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Production of colloidal organic carbon and trace metals by phytoplankton decomposition

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Abstract

Colloids are important intermediates and can considerably influence the biogeochemical cycling of organic carbon and trace metals in aquatic systems. Previous studies have determined the release of carbon and trace elements from decomposing phytoplankton debris, but the degradation of biogenic particles to colloids and their significance in metal and carbon cycling remain unknown. In this study, we measured the release of carbon and trace elements (Cd, Cr, Se, and Zn) from the debris of two phytoplankton (diatom *Thalassiosira pseudonana,* and dinoflagellate *Prorocentrum minimum*) into a colloidal phase (operationally defined as 5 kDa–0.2 μm). In general, Cd, Se, and Zn were released at a faster rate and Cr was released at a slower rate than the release of C from the decomposing debris into the dissolved phase (<0.2 μm). Release of trace metals appeared to closely follow C release. The release rate coefficients of Cd, Cr, Se, Zn, and C in the two phytoplankton debris with microbial addition were 1.67–1.68 d^{-1} for Cd, 0.147–0.239 d^{-1} for Cr, 0.712–0.845 d^{-1} for Se, 0.765–1.14 d^{-1} for Zn, and 0.443–0.512 d^{-1} for C. The percentages of trace elements (Cr, Se, Zn) and C associated with the colloidal phase were relatively high within the first 5 d of decomposition. After 5 d, the fraction of colloidal trace elements in the <0.2-μm dissolved phase was 5–10% for Cd, 13–52% for Cr, 13–28% for Se, 14–30% for Zn, and 16–41% for C in the diatom decomposing experiment; and 4–9% for Cd, 15–62% for Cr, 15–31% for Se, 3–27% for Zn, and 22–38% for C in the dinoflagellate decomposing experiment, respectively. The partitioning of metals in the colloidal phase was not greatly affected by the microbial activity. Although the overall percentage of organic carbon release was related to that of metals from decomposing debris, no significant correlation between the percentages of colloidal metals and colloidal organic carbon was observed, implying that organic C and metals have different partitioning behavior during their release from decomposing phytoplankton debris. Our study demonstrated that the decomposition of biogenic particles may contribute considerably to the production of colloids in marine environments.

Considerable interest has been generated regarding the biological control of trace metal cycling in aquatic systems (Whitefield and Turner 1987). Biological processes, such as uptake, transformation, exudation/excretion, and decomposition, are essential in controlling the fate of trace elements in marine systems. Many previous studies have extensively quantified the release of trace metals and carbon from decomposing biogenic debris, including phytoplankton and zooplankton debris and copepod fecal materials (Lee and Fisher 1992*a*,*b*, 1993; Fisher and Wente 1993; Reinfelder et al. 1993; Wang et al. 1996; Wang and Fisher 1998). Generally, it was found that metals that are most particle reactive remain bound to these particles for the longest time and are typically lost at rates slower than that of the organic carbon. Microbial activity has relatively little effect on the release of metals in the breakdown of biogenic debris from the particulate phase to the dissolved phase (Fisher and Reinfelder 1995). In these previous studies, the release of metals and C from degrading biogenic debris were generally quantified by measurements of metals or C in the particulate phase (e.g., >0.2 μm) and the traditionally defined dissolved phase. However, the traditionally defined dissolved phase contains a significant fraction of colloids (Benner et al. 1992; Guo et al. 1995; Martin et al. 1995), which are operationally defined as the size range between 1 nm and 0.2 μm (Buffle 1990). The production of colloidal organic carbon and colloidal trace metals during the decomposition of marine phytoplankton debris has not yet been quantified.

Marine colloids are mostly organic in nature and are composed of biopolymers and macromolecules (Benner et al. 1992; Bianchi et al. 1995; Aluwihare et al. 1997; Santschi et al. 1998). Organic colloids are polyfunctional and polydisperse (Buffle et al. 1998) and can strongly bind trace metals, thus playing a critical role in the biogeochemical cycling of trace metals in natural waters. Recent studies have consistently demonstrated that colloids are abundant in marine environments (Wells and Goldberg 1991; Guo and Santschi 1997*a*). However, the mechanisms and pathways in the production of marine colloids and their role in the biogeochemical processes of trace elements are still largely unknown.

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Whether colloids act as a sink or link for trace metals in aquatic system remains to be determined (Wang and Guo 2000).

Recently, there have been extensive field measurements of colloidal trace metals and C in different marine systems, mostly in coastal and estuarine regions (Dai et al. 1995; Martin et al. 1995; Sanudo-Wihelmy et al. 1996; Wells et al. 1998; Wen et al. 1999). The range of the fraction of metals in colloidal phases in different aquatic systems is generally large (Guo and Santschi 1997*a*; Santschi et al. 1999), which indicates that the nature and composition of organic ligands that bind trace metals vary greatly in different systems. Both biogenic and terrestrial origins of colloidal materials have been proposed as contributing to marine colloidal pool (Guo and Santschi 1997*a*; Bianchi et al. 1997; Opsahl and Benner 1997; Aluwihare and Repeta 1999). Nevertheless, the relative importance of different colloidal sources and their production and degradation mechanisms need to be better understood.

In this study, we quantified the release of organic C and trace elements (Cd, Cr, Se, and Zn) from decomposing phytoplankton debris into the dissolved phase (<0.2 μm) and further evaluated the partitioning of released elements between the colloidal (5 kDa–0.2 μm) and the ultrafilter-passing (<5 kDa) phases under controlled laboratory conditions. Radiotracer techniques were employed to trace the release of trace elements and carbon from the radiolabeled biogenic particles into the preultrafiltered low molecular weight (LMW) seawater containing no radiotracers.

Materials and methods

Axenic cultures of the diatom *Thalassiosira pseudonana* (clone 3H) and the dinoflagellate *Prorocentrum minimum* (clone CCMP 696) were obtained from the Provasoli-Guillard Phytoplankton Collection Center and maintained in f/2 medium (Guillard and Ryther 1962) at 18°C. To radiolabel the phytoplankton cells, early stationary phase cells were filtered, rinsed with filtered seawater, and resuspended into 2 liter filtered seawater (<0.2 μm) enriched with f/2 levels of N, P, Si, vitamin and f/20 levels of Co, Mn, Mo, and Fe, without the additions of Cu, Zn, and EDTA. The initial cell density was 10,000 cells ml^{-1} for *T. pseudonana* and 2,000 cells ml^{-1} for *P. minimum.* The culture was then spiked with radioisotopes ^{109}Cd (in 0.1 N HCl, 49.3 kBq L^{-1}, corresponding to 5.9 nM), ^{51}Cr(III) (in 0.1 N HCl, 49.3 kBq L^{-1}, corresponding to 0.12 nM), ^{75}Se (as selenite, in distilled water, 49.3 kBq L^{-1}, corresponding to 0.91 nM), ^{65}Zn (in 0.1 N HCl, 49.3 kBq L^{-1}, corresponding to 5.9 nM), and ^{14}C (as bicarbonate, 247 kBq L^{-1}). Because ^{109}Cd, ^{51}Cr, and ^{65}Zn were carried in acidic solution (0.1 N HCl), we added a microliter amount of 0.5 N Suprapur NaOH before the addition of radioisotopes to maintain the final pH at 8.0. The cultures were then grown for 6 d on a 14 : 12 h light : dark cycle at 18°C, as described above.

After 6 d growth, the cell density had reached 10^6 cells ml^{-1} (6.6 divisions) for *T. pseudonana* and 35,000 cells ml^{-1} (4.1 divisions) for *P. minimum.* A 1-ml water sample was removed for the radioactivity measurement (representing the radioactivity in the cells and in the dissolved phase). A 5-ml sample was filtered onto a 3-μm polycarbonate membrane, and the radioactivity in the filter was counted (representing the radioactivity in the cells). All cells were then collected by filtration onto 3-μm polycarbonate membranes, rinsed with filtered seawater, and resuspended in 2-liter preultrafiltered (<1 kDa) low molecular weight (LMW) seawater. The LMW seawater was collected by cross-flow ultrafiltration with a nominal molecular weight cutoff of 1 kDa (Amicon S10Y1, Guo and Santschi 1996; Wang and Guo 2000). The cells were resuspended twice to remove any weakly bound metals.

The resuspended cells were then distributed into four 500-ml flasks, each receiving 450-ml cell resuspension. The measured biomass in the cell suspension was 22 mg L^{-1} for *T. pseudonana* and 21 mg L^{-1} for *P. minimum.* These four identical flasks were then divided into two groups, one with microorganism treatment by adding 10 ml of microbial assemblages (0.2–1.0 μm) freshly collected from Port Shelter Pier, Hong Kong, and the other containing 10 ml of sodium azide at a final concentration of 50 mM. For each treatment there were two replicate bottles. The flasks were then placed in the dark at 18°C for 22 d. At time intervals, 1 ml of well-mixed solution was sampled for total radioactivity measurement representing radioisotopes in both particulate and dissolved phases. A 5-ml sample was filtered onto a 0.2-μm polycarbonate membrane and rinsed with LMW seawater; then the radioactivity in the membrane was counted (representing the radioactivity in the particulate phase, defined as >0.2 μm). Another 5-ml sample was pipetted into a centrifugal ultrafilter (Amicon, Centricon plus-20) with a molecular weight cutoff (MWCO) of 5 kDa. The sample was then centrifuged at 4,500 g for 15 min, as recommended by the manufacturer. Two milliliters of the ultrafiltrate was then counted for radioactivity. A final 5-ml sample was taken and acidified at pH < 2 (addition of 35 μl of 6 N HCl). The sample was then bubbled with N_2, and any released $^{14}CO_2$ was absorbed in 1 M NaOH, as described in Lee and Fisher (1992*a*). The ^{14}C radioactivity was then determined to quantify the fraction of ^{14}C transformed from organic to inorganic carbon phases. Total recovery of radioactivity by the end of experiments compared with the initial total amount of radioactivity was about 96–98%, 70–93%, and 80–94% for Cd, Cr, and Zn, respectively.

One piece of information critical to the success of this study was the apparent MWCO and the efficiency of colloidal retention by the centrifugal ultrafilter (Amicon Centricon plus-20) and the potential adsorption of radiotracers onto the membranes. To address these questions, three standard macromolecules with known molecular weights (vitamin B_{12} with a MW of 1.33 kDa and fluorescein tagged dextran with a MW of 3 and 10 kDa, respectively) were used to calibrate the Amicon centricon plus-20 ultrafiltration membrane (5 kDa). Concentrations of vitamin B_{12} in both permeate (<5 kDa) and retentate (>5 kDa) were measured by a spectrophotometer (Beckman DU-64), and concentrations of fluorescein labeled dextran (both 3 and 10 kDa) were quantified by a fluorescence detector (Waters 2487) as described previously (Guo et al. 2000). Possible sorption of radiotracers on the same ultrafiltration membrane (Centricon

Table 1. Retention of standard macromolecules by the Centricon ultrafilter (5 kDa) and the sorption of radiotracers (mean ± SD, $n = 2$).

Macromolecule or isotope	Molecular weight (kDa)	Percentage retained (%)	Percentage sorbed onto the membrane
Vitamin B_{12}	1.33	9–11	—
Dextran	3	75–78	—
Dextran	10	96–97	—
^{109}Cd	—	—	0.5 ± 0.1
^{51}Cr	—	—	1.0 ± 0.2
^{65}Zn	—	—	3.5 ± 0.6

plus-20) was also examined using LMW permeate solution containing radiotracers. The percentage of radiotracers (^{109}Cd, ^{51}Cr, ^{65}Zn) lost to the membrane was estimated from the recovery in the permeate.

The percentages of metals and C retained in the biogenic debris were calculated as the ratio of the radioactivity in the particulate phase (>0.2-μm filter) and the radioactivity in the water sample (including particles and water). The percentages of metals and C released were calculated as 100% minus percentage retained. The percentages of metals and C partitioning in the colloidal phase were calculated as the ratio of radioactivity in the colloidal particles to the radioactivity in the total dissolved phase (<0.2 μm):

$$\% \text{ in colloidal} = (A_t - A_P - A_u)/(A_t - A_P), \quad (1)$$

where A_t is the total radioactivity in the water sample, A_P is the radioactivity in the particulate phase (>0.2 μm), and A_u is the radioactivity in the permeate after ultrafiltration.

The gamma radioactivity of the samples was counted by a Wallac 1480 Na(Tl) gamma detector. Counts were related to spillover from a higher energy window to a lower energy window and counting efficiency. The gamma emission of ^{109}Cd was counted at 88 keV, of ^{51}Cr(III) at 320 keV, of ^{75}Se at 264 keV, and of ^{65}Zn at 1115 keV. To count the ^{14}C activity of the samples, we first added Solvable to solubilize the samples and then the cocktail (Gold Maxima). The radioactivity of ^{14}C was measured by a Beckman LSC with the external standard ratio method. To correct the effect of each gamma-emitting isotope on the counting of ^{14}C, a series of gamma radioisotope standards (with different amounts of radioactivity) were prepared and their spillovers on the counting of ^{14}C were measured, as described in Wells (1999). The spillover of each gamma isotope on ^{14}C counting in the samples was then subtracted using the linear working standard curve. Counting times were adjusted to result in a propagated counting error <5%.

Results

Calibration of ultrafiltration membrane—When the accuracy of the membrane's molecular weight cutoff (MWCO) was examined using the standard macromolecules, over 96% of a 10 kDa dextran was retained by our 5 kDa Centricon plus-20 ultrafilter (Table 1). This indicated that the Centricon plus-20 indeed had a retention or recovery rate up to the specification given by the manufacturer. However, this 5 kDa membrane also retained a notable fraction of lower MW molecules, i.e., ~10% of the 1.3 kDa vitamin B_{12} after correction for its sorption on the membrane and ~75% of a 3 kDa dextran under low concentration factor (Table 1). Similarly, Pantoja and Lee (1999) also reported a 93% retention of a 19 kDa protein standard by a 30 kDa membrane. Using molecular probes, Guo et al. (2000) concluded that the retention of lower MW molecules by ultrafiltration membranes is significant under low concentration factors, which may potentially lead to overestimation of the HMW fraction. Overall, our calibration results are consistent with those reported in the literature (Guo and Santschi 1996; Pantoja and Lee 1999; Guo et al. 2000).

Sorption losses of radiotracers to membrane using spiked LMW permeate solution were minimal (Table 1). On average, only less than 1% of spiked Cd and Cr was lost to the membrane, whereas the loss of Zn was 3.5%. These results indicated that the sorption of LMW radiotracers onto the membrane was insignificant. We did not measure the sorption of ^{75}Se onto the membrane.

Retention of metals and carbon in the phytoplankton debris—After 6 d growth, about 19% of C, 50% of Cd, 54% of Cr, 57% of Se, and 98% of Zn were associated with the diatom *Thalassiosira pseudonana*; and 24% of C, 73% of Cd, 18% of Cr, 29% of Se, and 93% of Zn were associated with the dinoflagellate *Prorocentrum minimum*. The percentage of C and metals retained in the decomposing algal cells are shown in Fig. 1. There was a rapid loss of both C and Cr after both species of algal cells were resuspended into the LMW seawater. About 24% of C in *T. pseudonana* and 44–47% of C in *P. minimum* were lost to the dissolved phase within the first hour. For Cr, about 29–39% in *T. pseudonana* and 30–31% in *P. minimum* was lost within the first hour. In contrast, Cd, Se, and Zn were retained in the algal cells for at least a few days before a significant loss from the cells was evident. The most significant loss of metals and carbon from the decomposing cells occurred within the first 7 d. For Cr, the percentage retained in the diatom debris essentially remained unchanged following an initial rapid loss.

The addition of microorganisms enhanced the release of C and all metals except Cr in both species of phytoplankton. The release of both Cd and Zn appeared to be most affected by the additions of microorganisms. We also monitored the free-living bacteria at the end of the experiment. However, the number of free-living bacteria was too low, about 6,600 cells ml^{-1} with microorganism addition, compared with 1,600 cells ml^{-1} with NaN_3 treatment. Our $^{14}CO_2$ measurements also showed that relatively little CO_2 was released by the decomposing algae, ranging from 2–8% of total organic carbon with microorganism addition to 0.4–2% with NaN_3 treatment in both species of phytoplankton debris.

According to Lee and Fisher (1992*a*, 1993), the release of metals and C by decomposing phytoplankton can be modeled by the following equation:

$$y = 100 \times (t + 1)^{-b}, \quad (2)$$

where y is the percentage of metals or C retained in the

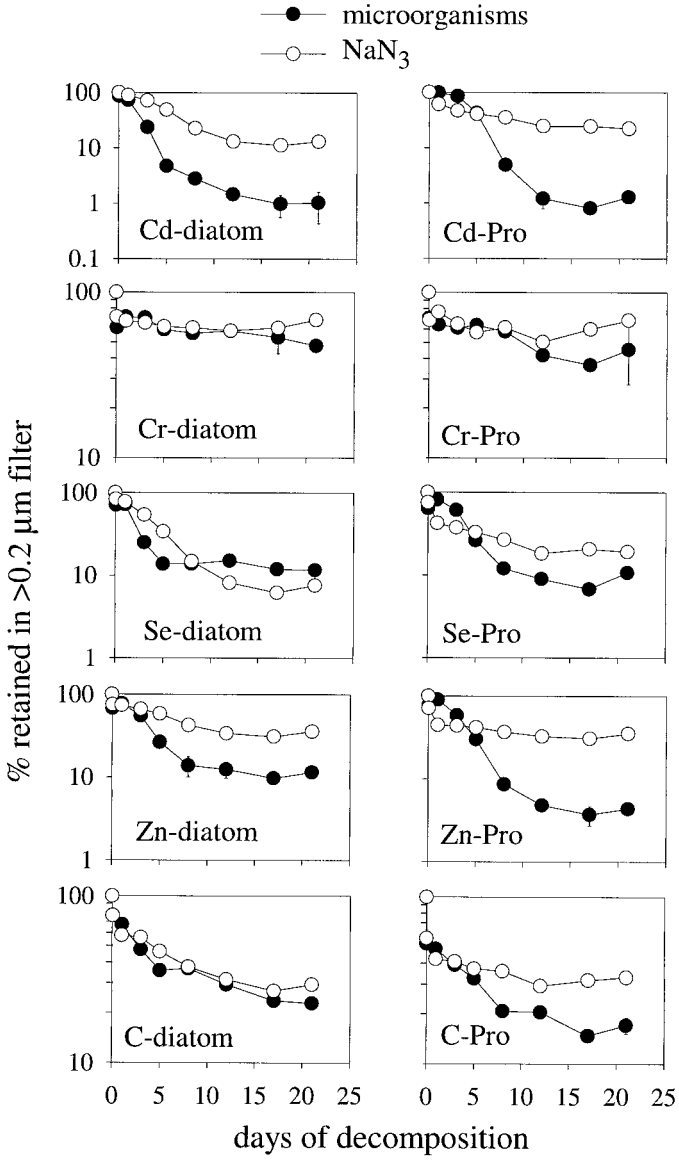

Fig. 1. The percentages of carbon and trace elements (Cd, Cr, Se, and Zn) retained in the decomposing diatom (*Thalassiosira pseudonana*) and dinoflagellate (*Prorocentrum minimum*, Pro) with microorganism addition or with sodium azide addition. Data presented are mean ± SD ($n = 2$).

particles at time t and b is the release rate coefficient. Values of b can be calculated from the linear regression of the log y and the log $(t + 1)$ for both replicates, and the mean and standard deviation of the two replicates are shown in Table 2. The retention half-lives ($t_{1/2}$) can be calculated as

$$t_{1/2} = \exp(0.693/b) - 1. \quad (3)$$

Thus, Cd had the highest release rate coefficient, followed by Zn > Se > C > Cr. The release was somewhat comparable between the diatom and the dinoflagellate debris, with a few exceptions noted. The release coefficient was reduced by 2.2–3.4 ×, 1.6–2.1 ×, 1.7 × (for *P. minimum* only), 2.2–3.8 ×, and 1.2–1.9 × for Cd, Cr, Se, Zn, and C, respectively, in the NaN_3 treatment than in the microorganism treatment in both phytoplankton. Se release in diatom debris was, however, enhanced by 1.3 × in the presence of NaN_3. The retention half times were 0.5 d for Cd, 17–110 d for Cr, 1.3–1.6 d for Se, 0.8–1.5 d for Zn, and 2.9–3.8 d for C in both debris with microbial addition; and 1.5–3.1 d for Cd, 460–1867 d for Cr, 1.1–3.1 d for Se, 6.7–9.3 d for Zn, and 5.4–11.4 d for C with NaN_3 addition, respectively.

The relationship between the percentage of metals released from the decomposing debris and the release of C in both phytoplankton is shown in Fig. 2. Significant correlation was observed between the percentage of C release and the percentage of metal release in both types of particles. The slope describing the linear relationship between the percentage of metals released and the percentage of C release was greater than 1 for Cd, Se, and Zn, which indicates that these metals were released at a faster rate than the release of C, particularly in *P. minimum.* For Cr, the slope was smaller than 1, indicating that its release was slower than the release of C from the cells.

Release of colloidal carbon and metals—The percentages of trace metals and C associated with the colloidal phase during the decomposition period are shown in Fig. 3. Only a small fraction of Cd was found in the colloidal phase, which indicates that most Cd was released into the LMW phase (<5 kDa). For *T. pseudonana,* the percentage of Cd associated with the colloidal phase was 0.5–9%. For *P. minimum,* high percentage of Cd (16–84%) was found in the colloidal phase during the early stage of decomposition with microorganism addition, but this percentage then dropped to 4–9% after 8 d. Similarly, only 2–8% of Cd was in the colloidal phase in the NaN_3 treatment. For Cr, Se, and Zn, the proportion of colloidal phase tended to decrease with

Table 2. Release rate coefficient (d^{-1}) of metals and C in the decomposing phytoplankton debris (diatom *Thalassiosira pseudonana,* and dinoflagellate *Prorocentrum minimum*). Mean ± SD ($n = 2$).

Treatment	Cd	Cr	Se	Zn	C
T. pseudonana					
With bacteria	1.68 ± 0.16	0.147 ± 0.042	0.712 ± 0.001	0.765 ± 0.048	0.443 ± 0.012
With NaN_3	0.765 ± 0.011	0.092 ± 0.014	0.925 ± 0.018	0.339 ± 0.021	0.373 ± 0.018
P. minimum					
With bacteria	1.67 ± 0.01	0.239 ± 0.052	0.845 ± 0.032	1.14 ± 0.05	0.512 ± 0.024
With NaN_3	0.491 ± 0.008	0.113 ± 0.002	0.492 ± 0.007	0.297 ± 0.007	0.275 ± 0.004

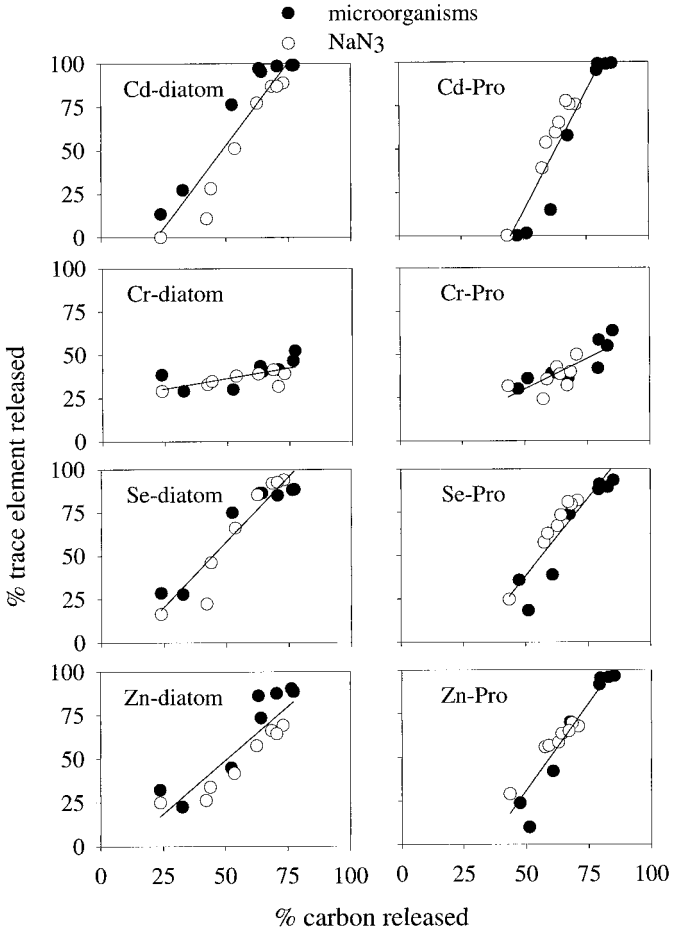

Fig. 2. The relationship between the percentage of trace elements and the percentage of C released from the decomposing phytoplankton (diatom *Thalassiosira pseudonana* and dinoflagellate *Prorocentrum minimum*, Pro) with microorganism addition or with sodium azide addition. Equations describing the relationship between the percentage of metals (y) and the percentage of C (x) released from the decomposing phytoplankton follow. For diatom *T. pseudonana* debris: Cd: $y = -42.1 + 1.9x$, $r^2 = 0.885$, $P < 0.001$; Cr: $y = 24.4 + 0.2x$, $r^2 = 0.449$, $P < 0.01$; Se: $y = -16.8 + 1.5x$, $r^2 = 0.891$, $P < 0.001$; Zn: $y = -12.0 + 1.2x$, $r^2 = 0.807$, $P < 0.001$. For dinoflagellate *P. minimum* debris: Cd: $y = -123.7 + 2.8x$, $r^2 = 0.884$, $P < 0.001$; Cr: $y = -5.6 + 0.7x$, $r^2 = 0.660$, $P < 0.001$; Se: $y = -52.1 + 1.8x$, $r^2 = 0.832$, $P < 0.001$; Zn: $y = -69.4 + 2.0x$, $r^2 = 0.895$, $P < 0.001$.

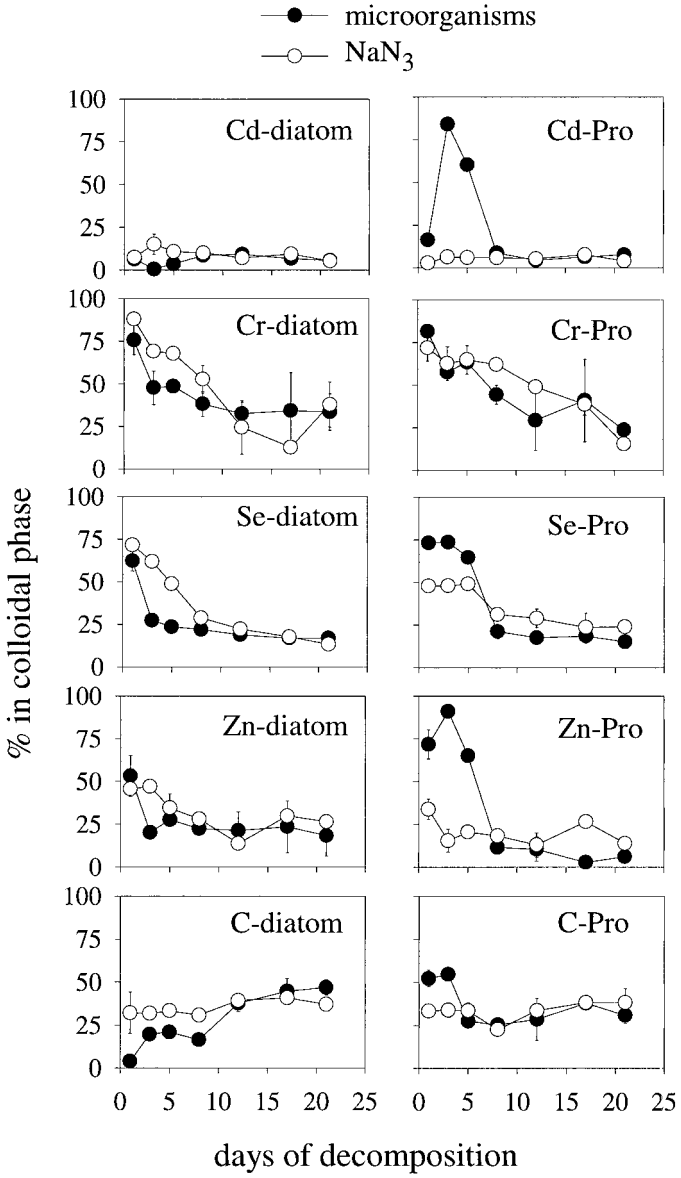

Fig. 3. The percentages of colloidal carbon and trace elements (Cd, Cr, Se, and Zn) released by the decomposing diatom (*Thalassiosira pseudonana*) and dinoflagellate (*Prorocentrum minimum*, Pro) with microorganism addition or with sodium azide addition. Data presented are mean ± SD ($n = 2$).

increasing time of decomposition. A higher percentage of metals was detected in the colloidal phase within the first 8 d, afterward it decreased and remained somewhat constant. For example, Cr was mostly in the colloidal phase within the first 8 d (48–88%). After 8 d, about 13–53% of Cr, 13–29% of Se, 14–30% of Zn in the diatom debris; and 15–49% of Cr, 15–31% of Se, 2–26% of Zn in the dinoflagellate debris was found in the colloidal phase.

For C, the percentage of colloidal C released from the diatom debris with microorganism addition increased with time of decomposition, from 4% on day 1 to 47% on day 21, whereas this fraction remained relatively constant during the course of experiment (31–41%) in the NaN_3 treatment. For *P. minimum* debris, 52–54% of dissolved organic carbon (DOC) was in the colloidal phase within the first 3 d, and this ratio then leveled off to 25–38% with microorganism addition. Similar to the finding for diatom debris, the percentage of colloidal carbon remained relatively constant (22–38%) in the NaN_3 treatment.

There was not a clear trend to indicate that the presence of microorganisms significantly affected the partitioning of metals into the colloidal phase. A high percentage of Cd, Se, Zn, and C was found in the colloidal phase within the first 5 d in the NaN_3 treatment, but the percentages of colloidal metals and carbon were comparable to those with microorganism treatment after 5 d.

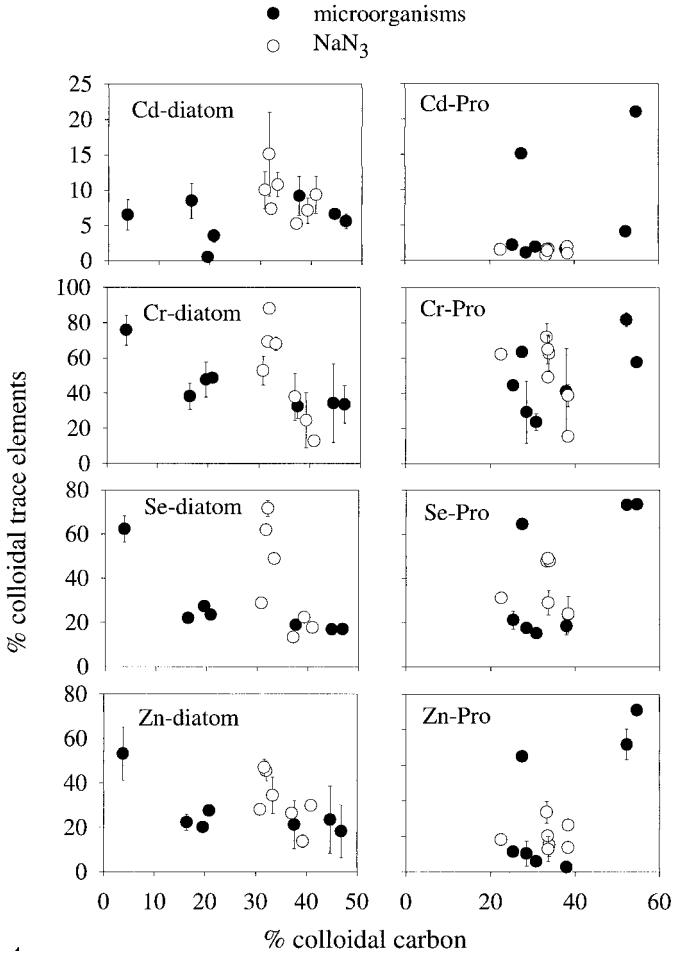

Fig. 4. The relationship between the percentage of colloidal trace elements and the percentage of colloidal C released from the decomposing phytoplankton (diatom *Thalassiosira pseudonana* and dinoflagellate *Prorocentrum minimum*, Pro) with microorganism addition or with sodium azide addition. Data presented are mean ± SD ($n = 2$).

The relationship between the percentage of metals and the percentage of C found in the colloidal phase is shown in Fig. 4. In general, there was no significant relationship between the percentage of colloidal metals and the percentage of colloidal C in both treatments.

Discussion

Retention of metals and carbon by decomposing phytoplankton—Lee and Fisher (1992*a*) emphasized that many previous studies investigated the decomposition of phytoplankton by killing the cells (by freezing or by heat). There have been relatively few studies that considered the release of metals and carbon from naturally decomposing cells (Lee and Fisher 1992*a*, 1993; Fisher and Wente 1993). These previous studies demonstrated that the most particle reactive elements are retained by planktonic debris for the longest periods. Temperature generally had a small effect on metal retention, indicating that the biological process such as microorganism activity may not directly release metals from the cells (Lee and Fisher 1992*a;* Fisher and Wente 1993). Similarly, Karl et al. (1988) indicated that biogenic particle decomposition can occur abiotically by fragmentation and dissolution. Free-living bacteria in seawater are mainly responsible for remineralization of dissolved organic carbon (Cho and Azam 1988; Karl et al. 1988).

Our results suggest that the release of Cd, Se, and Zn was faster than the release of organic carbon from the cells, similar to finding on Se by Fisher and Wente (1993). Fisher and Wente (1993) also indicated that other elements that they studied (Ag, Sn, Au, Am) were released at a slower rate than C, whereas Lee and Fisher (1992*a*, 1993) indicated that the release of Cd, Se, and Zn closely followed the release of C from the degrading diatoms regardless of the temperature and the level of microbial activity in the incubation system. Among the metals examined in our study, only Cr showed a slower release than C from phytoplankton debris.

The release rate coefficients of C determined in this study were similar to those found by Lee and Fisher (1992*a*, 1993). For example, we found a release rate coefficient of 0.443 d^{-1} for diatom debris and of 0.512 d^{-1} for dinoflagellate debris, compared to a release rate coefficient of 0.496 d^{-1} for diatom debris measured by Lee and Fisher (1992*a*) at the same temperature and with the addition of microorganism. However, the release rate coefficients of metals determined in this study were somewhat higher than those measured by Lee and Fisher (1992*a*). We found a coefficient of 1.68 d^{-1} for Cd, 0.712 d^{-1} for Se, and 0.765 d^{-1} for Zn in the diatom, compared to 1.19 d^{-1} for Cd, 0.484 d^{-1} for Se, and 0.345 d^{-1} for Zn determined by Lee and Fisher (1992*a*). Lee and Fisher (1992*a*, 1993) also examined the influences of the addition of different preservatives on the release of C and trace metals from diatom debris. Their data demonstrated that NaN_3 at 154 mM did not sufficiently inhibit the bacterial activity, in which up to 12% of total C was remineralized at 18°C. In contrast, Bidle and Azam (1999) indicated that NaN_3 at a concentration of 50 mM (concentration used in our study) strongly inhibited bacterial colonization in the diatom detritus. In our study, we found that the addition of 50 mM NaN_3 reduced the bacterial density in suspension by about 7 times. We did not specifically evaluate the effectiveness of NaN_3 on bacterial growth at different concentrations. The density of free-living bacteria was, however, too low in our experiment. The reason was not clear from our study. We did not quantify the density of bacteria in the detritus, which appeared to be low based on our measurements of $^{14}CO_2$ remineralization from the detrital particles. Thus, our data on the microbially mediated degradation of phytodetritus into the colloidal phase may not be representative of the expected response in a natural system because of the low bacterial biomass found in our microorganism treatment.

Our study demonstrated that the releases of carbon and trace elements by diatom debris (with siliceous frustules) was generally comparable to or lower than the release by dinoflagellate debris. Fisher and Wente (1993) measured the release of particulate organic carbon (POC, by CHN analysis) and found that the release of POC in the diatom (6–11%) was somewhat higher than the release of organic carbon in the dinoflagallete *P. minimum* (4–6%).

The release of trace metals by biogenic particles quantified using radiotracer technique is complicated by metal reequilibrium with the seawater medium following particle resuspension (Lee and Fisher 1992*a*). Fisher and Wente (1993) discussed the possibility of equilibrium partitioning in controlling the release of metals from decomposing cells. They suggested that much of the initial decline in cell radioactivity immediately following resuspension into unlabeled seawater is likely due to the rapid reequilibrium of the radioisotopes between the cells surfaces and the ambient water. However, repartitioning is less of a problem for metals that are mostly associated with the intracellular pool (Fisher and Wente 1993; Lee and Fisher 1993). All metals (except Cr) that we studied were mostly associated with the intracellular pool of algal cells. Consequently, reequilibrium was only likely for Cr, most of which was associated with the cell surface (Wang and Fisher 1996; Wang and Guo 2000). Consistently, these metals (except Cr) showed little release from the cells following resuspension into the radiotracer-free water. In addition, we removed the surface weakly bound metals by resuspending the algal cells twice in radiotracer-free water. The decomposition of phytoplankton debris was followed in LMW seawater without the presence of natural colloids to minimize the potential equilibrium between new colloids and background colloids. This was necessary for a realistic interpretation of the decomposition data.

The rapid release of C from resuspended cells into the DOC pool remained unanswered. It may be likely that the extracellular carbon was released into the ambient environment immediately following the resuspension of radiolabeled algae into the ^{14}C-free medium. For Cd, Se, and Zn, which were mostly associated with the intracellular pools, metals were released following 1–3 d of incubation, during which time the cells presumably began to decompose and the intracellular metals were released into the ambient water. Our study could not conclude that the releases of C and metals were directly coupled because the releases of elements were inherently affected by the time course of decomposition.

Release of colloidal carbon and metals—It should be noted that our experimental approach did not allow for exclusion of the possibility of colloidal coagulation, which may also affect the interpretation of experimental results. Although our previous study demonstrated that colloidal coagulation was extremely slow without the presence of particles (Wang and Guo 2000), it may be possible that the produced radiolabeled colloids resorbed onto the decomposing particles. This pathway was not examined in our study. Thus, our colloidal production data at best represented the net production of colloidal organic carbon and trace elements. The unidirectional flux of carbon and metals from larger particles to colloidal particles and, conversely, the unidirectional flux from colloidal particles to larger particles were not evident from our study. Further studies are required to examine the dynamic changes of metals between these two phases. A high concentration factor has been shown to eliminate the retention of LMW molecules during the ultrafiltration (Guo and Santschi 1996; Guo et al. 2000). Overestimation of colloidal fraction is less likely since the concentration factor used during the centrifugation was very high (i.e., centrifuge to near dryness).

In our study, we examined the partitioning of elements during the decomposition of unialgal cells over a relatively short period of time. It should be noted that the colloidal particles in natural systems may have different composition and origins (Aluwihare et al. 1997; Opsahl and Benner 1997). However, our measurements of the colloidal fractions of carbon and trace metals were comparable to many previous field measurements of colloidal association. For example, <10% of colloidal Cd was observed in estuarine waters of San Francisco Bay (Sanudo-Wilhelmy et al. 1996) and Narragansett Bay (Wells et al. 1998). The fraction of Zn associated with the colloids was also found to be <10% in both San Francisco Bay (Sanudo-Wilhelmy et al. 1996) and Narragansett Bay (Wells et al. 1998). Colloidal fraction was, however, much higher in other estuarine regions (Dai et al. 1995; Martin et al. 1995; Powell et al. 1996; Wen et al. 1999). For example, Wen et al. (1999) showed that 44% of Cd and 91% of Zn were in the colloidal phase (>1 kDa) in Galveston Bay. Our measurements of Se were also comparable to previous studies, for example, 30–60% as reported by Takayanagi and Wong (1984) and Santschi et al. (1987). There are few field measurements available for Cr to be compared with our data.

For comparison, colloidal organic carbon has been shown to represent 14–46% of total DOC in different coastal and oceanic waters (Guo et al. 1995). In our study, about 4–54% of phytoplankton-derived DOC was in the colloidal phase. After 5 d of decomposition, this fraction was relatively constant, ranging from 16 to 47% in the diatom and 25 to 38% in the dinoflagellate experiments. Thus, decomposition of phytoplankton debris may contribute to the production of colloids in seawater.

Various sources of colloidal origins have also been speculated, including exudation of peptides, terrestrial humic substances, and terrestrial organic matter. Aluwihare et al. (1997) and Aluwihare and Repeta (1999) demonstrated that colloidal organic matter (COM) was rich in acyl heteropolysaccharides (APS). These studies, along with others, concluded that a large fraction of persistent COM is produced by direct algal biosynthesis (Bianchi et al. 1995; Skoog and Benner 1997; Aluwihare and Repeta 1999). Wells et al. (1998) speculated that the primary sources for colloidal metal-complexing ligands in surface waters of Narragansett Bay are the predatory discharge of intracellular HMW components. Santschi et al. (1995) and Bianchi et al. (1997) found that samples collected from Galveston Bay water contained a high aromatic content, which is an indication of terrestrial humic substance, whereas samples collected from the open Gulf of Mexico had a more carbohydrate-like carbon composition that decreased from surface to deeper waters. Recently, Santschi et al. (1998) showed that an important fraction of colloidal organic matter consists of fibrillar material that is rich in polysaccharides, further suggesting the biological origins of colloidal materials.

The fraction of colloidal carbon and metals decreased with increasing time of decomposition. However, the dynamics of colloidal production remain unknown. Because Cr is a particle reactive metal, the decrease of colloidal Cr with time

(albeit the fraction of Cr in the >0.2-μm fraction remained somewhat constant) could result from both scavenging and degradation of colloidal Cr. Presumably, the production of new colloids may be slower than the destruction and coagulation of colloidal materials. This would need to be tested further. Several recent studies have demonstrated the assemblage of small colloids into the large particles (Honeyman and Santschi 1989; Wells and Goldberg 1993; Chin et al. 1998). Our study strongly highlights the importance of particle degradation from large particles to colloidal particles and further down to low molecular weight compounds, consistent with other previous studies (Amon and Benner 1994, 1996; Guo et al. 1996, 1997*b*).

Our study also indicated that there was no correlation between the colloidal carbon and colloidal metals in decomposing algal debris. Although the decomposition of metals followed the decomposition of C from the large debris (>0.2 μm) to small particles, metals and C were decoupled in their association with the colloidal particles, implying that the metals may selectively bind with specific ligands within colloidal organic carbon pool. Similarly, many field studies have not observed a close relationship between the percentage of trace metals and the percentage of organic carbon in the colloidal phase (e.g., Dai et al. 1995; Powell et al. 1996; Wen et al. 1999). There is, however, a general trend that both the percentage and concentration of colloidal organic carbon and colloidal metal decreased from estuarine to coastal and open oceanic regions (Guo et al. 1995; Wen et al. 1999).

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