALS AND METHODS

Channels setup for periphyton colonization

Periphyton was colonized in three artificial flow-through channels (86 cm long, 10.4 cm wide, and 10 cm high, polymethyl methacrylate) that had a premixing chamber for the incoming water [30]. Sixty-four microscope glass slides were fixed in pairs and vertically (long edge) in four rows in grooves along each channel to avoid the coverage of periphyton with sediment. Water flow in the channels was 4 L/min with a water depth of approximately 3.1 cm and a water column of approximately 0.5 cm above the slides. The channels were supplied with natural freshwater from the nearby Chriesbach stream containing low dissolved Cd background concentrations and the organisms for the colonization of periphyton. Illumination was provided by two photosynthetically active radiation lamps (Osram HQL [MBF-U] Deluxe 400 W) overhanging the channels. The photoperiod was 15:9 h dark:light, and the average light irradiance was 329 $\pm$ 59 μmol of photons/$m^2 \times s$.

Kinetic experiment

Before the experiment, periphyton was colonized for three weeks, which resulted in sufficient biomass to measure metal content and a homogeneous coverage of slides with periphyton. Experiments were run in the colonization channels using the same water flow, light intensity, and photoperiod conditions as during colonization. Before the start of the kinetic experiment, two solutions of 405 and 1620 nM Cd were prepared in 200-L polyethylene barrels (Semadeni). Cadmium nitrate from a concentrated standard solution (Mallinckrodt Baker) was added to Chriesbach stream water (Dübendorf, Switzerland), which was previously diluted 1:27 with nanopure water to avoid precipitation of cadmium carbonate. Solutions were equilibrated for 19 h to allow full equilibration of metals with natural ligands. To obtain nominal Cd concentrations of 5 and 20 nM in the channels, Cd solutions were added with a peristaltic pump (MCP SW 5.01, Ismatec), with a dilution of approximately 1:67, to plastic tubes supplying the channels with natural water. To ensure further mixing by turbulence of the Cd solutions with the natural water, a plate with three perforated vertical plates was placed in the premixing chamber of each channel. Periphyton was exposed in two channels for 26.4 h to nominal Cd concentrations of 5 and 20 nM in Chriesbach water to study the kinetics of uptake (exposure period). After Cd addition was halted, periphyton was exposed to the original water from the Chriesbach stream containing low dissolved Cd background concentrations for 21.2 h to investigate the release of Cd (postexposure period). One channel without Cd addition was used as the control. Dark periods started 6.6 and 3.8 h after the beginning of the exposure and postexposure period, respectively. Channels were covered with black plastic during this time to avoid any light influence on periphyton. Periphyton slides and water samples for dissolved metal concentrations were taken four times during the first 71 min after the start of the exposure period to examine the initial uptake of Cd in periphyton, four times during the subsequent exposure period, and once at the end of the postexposure period.

Clean trace metal handling

To avoid contamination of samples and equipment, plastic gloves (Semadeni) were used for all procedures. All channel equipment, barrels, polypropylene vials, bottles and beakers, DGT devices and filters, syringes, and filtration units were placed in 0.1 M HNO_3 for at least 24 h, properly rinsed with nanopure water, and when necessary, sealed in clean plastic bags. Cellulose nitrate filters (0.45 μm, Sartorius) for metal content in periphyton were boiled twice in 0.1 M HNO_3, rinsed with nanopure water, dried twice afterward at 50°C for 15 h in an oven, and then preweighed. Except for the sampling at the channels, all handling was performed in a clean bench.

Water sampling for dissolved metal concentrations

Water samples (14 ml) for determination of dissolved metal concentrations were collected at the end of the channels and filtered (0.45 μm filters, Millipore) into polypropylene tubes using a plastic syringe (BD Plastipak, 50 ml). Before sample collection, filters and syringes were thoroughly rinsed with water from the channel. Samples were acidified to 0.24 M with HNO_3 (65% suprapure) and kept at 4°C in the dark until analysis. Water samples from the Cd solutions were taken following the same procedure.

Preparation, sampling and processing of DGT devices

Labile metal concentrations in water were measured with DGT. The procedure for making DGT devices followed the recommendation by Zhang [28]. Nonrestricted diffusive gel (pore size $\approx$ 5 nm) with a thickness of 0.8 mm and resin hydrogel with a thickness of 0.4 mm were covered with acid-cleaned 0.45-μm cellulose nitrate filters and enclosed on a piston with a cap (high-density polypropylene). At the beginning of the exposure period, three DGT devices were placed at the end of each channel, floating on the water surface with the resin pointing down. At the end of the exposure period, they were removed and replaced with three new DGT devices for the postexposure period. For measurement of labile metal concentrations, the resin gel layer was removed, placed for 24 h in a 14-ml polypropylene vial containing 2 ml of 1.66 M HNO_3 (65% suprapure, Merck), and then diluted sevenfold. To calculate DGT-labile metal concentrations in water, average water temperatures during the time of deployment were used to obtain diffusion coefficients for free metal ions. The calculations for labile metal concentrations are described by Zhang and Davison [31], and the diffusion coefficients for free metal ions were measured by Hao Zhang (DGT Research, Lancaster, UK, personal communication).

Free Cd concentrations in water were estimated using the speciation program Visual MINTEQ [www.lwr.kth.se/english/OurSoftware/vminteq; 32]. Because of lack of information on dissolved organic carbon (DOC), DGT-labile Cd concentrations were assumed to correspond only to inorganic complexes and were used as input Cd concentrations for the calculation, together with average concentrations of major cations, anions and alkalinity, pH, and temperature for the

exposure (four samples) and postexposure period (two samples).

Periphyton sampling and processing

For metal analysis, six periphyton slides were sampled randomly from each channel and stored in a plastic box until further processing. Periphyton was scratched from the slides with a microscope slide into a plastic beaker containing 100 ml of filtered experimental water. To obtain homogeneous periphyton suspensions, stirred solutions were first mixed using a 5-ml pipette and then sonicated in an ultrasonic bath for 30 s. Sediment and particles were then allowed to settle, and the supernatant containing periphyton organisms and the matrix was transferred into a plastic beaker and filled with filtered experimental water to 150 ml. This procedure avoids Cd redistribution among algae and water during sample handling. From each suspension, three measurement replicates of 20 ml were filtered through cellulose nitrate filters to obtain dry weight and total metal contents. It was assumed that the soluble part of the matrix was removed from the organisms by this treatment procedure. The rest of the suspension was treated for 10 min with 1 ml of 0.26 M ethylenediaminetetraacetic acid (EDTA; 4 mM final concentration) to remove metals adsorbed to the cell walls. The remaining metal content is considered to be intracellular (nonexchangeable with EDTA) [21]. Three measurement replicates of 20 ml were then filtered to obtain intracellular metal contents. Filters were dried twice for 15 h at 50°C in an oven and weighed. Afterward, they were digested in Teflon® beakers with 4 ml of nitric acid (65% suprapure, Merck) and 1 ml of hydrogen peroxide (30% suprapure, Merck) for 24 min in a high-performance microwave digestion unit (MLS 1200 Mega). Solutions of digested filters were transferred into 25-ml Erlenmeyer flasks and diluted with nanopure water. The digestion procedure was also used for plankton reference material (CRM 414, Institute for Reference Materials and Measurements, European Commission) and blank filters. The measured metal content in periphyton was related to the measured dry weight.

Chlorophyll *a* was extracted from 5 ml of the suspension with ethanol, and concentrations were measured using the high-performance liquid chromatography method described by Murray et al. [33]. For determination of species composition, 5 ml from suspensions were fixed with 4% formaldehyde. It was determined semiquantitatively upon visual observation, using an inverted phase contrast microscope with a magnification of 640. A three-graded scale was used for the abundance (3, dominant; 2, moderately abundant; 1, rare).

Metal analysis

Metal concentrations were measured with high-resolution inductively coupled plasma mass spectrometry (Element 2, Thermo Finnigan). The accuracy of the measurements was checked using SLRS-4 reference water (National Research Council Canada; errors: Cd $< 8\%$, Zn $< 9\%$, Mn $< 6\%$, Cu $< 10\%$), TM-28.2 (National Research Council Canada; errors: Cd $< 6\%$, Zn $< 9\%$, Mn $< 9\%$, Cu $< 9\%$), and plankton reference material (errors: Cd $< 10\%$, Zn $< 7\%$, Mn $< 7\%$, Cu $< 10\%$).

Water chemistry

Water samples for DOC, alkalinity, major cation, and anion analysis were taken four times during the exposure and once at the end of the postexposure period from the tank supplying the channels with water. Concentrations of major cations were determined by inductively coupled plasma optical emission spectrometry (Ciros, Spectro SA), and anion concentrations were measured by ion chromatography (Metrohm). Alkalinity measurements were performed by titration (with HCl 0.1 M until pH 4.5), and DOC was obtained by combustion. Temperature and pH were measured at the times of periphyton sampling.

Modeling of intracellular Cd uptake and release in periphyton

Initial intracellular uptake rates (r_i) in periphyton were modeled linearly during the first 71 min of Cd exposure at both exposure concentrations. Kinetics in this model were dictated only by uptake. To model the intracellular Cd uptake during the whole exposure period, a nonlinear one-compartment model was used, which assumes that uptake and release follow first-order kinetics:

$$\{\mathrm{Cd_{periphyton}}\} = \frac{k_1}{k_2} \times [\mathrm{Cd_{water}}] \times \left(1 - e^{-k_2 t}\right) \quad (1)$$

where k_1 (L/g of dry weight $\times$ min) and k_2 (1/min) are Cd uptake and clearance rate constants for periphyton, respectively; $\mathrm{Cd_{periphyton}}$ (nmol of Cd/g of dry weight) is the intracellular concentration of Cd in periphyton; $\mathrm{Cd_{water}}$ (nmol of Cd/L) is the Cd concentration in water, and t (min) is the time of Cd exposure. The modeling was performed with the program GraphPad Prism 4.03 (GraphPad Software). Computed parameters were intracellular Cd concentrations in periphyton at the end of the exposure period ($\{\mathrm{Cd_{max,\ predicted}}\}$), represented by the term $\frac{k_1}{k_2} \times [\mathrm{Cd_{water}}]$ in Equation 1, and clearance rate constants (k_2). Uptake rate constants (k_1) were calculated from these parameters using dissolved and labile Cd concentrations in water ($\mathrm{Cd_{water}}$).

Average $\mathrm{Cd_{water}}$ during the exposure period were used with the k_1 to calculate average uptake rates ($\bar{r}_1$):

$$\bar{r}_{1,\ \mathrm{dissolved/labile}} = k_1 \times [\mathrm{Cd_{water}}] \quad (2)$$

Intracellular Cd concentrations in periphyton at the end of the postexposure period ($\{\mathrm{Cd_{end,\ experimental}}\}$) were compared with intracellular concentrations predicted ($\{\mathrm{Cd_{end,\ predicted}}\}$) with a first-order rate release model using k_2 obtained from the modeled uptake:

$$\{\mathrm{Cd_{end,\ predicted}}\} = \{\mathrm{Cd_0}\} \times \left(e^{-k_2 t}\right) \quad (3)$$

where $\mathrm{Cd_{end,\ predicted}}$ (nmol of Cd/g of dry weight) is the intracellular Cd concentration in periphyton at the end of the postexposure period, $\mathrm{Cd_0}$ (nmol of Cd/g of dry weight) is the intracellular Cd concentration in periphyton at the end of the exposure to Cd, k_2 is the calculated clearance rate constant, and t is the time of the postexposure period.

Bioconcentration factors (BCFs) at steady state (L/kg) were calculated for the two exposures by

$$\mathrm{BCF(I)_{dissolved/labile}} = k_1/k_2 \quad (4)$$

where k_1 and k_2 are the uptake and clearance rate constants, respectively, and k_1 is calculated with dissolved or labile Cd concentrations in water. The BCFs were also calculated for Cd exposed and control periphyton by

$$\mathrm{BCF(II)_{dissolved/labile}} = \{\mathrm{Cd_{intracellular}}\}/\{\mathrm{Cd_{water}}\} \quad (5)$$

Table 1. Dissolved, diffusion gradient in thin films (DGT)–labile, and free cadmium (Cd) concentrations in water; percentages of DGT-labile and free Cd; pH; and temperature during the exposure and postexposure periods in all channels[ab]

	Dissolved Cd (nM)	Labile Cd (nM)	% Labile Cd	Free Cd (nM)	% Free Cd	pH	Temp. (°C)
Exposure							
Control	0.12 ± 0.02	0.013 ± 0.015	11	0.009	8	7.93 ± 0.03	9.7 ± 0.4
5 nM Cd	5.8 ± 0.3	3.5 ± 0.6	60	2.5	43	7.97 ± 0.06	9.8 ± 0.4
20 nM Cd	25 ± 3	17.2 ± 0.4	69	12.2	49	7.94 ± 0.06	9.9 ± 0.4
Postexposure							
Control	0.20 ± 0.06	0.025 ± 0.006	13	0.018	9	7.92 ± 0.01	9.5 ± 0.1
5 nM Cd	0.21 ± 0.04	0.030 ± 0.024	14	0.021	10	7.90 ± 0.06	9.6 ± 0.1
20 nM Cd	0.25 ± 0.01	0.021 ± 0.000	8	0.015	6	7.92 ± 0.04	9.5 ± 0.1

[a] Values represent means ± standard deviation.
[b] Means represent eight samples: measurements for dissolved Cd concentrations, pH, and temperature during the exposure period; two samples during the postexposure period; and three DGT replicates for each period.

where $Cd_{intracellular}$ (nmol of Cd/kg of dry weight) is the intracellular Cd content in periphyton at the end of the exposure period and Cd_{water} (nmol of Cd/L) is either the dissolved Cd concentrations at the end of the exposure period or the labile Cd concentrations during the exposure period.

RESULTS

Dissolved and DGT-labile metal concentrations in water

Dissolved, DGT-labile, and free Cd concentrations in water, as well as percentages of DGT-labile and free Cd during the exposure and postexposure period for all channels, are summarized in Table 1. Average background concentrations of dissolved Cd in water of the control channel were low (0.12 ± 0.02 nM) during the exposure period. Two rain events during the second light:dark period elevated average concentrations to 0.2 ± 0.06 nM during the postexposure period. The percentages of DGT-labile (11–13%) and free Cd (8–9%) in the control channel were low during the whole experiment. In both Cd exposure channels, dissolved Cd concentrations remained constant during the exposure period (5 nM = 5.8 ± 0.3, 20 nM = 25 ± 3). Compared to the control, high and similar proportions of DGT-labile Cd (5 nM = 60%, 20 nM = 69%) and free Cd (5 nM = 43%, 20 nM = 49%) were determined in the Cd exposure channels. Shortly after the end of the exposure period, dissolved Cd concentrations were comparable to the control (20 nM exposure = 0.26 nM, 5 nM exposure = 0.18 nM, control = 0.16 nM). During the postexposure period, average concentrations of dissolved and DGT-labile Cd in the Cd exposure channels were as low as in the control.

Dissolved Zn, Cu, and Mn concentrations in water were similar in all channels during the exposure period (Zn = ~240 nM, Cu = ~32 nM, Mn = ~93 nM) and the postexposure period (Zn = ~463 nM, Cu = ~53 nM, Mn = ~125 nM), as shown in Table 2. The two rain events also elevated dissolved concentrations of these metals during the postexposure period. Percentages of DGT-labile Zn in water were around 36% during the whole experiment in all channels. Percentages of DGT-labile Cu increased with increasing Cd concentrations in water during the exposure period, namely, from 24% in the control to 28% in the 5 nM exposure and 33% in the 20 nM exposure. During the postexposure period, percentages of DGT-labile Cu were around 40% in all channels. Although labile Mn concentrations were not measured, data obtained in other studies with Chriesbach water (P. Bradac, unpublished data) showed that most of total dissolved Mn is labile.

During the exposure period, DOC was 2.68 ± 0.1 mg/L, calcium was 2.89 ± 0.06 mM, and alkalinity was 6.28 ± 0.15 mM (means of four samples). The rain events caused an increase of DOC to 3.07 mg/L and a decrease of calcium to 2.58 mM and alkalinity to 5.65 mM at the end of the postexposure period. Temperature and pH were similar and constant in all channels over the whole experiment.

Periphyton characterization

Chlorophyll *a* content of periphyton (expressed as mg of chlorophyll *a*/g of dry weight) was 4.9 ± 1.4 in the control, 4.3 ± 1 in the 5 nM exposure, and 5.5 ± 1.4 in the 20 nM Cd exposure. Semiquantitative microscopical analysis showed that periphyton was mostly composed of diatoms. The dominant species was *Nitzschia palea*; moderately abundant were *Achnanthes minutissima*, *Achnanthes lanceolata*, and *Gomphonema parvulum*; and rare were *Melosira varians*, *Cymbella affinis*, *Rhoicosphenia abbreviata*, *Nitzschia linearis*, *Nitzschia*

Table 2. Dissolved and diffusion gradient in thin films (DGT)–labile concentrations of zinc (Zn) and copper (Cu) and dissolved concentrations of manganese (Mn) in water, as well as percentages of DGT-labile concentrations during the exposure and postexposure periods in all channels[a]

	Dissolved Zn (nM)	Labile Zn (nM)	% Labile Zn	Dissolved Cu (nM)	Labile Cu (nM)	% Labile Cu	Dissolved Mn (nM)
Exposure							
Control	252 ± 65	94 ± 11	37	32 ± 4	7.7 ± 1.4	24	94 ± 6
5 nM Cd	214 ± 41	78 ± 19	36	31 ± 4	8.7 ± 1.9	28	91 ± 9
20 nM Cd	256 ± 41	92 ± 7	36	31 ± 3	10.2 ± 1.9	33	93 ± 7
Postexposure							
Control	463 ± 102	144 ± 19	31	53 ± 3	22.8 ± 5.3	43	126 ± 33
5 nM Cd	458 ± 116	173 ± 21	38	53 ± 5	22.4 ± 3.0	43	121 ± 22
20 nM Cd	468 ± 98	164 ± 41	35	54 ± 2	18.7 ± 1.8	35	127 ± 25

[a] Representation of values and means are as described in Table 1.

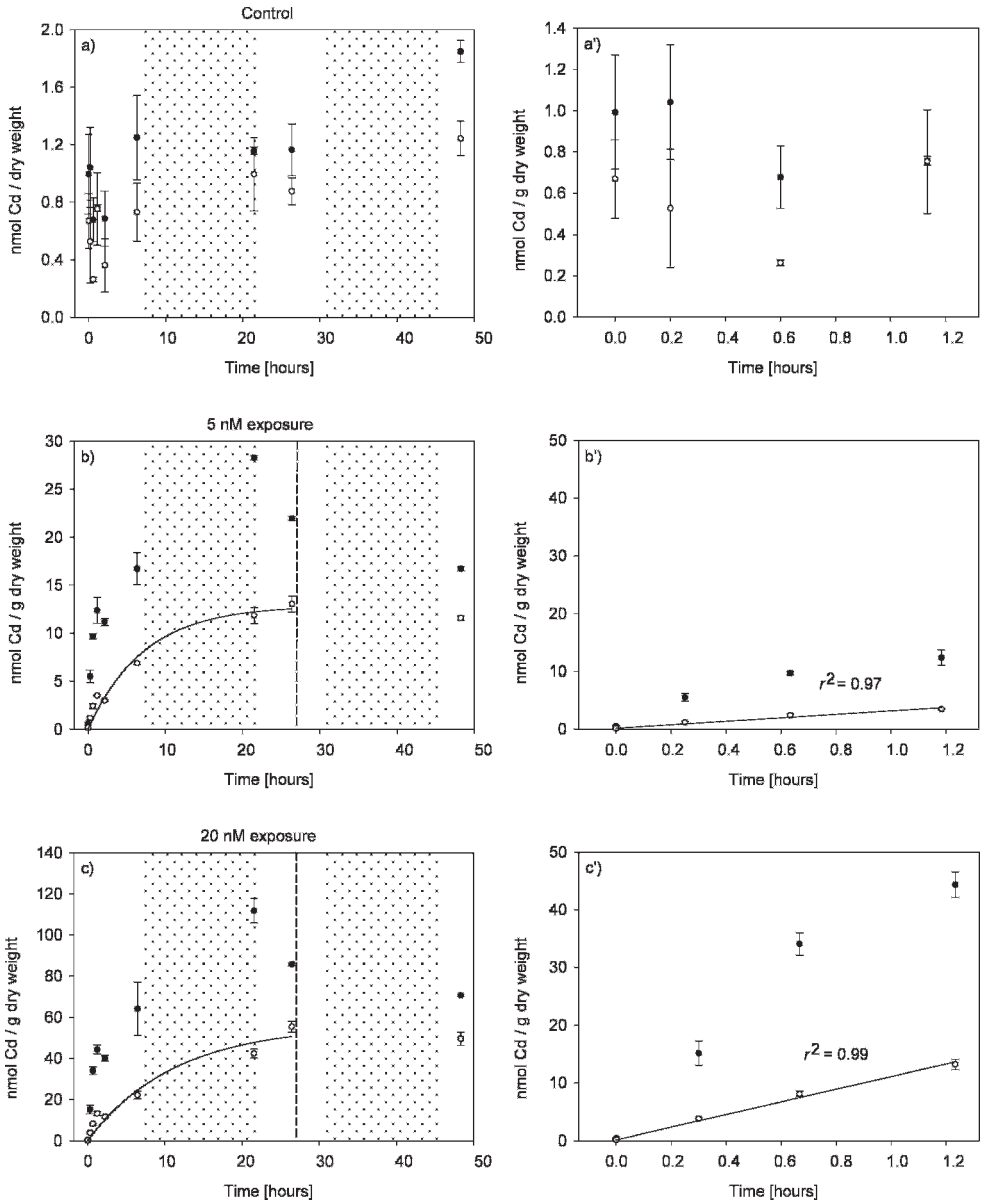

Fig. 1. Total and intracellular cadmium (Cd) concentrations (nmol of Cd/g of dry weight) in periphyton as a function of time (hours) during the exposure and postexposure period in all channels (**a**–**c**). The solid curves are the results from the nonlinear model for intracellular Cd uptake during the exposure period. Initial Cd uptake in periphyton during the first 71 min of Cd exposure (**a**′–**c**′). The solid lines represent the modeled linear increase. Black dots represent the total, white dots the intracellular Cd concentrations in periphyton, dashed lines the end of Cd exposure, and dotted rectangles the dark periods. Data points for Cd content in periphyton represent three replicates (means ± standard deviations).

acicularis, *Amphora ovalis*, *Cocconeis placentuala*, *Navicula rhynchocephala*, and *Fragilaria capucina*. Chlorophyll *a* content and species composition and abundance were similar in all channels and did not change after exposure to Cd.

Kinetics of Cd uptake and release in periphyton

Average total and intracellular Cd concentrations in control periphyton were low during the whole exposure period (total = 0.97 ± 0.23 nmol of Cd/g of dry weight, intracellular = 0.65 ± 0.25 nmol of Cd/g of dry weight). At the end of the postexposure period, total concentrations increased slightly to 1.9 ± 0.1 nmol of Cd/g of dry weight and intracellular increased to 1.2 ± 0.1 nmol of Cd/g of dry weight concomitantly to the elevation of dissolved Cd concentrations in water (Fig. 1a). Intracellular Cd concentrations increased steadily during the whole time of Cd exposure in the 5 and 20 nM treatments. In contrast, total Cd concentrations in both treatments increased steadily to the end of the first dark period but decreased afterward to the end of Cd exposure (Fig. 1b and c). At the end of the exposure period, Cd content in periphyton was 21.9 ± 0.2 nmol of Cd/g of dry weight in the 5 nM exposure and 85.7 ± 0.7 nmol of Cd/g of dry weight in the 20 nM exposure and intracellular was 13 ± 0.8 and 55.5 ± 2.7 nmol of Cd/g of dry weight, respectively. The intracellular Cd content of periphyton at the end of the exposure period was 15 times higher in 5 nM and 64 times higher in the 20 nM treatment when compared to the control. Total and intracellular Cd concentrations in periphyton increased rapidly during the first 71 min (initial uptake). The subsequent total and intracellular content increase for the rest of the exposure period was slower than the initial uptake. Total and intracellular Cd contents also increased during the dark period

Table 3. Modeled parameters for cadmium (Cd) uptake and release by periphyton

Parameter	5 nM Cd	20 nM Cd	Ratio 20:5
Initial uptake (linear)[a]			
Dissolved Cd (nmol/L)	6.1 ± 0.1	23.4 ± 0.5	3.8
Labile Cd (nmol/L)	3.5 ± 0.6	17.2 ± 0.4	4.9
r_i (nmol of Cd/g of dry wt × min)	0.05	0.18	3.6
Uptake and release model[b]			
$k_{1,\ dissolved}$ (L/g of dry wt × min)	0.0051	0.0033	0.65
$\bar{r}_{1,\ dissolved}$ (nmol of Cd/g of dry wt × min)	0.03	0.08	2.8
$k_{1,\ labile}$ (L/g of dry wt × min)	0.0084	0.0048	0.57
$\bar{r}_{1,\ labile}$ (nmol of Cd/g of dry wt × min)	0.03	0.08	2.8
k_2 (1/min)	0.0023	0.0015	0.65
$BCF(I)_{dissolved}$ (L/kg)	2.2×10^3	2.2×10^3	1
$BCF(I)_{labile}$ (L/kg)	3.6×10^3	3.2×10^3	0.88
$BCF(II)_{dissolved}$ ([nmol of Cd/kg of dry wt]/[nmol of $Cd_{dissolved}$/L])	2.5×10^3	1.9×10^3	0.76
$BCF(II)_{labile}$ ([nmol of Cd/kg of dry wt]/[nmol of Cd_{labile}/L])	3.7×10^3	3.2×10^3	0.86
r^2	0.96	0.95	—
$Cd_{max,\ predicted}$ (nmol of Cd/g of dry wt)	13	56	4.4
$Cd_{max,\ experimental}$ (nmol of Cd/g of dry wt)	13	56	4.3
$Cd_{end,\ predicted}$ (nmol of Cd/g of dry wt)	0.64	7.9	12.4
$Cd_{end,\ experimental}$ (nmol of Cd/g of dry wt)	11.6	49.6	4.3
Modeled half-life of Cd in periphyton (min)	301	466	1.6

[a] Dissolved and diffusion gradient in thin films (DGT)–labile Cd concentrations in water and initial uptake rates (r_i) in periphyton during the first 71 min of Cd exposure. Means ± standard deviations represent three samples for dissolved and three DGT replicates for labile Cd concentrations. Values for DGT-labile Cd concentrations are average concentrations from the exposure period.

[b] Experimental and modeled parameters for the uptake and release of Cd in periphyton. Parameters represent uptake rate constants (k_1), averaged uptake rates ($\bar{r}_1$), clearance rate constants (k_2), bioconcentration factors (BCFs) calculated from uptake and clearance rate constants (BCF[I]), and BCFs calculated from Cd concentrations in periphyton and water (BCF[II]). Some parameters were calculated with dissolved and labile Cd concentrations in water (subscript). Further parameters were the correlation coefficient from the model (r^2) and modeled and experimental Cd concentrations in periphyton at the end of the exposure period ($Cd_{max,\ predicted}$, $Cd_{max,\ experimental}$) and at the end of the postexposure period ($Cd_{end,\ predicted}$, $Cd_{end,\ experimental}$). Ratio 20:5 is the ratio of parameters between the 20 nM and the 5 nM Cd exposures.

in both treatments during the exposure period. Total and intracellular Zn, Cu, and Mn contents in periphyton during the exposure period were similar in all channels (data not shown). After Cd addition was halted, total and intracellular Cd concentrations in periphyton decreased slowly, whereas total contents showed a faster decrease than intracellular contents.

The rapid increases of intracellular Cd concentrations in periphyton during the first 71 min of Cd exposure were fitted to a linear uptake (initial uptake, Fig. 1b′ and c′). Initial intracellular uptake rates were 0.05 nmol of Cd/g of dry weight × min in the 5 nM exposure and 0.18 nmol of Cd/g of dry weight × min in the 20 nM exposure.

Percentages of adsorbed Cd were similar at both exposure concentrations during the whole experiment and ranged from 58 to 79% during the exposure period except for the last point, which had a percentage of around 38%. At the end of the postexposure period, the percentage of adsorbed Cd was around 30%, similar to the control.

Linear and nonlinear modeling of Cd accumulation in periphyton

Ratios of initial intracellular uptake rates (r_i) between the 20 nM and the 5 nM exposures were compared with ratios of dissolved and labile Cd concentrations in water. Ratios were 3.6 for initial uptake rates, 3.8 for dissolved Cd concentrations, and 4.9 for labile Cd concentrations (Table 3).

The nonlinear model fitted the data well ($r^2 = 0.96$ for 5 nM, $r^2 = 0.95$ for 20 nM). All modeled and calculated parameters are summarized in Table 3. Values of uptake rate constants $k_{1,\ dissolved/labile}$ and clearance rate constants k_2 were similar at both exposure concentrations, and values for k_1 were consistent for both calculations used. Ratios of averaged intracellular uptake rates ($\bar{r}_{1,\ dissolved}$, $\bar{r}_{1,\ labile}$) between the two treatments were 2.8 for both calculations. Averaged intracellular uptake rates were smaller than initial intracellular uptake rates. Values of BCFs were in the similar order of magnitude at both exposure concentrations and were consistent for all calculations used. Predicted ($\{Cd_{max,\ predicted}\}$) and experimental ($\{Cd_{max,\ experimental}\}$) intracellular Cd concentrations in periphyton at the end of the exposure period show equal values at both exposure concentrations. Comparisons of modeled intracellular Cd concentrations in periphyton at the end of the postexposure period ($\{Cd_{end,\ predicted}\}$) with the experimental values ($\{Cd_{end,\ experimental}\}$) show that Cd is released more slowly at both concentrations than predicted by the model.

DISCUSSION

Metal speciation in channels

Background concentrations of dissolved Cd in Chriesbach water were low, as was reported for other unpolluted sites [2,3]. The low percentages (11–13%) of DGT-labile Cd concentrations measured in the control channel indicate that most Cd was bound to nonlabile organic ligands or colloids. Higher percentages of DGT-labile Cd (70%) were measured in a freshwater stream with similar dissolved Cd but lower DOC concentrations than in the present study [2,3]. However, percentages of DGT-labile Cd were high in the Cd exposure channels, probably because Cd solutions added to the channels contained an excess of Cd compared to organic ligands and other metals. The short contact time (~40 s) of Cd solutions with organic ligands of Chriesbach water in the channels was too short to achieve a new, complete chemical equilibrium, resulting in higher percentage of labile Cd concentrations. The increase of labile Cu concentrations with increasing Cd concentrations might be due to competition of Cd with Cu for binding sites of nonlabile ligands. This effect was not

observed with Zn, maybe due to the high concentrations of DGT-labile Zn. These results show that elevated Cd concentrations can change the speciation of other metals.

Cd accumulation and kinetics in periphyton

Two uptake phases for the intracellular Cd uptake, a fast initial one and a slower subsequent one approaching steady state at the end of the exposure period, were observed in periphyton in both Cd exposure channels. The time course of adsorbed Cd shows a continuous binding of Cd to cellular binding sites, which become saturated toward the end of the exposure period. The fast initial linear Cd uptake during the first 71 min of Cd exposure is assumed to be a consequence of uptake only. The subsequent slower uptake phase suggests that some Cd is released and a steady state is reached. These results may be compared with Cd uptake kinetics in algae cultures under defined chemical conditions, in which uptake is only controlled by transport over the cell wall and membrane. The comparison shows that both the saturation of binding sites (adsorbed Cd) [13] and the uptake are faster than in periphyton. In the green alga *Selenastrum capricornutum* Printz, the initial uptake phase was 9 min and steady state was reached within 30 min at comparable free Cd concentrations [12]. Other than in free-floating algae, the matrix and the three-dimensional structure of the periphyton community may slow the diffusion of Cd to cellular binding sites of algae.

Few data on Cd accumulation kinetics in periphyton are available for comparison. Concentrations of Cd for the whole periphyton biofilm in a lake exposed to 0.8 nM Cd increased nonlinearly, approaching steady state after 11 d of exposure [26]. Hill et al. [20] demonstrated a linear increase of Cd in the whole periphyton biofilm exposed to 8.9 nM Cd in flow-through channels supplied with natural freshwater over 48 h. Comparisons among such studies are difficult, since various factors such as species composition and biomass, as well as Cd concentration and speciation, and other metals influence the uptake of metals in periphyton in natural freshwaters. Moreover, the diffusion of Cd from water to the algae of periphyton is influenced by the thickness and composition of the matrix, sediments and particles incorporated in the matrix and water current [20].

The intracellular uptake of Cd in periphyton could be modeled with a simple, nonlinear one-compartment model. However, the used model does not provide a distinction between the diffusion kinetics (diffusive layer, matrix, and cell wall) and the transport kinetics through the cell membrane. Uptake rate constants depend on diffusion of Cd through the diffusive boundary layer, the matrix, and the transport across the plasma membrane, and clearance rate constants depend on the binding to intracellular ligands and the transport across the plasma membrane. The consistent values of both constants show that they are independent of Cd concentrations in water. Stephenson and Turner [26] used a similar model for the accumulation of Cd in the whole periphyton biofilm exposed in lake water to 0.8 nM Cd and obtained a good fit. They found a similar uptake rate constant of 0.026 L/g of C $\times$ min (calculated from Stephenson and Turner [26]), when the present results were referred to a C content of 16%, as obtained in previous experiments.

Initial uptake rates increased with increasing Cd concentrations in water. Ratios of initial uptake rates were closer to ratios of dissolved Cd concentrations than to those of labile Cd concentrations in water. Due to only two Cd exposure concentrations used and high labile Cd concentrations being close to dissolved concentrations, no clear relationship could be obtained between Cd accumulation in periphyton and Cd speciation in water. Averaged uptake rates ($\bar{r}_{1,\ \mathrm{dissolved}}$, $\bar{r}_{1,\ \mathrm{labile}}$) within the same exposure were lower than initial uptake rates, resulting from the influence of Cd release during the exposure. Stephenson and Turner [26] found averaged uptake rates of 0.021 nmol of Cd/g of C $\times$ min at Cd concentrations of 0.8 nM. In the present study, they were 0.19 and 0.50 nmol of Cd/g of C $\times$ min in the 5 nM and 20 nM Cd exposures, respectively (a C content of 16% was assumed as obtained in previous experiments). These uptake rates are thus similar if related to the Cd concentrations in water.

Values of BCFs for the two Cd exposure concentrations were in the similar order of magnitude and consistent for all calculations used. Morin et al. [23] found BCFs in periphyton which were higher than in the present study, probably due to exposure in media without competing organic ligands and excess of other metals. The BCFs calculated from Cd concentrations in periphyton and water (Table 3) were higher in the control channel, i.e., $\mathrm{BCF(II)_{dissolved}} = 6.6 \times 10^3$ L/kg and $\mathrm{BCF(II)_{labile}} = 6.7 \times 10^4$ L/kg, than in the Cd exposure channels, showing a decrease in BCFs with increasing Cd concentrations. This slight decrease might be due to an active regulation of internal Cd concentrations, i.e., an active release of Cd at the two exposure concentrations. Decreases of BCFs with increasing Cd concentrations in water were also found for periphyton exposed to Cd concentrations of 89 and 890 nM [23] and were reported for different algal species [34].

Competition of other metals for Cd uptake

Despite the excess of total dissolved and labile Zn, Mn, and Cu concentrations in water, total and intracellular Cd concentrations in periphyton increased during the exposure to Cd in the 5 and 20 nM treatments and in the control during the two rain events. Competitions of these metals for Cd uptake have been demonstrated with algae cultures in defined media. Uptake of Cd was inhibited by Zn and Mn in several marine diatoms [9,35] and in *Scenedesmus vacuolatus* [36] and by Cu and Zn in *Chlamydomonas reinhardtii* [8]. Different uptake mechanisms in algae have been identified. In *S. vacuolatus*, for example, binding constants of Cd and Zn to transport sites had similar values [36] and the Zn transport system was assumed to be the main uptake path for Cd. Uptake of Cd in algae can also occur via the Mn transport system [9,35] or a divalent metal transporter [37] acting in the transport of Mn, Fe, and Cu. The efficient Cd uptake in the present study in the presence of an excess of competing metals indicates that Cd has a high affinity to transport sites of the plasma membrane. A faster Cd accumulation and higher Cd contents in periphyton at steady state would be expected in the absence of those metals [9,35].

Cd release

Total and intracellular Cd concentrations in periphyton decreased slowly after Cd addition was halted. Obtained clearance rate constants from the modeled uptake were higher when compared to published values for periphyton, being in the range of 2.03×10^{-4} [26] to 5.42×10^{-5} [27] per minute. Predicted intracellular Cd concentrations in periphyton at the end of the postexposure period using a first-order decrease model and calculated clearance rate constants (Table 3) were lower than measured, showing an overestimation of k_2 by the

model. Such differences at the end of a Cd-release experiment were also observed by Stephenson and Turner [26] for lake periphyton. The slow decrease and the overestimated clearance rate constants may be due to binding of Cd to intracellular ligands, e.g., phytochelatins. Phytochelatins are cysteine-rich metal-binding polypeptides that are strongly induced in algae by Cd even at low concentrations [38,39], with synthesis starting within minutes to hours after exposure [40]. Phytochelatins are only one regulation mechanism within the complex network of metal homeostasis, which shows interspecific differences and may thus result in different residence times of intracellular Cd concentrations in periphyton.

As for the Cd uptake, differences were observed when the release from periphyton is compared with the release from planktonic algae [8,12]. The slower release in periphyton might be due to retention of released Cd by the matrix and subsequent uptake by adjacent cells [27].

Environmental relevance

In terms of environmental relevance, data from the present study show that periphyton accumulates Cd rapidly at low Cd concentrations and that most Cd is retained in the cells when Cd exposure is halted. Although Cd content in periphyton can be reduced by release, growth dilution, and cell desorption [27], successive exposures to elevated metal concentrations in water may maintain high concentrations of Cd and other metals in periphyton. Due to its high toxicity and persistence, Cd will directly affect periphyton and indirectly affect organisms feeding on periphyton. Cadmium released back to the water from periphyton may also affect other aquatic organisms.

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REFERENCES

1. Dobson S. 1992. *Cadmium: Environmental Aspects*. World Health Organization, Geneva, Switzerland.
2. Xue HB, Sigg L. 1998. Cadmium speciation and complexation by natural organic ligands in freshwater. *Anal Chim Acta* 363:249–259.
3. Sigg L, Black F, Buffle J, Cao J, Cleven R, Davison W, Galceran J, Gunkel P, Kalis E, Kistler D, Martin M, Noel S, Nur Y, Odzak N, Puy J, Van Riemsdijk W, Temminghoff E, Tercier-Waeber ML, Toepperwien S, Town RM, Unsworth E, Warnken KW, Weng LP, Xue HB, Zhang H. 2006. Comparison of analytical techniques for dynamic trace metal speciation in natural freshwaters. *Environ Sci Technol* 40:1934–1941.
4. Morin S, Duong TT, Dabrin A, Coynel A, Herlory O, Baudrimont M, Delmas F, Durrieu G, Schafer J, Winterton P, Blanc G, Coste M. 2008. Long-term survey of heavy-metal pollution, biofilm contamination and diatom community structure in the Riou Mort watershed, South-West France. *Environ Pollut* 151:532–542.
5. Lehmann V, Tubbing GMJ, Admiraal W. 1999. Induced metal tolerance in microbenthic communities from three lowland rivers with different metal loads. *Arch Environ Contam Toxicol* 36:384–391.
6. Admiraal W, Blanck H, Buckert-De Jong M, Guasch H, Ivorra N, Lehmann V, Nystrom BAH, Paulsson M, Sabater S. 1999. Short-term toxicity of zinc to microbenthic algae and bacteria in a metal polluted stream. *Water Res* 33:1989–1996.
7. Tessier A, Turner DR. 1995. *Metal Speciation and Bioavailability in Aquatic Systems*. Wiley, Chichester, West Sussex, UK.
8. Kola H, Wilkinson KJ. 2005. Cadmium uptake by a green alga can be predicted by equilibrium modelling. *Environ Sci Technol* 39: 3040–3047.
9. Sunda WG, Huntsman SA. 2000. Effect of Zn, Mn, and Fe on Cd accumulation in phytoplankton: Implications for oceanic Cd cycling. *Limnol Oceanogr* 45:1501–1516.
10. Vigneault B, Campbell PGC. 2005. Uptake of cadmium by freshwater green algae: Effects of pH and aquatic humic substances. *J Phycol* 41:55–61.
11. Phinney JT, Bruland KW. 1994. Uptake of lipophilic organic Cu, Cd, and Pb complexes in the coastal diatom *Thalassiosira weissflogii*. *Environ Sci Technol* 28:1781–1790.
12. Errecalde O, Campbell PGC. 2000. Cadmium and zinc bioavailability to *Selenastrum capricornutum* (Chlorophyceae): Accidental metal uptake and toxicity in the presence of citrate. *J Phycol* 36: 473–483.
13. Campbell PGC, Errecalde O, Fortin C, Hiriart-Baer WR, Vigneault B. 2002. Metal bioavailability to phytoplankton: Applicability of the biotic ligand model. *Comp Biochem Physiol C-Toxicol Pharmacol* 133:189–206.
14. Orvoine J, Hare L, Tessier A. 2006. Competition between protons and cadmium ions in the planktonic food chain leading to the phantom midge *Chaoborus*. *Limnol Oceanogr* 51:1013–1020.
15. Croteau MN, Luoma SN. 2008. A biodynamic understanding of dietborne metal uptake by a freshwater invertebrate. *Environ Sci Technol* 42:1801–1806.
16. Stevenson RJ, Bothwell ML, Lowe RL. 1996. *Algal Ecology: Freshwater Benthic Ecosystems*. Academic, San Diego, CA, USA.
17. Wingender J, Neu T, Flemming H-C. 1999. What are bacterial extracellular polymer substances? In Wingender J, Neu T, Flemming H-C, eds, *Microbial Extracellular Polymeric substances: Characterization*, *Structure and Function*, 1st ed. Springer, Heidelberg, Berlin, Germany, pp 1–19.
18. Brooks BW, Stanley JK, White JC, Turner PK, Wu KB, La Point TW. 2004. Laboratory and field responses to cadmium: An experimental study in effluent-dominated stream mesocosms. *Environ Toxicol Chem* 23:1057–1064.
19. Selby DA, Ihnat JM, Messer JJ. 1985. Effects of subacute cadmium exposure on a hardwater mountain stream microcosm. *Water Res* 19:645–655.
20. Hill WR, Bednarek AT, Larsen IL. 2000. Cadmium sorption and toxicity in autotrophic biofilms. *Can J Fish Aquat Sci* 57:530–537.
21. Meylan S, Behra R, Sigg L. 2003. Accumulation of copper and zinc in periphyton in response to dynamic variations of metal speciation in freshwater. *Environ Sci Technol* 37:5204–5212.
22. Ivorra N, Hettelaar J, Kraak MHS, Sabater S, Admiraal W. 2002. Responses of biofilms to combined nutrient and metal exposure. *Environ Toxicol Chem* 21:626–632.
23. Morin S, Duong TT, Herlory O, Feurtet-Mazel A, Coste M. 2008. Cadmium toxicity and bioaccumulation in freshwater biofilms. *Arch Environ Contam Toxicol* 54:173–186.
24. Gold C, Feurtet-Mazel A, Coste M, Boudou A. 2003. Effects of cadmium stress on periphytic diatom communities in indoor artificial streams. *Freshw Biol* 48:316–328.
25. Croteau MN, Luoma SN, Stewart AR. 2005. Trophic transfer of metals along freshwater food webs: Evidence of cadmium biomagnification in nature. *Limnol Oceanogr* 50:1511–1519.
26. Stephenson M, Turner MA. 1993. A field study of cadmium dynamics in periphyton and in *Hyalella azteca* (Crustacea, Amphipoda). *Water Air Soil Pollut* 68:341–361.
27. Hill WR, Larsen IL. 2005. Growth dilution of metals in microalgal biofilms. *Environ Sci Technol* 39:1513–1518.
28. Zhang H. 1997. Practical guide for making gels for DGT and DET and practical guide for using DGT and DET. DGT Research, Lancaster, UK.
29. Zhang H, Davison W. 2000. Direct in situ measurements of labile inorganic and organically bound metal species in synthetic solutions and natural waters using diffusive gradients in thin films. *Anal Chem* 72:4447–4457.
30. Navarro E, Robinson CT, Wagner B, Behra R. 2007. Influence of ultraviolet radiation on UVR-absorbing compounds in freshwater algal biofilms and *Scenedesmus vacuolatus* cultures. *J Toxicol Env Health Part A* 70:760–767.
31. Zhang H, Davison W. 1995. Performance characteristics of diffusion gradients in thin films for the in situ measurement of trace metals in aqueous solution. *Anal Chem* 67:3391–3400.

32. Gustafsson JP. 2006. *Visual MINTEQ*, 2.51 ed. Royal Institute of Technology, Department of Land and Water Resources Engineering, Stockholm, Sweden.
33. Murray AP, Gibbs CF, Longmore AR, Flett DJ. 1986. Determination of chlorophyll in marine waters: Intercomparison of a rapid HPLC method with full HPLC, spectrophotometric and fluorometric methods. *Mar Chem* 19:211–227.
34. McGeer JC, Brix KV, Skeaff JM, DeForest DK, Brigham SI, Adams WJ, Green A. 2003. Inverse relationship between bioconcentration factor and exposure concentration for metals: Implications for hazard assessment of metals in the aquatic environment. *Environ Toxicol Chem* 22:1017–1037.
35. Sunda WG, Huntsman SA. 1996. Antagonisms between cadmium and zinc toxicity and manganese limitation in a coastal diatom. *Limnol Oceanogr* 41:373–387.
36. Topperwien S, Behra R, Sigg L. 2007. Competition among zinc, manganese, and cadmium uptake in the freshwater alga *Scenedesmus vacuolatus*. *Environ Toxicol Chem* 26:483–490.
37. Rosakis A, Koster W. 2005. Divalent metal transport in the green microalga *Chlamydomonas reinhardtii* is mediated by a protein similar to prokaryotic Nramp homologues. *Biometals* 18: 107–120.
38. Ahner BA, Price NM, Morel FMM. 1994. Phytochelatin production by marine phytoplankton at low free metal–ion concentrations: Laboratory studies and field data from Massachusetts Bay. *Proc Natl Acad Sci USA* 91:8433–8436.
39. Le Faucheur S, Behra R, Sigg L. 2005. Phytochelatin induction, cadmium accumulation, and algal sensitivity to free cadmium ion in *Scenedesmus vacuolatus*. *Environ Toxicol Chem* 24:1731–1737.
40. Morelli E, Scarano G. 2001. Synthesis and stability of phytochelatins induced by cadmium and lead in the marine diatom *Phaeodactylum tricornutum*. *Mar Environ Res* 52:383–395.