NOTE

Cadmium uptake and trophic transfer in coastal plankton under contrasting nitrogen regimes

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ABSTRACT: Biological processes (uptake, trophic transfer, regeneration/excretion, and decomposition) are critical in controlling the fate of bioactive metals in the ocean. Whether nutrient conditions can affect metal uptake in marine phytoplankton and its subsequent trophic transfer along planktonic food chains is little known. We demonstrate that Cd accumulation in marine diatoms is dependent on ambient nitrogen conditions and thus the physiological status of phytoplankton cells. Diatom cells Thalassiosira pseudonana and T. weissflogii inoculated under nitrogen-limited conditions accumulated considerably less Cd than cells maintained under nitrogen-enriched conditions. The intracellular partitioning of Cd in the diatoms was also positively related to the level of ambient N. Phosphate and silicate starvation did not affect Cd uptake in diatoms. The trophic transfer, quantified by measurement of the Cd assimilation efficiency (AE) from ingested diatoms in copepods Calanus sinicus, increased with an increase in the N quota of the diatom cells maintained in a semi-continuous culture. AEs were however comparable in copepods feeding on N-starved and N-enriched diatoms. There was a linear relationship between the Cd AE and the Cd distribution in the cytoplasm of diatoms. The Cd regeneration rate in copepods was independent of the N status of ingested diatoms. Our study suggests that the cycling of Cd in marine planktonic food chains may be depressed under nitrogen-limited conditions. Conversely, nitrogen enrichment leads to an increase in Cd uptake in phytoplankton cells and zooplankton, elevating the potential exposure of plankton to toxic metals. Thus, nitrogen-stimulated eutrophication may not only affect the cycling of carbon, nitrogen and phosphorus in aquatic ecosystems, but can also result in changes in the cycling of toxic metals in marine food webs.

KEY WORDS: Cadmium · Uptake · Trophic transfer · Nutrients · Biogeochemical cycling

Understanding the fate of trace metals and their interaction with marine plankton is essential to our knowledge of metal biogeochemistry in oceanic and coastal waters. Previous studies on the bioavailability (or uptake) of metals to marine phytoplankton have mainly focused on the control exerted by metal aqueous chemistry (Campbell 1995). Geochemical and biological processes (metal speciation, metal-metal antagonistic interaction, such as the Cd-Zn-Mn system), and environmental factors (pCO₂) are known to greatly affect Cd uptake in marine phytoplankton (Sunda 1994, Hare & Tessier 1996, Sunda & Huntsman 1996, Cullen et al. 1999). Biochemical processes leading to metal sequestration include the production of extracellular chelating compounds or porphyrin complexes (Moffett & Brand 1996, Hutchins et al. 1999b). Recent studies have also indicated that the pCO₂ in the ambient water is critical for the uptake of Cd and Zn by marine phytoplankton (Morel et al. 1994, Cullen et al. 1999). However, little is known about the interactions between nutrient and metal uptake in marine phytoplankton and their potential impacts on metal trophic transfer in planktonic food chains. There is only limited knowledge on the influence of coastal eutrophication on the uptake of metals by aquatic organisms (Currie et al. 1998, Rijstenbil et al. 1998, Wang & Dei 2001). Coastal eutrophication has been reported in many aquatic systems impacted by human activity (Smith et al. 1999). Considerable evidence indicates that coastal eutrophication has affected the cycling of carbon, nitrogen and phosphorus in these systems (Vitousek et al. 1997, Smith et al. 1999). Whether nutrient enrichment has affected the biological cycling of toxic metals remains essentially unknown. Circumstantial evidence suggests an interaction between macronutrients and trace metals in affecting the ecosystem dynamics (Breitburg et al. 1999). This interaction appears to be non-additive, i.e., the addition of trace elements greatly decreases the response of phytoplankton to macronutrient addition in mesocosm experiments (Breitburg et al. 1999).

In this study, we examined the influence of macronutrients on the biological uptake, trophic transfer and
regeneration of Cd in coastal plankton. Cd is both a toxic metal and a tracer of paleo-phosphate concentration in surface water, although a recent study has suggested that the Cd:P ratio in phytoplankton is dependent on the ambient pCO2, which may complicate the use of Cd as a tracer of paleo-phosphate concentration (Cullen et al. 1999). Our objectives were to examine the Cd uptake in diatoms influenced by macronutrient starvation or different levels of N enrichment. We further determined the assimilation efficiency (AE) and regeneration rate of Cd in marine copepods feeding on diatoms inoculated under different N conditions. AE is an important physiological parameter determining the trophic transfer of metals in marine food chains (Wang & Fisher 1999).

**Materials and methods. Metal uptake in diatom cells:** Two axenic cultures of coastal diatoms, *Thalassiosira pseudonana* (Clone 3H) and *T. weissflogii* (CCMP 1048), were used in the experiments. The cells were maintained in an f/2 medium (Guillard & Ryther 1962) at 18°C under a light illumination of 100 µE m−2 s−1 with a 14:10 h light:dark cycle. The seawater used in all experiments was collected 10 km off East Hong Kong, remote from any anthropogenic activity. 109Cd was used as a tracer for the uptake of stable Cd into the cells. Radioisotope addition corresponded to a Cd concentration of 2 nM. All nutrient stocks were prepared by being passed through a Chelex ion exchange resin to remove trace metals. All uptake experiments were conducted in 150 ml of 0.2 µm-filtered seawater maintained at 18°C, and held in acid-cleaned polycarbonate bottles under a light illumination of 100 µE m−2 s−1. Cd uptake in the diatoms was determined over a short-term exposure period, during which time there was a negligible decrease of nutrient concentration in the ambient water. The cells inoculated under different nutrient conditions were collected onto 3 µm polycarbonate membranes, rinsed, and resuspended in <0.2 µm-filtered seawater. The cells were then added to 150 ml of filtered seawater containing 18 nM of Cd and different additions of nutrients. Cell density in the medium was between 3 and 5 × 10^5 cells ml⁻¹ for *T. pseudonana*, and 10^5 cells ml⁻¹ for *T. weissflogii*. In each experiment, cell density was maintained at a similar level among the different treatments. At time intervals of 1, 2, 3 and 5 h, a 10 ml aliquot was filtered onto a 1 µm polycarbonate membrane, rinsed with filtered seawater, and the radioactivity of the cells counted. To measure the dry weight of the cells, the cells were filtered onto a pre-weighed glass-fiber filter, rinsed with ammonium formate, and dried at 80°C for 1 d. The protein content of the cells was measured using the bicinchoninic acid (BCA) method. Intracellular distribution of Cd was measured by the Ti washing technique (Hudson & Morel 1989, Hutchins et al. 1999a). The radioactivity of 109Cd was determined on a Wallac Wizard 1480 NaI gamma detector at 88 keV. Counting times were adjusted to result in propagated counting errors of <3%.

Three experiments were conducted to determine the influence of macronutrients on the Cd uptake in diatoms. Only the experimental macronutrient was added in the medium for Cd uptake measurement. The first experiment was designed to compare the uptake of Cd in nutrient-starved and nutrient-enriched cells. Diatom cells in the late exponential growth phase were filtered and starved of nitrate for 3 d, phosphate for 3 d, or silicate for 1 d before the experiments. The cells did not grow following these treatments, indicating that they were under starvation for a specific nutrient. Other macronutrients were added during the starvation of 1 specific nutrient. In another treatment (enriched cells), exponentially growing cells were transferred to nutrient-enriched water (882 µM of nitrate, 36.2 µM of phosphate, and 105.6 µM of silicate) for 2 d. Both the ‘starved’ and the ‘enriched’ cells were then suspended in either nutrient-depleted or -enriched filtered seawater for Cd uptake measurements. The second experiment was designed to compare the Cd uptake in diatoms at different ambient nutrient concentrations. The late exponentially growing diatom cells inoculated in a full f/2 nutrient medium were transferred to different nitrate concentrations (17.6, 58.7, and 176 µM) for 1.5 d, during which time the initial N concentrations declined by 20 to 60%, before the Cd uptake measurements were made at these nitrate concentrations. The third experiment was designed to compare the Cd uptake in diatoms maintained in semi-continuous cultures. Diatom cells were inoculated at 2 N levels (17.6 and 176 µM), and were transferred to a new medium containing the same concentration of N when they reached the mid-exponential growth phase. After 5 transfers, the cells underwent >10 divisions and Cd uptake was subsequently measured at these 2 N levels.

**Cd assimilation efficiency in copepods:** Diatom cells (*Thalassiosira weissflogii*) were inoculated in a semi-continuous culture at 2 different nitrate levels (17.6 and 176 µM) and radiolabeled with 109Cd, as described in Wang & Fisher (1996, 1998a). Additions of other nutrients were f/2 levels for P, Si, and vitamins, and f/20 levels for Mn, Co, Fe, and Mo. After 1 more transfer (in the presence of 109Cd) when the cells reached the exponential phase, the cells were collected before being fed to the copepods *Calanus sinicus*. In another experiment, the diatoms *T. pseudonana* and *T. weissflogii* were inoculated in f/2 levels of macronutrients and radiolabeled with 109Cd. When the cells reached the late exponential growth phase, they were collected and divided into 2 groups. One group was subjected to N starvation for 3 d (but in the presence of other
macronutrients), whereas the other group was enriched with an 1/2 level of nitrate and other macronutrients for 2 d. The cells were then collected by filtration before being fed to the copepods.

The radiolabeled diatoms were pulse-fed to the copepods for 20 min at a cell density of 20,000 cells ml\(^{-1}\) and a copepod density of 0.2 copepods ml\(^{-1}\) using methods described in Wang & Fisher (1998a, 1999). The copepods had originally been collected by net towing (500 µm) from Clear Water Bay, Hong Kong, and were acclimated in the laboratory for 1 d in the presence of diatom diets. After the pulse radioactive feeding, the copepods were removed with a mesh and their radioactivity was immediately counted for 2 min. Any feces produced during the radioactive feeding period were also collected and radioassayed. The total amount of \(^{109}\text{Cd}\) ingested was calculated as the sum of the radioactivity in the copepods and the radioactivity in egested feces after 20 min of pulse feeding. The copepods were subsequently depurated in non-radioactive waters for 2 d in the presence of the same diatom diet. The radioactivity retained in the copepods was determined at time intervals. The AE and regeneration rate were calculated as the \(y\)-intercept and the slope of the linear regression between the natural log of the percentage of Cd retained in copepods and the time of depuration (between 12 and 48 h), respectively (Wang & Fisher 1998a, 1999). The fraction of Cd in the algal cytoplasm was measured as described in Fisher et al. (1983) and Reinfelder & Fisher (1991).

**Results and discussion.** Nitrate starvation significantly reduced the uptake of Cd in the *Thalassiosira* spp. cells (Fig. 1). The uptake rate, calculated from the slope of Cd accumulation in diatom cells against exposure time (1 to 5 h), was about 4 times higher in diatom cells inoculated in N-enriched medium (176 µM) than in cells starved of N for 3 d (\(p < 0.05\), Student's \(t\)-test). When the N-starved cells were resuspended in non-radioactive waters for 2 d in the presence of the same diatom diet. The radioactivity retained in the copepods was determined at time intervals. The AE and regeneration rate were calculated as the \(y\)-intercept and the slope of the linear regression between the natural log of the percentage of Cd retained in copepods and the time of depuration (between 12 and 48 h), respectively (Wang & Fisher 1998a, 1999). The fraction of Cd in the algal cytoplasm was measured as described in Fisher et al. (1983) and Reinfelder & Fisher (1991).

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When the 2 diatoms (in the late exponential growth phase) were acclimated to 3 nitrate levels for only 1.5 d, the Cd uptake measured in these cells was significantly reduced, with a decrease in N concentration in both species (\(p < 0.05\), 1-way ANOVA) (Fig. 2). The uptake rate was 4.0 to 4.6 times higher at 176 µM than at 17.6 µM for both diatoms. In this experiment, we did not quantify the intracellular distribution of Cd.

These results were further confirmed by experiments using cells inoculated in a semi-continuous culture. In this experiment, Cd uptake was also significantly enhanced with increasing N concentration (\(p < 0.01\), \(t\)-test, Fig. 3). The calculated uptake rate was 4.1 times higher at 176 µM than at 17.6 µM. The cell dry weight was 1.2 times higher at 176 µM than at 17.6 µM. At 5 h, 80% of the diatoms' Cd was found in the intracellular compartment in cells maintained at 176 µM compared to 42% in cells maintained at 17.6 µM. At 24 h, 94% of the Cd was found in the intracellular pool at 176 µM compared to 72% at 17.6 µM.

These experiments therefore demonstrated that Cd uptake in phytoplankton cells is strongly related to the ambient N level, and add an important dimension to our current understanding of the processes controlling Cd uptake in marine phytoplankton. In our experi-
ments, we also found that, similarly to earlier studies (Harrison et al. 1990, Wang & Fisher 1996), the protein concentration in the cells increased with increasing N concentration (Table 1). It is likely that the concentration of ligands (e.g., phytochelatins: Ahner et al. 1995) available for Cd binding, transport and sequestration increases with increasing N levels, leading to an enhanced Cd uptake into the cells. In our study, we did not quantify the carbonic anhydrase activity in cells maintained under different N conditions, which has been recently shown to have Cd as a co-factor (Cullen et al. 1999). Alternatively, the increase in Cd uptake may be due to an increase in the cell growth rate arising from nitrate addition. Circumstantial evidence has indicated that cell growth may control Cd uptake. For example, Luoma et al. (1998) observed a significant increase in Cd concentration in a phytoplankton assemblage during blooms in San Francisco Bay. Our recent study also demonstrated that there was a significant correlation between Cd uptake and cell growth rate in different species of marine phytoplankton (Wang & Dei 2001). However, whether the cell growth rate directly affects Cd uptake in marine phytoplankton remains to be determined.

The interaction between nutrient and metal uptake of phytoplankton may have important implications for metal cycling in coastal and oceanic waters. The low biological uptake in phytoplankton cells under nitrogen-limited conditions suggests that the biological transport of Cd in marine planktonic food chains would be limited. Conversely, nitrogen enrichment (e.g., coastal eutrophication) may considerably enhance the Cd concentration in phytoplankton cells, and thereby exaggerate the potential toxicity of metals to phytoplankton. This was not tested in our study. An alternative argument would be that N-enriched cells are able to accumulate more intracellular detoxifying ligands than N-starved cells; consequently, toxicity could actually decrease despite the increased Cd uptake. Furthermore, the dependence of Cd uptake on ambient

![Fig. 2. *Thalassiosira pseudonana* and *Thalassiosira weissflogii*. Cd uptake in 2 diatoms following inoculation at different nitrate levels for 1.5 d. (●) Cells maintained at 17.6 µM nitrate; (○) cells maintained at 58.7 µM nitrate; (▼) cells maintained at 176 µM nitrate. Data are means ± SD (n = 2)](image)

![Fig. 3. *Thalassiosira pseudonana*. Cd uptake in diatoms inoculated at 17.6 and 176 µM nitrate in semi-continuous culture. (●) Cells maintained at 17.6 µM nitrate; (○) cells maintained at 176 µM nitrate. Data are means ± SD (n = 2)](image)

<table>
<thead>
<tr>
<th></th>
<th><em>Thalassiosira pseudonana</em></th>
<th><em>Thalassiosira weissflogii</em></th>
<th><em>Thalassiosira weissflogii</em></th>
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<td></td>
<td>N starv.</td>
<td>N enrich.</td>
<td>N starvation</td>
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<tr>
<td>Cd in diatom cytoplasm (%)</td>
<td>39.1 ± 1.4</td>
<td>41.0 ± 0.2</td>
<td>71.0 ± 0.4</td>
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<td>Cd AE (%)</td>
<td>40.2 ± 3.5</td>
<td>41.2 ± 2.9</td>
<td>90.4 ± 3.4</td>
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<tr>
<td>Cd regeneration rate constant (d⁻¹)</td>
<td>0.470 ± 0.042</td>
<td>0.483 ± 0.055</td>
<td>0.923 ± 0.039</td>
</tr>
<tr>
<td>Protein content (pg cell⁻¹)</td>
<td>2.85</td>
<td>4.63</td>
<td>49.3</td>
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Table 1. *Calanus sinicus*. Calculated assimilation efficiencies (AE) and regeneration rate constants of Cd in marine copepods feeding on diatoms inoculated under different nitrates conditions. Data are means ± SD (n = 2 to 3). Only 1 measurement of protein was made in each treatment.
nitrate concentration but not on phosphate concentration suggests that the Cd/P ratio in surface waters can be affected by the nitrate concentration in addition to the CO₂ partial pressure (Cullen et al. 1999).

The transfer of Cd in marine copepods *Calanus sinicus* was determined by measurements of Cd AE in cells inoculated at 2 nitrate concentrations in a semi-continuous culture. In this experiment, copepods depurated Cd at a faster rate following a pulse ingestion of ¹⁰⁹Cd-labeled N-poor diatoms than those fed with diatoms inoculated at 17.6 µM nitrate (Table 1). Moreover, in this experiment, we found that the distribution of Cd in the diatom cytoplasm, which critically affects metal assimilation in marine copepods (Reinfelder & Fisher 1991, Hutchins et al. 1995), was also 1.8 times higher for diatoms inoculated at 176 µM than for N-poor diatoms inoculated at 17.6 µM (Table 1). The ingestion activity of copepods was comparable for N-enriched cells (26 000 cells h⁻¹ copepod⁻¹) and N-poor cells (23 000 cells h⁻¹ copepod⁻¹). The regeneration rate of Cd (long-term physiological turnover rate) was however independent of the nutritional status of the cells ingested, but was relatively high (0.632 to 0.668 d⁻¹ for both treatments) and comparable to earlier studies (Wang & Fisher 1998a, b). This experiment suggested that Cd assimilation but not regeneration in copepods is controlled by the N quota of food particles, in contrast to the finding for marine mussels (Wang & Fisher 1996).

Comparable depuration was however found in copepods feeding on N-starved and N-enriched diatoms (data not shown). Although there was more Cd in the intracellular compartment in N-enriched diatoms than in N-starved diatoms during the short-term exposure period, there was no major difference in Cd distribution in the diatoms' cytoplasm between the 2 treatments (Table 1). This was probably due to the fact that ¹⁰⁹Cd was radiolabeled onto the diatoms before the cells were transferred for N starvation; thus, the metal distribution in diatoms was not greatly influenced by subsequent N starvation or N enrichment. The calculated Cd AEs were also comparable between the 2 treatments (Table 1). More Cd was found in the cytoplasm of diatom *Thalassiosira weissflogii* than in that of *T. pseudonana*. The measured AEs were >90% in copepods feeding on *T. weissflogii* and were considerably higher than in a separate experiment examining the influence of N level on Cd AEs (21 to 52%). There were also considerable differences in the Cd regeneration rate in copepods among the 3 experiments (Table 1). The difference in the total protein concentration in the diatoms appeared to have no consistent effect on Cd assimilation in the copepods.

The difference in Cd AEs among different experiments and between the 2 diatoms was primarily accounted for by the difference in Cd distribution in the cytoplasm of the diatoms. There was a significant linear relationship between the Cd AEs in the copepods and the Cd distribution in diatoms' cytoplasm (Fig. 5). Our study provides further evidence that metal distribution in phytoplankton critically affects the trophic transfer of metals in the marine planktonic food chain (Reinfelder & Fisher 1991, Hutchins et al. 1995). Previously, only Hutchins et al. (1995) demonstrated a significant relationship between the AE in copepods and the distribution in the diatoms' cytoplasm for a specific metal such as Fe. Thus, for metals such as Cd, which partitions mostly in the intracellular pool of diatoms, metal desorption within the copepod’s gut following food ingestion may not be important in controlling metal assimilation in the copepods.

Metal accumulation in marine copepods is related to the metal influx rate (controlled by metal AE, ingestion activity, and metal concentration in ingested cells) and the metal efflux rate (Reinfelder et al. 1998, Wang & Fisher 1996).
Fisher 1998a). Of the 4 aforementioned parameters, nitrogen limitation or enrichment can affect metal accumulation in marine copepods through its influence on metal uptake in phytoplankton and metal assimilation in copepods. Thus, an increase in N concentration can lead to an increase in Cd concentration in phytoplankton cells, and further to an increase in Cd influx in marine zoooplankton. Our study therefore highlights the dependence of Cd uptake and transfer on environmental nitrogen conditions, which has important implications for our understanding of metal dynamics in coastal waters. Thus, coastal eutrophication may potentially affect metal uptake, toxicity and trophic transfer in aquatic food chains. Given that metal pollution and nutrient enrichment can often occur simultaneously in coastal waters, there is a need to further study the interaction between toxic metals and macro-nutrients in controlling ecosystem dynamics and function.

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