A Structural Study of Cadmium Interaction with Aquatic Microorganisms

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The molecular mechanisms of cadmium toxicity for aquatic phototrophic microorganisms, reversible adsorption on the surface, and cellular uptake during growth were investigated by combining batch macroscopic experiments with atomic-level in situ Cd K-edge X-ray absorption fine structure spectroscopy. The following species were examined: marine planktonic (Skeletonema costatum, Thalassiosira weissflogii) and freshwater periphytic (Navicula minima, Achnanthiscum minutissimum) diatoms, cyanobacteria (Gloeocapsa sp.), anoxygenic phototrophic bacteria (Rhodopseudomonas palustris), and freshwater diatom-dominated biofilms. Upon short-term adsorption at the freshwater diatoms and cyanobacteria, cell surface from a NaNO3 or NaCl solution, Cd is octahedrally coordinated by oxygen at an average distance of 2.27 ± 0.02 Å and is associated with carboxylate groups. The atomic environment of cadmium incorporated into freshwater diatoms during long-term growth (operationally defined as Cd nonextracted by EDTA) is similar to that of adsorbed metal in terms of Cd–O first-shell distances and coordination numbers. Contrasting speciation is found for Cd incorporated into marine diatoms and adsorbed onto phototrophic anoxygenic bacteria R. palustris, where Cd is coordinated with three to five oxygen/nitrogen atoms and one to three sulfur atoms in the first atomic shell, likely in the form of cysteine/hystidine complexes or Cd–thiolate clusters. The Cd association with sulfhydryl groups in marine planktonic diatoms and anoxygenic bacteria is an important feature of Cd binding which can be useful for assessing the bioavailability of this metal.

Introduction

At the present time, serious concerns at the European level are raised regarding the pollution of French hydrosystems (Lot, Gironde) and estuaries of the Atlantic coast by various toxic metals (Zn, Pb, As, Hg, and, in particular, Cd) originating from industrial activity located in the middle part of the Lot River. Over the past 10 years, a group of geochemists and ecotoxicologists has been performing a thorough study of Cd migration, speciation, and biouptake along the pollution gradient in a pilot site of the Lot River basin down to the Gironde estuary in southwest France (e.g., refs 1-4). Because the first step in metal uptake by biota is the adsorption of aqueous ions or complexes on external layers of the cell wall, the study of these “primary” adsorption processes is crucial for modeling the impact of aquatic organisms on metal transport in natural settings and the ecotoxicological consequences. In this study, we focus on Cd interactions with the first and most important biological component of trophic chains, the periphytic diatoms and photosynthetic bacteria grown under controlled laboratory conditions.

The molecular-level environment and speciation of metals such as zinc in diatom cultures have been recently characterized by X-ray absorption fine structure (XAFS) spectroscopy (5) combined with macroscopic and isotopic characterization. The present study is aimed at gaining further insight on metal speciation in diatom and other microorganism cultures by looking at another very important environmental pollutant, cadmium, which is often considered as biological analog of Zn, capable of substituting for Zn in some macromolecules and activating phytochelatin and glutathione production (7). A fairly large amount of data has been collected on the mechanisms of Cd toxicity for marine diatom cultures, Cd uptake, and its influence on their growth (8). Cadmium toxicity is usually explained by blocking and reducing the thiol sites on proteins. Similar to zinc and other metals, cadmium can interact with both high-affinity (phosphoryl, sulfhydryl) and low-affinity (carboxyl) sites on cell surfaces, depending on its concentration in solution. Rigorous analysis of these chemical processes requires, however, knowledge of the identity and molecular structure of metal complexes both on the surface and inside of the cells. Such information can be gained using in situ XAFS spectroscopy. For example, XAFS spectroscopy proved to be capable of distinguishing changes in the coordination and local environment of Cd attached to bacteria (9, 10) and on mineral surfaces (11, 12). However, determining the speciation of Cd associated with biological surfaces, notably at concentrations and biomass-to-metal ratios approaching those of the natural environment, remains unprecedented in the case of diatoms, their biofilms, and other phototrophic microorganisms.

The present study addresses several important open questions on the mechanisms of Cd interaction with aquatic biota: (1) Does the chemical status of reversibly adsorbed Cd depend on exposure time, pH, and its concentration in solution? (2) How can one relate Cd surface speciation inferred from a macroscopic thermodynamic approach with that directly observed by XAFS? (3) What is the difference in the chemical forms of cadmium adsorbed on the surface and incorporated inside the cells during their growth? (4) How specific is the Cd chemical environment between different diatom species, phototrophic bacteria, or natural biofilms? It is anticipated that answering these questions should provide a molecular-level basis for the quantitative and predictive modeling of Cd biogeochemical fluxes and their bioavailability in natural aquatic systems under various environmental conditions.

Materials and Methods

Monospecific cultures of marine planktonic Skeletonema costatum (SC), Thalassiosira weissflogii (TW), and freshwater periphytic Achnanthiscum minutissimum (AMIN) and Navicula minima (NMIN) diatoms were prepared as described previously (6, 13). Diatoms were cultured over 1–2 weeks to
TABLE 1. Samples Used for XAFS Studies

<table>
<thead>
<tr>
<th>sample, new name</th>
<th>type of interaction</th>
<th>medium, conditions</th>
<th>duration of exposure</th>
<th>pH</th>
<th>[Cd(^{2+})](_{av}) (\mu)M (solution)</th>
<th>[Cd] in biomass, mmol/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMIN-1</td>
<td>incorporation</td>
<td>Dauta, light</td>
<td>76 h</td>
<td>9.4</td>
<td>0.44(^b)</td>
<td>4.5</td>
</tr>
<tr>
<td>AMIN-2</td>
<td>adsorption</td>
<td>0.01 M NaNO(_3)</td>
<td>30 min</td>
<td>7.8</td>
<td>4.4</td>
<td>106</td>
</tr>
<tr>
<td>NMIN-1</td>
<td>adsorption</td>
<td>0.01 M NaNO(_3), dark</td>
<td>1 h</td>
<td>7.7</td>
<td>32</td>
<td>88</td>
</tr>
<tr>
<td>NMIN-2</td>
<td>adsorption</td>
<td>Dauta, dark</td>
<td>40 min</td>
<td>7.6</td>
<td>60</td>
<td>115</td>
</tr>
<tr>
<td>NMIN-3</td>
<td>adsorption</td>
<td>Dauta, light</td>
<td>3–5 min</td>
<td>6.55</td>
<td>2.3</td>
<td>7.2</td>
</tr>
<tr>
<td>NMIN-4</td>
<td>incorporation</td>
<td>0.1 M NaNO(_3), light</td>
<td>24 h</td>
<td>6.95</td>
<td>8.8(^b)</td>
<td>31</td>
</tr>
<tr>
<td>NMIN-5</td>
<td>incorporation</td>
<td>Dauta, light</td>
<td>77 h</td>
<td>8.1</td>
<td>9(^c)</td>
<td>6.6</td>
</tr>
<tr>
<td>NMIN-6</td>
<td>incorporation</td>
<td>Dauta, light</td>
<td>120 h</td>
<td>8.2 \pm 0.2</td>
<td>0.88 \pm 0.26(^b)</td>
<td>8.8</td>
</tr>
<tr>
<td>TW-1</td>
<td>adsorption</td>
<td>0.1 M NaNO(_3), dark</td>
<td>72 h</td>
<td>7.68</td>
<td>53</td>
<td>26.5</td>
</tr>
<tr>
<td>TW-2</td>
<td>adsorption</td>
<td>0.7 M NaCl, dark</td>
<td>40 min</td>
<td>7.7</td>
<td>680</td>
<td>88</td>
</tr>
<tr>
<td>SC-1</td>
<td>adsorption</td>
<td>0.7 M NaCl, dark</td>
<td>40 min</td>
<td>8.66</td>
<td>442</td>
<td>53</td>
</tr>
<tr>
<td>SC-2</td>
<td>adsorption</td>
<td>f/2, light</td>
<td>72 h</td>
<td>8.61</td>
<td>9(^c)</td>
<td>4.4—26.5</td>
</tr>
<tr>
<td>SC-3</td>
<td>adsorption</td>
<td>0.1 M NaNO(_3), light</td>
<td>96 h</td>
<td>6.54</td>
<td>19</td>
<td>57.5</td>
</tr>
<tr>
<td>H(_2)SiO(_4) silicic acid</td>
<td>adsorption</td>
<td>0.01 M NaNO(_3)</td>
<td>1 h</td>
<td>7.2</td>
<td>3600</td>
<td>133</td>
</tr>
<tr>
<td>SiO(_2) vitreous SiO(_2)</td>
<td>adsorption</td>
<td>0.01 M NaNO(_3)</td>
<td>1 h</td>
<td>7.25</td>
<td>25</td>
<td>50</td>
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<tr>
<td>Gloeocapsa sp.</td>
<td>adsorption</td>
<td>0.1 M NaNO(_3), dark</td>
<td>40 h</td>
<td>6.46</td>
<td>24</td>
<td>13</td>
</tr>
<tr>
<td>R. palustris</td>
<td>adsorption</td>
<td>0.01 M NaNO(_3), dark</td>
<td>18 h</td>
<td>5.96</td>
<td>66</td>
<td>3.8</td>
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<tr>
<td>Biofilm-1</td>
<td>adsorption</td>
<td>0.01 M NaNO(_3), dark</td>
<td>16 h</td>
<td>6.99</td>
<td>0.88</td>
<td>4.6</td>
</tr>
<tr>
<td>Biofilm-2</td>
<td>adsorption</td>
<td>0.01 M NaNO(_3), dark</td>
<td>2 h</td>
<td>5.99</td>
<td>177</td>
<td>240</td>
</tr>
</tbody>
</table>

\(^a\) All samples were freeze-dried prior the analysis. Achnanthidium minutissimum = AMIN; Navicula minima = NMIN; Skeletonema costatum = SC; Thalassiosira weissflogii = TW; 95 P = Rhodopseudomonas palustris; GI = Gloeocapsa sp. f-6gl. Dissolved organic carbon concentration in all samples was below 10 mg/L. All solutions were strongly undersaturated with respect to any individual solid phase of Cd. \(^b\) The value was maintained constant within \(\pm 10\)–20% (unless indicated) by adding fresh nutrient solution in the course of growth, for Cd concentration in solution. \(^c\) The initial Cd concentration in solution.

A concentration of \(\sim 10^7\) cells/L at 20 °C in a sterile Dauta (freshwater) or f/2 (seawater) medium at pH \(\sim 7.6–8.2\) under continuous aeration and harvested from the late exponential-stationary growth phase. Samples for adsorption experiments were grown in Cd-free media at \([\text{Cd}]_{\text{tot}} < 10^{-3}\) M. Samples for incorporation experiments (EDTA-nonextracted, see below) were grown under controlled Cd concentrations (\([\text{Cd}]_{\text{tot}} = 0.5–10\) nM), as verified by periodic sampling. Cd analysis, and aliquot addition, to keep the concentration (freshwater) or f/2 (seawater) medium at pH constant (Table 1). Anoxygenic photosynthetic purple bacteria *Rhodopseudomonas palustris* (95-P) and mesophilic cyanobacteria *Gloeocapsa* sp. f-6gl were grown to the stationary phase on mineral Pfenning and D media, respectively (14). Natural biofilms were grown on glass supports deployed in the river channel for 2–4 weeks in the tributaries of the Lot River in southwestern France (Riou Viou and Riou Mort, refs 3, 4). The supports were colonized by 2-mm-thick biofilm composed of a compact association of different species of freshwater phytiocytic diatoms with <10% of bacteria, fungi, protozoa, their exudates, and mineral suspended matter. The biomass of live bacterial and diatom cells suspension and biofilms was quantified by its humid (centrifuged 25 min at 4500 g) and dry (lyophilized or freeze-dried) weights. Before the adsorption experiment, cells were rinsed three times in appropriate 0.01 M NaNO\(_3\) or 0.7 M NaCl solution using centrifugation at 4500 g (500 mL of solution for 1 g of wet biomass) to remove, as possible, the adsorbed ions and organic cell exudates from the surface.

Cadmium adsorption experiments were designed to provide quantitative characterization of metal binding by the microorganism surface in a wide range of pH's and Cd(II) concentrations in solution. For this purpose, two types of experiments were performed: (i) adsorption at a constant initial cadmium concentration in solution as a function of pH (pH-dependent adsorption edge) and (ii) adsorption at a constant pH as a function of cadmium concentration in solution (adsorption isotherm). All solutions were undersaturated with respect to any solid cadmium oxide, hydroxide, or carbonate phase as verified by calculations using the MINTEQA2 computer code and corresponding database (15). Details of analytical and experimental procedures of Cd adsorption and verification of adsorption reversibility are provided in the Supporting Information (SI-1).

All investigated samples are listed in Table 1. The short-term adsorption experiments were performed as described in SI-1. The long-term growth samples result from culturing the cells in nutrient media with added Cd. For all growth (incorporation) experiments, prior to the analysis, the cells collected via centrifugation were rinsed in a proper electrolyte (Cd-free NaNO\(_3\) or NaCl) and subsequently in a 0.01 M EDTA solution for 10 min in order to remove Cd that was reversibly adsorbed on most surface envelopes (e.g., refs 16–18). In this work, we will operationally define the intracellular metal as Cd not extracted by EDTA (see details in SI-1).

In all XAFS experiments, only freeze-dried biomass was used. The wet centrifuged biomass was submersed into liquid nitrogen and frozen at \(-80^\circ\)C before lyophilization. Our previous study demonstrated identical chemical status of a metal such as Zn in wet cells and in freeze-dried samples (5); however, the latter allows a significantly higher concentration of metal without increasing the level of initial Cd loading and thus provides higher sensitivity for XAFS analysis.

XAFS spectra (including the X-ray absorption near-edge structure region or XNEXS, and the extended X-ray absorption fine structure region or EXAFS) of Cd-bearing solids, aqueous solutions, and freeze-dried cells were collected in the transmission or fluorescence mode (depending on Cd concentration) at the Cd K edge \((\sim 26.71\) keV) over the energy range 26.3–27.6 keV on the BM29 bending-magnet beamline at the European Synchrotron Radiation Facility. Analysis of XAFS spectra (see below) of the same samples collected at ambient- and low-temperature conditions (e.g., NMIN-1, SC-1; see Table 2) yielded structural parameters identical within errors, demonstrating that structural disorder largely dominates over thermal motions in most microorganism samples and, thus, the temperature has no significant effect on the Cd atomic environment. The description of the XAFS reduction procedure can be found elsewhere (5, 19), and additional details about the data collection procedure are given in the Supporting Information (SI-2).
Previous macroscopic and spectroscopic measurements suggest that the diatom cell wall structure can be viewed as a layer of amorphous silica (frustule) attached to a protein template from the interior of the cell and covered by a polysaccharide layer bearing negatively charged $-\text{COO}^-$ moieties (13, 21). Therefore, the metal speciation at the outermost diatom cell walls resulting from short-term adsorption is likely to be controlled by $-\text{COO}^-$–Cd $^+$ surface complexes, similar to Zn (5, 6), and solely carboxylate moieties were found to be sufficient to provide an adequate fit to the sorption data (21). Indeed, the order of Cd binding affinity to the diatoms’ cell wall, $S. costatum > N. minima > A. minutissimum > T. weissflogii$, follows the trend established earlier for Zn (6), in agreement with the decrease of carboxylate group concentration in the surface layers (13).

**Cd Chemical Status in Cultures.** Freshwater Diatoms, Cyanobacteria, and Their Biofilms. XAFS spectra are shown in Figure 2, and derived structural parameters are listed in Table 2. XANES spectra of freshwater species, $A. minutissimum$ (AMIN) and $N. minima$ (NMIN), at a pH between 7 and 9 exhibit shapes and energy positions very close to those of...
of crystalline Cd acetate, oxalate, and citrate salts, and Cd nitrate and acetate aqueous solutions (Figure SI-1 of Supporting Information), suggesting an octahedral coordination for Cd$^{2+}$, likely with oxygen atoms. This is quantitatively confirmed by the EXAFS analysis showing that both adsorbed (samples NMIN-1, NMIN-2, NMIN-3, and AMIN-2) and incorporated (NMIN-4, NMIN-5, NMIN-6, and AMIN-1) cadmium is surrounded by $6 \pm 1$ oxygen/nitrogen atoms in the first coordination shell with Cd–O distances ranging from 2.25 to 2.28 Å (Table 2). The metal incorporated in the freshwater diatom cells during their growth exhibits similar, within errors, mean distances (2.28 ± 0.01 Å as averaged over the studied samples) as the reversibly adsorbed cadmium (2.27 ± 0.02 Å). Both types of samples exhibit EXAFS and FT amplitudes very similar to those of acetate solids and solutions (Figure SI-2) in which Cd is bound to acetate ligand C2 via both oxygen and nitrogen atoms, resulting in a Cd–C distance of 2.7 Å and a collinear multiple scattering feature Cd–C–Cd from the C2 atom in the acetate molecule, visible on the Fourier transform of some NMIN samples and Cd acetate solutions (Figure SI-1). This relatively short Cd–C distance (2.7–2.8 Å) detected in some adsorbed samples (e.g., NMIN-1, AMIN-2) as compared to Cd–C distances in oxalate and citrate (R_Cd-O ∼ 3.0–3.2 Å, see Supporting Information for literature references and full structural parameters), in which Cd is bound to one oxygen atom of the COO moiety, further confirm that Cd is likely to form bidentate linkages with both oxygen atoms of the COO ligand in the studied cultures. Similar structures were reported for Cd adsorbed on Bacillus subtilis bacterial cells (9). No other, heavier contributions like Cd–C–O via Cd–O–C chemical linkages, in agreement with previous findings (9).

Note that, owing to the similarity of O and N backscatterer phases and amplitudes and Cd–O and Cd–N distances in most Cd–organic compounds, it was impossible to distinguish between these neighbors in the first coordination shell by EXAFS analysis. No first-shell splitting (i.e., the presence of two distinct subshells having different Cd–O/N distances), which might be indicative of the presence of different O- and N-binding groups, could be resolved in our samples, likely because of the limited spectral statistics (a minimal distance resolution for similar backscatterers, $\Delta R = \pi/2\Delta k$, is $\sim 0.20$ Å for most spectra). The Cd–O/N distances found in this study for diatom samples are in the range corresponding to Cd–O distances in cadmium phosphates and, citrate hydrated salts, in which Cd is typically coordinated to six O atoms from carboxylic groups both in mono- and bidentate fashion, and, eventually, water molecules ($R_{\text{Cd-O}} \sim 2.23–2.35$ Å, see Table SI-2 for details and literature references). At the same time, the first-shell distances of diatom samples are similar to those for Cd adsorbed on both hydrous and anhydrous amorphous silica (SiO$_2$·$n$H$_2$O and SiO$_2$, vitreous, respectively), where Cd is found in a 6-fold coordination with oxygen at 2.26–2.28 Å (Table 2, samples H$_4$SiO$_4$·$n$H$_2$O and SiO$_2$). For diatom cultures, our macroscopic measurements prove the dominance of organic (carboxylate-like from polysaccharide/protein layer) rather than inorganic (silanol from frustule) ligands in Cd binding by the diatom cell wall, as evidenced from a comparison of Cd adsorption on whole cells and isolated cell frustules (SiO$_2$·$n$H$_2$O), ref. 21. Therefore, although EXAFS allows both carboxylate and silanol binding, the first groups are most likely candidates for the Cd environment on diatoms’ surfaces as follows from the surface complexation approach.

Second-shell features for Cd in most adsorption samples are too weak to allow the extraction of quantitative parameters from EXAFS fitting. Comparative analyses of the amplitudes, phases, and positions of these features for NMIN adsorbed samples (NMIN-1, NMIN-3) reveal some similarities with those in Cd acetate solids and solutions (Figure SI-2) in which Cd is bound to acetate ligand C2–C1O via both oxygen atoms, resulting in a Cd–C1 distance of 2.7 Å and a collinear multiple scattering feature Cd–C1–C2 from the C2 atom in the acetate molecule, visible on the Fourier transform of some NMIN samples and Cd acetate solutions (Figure SI-1). This relatively short Cd–C1 distance (2.7–2.8 Å) detected in some adsorbed samples (e.g., NMIN-1, AMIN-2) as compared to Cd–C distances in oxalate and citrate ($R_{\text{Cd-C}} \sim 3.0–3.2$ Å, see Supporting Information for literature references and full structural parameters), in which Cd is bound to one oxygen atom of the COO moiety, further confirm that Cd is likely to form bidentate linkages with both oxygen atoms of the COO ligand in the studied cultures. Similar structures were reported for Cd adsorbed on Bacillus subtilis bacterial cells (9). No other, heavier contributions like Cd–C–O via Cd–O–C chemical linkages, in agreement with previous findings (9).

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neutral pH from a solution with [Cd\(^2+\)]\(_{aq}\) = 8.8 \mu M. This sample has a pronounced second-shell feature which was modeled with 1 ± 0.5 P or Si atoms at ~3.38 Å from Cd. Similar Cd–P structures were also reported for Cd adsorbed on \(B.\ subtilis\) cells in acidic solutions (9) and on thermophilic \(A.\ flavithermus\) at pH > 7 (10). Note that these features are also very close to those found for Cd adsorbed on amorphous silica, with Cd–Si distances of 3.36 Å and coordination numbers close to 1 (sample H\(\text{SiO}_4\), Table 2). Similarly to the failure of distinguishing between O and N in the first Cd shell (see above), it is impossible to distinguish, within the resolution of our measurements, the P of phosphoryl ligands inside the cell versus the Si of the cell walls as the major binding sites for incorporated cadmium in freshwater diatoms. However, it is known from literature data that the contribution of silica frustule to overall Cd binding by diatom cells is less than 10% (21), which is below the detection limits of our EXAFS analysis for Si second-shell neighbors (±0.2 Si atoms). Therefore, within the resolution of EXAFS analysis, we submit Cd storage inside the freshwater diatom cells in the form of carboxylate and phosphoryl rather than silanol ligands. A similar conclusion has been reached for Zn interacting with diatom surfaces (5). Although the EXAFS data do not allow the resolution of stoichiometry of Cd binding to the surface carboxylates (bidentate mononuclear versus bidentate binuclear complexes), our macroscopic adsorption results suggest mononuclear rather than binuclear binding.

Two samples of natural biofilms having different Cd loadings (520 and 27 000 ppm) demonstrated the first shell of Cd to be consistent with 6O/N at 2.25–2.27 Å and likely the presence of P and C atoms in the second shell at ~3.4 and ~2.9 Å, respectively. This result corroborates our finding on freshwater diatoms that exhibit predominantly carboxylate group binding for Cd, with some eventual phosphoryl contribution.

The cadmium atomic environment described above was found to be independent of both the duration and level of exposure and the type of diatom species (AMIN or NMIN). This result is consistent with the surface complexation model of Cd interaction with diatom cell walls (21) and suggests that reversible adsorption of this metal on freshwater diatom surfaces is not likely to be coupled with cysteine-bearing ligands or Cd–histidine complex production; rather, the fixation of Cd on the outer polysaccharide matrix prevents its further uptake through the cell membrane. Therefore, we suggest that complexation with carboxylate surface groups is the primary process responsible for metal binding on \(N.\ minima\) cell walls. This corroborates results of previous investigations that have concluded that carboxyl and phosphoryl sites play dominant roles in metal biosorption by marine algae (22), bacteria (10), and bacterial Biofilm (23). NMR studies using \(^{103}\text{Cd}\) also confirm the dominance of Cd–carboxylate complexes for algae and plant species, especially at high metal loadings (24, 25).

**FIGURE 2.** Normalized \(k^2\)-weighted EXAFS spectra at the Cd K edge of selected microorganism samples and reference solids and solutions (left), and their corresponding Fourier transform magnitude (right, FT transform \(k\) range = 2.1–10.0 Å\(^{-1}\), Kaiser–Bessel window function with \(dk = 3.0\); not corrected for phase shift). Vertical dashed lines on the left graph show changes in phase of EXAFS spectra depending on the dominant first shell backscattering atoms (O/N versus S/Cl) whereas those on the right graph denote approximate positions of different backscattering atoms around Cd (O, N, S, Cl, Si, P; C\(_1\) and C\(_2\) next-nearest carbon atoms; MS = linear multiple scattering path within the Cd–C\(_1–C_2\) carboxyl moiety typical of Cd–acetate complexes). Spectra are shifted along the \(y\) axis for clarity.

**Marine Diatoms and Anoxicogenic Photosynthesizing Bacteria.** Samples resulted from the long-term adsorption of Cd onto marine (\(S.\ costatum, SC-3\)) and estuarine (\(T.\ weissflogii, TW-1\)) diatoms in 0.1 M NaNO\(_3\) exhibit XANES spectra similar to those of Cd alginate, acetate, and freshwater NMIN species, thus suggesting the presence of oxygen in the first and probably carbon in the second coordination shells. EXAFS modeling is consistent with 6 ± 1 O at ~2.27 ± 0.02 Å in the first Cd atomic shell. Reversible short-term (~40 min) adsorption of Cd from 0.7 M NaCl solution on marine diatoms (samples TW-2 and SC-1) revealed the presence of about one to two Cl atoms originating from initial CdCl\(_2\) salts in the NaCl media (see details of treatment in SI-2).

In contrast to adsorption samples, the long-term growth of \(S.\ costatum\) in the presence of a high Cd concentration (9 \mu M, sample SC-2) yields a shape and energy position of its
Rings with oxygen is a larger size of the Cd ion (2.30 Å for [6]Cd). The carboxyl ligand (erentially monodendate bonds with the hydroxyl group of strain energy and renders [4]Zn chelates unstable. In the angular distortion in the formed chelate which gives rise to Zn incorporation into marine diatom A. minutissimum and N. minutissimum compared to marine species S. costatum. Indeed, while the phytochelatin production by marine diatoms in response to metal stress is a well-established phenomenon, the quantitative characterization of phytochelatin production by freshwater diatoms, cyanobacteria, and phototrophic anoxygenic bacteria due to the presence of Cd in an external solution is largely unknown and should be a topic of future studies.

A particular environment of adsorbed Cd (O/N and S in the first shell, P in the second shell) is revealed in an anoxic photosynthetic bacterium (Rhodopseudomonas palustris) where Cd binds on average with 3.5 ± 0.5 O/N and 1.0 ± 0.3 S in the first shell and 0.7 ± 0.3 P in the second shell, demonstrating the likely presence of phosphoryl ligands. Thus, the atomic environment of adsorbed Cd in R. palustris is different from Cd adsorbed on freshwater diatom N. minutissimum but similar to Cd incorporated into marine diatom S. costatum. Note that the binding of Cd to S-bearing groups has been previously observed by EXAFS for Gram-negative bacterial surfaces Shewanella oneidensis (30), whereas Zn binding to sulphydryl sites by heterotrophic Gram-negative bacteria is reported by Guiné et al. (31).

Comparisons of results of the present study with those of Zn adsorption and incorporation into diatoms demonstrate general similarities of Zn and Cd molecular interactions with phototrophic aquatic microorganisms. Both for freshwater diatoms and their biofilms, carboxylate binding is the main molecular environment for Zn and Cd. In contrast, upon adsorption and/or incorporation from an aqueous solution into diatoms, Zn changes its coordination from octahedral to tetrahedral (5), whereas Cd remains six-coordinated (this study). There are also differences in the main type of binding to C–COOH ligands. If [80]Cd exhibits essentially bidentate linkages with the oxgens of carboxylate, [10]Zn forms preferentially monodendate bonds with the hydroxyl group of the carboxyl ligand (5). The main reason for this difference is a larger size of the Cd ion (2.50 Å for [10]Cd–O vs 2.00 Å for [14]Zn–O), which allows the formation of Cd for stable chelate rings with oxygen—metal—oxygen angles close to 90°, whereas the tetrahedral coordination of the smaller Zn induces an angular distortion in the formed chelate which gives rise to strain energy and renders [10]Zn chelates unstable. In the presence of larger and more covalent than O/N ligands, like thiols, Cd is likely to adopt a tetrahedral coordination, as it is found in Cd sulfide and cysteine (this study), and phytochelatine and synthetic peptide complexes (32).

Note that, at the high Cd concentrations used in this study to collect a sufficient EXAFS signal, it is inevitable that the majority of the Cd will be bound nonspecifically to the surface, perhaps masking the true physiological response of the cell to metal ions actually entering the cytoplasm. However, the typical metal-to-ligand ratio used in our experiments is 0.1–1 mg/L of Cd per 10 g/L of biomass. These ratios are not very far from what one can expect in natural settings, having 0.1–1 µg/L of dissolved Cd and 10–100 mg/L of biomass (10^6 to 10^7 cell/L, typical concentrations of plankton in natural waters). Our measurements are pertinent to the polluted site in the southwest of France (tributary of the Lot river). In this river, much higher Cd concentrations, between 10 and 40 µg/L in the dissolved phase and 100–500 mg/kg in the particulate, are reported throughout the year (33). This range of concentrations is consistent with the one used in the present study (e.g., Table 1, sample AMIN-1). The typical range of Cd concentration in polluted river biofilms is between 500 and 1000 mg/kg (4, 34). Therefore, knowledge of the atomic environment of adsorbed and incorporated cadmium gained in the present study will help to identify the structural factors controlling the Cd toxicity for autotrophic organisms. Such a molecular-level understanding will be the key to establishing a sound scientific basis for determining the quality status of water reservoirs and for developing reliable predictive models of the impact of pollutants on aquatic ecosystems.

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**Supporting Information Available**

Chemical analyses, adsorption procedure, EDTA treatment of incorporation samples, and adsorption reversibility tests are given in SI-1. Details of the EXAFS spectra collection and reduction procedure, XANES spectra of investigated samples, and imaginary part of the Fourier transform of EXAFS spectra of selected microorganisms samples and reference compounds are presented in SI-2. This material is available free of charge via the Internet at http://pubs.acs.org.

**Literature Cited**


