Accumulation of Cadmium in Periphyton under Various Freshwater Speciation Conditions

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The relationship between cadmium speciation and accumulation in periphyton was examined at environmentally relevant Cd concentrations under natural freshwater conditions. Periphyton was exposed in artificial recirculating channels containing natural freshwater to two Cd concentrations (20 and 40 nM), for which speciation was modified by the addition of a synthetic organic ligand (nitrilotriacetate, NTA). Labile metal concentrations were measured with the technique of diffusion gradient in thin-films (DGT) and major Cd species were estimated by modeling. Total and intracellular Cd content in periphyton increased within both Cd exposure concentrations with NTA additions and were related to an increase in DGT-labile Cd, which was caused by the competition of NTA with probably colloidal species. Bioaccumulation was thus not controlled by the free Cd concentrations, as predicted by the free ion activity model, but by the diffusion of labile Cd−NTA complexes, which constituted a large fraction of DGT-labile Cd. These findings confirm the importance of labile species for Cd accumulation in periphyton under freshwater conditions, as predicted by models considering diffusion and uptake kinetics.

Introduction

Bioavailability and hence uptake of essential and nonessential trace metals to aquatic organisms depend on the chemical speciation of the metals as well as on their concentrations (*1, 2*). Models have been developed to relate biouptake to metal speciation in water. The free ion activity model (FIAM) and the derived biotic ligand model (BLM) are equilibrium-based models. The FIAM predicts that uptake is controlled by the free metal ion concentration in solution in presence of ligands (*3*). The applicability of the FIAM has been demonstrated for algae in defined media (*4*−*6*), but exceptions have also been found (*7*−*9*). The BLM (*9, 10*) emphasizes the role of metal binding to the biotic ligand and the competition among cations and ligands. Using FIAM and BLM, it is assumed that (1) equilibrium is attained between the metal species in the bulk solution and metal bound to the biotic ligand, and (2) the diffusion flux of metal species from the bulk solution to the plasma membrane is faster than the uptake flux. The dynamic aspects of metal complexes, i.e., their mobility and lability are considered in models based on diffusion and internalization fluxes. If diffusion of free metal ions from the bulk solution to the plasma membrane is rate limiting, metal uptake can be controlled by the labile metal species (*11, 12*). Some examples of diffusion limitation of metal uptake have been shown in the case of silver uptake by algae in defined media (*13*) and of copper uptake by periphyton in natural freshwaters at very low free metal ion concentrations (*14*).

The aim of the present study was to investigate which cadmium species control accumulation in periphyton under freshwater conditions. Modification of Cd speciation was achieved by adding different concentrations of an artificial organic ligand (nitrilotriacetic acid, NTA) to natural freshwater containing added Cd concentrations close to the environmental range. It was expected that NTA would increase labile and decrease free Cd concentrations. NTA forms complexes with Cd which are fully labile with respect to the DGT (diffusion gradients in thin films) technique (*15*). Free Cd concentrations and other major Cd species were estimated with a speciation program, based on equilibrium with natural organic matter (NOM) and NTA. Experiments were carried out in artificial recirculating channels. Total and intracellular Cd concentrations in periphyton were related to total dissolved, DGT-labile, and free Cd concentrations in water. The results were compared to the predictions of the equilibrium-based and nonequilibrium-based models.

Materials and Methods

Periphyton Colonization. Prior to the experiment, periphyton was colonized on glass slides in two artificial channels (*16*) for ten weeks and four DGT devices were deployed for 5.8 days. The channel setup is described in detail in *17*. The channels were continuously supplied with freshwater from the nearby Chriesbach stream (Duebendorf, Switzerland) containing low dissolved Cd background concentrations and the organisms for the colonization of periphyton. Water flow in the channels was 10 L min^{-1}. Illumination was provided by two photosynthetically active radiation (PAR) lamps (Osram HQL Deluxe 400 W) overhanging the channels. The photoperiod was 15 h darkness, 9 h light and the average light intensity was 341 ± 48 μE m^{-2} sec^{-1}.

Experimental Design. Some DGT devices and periphyton slides were sampled from the channels running with streamwater without Cd and NTA addition before the start of the exposure experiment and used as controls.

Periphyton slides were exposed to added Cd and NTA in streamwater in artificial channels with the water running in a closed circuit. Water flow was 6 L min^{-1}, light intensity was 367 ± 24 μE m^{-2} sec^{-1}, and illumination was continuous. Prior to the experiment three exposure solutions with 20 nM Cd (NTA: 0, 2×10^{-6}, 8×10^{-6} M) and three solutions with 40 nM Cd (NTA: 0, 2×10^{-6}, 8×10^{-6} M) were prepared in 20 L canisters (PE-HD), by adding cadmium nitrate from a concentrated standard solution (J. T. Baker) to Chriesbach streamwater. Canisters were sealed and equilibrated for 16 h at constant temperature to allow full equilibration of metals with natural ligands and NTA. For the experiment solutions were purged with an air mixture (0.5% CO_2, 21.5% O_2, 78% N_2) to maintain constant pH, first for 30 min alone and then for 60 min with the channels running without periphyton slides and DGT devices. Afterward 12 periphyton slides were fixed vertically in each channel and 8 DGT devices were placed at the end of each channel, floating on the water surface with the resin pointing down.

Clean Trace Metal Handling. To avoid contamination of samples and equipment, plastic gloves (Semadeni) were used for all procedures. Samples and the material for sampling were sealed in plastic bags. The experimental system was

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protected by plastic coverings. Components of the channel system, boxes for periphyton transport, polypropylene vials, bottles and beakers, DGT devices, holders and filters, syringes, and filtration units were placed in 0.1 M HNO_3 for at least 24 h and then properly rinsed with nanopure water. Cellulose nitrate filters for metal content determination in periphyton were boiled twice in 0.1 M HNO_3, rinsed with nanopure water, and dried twice at 50 °C for 15 h in an oven. Except for the sampling at the channels all handling was performed in a clean bench.

Sampling. Six periphyton slides for total and intracellular metal content, as well as for chlorophyll-*a* content and species composition, and 4 DGT devices for labile metal concentrations in water were sampled after 3 and 6 h exposure. Water samples (14 mL) for dissolved metal concentrations were taken every hour at the beginning of the channels and filtered (0.45 μm filters, cellulose nitrate, Millipore) into polypropylene tubes using a plastic syringe (BD Plastipak, 50 mL). Filters and syringes were previously thoroughly rinsed with water from the channels. Samples were acidified to 0.24 M with HNO_3 (65% suprapure, Merck) and kept at 4 °C in the dark until analysis. Temperature and pH were measured manually at the same times. Water samples for alkalinity, major cation, and anion analysis were taken at the start of the experiment and after 3 and 6 h of exposure in each channel. A sample for natural organic matter (NOM) characterization (see below) was taken in the 20 nM Cd exposure channel without NTA after 3 h. Water samples for total organic carbon content (TOC) were taken from the other channels.

Water for dissolved metal concentrations was also sampled in the control channels during the time of DGT deployment with temperature and pH being measured at the same times.

NOM Analysis and Modeling of Metal Speciation. Natural organic matter was analyzed in the streamwater by size-exclusion chromatography in connection to online, high-sensitivity organic carbon detection (LC-OCD) (*18, 19*). Fractions determined by this method are biopolymers (polysaccharides, proteins), humic substances (humic and fulvic acids), degradation products of humic substances (building blocks), low molecular weight humics (degradation products of humic substances), low molecular weight acids (mono- and dicarboxylic acids), and neutrals/amphiphilics (amino acids, ketones, aldehydes, alcohols). Characterization of molecular weight (based on retention time in the SEC column) and aromaticity (expressed as UV-absorption per concentration of DOC (m^{-1} L mg^{-1})) of humic substances allowed further differentiation between fulvic and humic acids. Concentrations of characterized humic substances were used together with dissolved concentrations of metals (Cd(II), Zn(II), Mn(II), Cu(II), Pb(II), Fe(III)), alkalinity, major cations (Na^+, K^+, Ca^{2+}, Mg^{2+}), and anions (Cl^-, SO_4^{2-}, NO_3^-, o-PO_4^{3-}), as well as pH and temperature to calculate concentrations of free metal ions, inorganic metal complexes, NTA−metal complexes and fulvic acid−metal complexes using the program vMINTEQ (*20*) with the Stockholm humic acid model (*21*). Calculations were performed with the assumption of Fe being present as dissolved Fe(III).

Determination of Labile Metals by DGT. Labile metal concentrations in water were measured with DGT. DGT-labile species include free metal ions, inorganic complexes, and labile organic complexes according to the dynamic characteristics of this method (*12*). DGT-devices were made following the procedure described by Zhang (*22*), as described in detail in *17*. For measurement of labile metal concentrations, the resin gel layer was removed and 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 7-fold. The metal concentrations were then measured by inductively coupled plasma mass spectrometry (ICP-MS).

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 followed the procedure by Zhang and Davison (*23*). Percentages of DGT-labile metal concentrations are related to averaged dissolved metal concentrations over the time of DGT deployment.

Since concentrations of dynamic species are determined using DGT and the concentrations of modeled metal species are based on equilibrium conditions, values cannot be compared directly. DGT-labile metal species can be calculated from the modeling results (Me_{labile}) by including diffusion coefficients of metal complexes:

$$Me_{labile} = \sum (Me^{z+} + Me_{inorg} + 0.2x(Me_{FA}) + 0.7x(Me_{NTA}))$$

where M^{z+} is the free metal ion concentration, M_{inorg} are inorganic metal complexes, M_{FA} are metal−fulvic acid complexes, and M_{NTA} are metal−NTA complexes. Factors of 0.2 and 0.7 are ratios of diffusion coefficients of the corresponding Cd complexes when compared to diffusion coefficients of free metal ions (*15, 24*).

Periphyton Processing. To avoid dehydration and contamination of periphyton slides, they were stored in a plastic box until further processing. Periphyton was scratched from six slides with a microscope slide into a plastic beaker containing 100 mL of filtered experimental water to avoid redistribution of Cd among algae and water. Sediment and particles were then allowed to settle, and three replicates of the supernatant containing periphyton organisms were filtered through cellulose nitrate filters (Millipore) to obtain dry weight and total metal contents. The rest of the suspension was treated for 10 min with 4 mM EDTA (ethylenediaminetetraacetate) to remove metals adsorbed to the cell walls. The remaining metal content is considered to be intracellular (EDTA-nonexchangeable). Three measurement replicates were then filtered to obtain intracellular metal contents. Filters were dried twice for 15 h at 50 °C in an oven, weighed, and digested 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, MLS GmbH, Leutkirch, Germany) (*17*). The measured metal content in periphyton was related to the measured dry weight.

Chlorophyll-*a* was extracted from 5 mL of the periphyton suspensions with ethanol (*25*) and concentrations were measured using the HPLC method described by Murray et al. (*26*). For determination of species composition, 5 mL of periphyton suspensions was fixed with 4% formaldehyde and examined using an inverted phase contrast microscope with a magnification of 640, to provide a semiquantitative estimate.

Analytical Methods. Metal concentrations in water, DGT probes, and periphyton were measured with high-resolution inductively coupled plasma mass spectrometry (HR-ICP-MS) (Element 2, Thermo Finnigan). The accuracy of the ICP-MS measurements was checked in each run using the reference waters SLRS-4 (National Research Council Canada, errors: Cd < 11%, Zn < 12%, Mn < 8%, Cu < 11%, Pb < 12%, Fe < 8%) and TM-28.2 (National Research Council Canada, errors: Cd < 5%, Zn < 11%, Mn < 6%, Cu < 7%, Pb < 8%, Fe < 5%). Plankton reference material was used to control the digestion of periphyton (CRM 414, Institute for reference materials and measurements, European Commission, Belgium, errors: Cd < 12%, Zn < 15%, Mn < 13%, Cu < 13%, Pb < 14%). Metal concentrations in periphyton are expressed per dry weight (dw) and are given as averages of three measurement replicates with standard deviation.

Concentrations of major cations were determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Spectro) and anion concentrations were measured

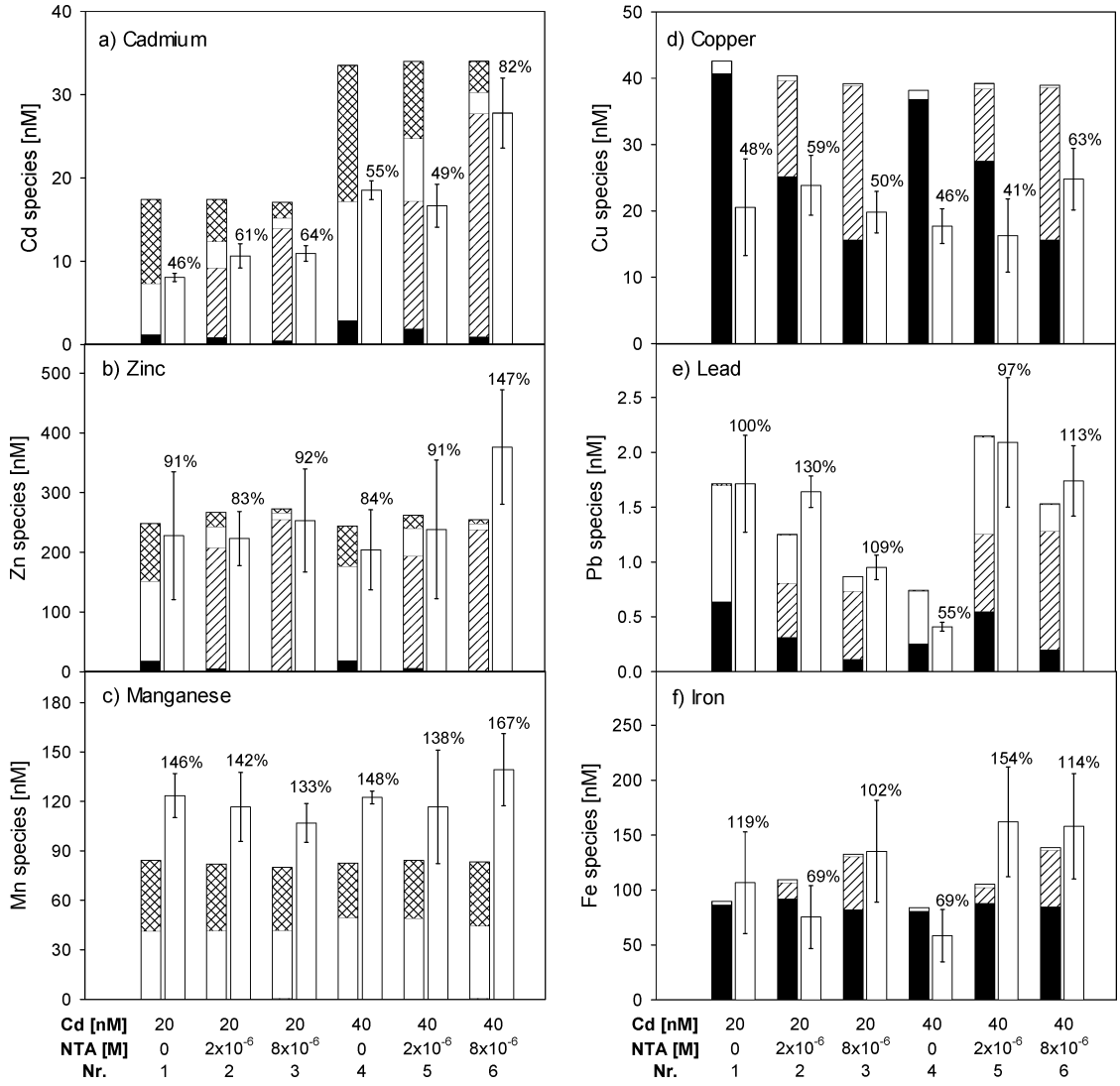

FIGURE 1. Dissolved metal concentrations in exposure channels (No. 1−6) after 6 h (stacked bars) with concentrations of modeled metal species (free metal ions (checkered texture), inorganic complexes (white texture), NTA−metal complexes (diagonal texture), and fulvic acid−metal complexes (black texture)). DGT-labile metal concentrations in water of exposure channels after 6 h (plain white bars), as well as percentages of DGT-labile metals. Values of DGT measurements represent means ± standard deviations of 4 replicates.

by ion chromatography (Metrohm). Alkalinity measurements were performed by titration (with HCl 0.1 M until pH 4.5).

Results

Dissolved and DGT-Labile Metal Concentrations. Dissolved metal concentrations in the control channel were 0.11 ± 0.05 nM Cd (DGT-labile 43%), 182 ± 119 nM Zn (DGT-labile 46%), 95 ± 13 nM Mn (DGT-labile 82%), 38 ± 19 nM Cu (DGT-labile 45%), 0.33 ± 0.22 nM Pb (DGT-labile 35%), and 134 ± 33 nM Fe (DGT-labile 49%). Dissolved Cd concentrations were similar and constant in the three exposure channels with 20 nM Cd (17.3 ± 0.22 nM) as well as in the three channels with 40 nM Cd (33.8 ± 0.6 nM) (average of 6 samples ± standard deviation) (Figure 1). Dissolved concentrations of other metals were similar and constant in all exposure channels, namely Zn (258 ± nM), Mn (83 ± 2 nM), Cu (40 ± 2 nM), and Pb (1.4 ± 0.5 nM). Dissolved Fe concentrations increased in both Cd exposures with increasing NTA concentrations (87 ± 4 nM (no NTA), 107 ± 3 nM (NTA: 2×10^{-6} M), 136 ± 10 nM (NTA: 8×10^{-6} M)). Percentages of DGT-labile Cd changed in the presence of NTA, but the effect was different in the two Cd exposures (Figure 1). DGT-labile Cd was similar to the control channel (43%) in the 20 nM and 40 nM Cd exposure without NTA and the 40 nM Cd exposure with 2×10^{-6} M NTA (~50%). But DGT-labile Cd increased to around 62% in the 20 nM Cd exposures in the presence of NTA and to 82% in the 40 nM Cd exposure with the highest NTA concentration. Compared to the control channel percentages of DGT-labile metals were higher in the Cd exposure channels for Zn (~98%), Mn (~146%), Pb (~101%), and Fe (~104%), and similar for Cu (~51%) (Figure 1). Some DGT-labile concentrations of Zn, Pb, and Fe exceeded dissolved concentrations probably due to short DGT deployment times and contamination. DGT-labile Mn was higher than the measured dissolved Mn, probably due to Mn(II) losses by oxidation upon filtration in the dissolved concentrations.

In the control channel alkalinity was 4.86 mM, calcium was 2.34 mM, pH was 8.0 ± 0.26, and temperature was 11.2 ± 1.8 °C. These parameters were similar and constant in all Cd exposure channels, namely alkalinity was 5.97 ± 0.01 mM, calcium was 2.77 ± 0.02 mM, pH was 8.28 ± 0.08, and temperature was 11.9 ± 0.2 °C.

NOM Characterization. Measured total organic carbon (TOC) concentrations were 2.18 ± 0.03 mg C (average of 4 samples) in the channels with NTA = 2×10^{-6} M and 2.60

TABLE 1. Total and Intracellular Cd Concentrations in Periphyton (nmol Cd g dw^{-1}) after 3 and 6 h Exposure (Average and Standard Deviation of Three Replicates)

	3 hour exposure		6 hour exposure	
exposure	total Cd	intracellular Cd	total Cd	intracellular Cd
20 nM Cd/0 M NTA	5.8 ± 1.0	2.9 ± 0.3	7.6 ± 1.1	3.5 ± 0.3
20 nM Cd/2 × 10^{-6} M NTA	7.5 ± 0.6	3.7 ± 0.4	7.3 ± 0.7	3.6 ± 0.1
20 nM Cd/8 × 10^{-6} M NTA	7.0 ± 1.1	2.9 ± 0.2	10.9 ± 1.2	4.9 ± 0.5
40 nM Cd/0 MNTA	14.6 ± 2.7	6.3 ± 0.5	15.7 ± 3.6	5.6 ± 0.8
40 nM Cd/2 × 10^{-6} M NTA	15.5 ± 2.1	5.0 ± 0.3	16.5 ± 5.2	5.6 ± 1.4
40 nM Cd/8 × 10^{-6} M NTA	12.4 ± 0.8	5.7 ± 1.8	18.4 ± 2.5	9.2 ± 1.8

± 0.02 mg C L^{-1} (average of 8 samples) in the channels with NTA = 8 × 10^{-6} M. Chromatographic fractions of hydrophilic dissolved organic carbon (DOC) determined in the stream-water without NTA (2.20 mg C L^{-1}) showed that humic substances represented the major fraction of DOC with 48%. Their average molecular weight was 522 g mol^{-1} and aromaticity was 2.9 L mg^{-1} m^{-1}, characteristic for fulvic acids. The other fractions were present at lower concentrations, namely neutrals (14%), building blocks (11%), low molecular weight humics (6%), and biopolymers (5%).

Modeled Metal Species. Modeling (stacked bars in Figure 1) was performed with the assumption that Fe was present as dissolved Fe(III), since the DGT measurements showed that most Fe was present in labile form. In both Cd exposures, increasing NTA concentrations decreased percentages of free Cd ions (20 nM Cd: 58, 29, 11%; 40 nM Cd: 49, 27, 11%), inorganic complexes and Cd−fulvic acid complexes, but increased percentages of Cd−NTA complexes (Figure 1), as well as of the other metal−NTA complexes. The modeled Mn speciation was not affected by NTA. Zn was in all cases the most abundant of the trace metal NTA-species, followed by Fe, Cu, and Cd. Pb−NTA and Mn−NTA complexes were present in low concentrations. A large fraction of NTA was bound to Ca, namely 1.7 μM at the low and 7.5 μM at the high NTA concentration.

Calculated labile Cd concentrations were 151% (20 nM Cd, no NTA), 126% (20 nM Cd, 2 × 10^{-6} M NTA), 114% (20 nM Cd, 8 × 10^{-6} M NTA), 141% (40 nM Cd, no NTA), 140% (40 nM Cd, 2 × 10^{-6} M NTA), and 90% (40 nM Cd, 8 × 10^{-6} M NTA) of DGT-labile Cd concentrations. The best agreement between calculated and measured labile Cd concentrations was found at the highest NTA concentrations.

Periphyton Characterization. Chlorophyll-*a* content of periphyton was 4.1 ± 0.3 mg Chl a g dw^{-1} in the control channels and 5.6 ± 1.6 mg Chl a g dw^{-1} in the Cd exposure channels. Semiquantitative microscopical analysis showed that periphyton was dominated by diatoms (*Achnanthes*, *Cocconeis*, *Cymbella*, *Gomphonema*, *Navicula*, *Nitzschia*). Green algae (*Ulothrix*) and Cyanobacteria (*Chamaesiphon*, *Lyngbya*) were less abundant.

Metal Accumulation and Speciation. Cd concentrations in control periphyton were 2.3 ± 0.4 nmol Cd g dw^{-1} for total and 1.2 ± 0.1 nmol Cd g dw^{-1} for intracellular content. Cd accumulation in periphyton was influenced by the addition of NTA at both Cd exposure concentrations (Table 1). Differences in total and intracellular Cd content between 3 and 6 h exposure were small, with exception of the two exposures with the highest NTA concentrations. In presence of 8 × 10^{-6} M NTA, total Cd content increased from 3 to 6 h by 56% in the 20 nM Cd exposure, by 48% in the 40 nM Cd exposure and intracellular Cd content by 68% and 62%, respectively. After 6 h exposure, total and intracellular Cd concentrations in periphyton were higher in both Cd exposures with the highest NTA concentration, when compared to the exposures with lower NTA concentrations or no NTA addition.

Total and intracellular Cd contents in periphyton were plotted as a function of DGT-labile and free Cd concentrations in water (Figure 2). Linear relationships of both Cd total and intracellular contents with DGT-labile Cd concentrations were observed after 6 h exposure (Figures 2a and a'), whereas no correlations were found in the case of free Cd concentrations (Figures 2b and b'). This relationship indicates that Cd accumulation is related to the DGT-labile Cd species. The correlation was weaker after 3 h exposure (data not shown).

Discussion

Metal Speciation in Channels. Under constant dissolved Cd concentrations, the addition of NTA changed the Cd speciation at both exposure concentrations by increasing DGT-labile and simultaneously decreasing calculated free Cd^{2+} concentrations.

Using DGT with nonrestricted gels, the labile metal species are detected, which are free metal ions, inorganic metal complexes, and a fraction of the organic metal complexes, including here the NTA−metal complexes (*15, 27*). Metals bound to high molecular weight organic ligands, to colloidal metal oxides (Fe, Al, Mn), or to strong binding sites of organic ligands are not measured (nonlabile or inert species).

NTA is a strong organic ligand ($\log(K_{CdNTA^-})$ = 11.04) and calculated complexing coefficients for Cd (α_{CdNTA} = K × $[NTA^{3-}]$) were 4.4 for 2 × 10^{-6} M NTA and 19 for 8 × 10^{-6} M NTA. NTA competed for Cd bound to complexes which were not measured by DGT probably because of larger size (<0.45 μm) and slow diffusion, after equilibration for 16 h, and thus increased DGT-labile Cd concentrations at both exposure concentrations. DGT-labile Cd concentrations at the 20 nM Cd exposure did not increase when NTA concentrations were increased from 2 × 10^{-6} M to 8 × 10^{-6} M. This indicates that the remaining nonlabile Cd was bound to ligands with complexing coefficients higher than 19 or more probably to slowly exchanging colloidal species. The dissociation of metals from particulate complexes (>0.45 μm, nonlabile) due to NTA addition was observed for Fe, for which dissolved concentrations increased with increasing NTA concentrations.

NTA did not increase DGT-labile concentrations of Zn and Mn since both metals were already mostly present in labile form without NTA. A high proportion of Cu (~49%, ($\log(K_{CuNTA^-})$ = 14.28)) was bound to nonlabile ligands, which did not exchange with NTA. Complexing coefficients (α_{CuNTA}) were 7.7 × 10^3 for 2 × 10^{-6} M NTA and 3.4 × 10^4 for 8 × 10^{-6} M NTA. Cu was thus bound to natural ligands with complexing coefficients higher than 3.4 × 10^4 or to slowly exchanging colloidal species.

The addition of NTA decreased modeled free Cd concentrations due to complexation with NTA. However, it is likely that free Cd^{2+} concentrations were overestimated in these calculations, since other organic ligands, which may be present in some of the fractions identified with LC-OCD, were not included in the model. Furthermore, the DGT-

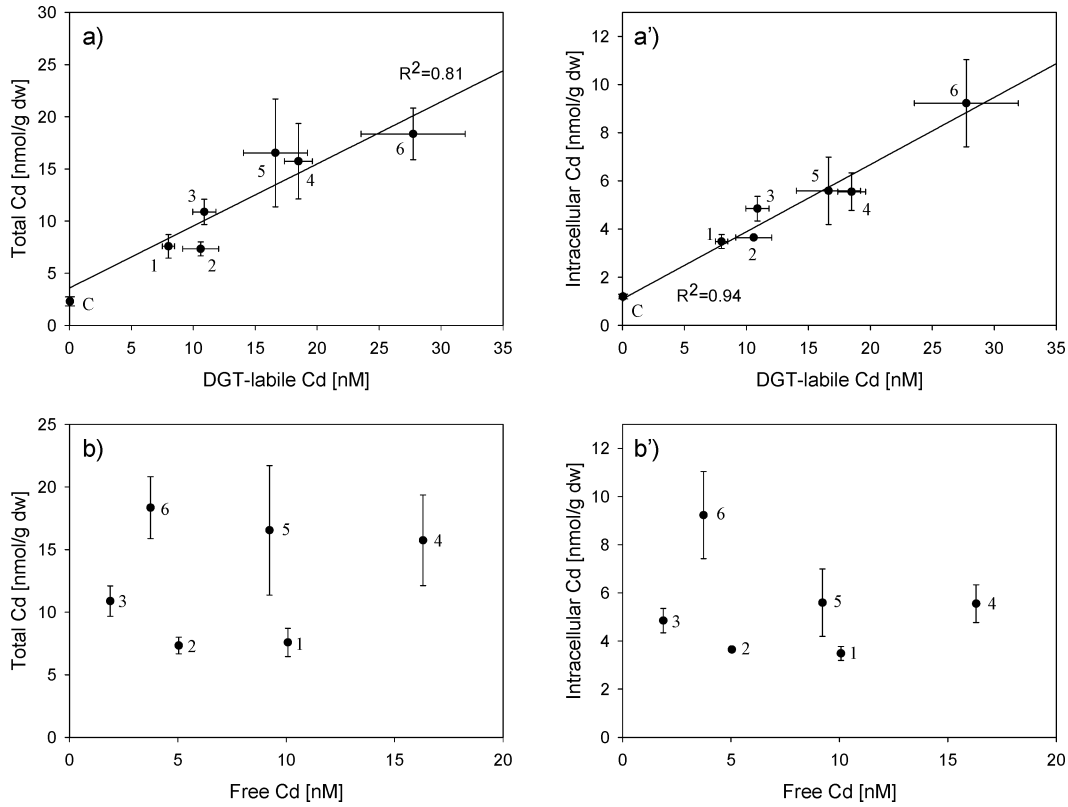

FIGURE 2. Total and intracellular Cd concentrations in periphyton as a function of DGT-labile (a, a') and free Cd (b, b') concentrations in water after 6 h exposure. The numbers represent the various exposures as shown in Figure 1 and C the control. Linear regression (solid line) for Cd content in periphyton and DGT-labile Cd concentrations was performed. Values represent means ± standard deviation. Data points for metal content in periphyton represent three replicates and DGT-labile concentrations four replicates.

measurements indicate that nonlabile Cd species were present, which would also lead to lower free Cd^{2+}. With increasing NTA concentrations, calculated labile Cd concentrations agreed better with DGT-labile concentrations at both Cd exposures. Since nonlabile ligands are not included in the model, but decrease the measured DGT-labile species, calculated labile Cd concentrations are higher than DGT-labile concentrations without the addition of NTA. The increase of labile species with NTA addition leads to a better agreement between calculated labile Cd and measured DGT-labile Cd.

Accumulation and Speciation. Total and intracellular Cd contents of periphyton increased in the presence of NTA and were related to Cd speciation in water, in particular to the DGT-labile species (Figure 2). The relationship of Cd in periphyton with DGT-labile Cd was more evident after 6 h exposure time, which may be due to more precise data at the longer exposure times of DGT devices and periphyton. Both the increase of Cd content in the presence of NTA and the correlation with DGT-labile Cd indicate that the labile Cd species play an important role for Cd uptake in periphyton. Since modeled free Cd concentrations decreased with NTA addition, the increase in Cd content appears to be related to a more efficient diffusion of Cd−NTA complexes in comparison to the nonlabile species which were exchanged with NTA.

Cd content in periphyton increased at the highest NTA concentration in both Cd exposures between 3 and 6 h, whereas it remained fairly constant in the other exposures (Table 1). This effect might be due to a slower diffusion of Cd−NTA complexes through the matrix of periphyton than diffusion of free Cd ions or inorganic complexes. It has been shown for diffusion layers of DGT, which might be comparable to the matrix, that the diffusion coefficient of Cd−NTA complexes is 70% of that of the free metal ion (*15*).

The present results differ from predictions by equilibrium based models, i.e., the FIAM and BLM, which assume equilibrium between metal species in the bulk solution and metals bound to transport sites of the plasma membrane. Nonequilibrium based models consider diffusion fluxes of metal species (J_{diff}) and internalization fluxes (J_{int}) (*11, 12*). If the internalization flux of metals across the cell membrane (J_{int}) is rate-limiting, uptake depends on the free metal ions. However, labile metal species control bioaccumulation in the case of diffusion limitation of free metal ions. The diffusion flux of Cd^{2+} is calculated for the present conditions in periphyton using a diffusion layer thickness of 1×10^{-4} to 1×10^{-3} m, the diffusion coefficient for Cd^{2+} $D_{Cd} = 6 \times 10^{-10}$ m^2 s^{-1} (18 °C) (*28*), and the modeled Cd^{2+} concentrations. Diffusion fluxes are then $J_{diff} = 1 \times 10^{-12}$ to 6×10^{-11} mol m^{-2} s^{-1}. Uptake fluxes are estimated using calculated surface areas of the periphytic algae (*29*) and obtained intracellular Cd contents after 3 and 6 h. Uptake fluxes are then $J_{int} = 2 \times 10^{-14}$ to 2.4×10^{-13} mol m^{-2} s^{-1} (Supporting Information Table S1). They are thus close to the lower estimated diffusion fluxes. Considering that Cd^{2+} is likely overestimated and that there are large uncertainties in the calculated uptake fluxes, diffusion limitation of Cd^{2+} is thus possible. The present results, as well as a previous study on accumulation kinetics (*17*) indicate that diffusion of metal species through the matrix of periphyton may be rate-limiting for Cd uptake by periphyton. In a similar way, uptake of Cu by periphyton appeared to be related to the labile Cu species (*30*). Diffusion of metal species through the gel layer in the DGT method is a process similar

to diffusion through the matrix layer of periphyton, so that the DGT method may be specially suited for available metal species in the case of periphyton.

The present results confirm that dynamic metal species can control bioaccumulation and that they need to be included in models that predict bioavailability, especially in natural aquatic systems where chemical equilibrium is almost never achieved. Various dynamic speciation sensors are available nowadays, which may be useful for the determination of dynamic species in natural waters (*12*).

Environmental Relevance. The present study shows that an anthropogenic ligand can increase the bioavailability of a nonessential metal by exchange with species, which would not be bioavailable to aquatic organisms. Concentrations of NTA in surface waters have been measured in the nanomolar range (*31, 32*). NTA may not play an important role in affecting Cd speciation in natural waters at these low concentrations. However, other anthropogenic as well as natural ligands forming labile complexes (e.g., small organic acids, amino acids) may be present at elevated concentrations, for example due to inputs from sewage effluents. The labile complexes may then contribute to the metal flux toward algae of periphyton, and indirectly to metal transfer over the food chain to organisms consuming periphyton. The present study confirms the importance of considering dynamic species for metal bioavailability.

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Supporting Information Available

Details about the calculation of diffusion and uptake fluxes. This material is available free of charge via the Internet at http://pubs.acs.org.

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