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Influence of microalgal biomass on absorption efficiency of Cd, Cr, and Zn by two bivalves from San Francisco Bay

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Abstract

The bioavailability to clams (*Potamocorbula amurensis* and *Macoma balthica*) of Cd, Cr, and Zn from suspended particulate material (SPM) collected during a phytoplankton bloom was compared to bioavailability from SPM dominated by resuspended sediments. Bioavailability was also compared among mudflat sediments amended with different levels of living benthic microalgae. Bioavailability was defined by absorption efficiencies determined using pulse-chase protocols, modified for studying natural particle assemblages. The partitioning of Cd and Zn to particles (K_d) increased as the microalgae biomass (Chl *a*) increased in the particle assemblages; partitioning of Cr was less affected by the algal biomass. The clams fed particle assemblages enriched with microalgae absorbed Cd and Zn with significantly greater efficiency than did the clams fed algae-poor particles. This was partially explained by the greater occurrence of Cd and Zn in the cytosolic fraction of the particle assemblages that were microalgae enriched, as well as by the efficient absorption of cytosolic material by the clams. Among metals, Zn was most efficiently absorbed by both clams, and Cr the least. *M. balthica* absorbed Zn more efficiently from all types of food particles (39–82%) than did *P. amurensis* (13–50%). *P. amurensis* absorbed Cd with greater efficiency from the bloom SPM (44–48%) than did *M. balthica* (13–21%), but the two clams absorbed Cd similarly from benthic microalgae (26–51%). The addition of microalgae to complex natural particle assemblages clearly affected the bioavailability of associated metals, so studies using sediments (or suspended particulate material) that do not include a realistic living food component could underestimate metal bioavailability from particle ingestion.

Aquatic organisms can accumulate metals from both dissolved phases and from ingestion of particulate material (Luoma 1989; Fisher and Reinfelder 1995; Wang et al. 1996; Thomann et al. 1995). The bioavailability of metals to organisms from each source is influenced by complex physicochemical processes. Factors affecting the bioavailability of dissolved metals include pH, temperature, salinity, dissolved organic carbon (DOC), and redox chemistry, all of which influence metal speciation (Sunda and Guillard 1976; Campbell and Tessier 1989). Studies that have demonstrated the significance of metal bioaccumulation via ingestion have often employed pure cultures of bacteria or algae (Reinfelder and Fisher 1991, 1994; Decho and Luoma 1991; Wang et al. 1996) or uniform types of particles (Luoma and Jenne 1977; Harvey and Luoma 1985; Decho and Luoma 1994). Studies of processes influencing metal bioavailability from sediments or suspended particles have usually emphasized geochemical influences (Luoma and Bryan 1978; Maloney 1996; Gagnon and Fisher 1997). Sediment bioassays, which are widely used to determine contaminant bioavailability or toxicity, are principally interpreted on the basis of the geochemical characteristics of the sediment used (e.g. DiToro et al. 1990).

Less attention has been paid to the influence on metal bioavailability of the dynamic living fraction of the complex particle assemblages that occur in sediments or water column, even though carbon can be better absorbed from living cells than from detritus, and living cells often constitute a main food source in such natural assemblages for suspension-, detritus-, and deposit-feeding organisms (Lopez and Levinton 1987). Some studies indirectly suggest that metals associated with the living component of sediments may be of relatively high bioavailability via ingestion. Decho and Luoma (1991, 1994) reported that benthic bivalves absorbed Cr with much greater efficiency when they were fed on Cr-labeled bacteria cultures than on uniform assemblages of Cr-labeled inorganic particles. Greater Cd and Zn assimilation by the blue mussel *Mytilus edulis* from pure phytoplankton cultures than from resuspended Long Island Sound suspended particulate material (SPM) was observed by Wang and Fisher (1996) and Wang et al. (1996). Bruner et al. (1994) reported that the bioavailability of some organic pollutants including DDT and PCB to the zebra mussel, *Dreissena polymorpha,* was significantly greater when they ingested contaminated cultures of microalgae as compared to contaminated suspended sediments. We directly studied the bioavailability of metals from complex natural assemblages of particles with different abundances of microalgae, one of the most important of the living components of such assemblages.

Phytoplankton blooms have an important influence on the composition of SPM and the partitioning of trace metals (K_d) between dissolved and particulate phases (Wrench and Measures 1982; Sharp et al. 1984; Luoma et al. 1988). South San Francisco Bay (SFB) has a well-defined, seasonal phytoplankton bloom (Cloern 1996) that removed Cd, Ni, and Zn from the water column as they were preferentially taken up by the plant cells (Luoma et al. 1988). Animals such as bivalves exploit this food source; rapid growth and reproduction often accompany the South SFB bloom (Thompson and Nichols 1988). Similar algal blooms occur in sediments (Thompson and Nichols 1988) and are also accompanied by

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Table 1. Characteristics of the particles, experimental media, and clams used for the feeding experiments. The bloom particles were collected from South San Francisco Bay on 7 March (Exp. 1) and the nonbloom particles on 22 September 1995 (Exp. 2). The mudflat particles (surface-oxidized sediments, concentrated benthic microalgae, and mixture of these two particles) were collected from Palo Alto, South San Francisco Bay, on 6 October 1995 (Exp. 3). Chl *a* is shown as concentrations in the particles and suspended load in the feeding media.

	Water column: Exp. 1 and 2		Mudflat: Exp. 3		
	Bloom	Nonbloom	Sediment	Sediment and microalgae	Microalgae
Particle assemblage					
Chl *a* (μg g^{-1})	400	50	12	1,200	5,300
Experimental media					
Chl *a* (μg liter^{-1})	12	1	2	109	197
Dry wt (mg liter^{-1})	30	26	140	88	37
Salinity (‰)	15	21	21	21	21
Clams					
Avg shell length (mm)					
P. amurensis	8.6	12.9	14.1	14.2	14.5
M. balthica	17.3	17.7	19.8	19.4	18.9
Avg dry wt (mg)					
P. amurensis	28	27	32	33	36
M. balthica	106	116	163	154	142

growth responses in consumer species (Miller et al. 1996). Field studies indicate that changes in metal body burdens may coincide with seasonal changes in the feeding behavior of consumer species (Snyder and Hendricks 1995). Changes in metal exposures of bivalve consumers could occur during algal blooms if the algae is selectively grazed, the algae is either enriched or depleted with metals compared to the abiotic SPM, or the form of the metal in the algae is different from that in nonliving SPM.

We hypothesized that bivalves ingesting a natural assemblages of particles enriched with fresh algal cells will accumulate metals more efficiently than do bivalves exposed to particles dominated principally by inorganic material. To test this idea we conducted radiotracer experiments comparing the bioavailability of Cd, Cr, and Zn to two benthic bivalves. We fed the bivalves suspended particles collected during the seasonal phytoplankton bloom in South SFB and, for comparison, particles collected during a season when phytoplankton productivity was low and the SPM was dominated by resuspended sediments (Cloern 1996). We also tested the influence of a microalgal bloom by comparing metal absorption from surface sediments collected from a mudflat with absorption from the surface sediments amended with different densities of natural benthic microalgae. The three metals were chosen because Cd and Zn tend to penetrate the cytoplasm of algal cells (Reinfelder and Fisher 1991) whereas Cr is more surface-reactive. Two dominant species of benthic bivalves in SFB, *Macoma balthica* and *Potamocorbula amurensis,* were compared to test whether organisms with different feeding, digestion strategy, and gut passage time show different metal absorption ability. *M. balthica* is primarily a deposit-feeder and facultatively a suspension-feeder with a gut residence time of ~72–96 h. It inhabits primarily the intertidal zone of estuaries such as SFB (Harvey and Luoma 1984; Hummel 1985). *P. amurensis* is an invader species to SFB from eastern Asia and became an abundant benthic bivalve in the bay after 1986 (Carlton et al. 1990). This euryhaline filter-feeding clam has a very rapid filtration rate (Werner and Hollibaugh 1993) and a rapid gut passage time (~24 h) (Decho and Luoma 1991), and is found in a variety of substrates in both the intertidal and subtidal zone of the bay (Brown and Luoma 1995).

To determine the bioavailability of metals to clams we adapted radiotracer protocols (Reinfelder and Fisher 1991; Luoma et al. 1992) to measure absorption efficiency (AE), which measures the physiological absorption of metals in soft tissues of animals following ingestion of food.

Materials and methods

Three sets of experiments were conducted to determine bioavailability of metals associated with the different particle assemblages (Table 1). Exp. 1 was conducted with SPM collected during the spring phytoplankton bloom in South SFB in 1995 when SPM was enriched with fresh phytoplankton biomass. Exp. 2 was conducted in the fall when SPM was dominated by resuspended inorganic particles, and Chl *a* concentrations were low. Exp. 3 compared the bioavailability of metals among sediments collected from the oxidized surface layer of an intertidal mudflat, a concentrated assemblage of benthic microalgae, and a mixture of the surface sediments and the microalgae.

Experimental particle assemblages—Suspended particulate matter from the water column: The natural particle as-

semblages and seawater used for Exp. 1 and 2 were collected at USGS station 30 in South SFB on 7 March and 22 September 1995, respectively. Hydrographic surveys under way at the time (Edmonds et al. 1996) verified that the earlier collection coincided with the annual phytoplankton bloom in South SFB (Cloern 1996). The later collections were conducted when SPM was dominated by resuspended particles during the seasonal period of persistent diurnal winds and low phytoplankton production in South SFB. Seawater was immediately brought into the laboratory on the day of collection and kept in the dark at 10°C. On the following day, the seawater was transferred to each of three screw-capped 2-liter Erlenmeyer flasks. Two flasks each received 66 kBq (1 μCi = 37 kBq) of carrier-free ^{109}Cd(II) (1 pM), 300 kBq of ^{51}Cr(III) (140 pM), and 73 kBq of carrier-free ^{64}Zn(II) (15 pM). All the isotopes were in 0.1 N HCl and were purchased from Amersham. The spiked Cd and Zn levels were two orders of magnitude lower and Cr level was an order of magnitude lower than dissolved metal concentrations in SFB (Flegal et al. 1991). Immediately after isotope addition, pH of the seawater was adjusted to 8.0 by addition (microliter quantity) of 0.1 N NaOH. The remaining flask was used as a parallel control to measure dry weight and Chl *a* content at the end of the incubation. The isotope-spiked media were then incubated at 18 ± 1°C for 3 d. During the incubation with isotopes, the flasks were shaken twice a day. The sample collected in March (Exp. 1) was incubated on a light/dark cycle (14 : 10 L/D) to maintain phytoplankton biomass in the water. The sample collected in September (Exp. 2) was incubated in the dark to minimize growth of photosynthetic organisms.

The biomass of phytoplankton in the adjacent stations (Sta. 27 and 30) on 7 March 1995 was dominated by diatoms *Thalassiosira rotula* (~80%), *Ditylum brightwelii,* and *Skeletonema costatum,* while some *Rhodomonas salinas* was found on 22 September 1995 (Edmonds et al. 1996). The composition of phytoplankton species could have changed during the transportation and incubation due to processes such as the mortality of sensitive taxa, nutrient depletion, and grazing. No attempt was made to measure the species composition of phytoplankton after the incubation.

Particles collected from the mudflat: On 6 October 1995, oxidized surface sediment and benthic microalgae were collected from the Palo Alto mudflat in South SFB for Exp. 3. The surface brown-colored layer of sediment (<1 cm in depth) was collected after the uppermost surface biomass was gently scraped off. The benthic microalgae was concentrated from the mudflat using the method of Khechfe (1997). Briefly, two layers of acid-cleaned 63-μm Nytex meshes (12 × 12 in.) were laid out on the mudflat where benthic microalgae were visually abundant. After 0.5–1 h, the microalgae had migrated up onto the meshes; the top layer of mesh was then removed into acid-cleaned 1-liter polyethylene bottles. The dominant algal species on the mudflat in the fall of 1995 were *Achnanthes hungarica, Cylindrotheca gracilis, Navicula* sp., and *Nitzschia* sp. (Khechfe 1997). No attempt was made to enumerate other organisms such as bacteria, protozoa, and crustaceans, but protozoa and crustaceans were not visibly abundant. Throughout the text, *microalgae* is used to describe the living biomass in the particles collected from water column or mudflat that could have contained some living organisms other than algae.

After being brought into the laboratory, the surface sediment was resuspended, vigorously mixed into a 200-ml cylinder using 0.4-μm filtered 21‰ seawater made by diluting the seawater collected from a coastal site near the Long Marine Facility (Univ. of California at Santa Cruz) with Milli-Q water. The suspension was allowed to settle for 1 min, the overlying seawater with unsettled particles was transferred through 63-μm mesh to a 100-ml beaker, and this sediment was then resuspended into a separate 4-liter flask. The microalgal concentrate was resuspended in similar water in a 4-liter flask. Each flask was spiked with 260 kBq of ^{109}Cd, 414 kBq of ^{51}Cr, and 196 kBq of ^{64}Zn. These samples were incubated as described previously. Following 3 d radiolabeling of the two media, a mixture of microalgae and sediment was constructed by mixing equal amounts of the sediment and microalgae media.

Experimental animals—About 40–80 *M. balthica* were collected from the same mud-flat as the benthic microalgae, 4 d prior to each feeding experiment. About 40 *P. amurensis* were collected from the RV *Polaris* with a Van Veen grab from North SFB at USGS Sta. 8.1 for Exp. 1 and at USGS Sta. 12.5 for Exp. 2 (Brown and Luoma 1995). An additional 80 *P. amurensis* were collected from South SFB at USGS Sta. 30 for Exp. 3. Upon returning to the laboratory, the clams were gradually acclimated for a week to the temperature, salinity, and food concentration used in experimental conditions. The clams were depurated for 2 d before the feeding experiments, and the particles adhering to the shell were removed. Experiments were conducted at 10°C (the salinities shown in Table 1). Shell length and dry weight of the clam tissue were determined after the feeding experiments (Table 1).

Pulse-chase experiments—The pulse-chase method was used to determine absorption efficiencies of metals from ingested particulate materials (Fisher and Reinfelder 1991; Decho and Luoma 1991; Luoma et al. 1992; Wang and Fisher 1996). Each of the two clam species was randomly divided into groups of three animals and each group was placed in a feeding chamber. *P. amurensis* was fed radioactive food for 1 h and *M. balthica* was fed for 2.5 h, so that each species ingested a measurable bolus of radioactivity but did not defecate radioactive feces during the "hot feed" (Decho and Luoma 1991). After the pulse feeding, clams were placed in uncontaminated feeding chambers designed to minimize reingestion of feces, and depurated in isotope-free seawater containing unlabeled food of the same type used in the pulse of radiolabeled feeding. *P. amurensis* was depurated for 24 h and *M. balthica* for 72 h. During this time >92% of unabsorbed radioactivity was egested from the digestive tract of the clams (Decho and Luoma 1991). The radioactivity retained in the tissues was defined as absorbed. The unlabeled water was changed periodically during the "cold feed" to prevent reabsorption of dissolved radioactivity released from feces and shell surface and to remove metabolic waste. Whole-body radioactivity remaining in the clams and egested radioactivity in feces were determined at

0, 2, 3, 6, 9, 12, and 24 h for *P. amurensis* and at 0, 3, 5, 8, 11, 23, 30, 46, 54, and 72 h for *M. balthica.* During this procedure animals were nondestructively counted for radioactivity and returned to the cold feeding chamber for continuous depuration.

After the suspensions were radiolabeled, it was not possible to separate by filtration and then completely recover the radiolabeled natural particulate assemblage (as can be done in pure algal cultures), so the clams were fed labeled food in labeled media for the hot feed. It was therefore necessary to correct for potential uptake from solution during the hot feed. This was done by exposing subsets of each clam species to an aliquot of 0.4-μm filtered radiolabeled suspension for the same time as the hot feed (1 h for *M. balthica,* 2.5 h for *P. amurensis*).

Radioactivity was determined with a gamma counter equipped with a 3-in. well-type NaI crystal detector. Photon emissions of ^{109}Cd were determined at 88 keV, ^{51}Cr at 320 keV, and ^{65}Zn at 1,115 keV. The counting efficiencies of ^{109}Cd, ^{51}Cr, and ^{65}Zn were 37.2 , 4.6, and 5.8 %, respectively. The counting times for all samples were 1–5 min, and propagated counting errors were generally <5%. All the dpm values were corrected for decay and expressed in terms of unit dry weight of soft tissue.

Absorption efficiency—Immediately after hot feeding, the radioactivity of all groups of live clams was determined nondestructively. This value represented the initial (t = 0) whole-body (tissue + shell) activities taken up from each source (solution and food). Selected clams were also sacrificed at t = 0 to determine radioactivity in the soft tissue and shell before depuration. At the end of the depuration the clams were dissected and radioactivity in soft tissue and on shell was determined.

Absorption efficiency (AE_1) was calculated using initial tissue activities from the whole-body analysis at t = 0 corrected for the activity on the shell ($C^*_{t=0}$):

$$\% \text{ AE}_1 = \frac{(C_{24} - C_{\text{diss}})}{(C^*_{t=0} - C_{\text{diss}})} \times 100, \quad (1)$$

where C_{24} is dpm in *P. amurensis* soft tissue after 24 h of depuration (72 h for *M. balthica*) and C_{diss} is dpm in soft tissue after 1 h (2.5 h for *M. balthica*) uptake from dissolved media. A ratio of radioactivity in soft tissue to activity in whole clams was determined from the subset of clams dissected immediately after the hot feed. To estimate $C^*_{t=0}$ the ratio was multiplied by the initial radioactivity in the clams later carried through for depuration.

To ensure that radioactivity in the shell did not bias whole-body determinations of AE, a second method of calculation was compared to the above. This method (AE_2) employed a mass-balance approach to determine the initial ingested activity;

$$\% \text{ AE}_2 = \frac{(C_{24} - C_{\text{diss}})}{\left[\left(\sum C_{\text{feces}}\right) + (C_{24} - C_{\text{diss}})\right]} \times 100, \quad (2)$$

where $\Sigma\ C_{\text{feces}}$ was the sum of all activity in feces collected during the 24 (72) h depuration. C_{diss} was subtracted from C_{24} to correct metal absorption from dissolved phase. Metal loss from C_{diss} during depuration (24 h for *P. amurensis,* 72 h for *M. balthica*) was assumed to be negligible.

Fractionation of particle assemblages—The radiolabel taken up by the suspended sediments collected during the bloom, the benthic microalgae, and the mud-flat sediments were fractionated into a supernatant fraction and three pelletized fractions (Fisher et al. 1983; Reinfelder and Fisher 1991). Nonbloom SPM and amended sediments were not fractionated. Particles were collected on a 0.4-μm Nuclepore filter from the radiolabeled media and washed with buffered 10^{-3} M EDTA in filtered seawater (for 2 min). The cells were then lysed by resuspending in deionized water (pH 8.0) followed by freezing. The lysed particles were centrifuged at 10°C and resulted in pellet 1 (750 × g, 5 min), pellet 2 (2,000 × g, 15 min), and pellet 3 (10,000 × g, 15 min). The supernatant was removed after the third centrifugation. In pure biological samples, the supernatant, termed hereafter the cytosol, would contain radioactivity from intracellular solution as well as endoplasmic reticulum, ribosomes, and Golgi complexes (Sheeler 1981). Pellets 2 and 3 would contain mitochondria, lysosome, and peroxisomes. Metals in the supernatant plus pellets 2 and 3 are herein termed cytoplasmic metal. Pellet 1 included activity associated with cell walls, plasmalemmae, nuclei, and other cell debris. It was possible that some of the metals could have redistributed during fractionation procedure; however, redistribution of metals in a similar fractionation procedures was reported to be minimal (Viarengo et al. 1988; Roesijadi and Klerks 1989). The components of natural particle assemblages included in these fractions would be more complex than fractionated pure phytoplankton. Nevertheless, the approach provides an operational definition of metal fractionation that may be relevant to bioavailability.

Results

Experimental particle assemblages—The assemblage of suspended particles collected during the spring bloom contained 8 times more Chl *a* than did the particulate material collected during the fall nonbloom conditions (Table 1). The Chl *a* content of the mudflat sediments was lower than the nonbloom SPM. Enriching the mudflat sediments with microalgae increased Chl *a* content 100-fold. The highest Chl *a* concentrations were found in the microalgal concentrate.

Metal partitioning on particles—After 3 d of labeling, ^{51}Cr was the most (>78%) sorbed and ^{109}Cd was the least (< 50%) sorbed of the metals, to all types of particles (Fig. 1). The partition coefficient (K_d) for Cr was greater than that for Zn; Cd had the lowest K_d in each treatment. Partition coefficients varied widely among particle assemblages, especially for Cd and Zn. Particles from the water column had a higher K_d (4–5 × 10^5) for Cr than those (8–9 × 10^4) from the mudflat. K_d values for Cd and Zn were highest for particle assemblages enriched with microalgae. For example, the Cd and Zn values for the particles collected from the mudflat followed the order: unamended surface sediment (5 × 10^2 for Cd, 7 × 10^3 for Zn) < mixed particles (2 × 10^3 for Cd, 2 × 10^4 for Zn) < benthic microalgae (1 × 10^4 for

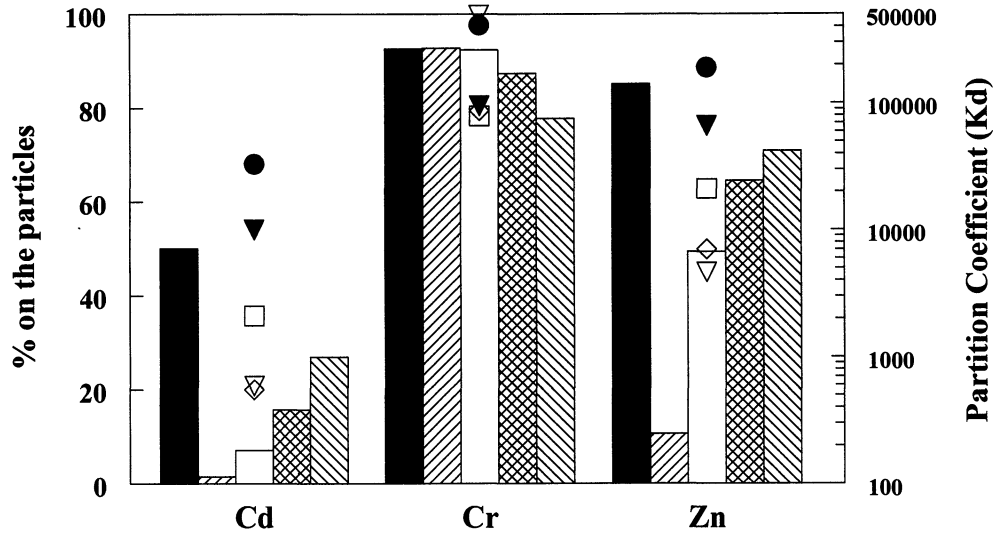

Fig. 1. Percentages partitioned to particles (bar) and partition coefficient (symbol) of Cd, Cr, and Zn for the particle assemblage collected from the water column or a mudflat after 3 d labeling with radioisotopes. Spring bloom particles, solid bar or ●; fall nonbloom particles, left slashed bar or ▽; unamended surface sediment, empty bar or ◇; sediment amended with benthic microalgae, hatched bar or □; concentrated benthic microalgae, right slashed bar or ▼.

Cd, 7×10^4 for Zn), increasing with the increase in Chl *a* concentration (Table 1).

The partitioning of radioactivity among different pools after centrifugation of lysed particles is shown in Table 2. The cytosol contained 38–63% of the Cd and Zn in the SPM collected during the bloom or the microalgae concentrated from the mudflat. Only 8–10% of the Cd and Zn in microalgae-poor sediment was in the cytosol. The cytosol contributed the largest proportion of metals to the cytoplasmic fraction (supernatant plus pellets 2 and 3) in the algae-rich treatments. The largest proportion of the cytoplasmic fraction in the algae-poor fraction came from pellet 2. Little Cr was found in the supernatant in any treatment, although the highest proportion was fractionated to the cytosol in particle assemblages enriched with microalgae.

Absorption efficiency—Corrections for uptake from dissolved phase and shell adsorption: Tables 3 and 4 show the details of the pulse-chase experiments. The tables illustrate the contribution of uptake from dissolved phase and adsorption to shell, both of which might bias determination of absorption efficiency in experiments where animals are fed labeled food in labeled media (i.e. experiments with natural assemblages of particles). In most experiments, uptake of dissolved ^{109}Cd contributed <10% to either the initial bolus of radiolabel taken up or the tissue burden retained after egestion. The contribution of dissolved Zn uptake was <7% in most experiments. The contribution of dissolved Cr was <1% in all experiments. Generally, both methods of calculating AE for Cr and Zn (AE_1 and AE_2) yielded similar results ($P < 0.05$; Student's *t*-test). Where AE_1 and AE_2 were similar, it was also likely that other biases that might disturb the mass balance (e.g. desorption from fecal pellets during egestion and uptake of resultant dissolved radiolabel) were unimportant.

In a few instances, however, dissolved uptake was important. Clams exposed to nonbloom SPM or to unamended surface sediment obtained 17–68% of their initial tissue Cd from dissolved sources. *M. balthica* fed nonbloom SPM obtained 27% of their tissue Zn from solution. In each of these treatments, only a small proportion of the radiolabel was bound by the particulate material, so the dissolved concen-

Table 2. Percentage distribution of Cd, Cr, and Zn in different fractions of particles used for the pulse-chase feeding experiments. Metals fractionated in the supernatant pool were considered to be cytosolic extract. Pellet 1 contained inorganic material, detrital particles, cell membranes, granules, and cell debris. The cytoplasmic fraction was defined as the sum of pellet 2, pellet 3, and cytosol.

	Spring bloom			Benthic microalgae			Surface sediment		
	Cd	Cr	Zn	Cd	Cr	Zn	Cd	Cr	Zn
Pellet 1	28.8	60.7	37.0	46.2	73.1	40.4	82.3	67.4	72.8
Cytoplasm	71.2	39.3	63.0	43.8	26.9	59.6	17.7	32.6	27.2
Pellet 2	7.2	15.4	24.5	7.6	7.4	0.4	7.3	22.9	14.3
Pellet 3	0.7	8.4	0.4	8.3	3.2	0.6	2.4	6.0	2.6
Cytosol	63.3	15.5	38.2	37.8	16.3	58.6	7.9	3.7	10.2

Table 3. Data used to determine absorption efficiency (AE) of metals from the particles in the water column collected during the spring bloom or fall nonbloom season. The mean activity values and standard deviations (dpm/g dry wt/1,000) were determined from four groups of three individual clams. Tissue activity at $t = 0$ was estimated by the whole-clam activity and the ratio of tissue to whole-clam activity. (t = final was tissue activity after egestion of undigested radioactivity [24 h for *P. amurensis,* 72 h for *M. balthica*]; n.d., not determined).

		Whole-clam ($t = 0$)	Tissue to whole-clam ratio	Estimated tissue ($t = 0$)	Uptake from water	Tissue (t = final)	Sum of feces	AE_1	AE_2
P. amurensis									
Spring bloom									
Cadmium	Mean	857	0.72	617	11	280	290	44.5	48.2
	SD	57	0.05	41	0	38	47	6.4	5.0
Chromium	Mean	13,104	0.48	6,291	26	361	6,826	5.4	4.8
	SD	1,833	0.02	880	10	24	1,400	0.5	0.7
Zinc	Mean	4,816	0.50	2,421	15	997	1,859	41.2	35.2
	SD	384	0.02	193	2	91	364	6.1	6.3
Fall nonbloom									
Cadmium	Mean	61	0.57	35	6	12	14	20.8	31.3
	SD	4	0.07	2	1	2	4	7.0	12.6
Chromium	Mean	19,367	0.89	17,154	19	291	14,920	1.6	1.8
	SD	1,350	0.06	1,196	4	33	476	0.1	0.3
Zinc	Mean	1,210	0.65	791	53	310	390	34.6	39.6
	SD	84	0.05	55	4	41	31	3.5	5.1
M. balthica									
Spring bloom									
Cadmium	Mean	528	0.96	505	10	75	265	13.1	20.7*
	SD	154	0.10	147	2	22	116	1.6	5.1
Chromium	Mean	14,057	0.88	12,400	13	435	11,409	3.7	3.8
	SD	5,639	0.07	4,976	6	89	4,661	0.7	0.7
Zinc	Mean	3,569	0.81	2,905	29	2,014	966	69.9	68.2
	SD	1,176	0.05	957	9	593	414	3.7	4.8
Fall nonbloom									
Cadmium	Mean	19	0.32	6	4	n.d.	n.d.	n.d.	n.d.
	SD	2	0.05	1	0	n.d.	n.d.	n.d.	n.d.
Chromium	Mean	2,020	0.80	1,624	5	87	1,615	5.0	4.8
	SD	107	0.06	87	2	21	93	1.1	1.0
Zinc	Mean	255	0.34	88	22	57	36	51.4	47.4
	SD	16	0.07	5	1	6	9	11.3	2.0

* AE determined by two methods was significantly different ($P < 0.05$; t-test).

tration was high in the hot feed (Fig. 1). The resultant AE for Cd was not reliable because the amount of ^{109}Cd retained after egestion was largely influenced by the uptake from dissolved ^{109}Cd. Therefore, no AE is reported for these treatments. Generally, AE_2 was greater for Cd than AE_1 probably due to desorption or leaching of Cd from fecal material during depuration. For this reason, AE_1 was used to compare and discuss the results (*see* discussion).

A significant amount of radioactivity accumulated on the shells during the pulse exposures, so the correction for shell adsorption was also important. Shell contamination in AE experiments with pure cultures of phytoplankton is typically attributed to uptake of radionuclides from dissolved phase (Wang and Fisher 1996). However, in the present experiments, the shells of clams exposed to the medium with natural food particles obtained 1.3–9.2 times more Cd, 7–46 times more Cr, and 2–29 times more Zn than did the shells of clams exposed to dissolved metals only (data not shown). Thus, most shell activity was the result of adherence of radiolabeled particles to the shells, even though the shells were rinsed with unlabeled seawater. Generally, less particle adherence occurred and the ratio of tissue to whole clam activity was highest for clams exposed to the bloom SPM and the concentrated benthic microalgae. The adherence of particles to bivalve shells must be accounted for when AE is determined from natural particle assemblages that contain a substantial nonalgal component.

Comparison of AE among particle assemblages: In general, increases in living cellular materials in the particulate assemblage (i.e. greater Chl *a*) were accompanied by increased absorption efficiencies (Figs. 2, 3). Clams absorbed significantly more Cd and Zn from the particles collected during the spring bloom than from particles collected during the nonbloom season, where comparisons were possible

Table 4. Data used to determine absorption efficiency (AE) of metals by the clams fed the particles collected from Palo Alto mudflat, South SFB. The mean activity values (dpm/g dry wt/1,000) and standard deviations were determined from four groups of three individual clams. Tissue activity at $t = 0$ was estimated by the whole clam activity and the ratio of tissue to whole clam activity. (t = final was tissue activity after egestion of undigested radioactivity [24 h for *P. amurensis,* 72 h for *M. balthica*]; n.d., not determined.)

		Whole clam ($t = 0$)	Tissue-to-whole clam ratio	Estimated tissue ($t = 0$)	Uptake from water	Tissue (t = final)	Sum of feces	AE_1	AE_2
P. amurensis									
Cadmium									
Sediment	Mean	232	0.55	127	23	n.d.	n.d.	n.d.	n.d.
	SD	23	0.06	12	10	n.d.	n.d.	n.d.	n.d.
Sed+Micro	Mean	449	1.02	458	28	63	183	8.1	15.7*
	SD	91	0.12	93	10	9	27	1.3	2.1
Microalgae	Mean	429	1.06	454	28	139	134	25.7	44.6*
	SD	23	0.02	24	10	26	8	5.1	7.0
Chromium									
Sediment	Mean	8,722	0.80	6,972	17	139	5,170	2.0	2.5
	SD	2,348	0.06	1,876	5	14	709	0.6	0.5
Sed+Micro	Mean	8,687	0.91	7,926	17	143	7,637	1.6	1.6
	SD	1,420	0.05	1,296	5	35	1,020	0.2	0.2
Microalgae	Mean	5,687	0.83	4,743	17	565	3,961	11.6	12.2
	SD	478	0.04	398	5	100	174	1.8	2.3
Zinc									
Sediment	Mean	2,007	0.63	1,267	22	183	828	13.3	16.3
	SD	266	0.04	167	4	28	160	3.3	1.8
Sed+Micro	Mean	2,284	0.82	1,867	22	340	1,388	17.3	18.6*
	SD	383	0.07	314	4	47	197	0.8	0.1
Microalgae	Mean	1,929	0.85	1,638	22	676	645	40.5	50.4*
	SD	105	0.02	90	4	76	79	5.0	5.8
M. balthica									
Cadmium									
Sediment	Mean	43	0.43	19	7	n.d.	n.d.	n.d.	n.d.
	SD	10	0.12	4	3	n.d.	n.d.	n.d.	n.d.
Sed+Micro	Mean	78	0.90	71	7	12	23	8.2	18.8*
	SD	6	0.13	5	3	2	2	2.3	5.9
Microalgae	Mean	115	0.68	78	7	30	22	32.6	51.0*
	SD	3	0.22	22	3	5	1	7.1	6.4
Chromium									
Sediment	Mean	2,580	0.72	1,848	1	43	1,624	2.7	3.0
	SD	1,272	0.04	911	1	15	913	1.0	1.0
Sed+Micro	Mean	1,543	0.95	1,465	1	46	1,439	3.0	3.1
	SD	70	0.06	65	1	6	222	0.5	0.7
Microalgae	Mean	1,278	0.80	1,020	1	211	909	20.5	18.7
	SD	93	0.19	74	1	35	80	2.0	1.9
Zinc									
Sediment	Mean	488	0.44	214	7	93	148	41.8	38.8
	SD	186	0.07	81	2	36	81	2.9	4.3
Sed+Micro	Mean	416	0.73	302	7	164	129	53.2	55.3
	SD	22	0.08	17	2	5	28	2.5	4.6
Microalgae	Mean	440	0.71	312	7	259	64	82.4	79.5
	SD	34	0.21	24	2	24	2	2.1	2.0

* AE determined by two methods was significantly different ($P < 0.05$; t-test).

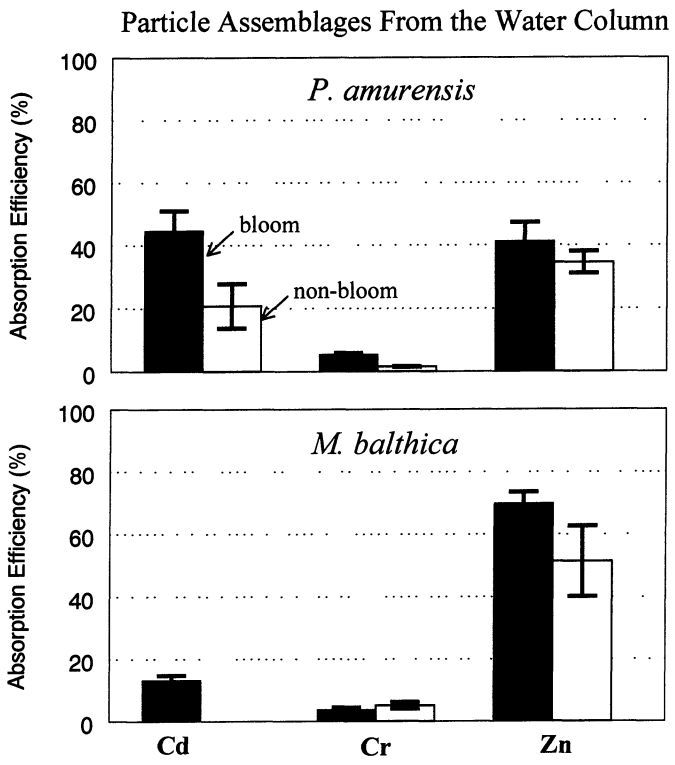

Fig. 2. Absorption efficiencies of Cd, Cr, and Zn by *Potamocorbula amurensis* and *Macoma balthica* fed particles collected during the spring phytoplankton bloom in 1995 in San Francisco Bay (solid bar) or particles collected in the fall 1995 during nonbloom conditions (empty bar). Values shown are means ±SD (n = 4). All metal AE values were significantly different ($P < 0.05$) between the two food types and between the two clams fed the same food type. AE of Cd in *M. balthica* for nonbloom conditions was not determined.

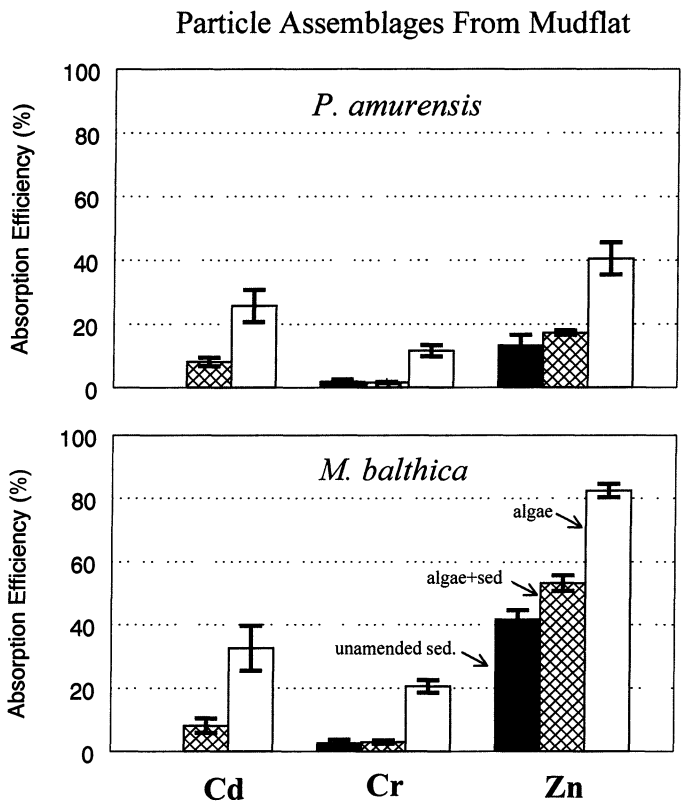

Fig. 3. Absorption efficiencies of Cd, Cr, and Zn by *Potamocorbula amurensis* and *Macoma balthica* fed on unamended sediment (solid bar), sediments amended with microalgae (hatched bar), or concentrated benthic microalgae (empty bar). Values shown are means ±SD (n = 4). All metal AE values were significantly different ($P < 0.05$) among the three food types except for the Cr AE between unamended sediment and amended sediment; AE values between the two clams fed the same food type were all significantly different except for Cd treatments and Cr for unamended sediment. AE of Cd for unamended sediment was not determined.

(Fig. 2). The AE of Cr by *P. amurensis* was significantly lower for nonbloom particles than for bloom particles, although the AE of Cr was low in all cases compared to that of Cd and Zn. Among the three types of mudflat particles, the clams fed concentrated benthic microalgae had the greatest absorption of all three metals (Fig. 3).

Absorption efficiencies of Cd, Cr, and Zn by *P. amurensis* were strongly related to the proportion of metal associated with the cytosol or the cytoplasm in the three particle assemblages that were fractionated (Fig. 4). Regardless of the metal, as the proportion of cytosolic metal increased, the proportion of metal absorbed by *P. amurensis* increased (R^2 = 0.88, $P < 0.001$; t-test for correlation coefficient; AE = 1.2 + 0.72(% cytosol)). The proportion of metal fractionated to the cytosol was greatest in the particles enriched with microalgae. Apparently, adding living microalgae to a particle assemblage increased AE because an increased proportion of the metal was in a bioavailable cytosolic form (extractable by lysing the living cells). The x-intercept of the relationship with the cytoplasmic metal fraction (R^2= 0.83, $P < 0.01$; AE = −18.7 + 0.92(% cytoplasm)) suggested that, especially in the algae-poor treatment, a fraction of cytoplasmic metal was not available for absorption (Fig. 4).

Absorption efficiencies of Cd, Cr, and Zn by *M. balthica* were not significantly related to the percentage of metal fractionated to the cytosol (R^2 = 0.23, $P > 0.05$) or to the cytoplasm (R^2 = 0.58, $P > 0.05$; Fig. 4). Cadmium was 63% fractionated to the cytosol in the SPM collected during the bloom, but Cd was absorbed with only 13% efficiency by *M. balthica.* This treatment greatly influenced the significance of the relationship of AE with cytosolic metal in *M. balthica.*

M. balthica absorbed Zn more than twice as efficiently as did *P. amurensis* in all experiments. Most notably, *M. balthica* absorbed Zn with 80% efficiency from the benthic microalgal concentrate. *M. balthica* also absorbed Cr with greater efficiency than did *P. amurensis.* In contrast, *P. amurensis* absorbed Cd with greater efficiency from the bloom SPM than did *M. balthica* (Fig. 2), but the two species absorbed Cd similarly from benthic microalgae.

Discussion

In this paper we have demonstrated that when microalgae are abundant (e.g. during blooms), the clams *P. amurensis*

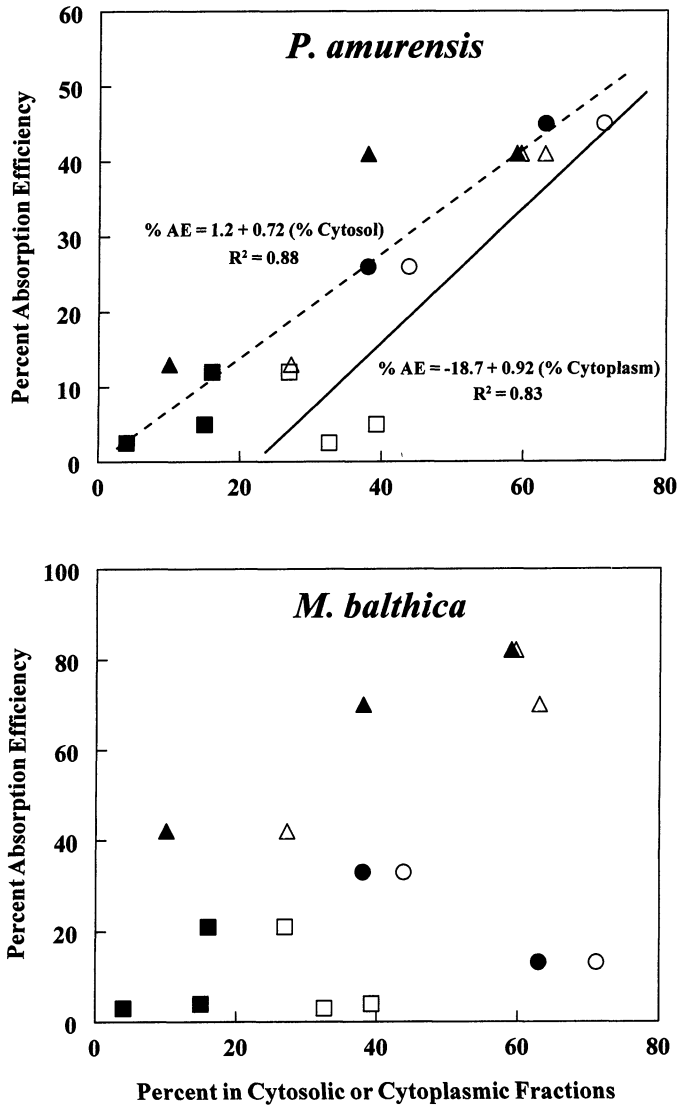

Fig. 4. Variation of absorption efficiency by *Potamocorbula amurensis* and *Macoma balthica* from three different particle assemblages (spring bloom SPM, unamended surface sediment, and benthic microalgae concentrate) as a function of the percent of Cd (circle), Cr (square), or Zn (triangle) in the cytosolic (solid symbols) or cytoplasmic fractions (empty symbols).

and *M. balthica* accumulate Cd and Zn with greater efficiency from ingestion than when the sediments or SPM are poor in microalgae. Absorption of Cr was also increased when a larger living biomass was present in the particle assemblage, but less so than that of Cd and Zn. Based on changes in absorption efficiency alone, phytoplankton blooms may result in as much as a twofold increase in Cd and Zn bioaccumulation from food suspensions, although the extent of the effect is both metal and species specific. This means that microalgal blooms may be a period of increased exposure of benthos to contaminants like Cd and Zn and a time when the risk of adverse effects increases. It also means experiments or bioassays using sediments or SPM that do not include a realistic living food component in the particle assemblage could underestimate bioavailability from particle ingestion.

One reason that the presence of microalgae enhances Cd and Zn bioavailability from particulate material may be that these metals are accumulated into the cytoplasm of the plants more efficiently than are more surface-reactive metals (as demonstrated by Reinfelder and Fisher 1991, 1994). Thus, particle assemblages with a greater microalgal biomass have more Cd and Zn in cytosolic form (extractable by cell lysing and centrifugation). In previous studies, absorption efficiencies by copepods increased with the proportion of metal in the cytoplasmic fraction of pure algal cultures (comparisons by Reinfelder and Fisher [1991] were among metals). In molluscs this relationship is more complex (Wang and Fisher 1996; Wang et al. 1996), but cytosolic metal is undoubtedly one of the important fractions of bioavailable metal in their food. Additional important organism-level biological processes include chemical reactions in the digestive fluids or the digestive tract (Fisher and Teyssié 1986; Mayer et al. 1996) and the characteristics of digestive processing (Decho and Luoma 1994, 1996).

Reinfelder and Fisher (1991, 1994) also found that the cytosolic fractions contained most of the cytoplasmic metal in the algal cultures. The same was true for the natural assemblage of particles enriched with algal cells in the present study. However, the cytosolic fraction of metals in microalgae-poor assemblages constituted only a small proportion of the cytoplasmic fraction (for surface-reactive Cr, this was true of all particle types). In natural assemblages of particles without large microalgal populations, pellets 1 and 2 may be dominated by material other than microalgae (e.g., inorganic particles, colloids, humic substances). These appear to be fractions unavailable for absorption by clams (*see* also Decho and Luoma 1994). Our data also show that a small proportion of the metals was found in the cytosolic extract from freshly collected surface sediments relatively poor in microalgae, suggesting the existence of some living material with a potential influence on metal bioavailability in all freshly collected natural sediments.

Although increased microalgal biomass seems to increase bioavailability of metals such as Cd and Zn to a variety of bivalves, differences in absorption efficiency also occur among these consumer species. *M. balthica* absorbed Zn more efficiently from all types of food particles than did *P. amurensis* in our experiments. The AEs of Zn for *M. balthica* were also 1.6–5 times higher than the values determined for *M. edulis* (Wang et al. 1996). In contrast, *P. amurensis* absorbed Cd with greater efficiency from the bloom SPM than did *M. balthica* (although the two species absorbed Cd similarly from benthic microalgae). To the extent that bioaccumulation is controlled by uptake from food (Wang et al. 1996; Thomann et al. 1995), the present experiments indicate that *M. balthica* will bioaccumulate Zn more efficiently than will *P. amurensis* or *M. edulis,* and thus better reflect changes in environmental concentrations of that element, whereas *P. amurensis* should be a better indicator of Cd contamination in estuarine environments. Brown and Luoma (1995) came to similar conclusions based on field collections where *M. balthica* and *P. amurensis* co-occur.

The reasons for the differences in absorption efficiencies among species are not fully understood, but it is clear that species-specific biological factors influence at least some aspects of metal absorption (and bioavailability) from food. For example, the short gut residence time (24 h) of *P. amurensis* compared to *M. balthica* could contribute to the more direct dependence of metal uptake on cytosolic metal in the former species (i.e. more noncytosolic metal might be absorbed during longer digestive processing). Different AEs from the natural particle assemblages could also result if *M. balthica* selectively ingested different particles from an assemblage than did *P. amurensis,* a consideration that can not be included in studies with pure cultures or uniform particle types. Such biological considerations should be more fully incorporated into conceptual models of factors affecting metal bioavailability.

A change in metal absorption efficiency is not the only aspect that could affect metal bioavailability as microalgae populations change in a particle assemblage. In our experiments, the partitioning of Cd and Zn to particles (K_d) differed by orders of magnitude as the biomass changed in the particle assemblage, so exposure concentrations from the different sources can change as microalgal biomass changes. Metal concentrations of the biological particles within the bulk particles may also differ from concentrations in the particle assemblage as a whole. Luoma et al. (1998) found that Cd concentrations in SPM in South SFB increased from 0.25 to 0.55 μg g^{-1} during a phytoplankton bloom, and calculated Cd concentrations in algal cells of 1.25 μg g^{-1}. In contrast Zn concentrations in algal cells were calculated to be about one-half the total Zn concentration in the SPM, and Ni concentrations were ~4 μg g^{-1}, <10% of the concentration in SPM. In these circumstances, organisms that selectively feed on benthic microalgae or phytoplankton could have higher Cd exposures and lower exposures to Zn or Ni than organisms that feed on mixtures of detritus and microalgae or nonselective deposit-feeders (in terms of total metal concentration). Exposures could vary if feeding selectivity or the relative abundance of the living component of the diet changes with changes in microalgal populations.

Cr is a more particle-reactive element than is Cd or Zn, as noted by the high K_d values for Cr found in this study. The low cytoplasmic Cr suggests that most Cr partitions to the surface of phytoplankton cells with only minute fractions penetrating into the cytoplasm. Absorption of Cr from particles is low compared to other elements (<20% in our experiments; <1–10% by *M. edulis* observed by Wang et al. 1997; <10% from humic substances by Decho and Luoma 1994). Nevertheless, particle assemblages enriched with microalgae showed increased cytosolic Cr and increased AE. Decho and Luoma (1991, 1996) reported that *P. amurensis* and *M. balthica* can absorb up to 90% of the Cr from pure cultures of bacterial cells. In our experiments bacteria could have been concentrated along with benthic microalgae, thus enhancing the AE. Even small changes in Cr absorption can have implications for Cr bioaccumulation (Wang et al. 1997).

Potential experimental limitations—The pulse-chase method is a substantially improved approach for determining absorption efficiencies of trace elements (Luoma et al. 1992; Wang et al. 1996). However, the use of radioisotopes as tracers for stable metals in natural particle assemblages requires careful experimental design and interpretation. One assumption is that radioisotopes closely follow stable metals in all reactions. The 3 d of radiolabeling used in the present study was probably not long enough for the radioisotopes to fully equilibrate with all particulate forms of the metals (especially inorganic forms). A short equilibrium time could result in preferential partitioning of isotopes to easily exchangeable pools of the experimental sediments (Jannasch et al. 1988; Balistrieri and Murray 1984). Preliminary studies showed that >90% of the ^{51}Cr labeled to SPM for 6 d was released in 0.6 N HCl after 2 h, while the same extraction methods yielded ~8% of total Cr from the natural SPM in San Francisco Bay. In contrast, 100% of both ^{109}Cd and stable Cd were released from the SPM by 0.6 N HCl. Other studies also report rapid equilibration of Cd to various marine particles (Nyffeler et al. 1984; Li et al. 1984), as well as association of Cr with fractions not extracted by 0.6 N HCl (Hornberger et al. in press), that could include sulfide, oxide, or silicate minerals (Loring 1979). The pH of gastric juices of bivalves averages 5.2–6 (Owen 1966) and would not extract as much natural Cr from sediments as would total chemical extraction. Thus, the ^{51}Cr AE determined from experimental sediments could overestimate the AE from many natural particle assemblages (*see* also Wang et al. 1997). Experiments probably did simulate recently introduced or a more labile pool of Cr on the food particles, however.

The equilibration time required to uniformly label the living components of the sediments including algae and bacteria is less of a problem. If these organisms undergo a few divisions during labeling, most new cell components will be formed with materials in equilibrium with radioisotopes. Thus, our AE values determined for algal-rich particles were probably close to the actual AEs for similar foods in nature. This means that the difference in metal bioavailability between algal-rich and algal-poor particle assemblages could be larger than shown here.

Some artifacts may occur in AE experiments with natural particle assemblages that are less problematic in experiments with pure cultures of algae or bacteria. A correction for uptake of dissolved isotope during pulse exposure was especially important in a few treatments in the present study because of low adsorption of radiolabel onto some types of experimental particles. The inability to separate and fully recover natural particles from the labeling media necessitated that pulse exposures occur in labeling media, which increased the possibility of dissolved isotope uptake. Adherence of nonliving particles to shells also required correction.

Release of Cd and Zn by microbial degradation or by leaching of soluble material from feces is another potential source of bias in AE experiments. Cd and Zn are released faster than other particle-reactive elements, including Cr, from both copepod fecal pellets and decomposing phytoplankton cells (Lee and Fisher 1992*a,b*). Clam feces are more loosely bound and more easily broken than copepod fecal pellets, which are encapsulated with peritrophic membrane. Therefore, release of Cd and Zn, but not Cr, from fecal materials of clams was possible, especially where food

included labile cell materials as compared to food that was dominated by sediments. The most important implication of this problem is that total ingested activity could be underestimated if it was determined by the mass balance method (i.e. ingested radioactivity equals the sum of tissue activity after depuration and radioactivity on the fecal pellets egested during the depuration). The result would be an overestimation of AE by our AE_2 in some treatments.

Conclusions

We have demonstrated that clams (*P. amurensis* and *M. balthica*) ingesting food enriched with either algal particles from the water column during a bloom or benthic microalgae accumulate Cd and Zn with greater efficiency than do clams ingesting particles poor in living microalgal biomass. The partitioning of Cd and Zn to particles (K_d) also increased as the microalgae biomass increased. Partitioning of Cr was less affected by the algal biomass. The greater absorption of metals from algal-rich diets occurred in both clams. At least in *P. amurensis,* it was partially explained by greater penetration of metals into the cytoplasm of algal cells and efficient absorption of cytosolic material by the clams.

Zinc was most efficiently absorbed by both clams, and Cr was the least available for absorption. The two clams appeared to use different strategies for absorbing metals. *M. balthica* absorbed Zn more efficiently from all types of food particles than did *P. amurensis. P. amurensis* absorbed Cd with greater efficiency from the bloom SPM than did *M. balthica,* but the two species absorbed Cd similarly from concentrated benthic microalgae assemblages. The results explain why *M. balthica* is a better indicator of Zn and *P. amurensis* of Cd contamination in estuarine environments (Brown and Luoma 1995). Anthropogenic metals introduced recently to the environment could be more bioavailable than those originating geologically or equilibrated with residual fractions in sediments. Recently introduced Cr is more associated with labile/easily exchangeable pools than is natural particulate Cr from San Francisco Bay. Recently introduced metals may also associate more readily with living materials in particle assemblages. Studies of metal bioavailability from sediments or SPM that do not include a realistic living particulate component could underestimate bioaccumulation and the dose of metal that the experimental organism would receive under equivalent conditions in nature.

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