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Geochemical Influences on Assimilation of Sediment-Bound Metals in Clams and Mussels

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A series of experiments was performed to evaluate the extent to which Cd, Co, Ag, Se, Cr, and Zn bound to sediments with different geochemical properties could be assimilated by the mussel *Mytilus edulis* and the clam *Macoma balthica*. Oxidized and reduced radiolabeled sediments were fed to suspension-feeding animals, the depuration patterns of the individuals were followed by γ-spectrometry, and the assimilation efficiencies (AEs) of ingested metals were determined. AEs from geochemically diverse sediments typically varied less than 2-fold and ranged from 1% for Cr to 42% for Zn. Metals were assimilated from anoxic sediment by both animals; Ag, Cd, and Co AEs in *M. balthica* were 9−16%, 2-fold lower than from oxic sediment, but in *M. edulis* AEs were about two times greater from anoxic sediment for all metals but Ag. For oxic sediment, Cd and Co AEs in *M. edulis* decreased 3−4-fold with increased sediment exposure time to the metals with smaller but significant effects also noted for Zn and Se but not Ag. A less pronounced decrease in AE for *M. balthica* was evident only after 6 months exposure time. Sequential extractions of the oxidized sediments showed a transfer of metals into more resistant sediment components over time, but the rate did not correlate with a decrease in metal AEs. Comparing the two bivalves, TOC concentrations had an inconsistent effect on metal AEs. AEs of metals from bacteria-coated glass beads were slightly higher than from humic acid-coated beads, which were comparable with whole-sediment AEs. There was correspondence of AE with desorption of Ag, Cd, Co, and Se (but not Zn) from sediments into pH 5 seawater, measured to simulate the gut pH of these bivalves. The results imply that metals associated with sulfides and anoxic sediments are bioavailable, that the bioavailability of metals from sediments decreases over exposure time, that organic carbon content generally has a small effect on AEs, and that AEs of sediment-bound metals differ among species.

Introduction

Trace metal concentrations in coastal sediments are typically orders of magnitude greater than concentrations in overlying waters and constitute an enriched pool of metal potentially available for organisms (*1, 2*). In urban estuaries and coastal sedimentary deposits, particle-reactive trace metals (that is, metals that are highly enriched in particles relative to ambient seawater) are found in concentrations that are well above historical background levels. These sediments constitute an important food source for many deposit- and suspension-feeding estuarine animals. To assess the potential impact of sediment contamination on marine ecosystems, the degree to which sediment-bound metals are assimilated into animal tissues must be quantified, and the mechanisms that control bioavailability must be understood.

Dissolved and particulate sources can contribute to metal accumulation in bivalve molluscs. Uptake from particles is a function of the assimilation efficiency (AE), metal concentration in the ingested particle, and the feeding rate. AE reflects the bioavailability of a metal for a given animal under a specified set of conditions; it represents a first-order process that can be compared among different organisms and between trace elements and has been successfully used as a parameter in modeling metal concentrations in marine bivalves (*3, 4*). AE is experimentally determined by pulse-feeding radioactive metal-labeled particles to an animal and measuring the fraction of ingested metal retained by the animal after the gut has been completely purged with a nonradioactive food. Sensitivity analyses of model parameters have shown that AE can strongly affect the extent of metal accumulation in mussels (*4, 5*).

In this study, we used the mussel, *Mytilus edulis* and the clam *Macoma balthica* to evaluate the AE of ingested Ag, Cd, Co, Cr, Se, and Zn from diverse sediments and individual sediment components. *M. edulis* is a common marine suspension-feeding mussel, and *M. balthica* is an infaunal facultative deposit feeder with suspension-feeding capabilities. Both species are broadly used in water quality monitoring programs (*3, 6*). The metals examined were chosen to contrast elements with different geochemical properties, including sulfur-seeking metals (e.g., Ag, Cd), which would rapidly coprecipitate in anoxic sediments with Fe_xS_y, and oxygen-seeking metals (e.g., Co, Cr), which may be expected to associate with iron/manganese oxides. Previous experiments with marine bivalves have used nonquantitative methodologies to evaluate bioavailability from sediments (*2*) or have measured AE from a limited range of sediments or natural seston (Se: ref *3*; Cr: ref *7*; Cd, Co, Se, Zn, Ag, Am, and Cr: ref *4*; Cd, Cr, and Zn: ref *8*; Cd, Co, and Ag: ref *9*). In our study, we addressed the influence of geochemical properties (e.g., redox condition, organic richness, clay and iron oxide content, bacteria, and humic material) on AE and test some of the prevailing hypotheses about factors that affect bioavailability from sediments.

Metals in sediments undergo complex geochemical reactions that are dependent on a variety of processes in different regions of the sediment column. One very distinct and dynamic geochemical boundary within sediment is the oxic/anoxic interface. Metal phase associations and behavior are significantly different in these two zones. Metal distributions in anoxic sediment are dominated by sorption to and substitution within authigenic Fe_xS_y phases, and metal bioavailability appears to be strongly controlled by the typically strong binding properties of these sulfide phases (*10−13*). A recent body of work shows how the high binding strength of reactive acid volatile sulfides (AVS) can control pore water concentrations of metals such as Ag, Cd, Cu, Ni, and Zn. In these studies, AVS consistently limits metal toxicity to invertebrates (*14−17*) in those anoxic and suboxic sedi-

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ments where AVS molar concentrations exceed molar concentrations of extractable metals. Effects of AVS on metal bioaccumulation are somewhat more ambiguous (*10*). Nearly all these experiments were conducted with very elevated metal concentrations, and none have examined the relationship between dietary metal assimilation and AVS binding. In this study, we determine whether metals are assimilated from anoxic sediments and compare AE in anoxic sediment to AE from oxic sediments of similar character.

Early field and microcosm studies suggested that the complex geochemistry of oxidized sediments resulted in complex effects on metal bioavailability. Summaries of these studies concluded that geochemical influences on metal bioavailability from oxidized sediments were diverse and metal-specific (*1, 2, 18, 19*). Generalizations were possible within some types of ecosystems but were difficult to develop across metals or across ecosystems. Some recent studies are more optimistic about the occurrence of consistent geochemical influences on availability. It is purported that total organic carbon (TOC) consistently reduces metal bioavailability (toxicity) in oxic sediments where AVS does not preclude accumulation of elevated pore water metal concentrations (*20*). However, other studies show that metal assimilation in marine bivalves *increases* in oxic sediments with a greater abundance of living (organic) microflora in sediment (*8*). Some studies also purport that the bioavailable fraction of metals includes predominantly the more labile forms of metals, such as those easily removed during a weak acid extraction (*2, 21, 22*) or extraction by an animal's "gut juices" (*23*). A simple relationship between binding strength of metals for sediment particles and AE has been reported (*9*), but the universality of this effect remains to be tested.

In this study, we further tested the consistency of the effects of organic carbon and binding strength on the assimilation of Ag, Cd, Co, Cr, Se, and Zn. We compared AE between two natural sediments of varying TOC concentration. We amended the organic-rich sediment with iron and clays and ashed it to test effects on AE of increasing the inorganic component of the sediment. We also specifically evaluated AE when animals were fed purely organic types of particles (bacteria or humic substance-coated beads) and purely inorganic synthetic particles (iron oxide, calcium carbonate). To assess the influence of binding strength, we evaluated whether AE correlates with reduced-pH or surfactant-enhanced desorption from the amended sediments.

Trace metal bioavailability in sediments may change as sediments are transformed in response to physical, chemical, and biological processes (e.g., redox cycling, delivery of fresh organic matter from the water column, bioturbation, resuspension events, etc.) (*1, 8, 24*). One result may be that the length of time a trace metal resides in a sediment may affect its bioavailability. Few studies have attempted to quantify this effect for metals (*13*), so we have also evaluated how time affects the processes that determine metal bioavailability from sediment.

Materials and Methods

The overall approach we used involved radiolabeling sediments with γ-emitting isotopes of the metals; these sediments were then fed to *M. edulis* and *M. balthica* over a short period, after which the retention of radiotracers in individual animals was monitored. Once the gut was emptied (3 d), the metal remaining in the animal was considered assimilated (*25, 26*). Concurrently, desorption of metals from radiolabeled sediment or the partitioning of metals among operationally defined sediment phases was measured.

Several batches of *M. balthica* with shell lengths ranging from 1.3 to 2.7 cm, collected from a mud flat in south San Francisco Bay (SFB) near Palo Alto, CA, were acclimated in the laboratory to 16 °C and fed a diet of diatoms (*Thalassiosira pseudonana*) for at least 1 wk prior to experiments. *M. edulis* individuals, shell length of 3.0–3.5 cm, were collected from a jetty in Long Island Sound (LIS), near Flax Pond in Oldfield, NY, and acclimated in the laboratory as described above. All experiments used 0.2-μm filtered seawater with a salinity adjusted to 30 psu.

Characterization of Sediments. Surface sediments used in this study were scraped from the upper 1–2 cm in mud flats adjacent to the sites where the bivalves were collected. Sediments were sieved to 63 μm and kept at 2 °C (for 2 week–6 month for SFB sediment; 1 d–2 week for LIS sediment) prior to radiolabeling (*27*). Batches of wet sediment ($\phi = 0.8$) of 25–200 mg (dry wt) were suspended in 1–10 mL of 0.2-μm filtered seawater (deoxygenated seawater for anoxic treatments) for at least 2 d before radiolabeling (described below). Sediment grain size distribution was measured on a sedigraph, and percent loss on ignition and porosity were determined. Approximately 5 g of wet sediment was dried in quartz crystal crucibles at 85 °C and ashed at 450 °C for 4 h, then deionized water was added (to partly account for loss of water from the crystal lattice of clay minerals), and the sediment was dried again at 85 °C. SFB and LIS sediment had approximately 0.6% and 5% organic carbon content, respectively, and the grain size distribution was similar between the two with 5–10% more clay in the SFB sediment. SEM micrographs showed an abundance of benthic diatom tests in both sediments, with LIS sediment having about twice the amount as SFB sediment. Background 1 N HCl-extractable metal concentrations in SFB sediment were (μg g^{-1}) 0.3 for Ag, <0.2 for Cd, 4.9 for Cr, 0.5 for Se, and 49 for Zn (*28*). Total metal concentrations (μg g^{-1} dry wt) in Flax Pond sediments were <0.2 for Cd, 31 for Cr, and 20–51 for Zn (*29, 30*).

Oxic and Anoxic Sediments. To compare the AEs of oxic and anoxic organic-rich and organic-poor sediments, wet oxic SFB and LIS sediments (70 mg dry wt) were suspended into 1 mL of filtered seawater in polycarbonate tubes, exposed continuously to air, and radiolabeled for 14 d. For the anoxic treatment, wet suboxic to anoxic LIS sediment (approximately 70 mg dry wt) was placed into 1 mL of deoxygenated seawater and then gently bubbled with N_2 gas for 2 h. All anoxic sediment treatments were continuously maintained and manipulated in a glovebag under N_2 gas. The pH was adjusted to 8.0 with NaOH. The suboxic sediment was incubated under anoxic conditions for 7 d after which the sulfidic sediment (evident by black color) was radiolabeled with microliter additions of radioisotopes, pH corrected to 8.0 with NaOH, bubbled with N_2 gas, and then tightly capped for 14 d. Periodically the pH and O_2 levels (using an oxygen probe, Microprobes, Inc.) in the anoxic treatments were measured. Sulfide concentrations were not directly measured in these experiments.

Sediment Aging. To measure the effect of labeling length on AE, SFB sediment (70 mg dry wt) was suspended in 1 mL of filtered seawater in six polycarbonate test tubes. One treatment immediately received trace amounts of ^{110m}Ag, ^{109}Cd, ^{57}Co, ^{75}Se, and ^{65}Zn (described below), and the remaining sediments were similarly labeled at various time intervals up to 35 d. This method reduced experimental artifacts by ensuring that each batch of sediment would differ from the other batches only in the metal contact time. In addition, SFB sediment was labeled with ^{110m}Ag, ^{109}Cd, and ^{57}Co for 6 months. Sediments were swirled daily and remained well oxidized. Small amounts of deionized water were added to maintain constant salinity during the labeling period. Sequential chemical extraction of the suite of aged treatments, added as wet sediment, was performed 1 d after pulse feeding to the bivalves using a method modified from Tessier et al. (*21*) with a sediment (dry wt):extractant ratio of 100 mg:1 mL. Briefly, at the end of the radiolabeling period, all aged treatments were centrifuged at 800*g* for 20 min, and the overlying water was decanted. Filtered seawater was added to the pellet, and the solid sediment was resuspended, shaken vigorously for 10 min, and then recentrifuged. The supernatant was filtered through a 0.2-μm filter; this fraction was called the "rinse." Following the rinse step, extractions were performed sequentially using 1 M $MgCl_2$ (exchangeable); 1 M sodium acetate, pH 5 (weak acid); 0.04 M $NH_2OH{\cdot}HCl$ in 25% acetic acid (reducible); and 0.02 M HNO_3 in 30% H_2O_2 adjusted to pH 2 followed by 3.2 M ammonium acetate in 10% HNO_3 (strong acid and oxidant). Less than 0.1% of the initial radioactivity remained associated with the sediment after this final extraction.

Sediment Augmentation. To assess the effects of iron oxide and clay content on AE, another batch of freshly collected LIS sediment (35–70 mg dry wt) was suspended in 2 mL of filtered seawater, and three treatments were prepared: one natural sediment (NS); one NS augmented with 4% iron oxide, added as 2 M $Fe(NO_2)_3{\cdot}6H_2O$; and another NS augmented with 50% (dry wt) montmorillonite clay (Sigma, Inc.). The pH of the slurry was immediately adjusted to 8.0 with microliter additions of 1 N NaOH and was periodically readjusted during the 6-d radiolabeling period (see below). Sediment remained well oxidized during the labeling period.

Synthetic Particles. For experiments exploring the influence of individual sediment components, synthetic particles were prepared in the following manner. Pure $CaCO_3$ (Sigma) was pulverized, and particles in the 5–14-μm range were collected by multiple filtrations. Particles were resuspended and allowed to settle for 1 h. The overlying water was decanted twice to fully remove particles <5 μm. A total of 25 mg of particles was resuspended into 10 mL of filtered seawater, and the pH was adjusted to 8.0. Pure silica beads (5–10 μm) were washed in 1.2 M HCl for 1 d, rinsed three times in deionized water, and then ashed for 4 h at 450 °C. For sediment-derived bacteria-coated beads, bacteria were removed from the sediment using a technique modified from Ellery and Schleyer (*31*). Approximately 10 g of wet LIS sediment was added to 200 mL of filtered seawater, homogenized for 8 min, and then placed into a sonication bath for 6 min. The slurry was filtered through 3-μm membranes, and 100 mg of acid-washed silica beads were added as a substrate for bacterial growth. Soy growth media (Aldrich) was added to 2 mL of the bacteria-rich filtrate, and bacteria were allowed to grow for 3 d following a method modified from Taylor and Gulnick (*32*) before radiolabeling (the milky white culture had a C:N ratio of 4.1, typical of bacteria). Humic-coated silica beads were made by placing 100 mg of clean beads into 2 mL of 0.2-μm filtered stock of organic-rich filtered seawater derived from *Spartina alterniflora* debris that had been leached for 2.5 yr. The solution contained 67 mg/L DOC (measured by Shimadzu TOC 5000 analyzer). The coated beads were removed from both solutions (bacteria-amended or *S. alterniflora* leachate) after 2 d, collected on a 5-μm filter, and resuspended into 10 mL of filtered seawater. Iron oxide-coated silica beads were prepared following the methods of Decho and Luoma (*33*). Ashed natural sediment was prepared by heating 1 g of LIS sediment to 450 °C for 6 h. The sediment was then pulverized and size fractionated to approximately 3–10 μm by settling and filtration. All of the individual sediment component particles were radiolabeled for 1 d.

Radiolabeling and Pulse-Feeding. In all particle-labeling procedures, microliter amounts of radioisotopes were added together (as Ag + Cd + Co, or Se + Cr + Zn, or Ag + Cd + Co + Se + Zn) per 10 mg of particles as follows: 1.4 kBq (19 ng) of ^{110m}Ag (in 0.1 N HNO_3), 10 kBq (270 ng) of ^{109}Cd (in 0.5 N HCl), 1.7 kBq (23 pg) of ^{57}Co (in 0.1 N HCl), 2.5 kBq (4.5 ng) of ^{75}Se (in water), 1.3 kBq (14 ng) of ^{65}Zn (in 0.1 N HCl), and 1.5 kBq (0.25 ng) of $^{51}Cr(III)$ (in 0.1 N HCl). All acid additions were neutralized with 0.1 N NaOH, and a pH of 7.9–8.2 was maintained in all treatments.

After radiolabeling, sediments or particles were centrifuged for 20 min at 800*g* (a force that did not lyse cellular material), and the supernatant was removed by pipet. Sediment was resuspended into 1 mL of filtered seawater (deoxygenated for the anoxic treatment) and then added in pulses of 250 μL every 5 min (*M. edulis*) or 20 min (*M. balthica*) to beakers with 2 L of filtered seawater containing 5 or 10 actively feeding mussels or clams; the total feeding time was 20 min for *M. edulis* and 80 min for *M. balthica*. The resulting particle concentration was 2–4 mg L^{-1} (low enough to prevent pseudofeces production by the animals). For the anoxic treatments, the total radioactive pulse-feeding time was held to 5 or 10 min for *M. edulis* and *M. balthica*, respectively. This time period is short enough to preserve >80% of reactive FeS (*34, 35*) and ensure that the particles initially entering the digestive system are anoxic. During pulse feeding, animals rested on mesh held 4 cm off the bottom, and particles were maintained in suspension by magnetic stirrers. In all AE experiments, loss of radioisotope from particles into the dissolved phase was monitored (*36*), and the uptake of dissolved metal into the animals was determined by suspending 2–4 animals in the filtrate derived from the pulse-feeding treatments. Two individual animals from each treatment were sacrificed after the radioactive-feeding period to determine the distribution of radioisotopes between the shell and soft tissues (for *M. edulis* and *M. balthica*, typically <1% and <5% was on the shell). Statistical significance of AE results was determined by a two-population *t*-test on treatments within an experiment.

Immediately following pulse feeding, all individuals were rinsed with a saturated solution of EDTA followed by filtered seawater and then nondestructively radioassayed. To follow the depuration of radiotracers from the animals over time, the animals were then placed into polypropylene beakers, which were held within a 20-L recirculating aquarium (*37*) and were continuously fed a diet of nonradioactive diatoms (*T. pseudonana*) or fine-grain sediment particles (*7*) mixed with diatoms.

Desorption Experiments. Experiments were also conducted to relate metal accumulation in the bivalves with metal desorption from sediment particles. Approximately 10 mg of radiolabeled sediment from the NS, iron oxide, and montmorillonite-augmented experiment was pipetted into each of four 500-mL flasks containing filtered seawater adjusted to either pH 5 (to simulate the gut acidity of these bivalves, ref *38*) or pH 8 and seawater with surfactant (Liquinox) added (contact angle 65°, midrange for deposit-feeding animals; ref *39*). Desorption from particles into the dissolved phase was determined (*40*) at 3, 6, 18, 24, and 48 h, time periods that encompass the gut transit times of these animals (*25, 26*).

Radioanalysis. Radioactivity in all samples (water, sediment, and bivalves) was determined noninvasively with intercalibrated γ-counters using large well NaI(Tl) γ-detectors. The γ-emissions of ^{110m}Ag were detected at 658 keV, of ^{109}Cd at 88 keV, of ^{57}Co at 122 keV, of ^{51}Cr at 320 keV, of ^{75}Se at 264 keV, and of ^{65}Zn at 1115 keV. Counting times were 2–5 min and were sufficient to yield propagated counting errors <5%. All analyses were related to appropriate standards and were calibrated for both Compton effects and radioactive decay.

Results

Mytilus edulis. *Anoxic Sediment.* At the end of the 14-d radiolabeling period, >99% of the metals in the anoxic sediment were associated with particles (except for Co, which was 98%). Detectable assimilation of all metals from anoxic sediments was observed, although the AEs varied widely

TABLE 1. Assimilation Efficiency (AE) of Trace Elements from Various Sediment and Particle Types by *Macoma balthica* and *Mytilus edulis*^{a}

	Ag	Cd	Co	Cr	Se	Zn
Oxic vs Anoxic (LIS) Sediment						
Macoma balthica						
oxic	20 ± 5.3	21 ± 2	28 ± 5	nd	nd	nd
anoxic	11 ± 1.7*	9 ± 2.1*	16 ± 5.8*	nd	nd	nd
Mytilus edulis						
oxic	12.8 ± 4.1	15.7 ± 2	9.4 ± 1.8	0.7 ± 0.1	13.6 ± 3.8	21.8 ± 5
anoxic	4.6 ± 2*	35.4 ± 6.5*	15.6 ± 5.8	4.7 ± 0.2*	21.2 ± 5*	31.6 ± 3.5*
Organic-Rich vs Organic-Poor Sediment						
Macoma balthica						
organic-rich	21 ± 3.5	23 ± 1.7	30 ± 4.4	19.6 ± 8	21.2 ± 5	32 ± 6.7
organic-poor	18 ± 3.6	9.7 ± 3.2*	14 ± 5.7*	18.7 ± 7.8	27 ± 2.3*	36 ± 3.1
Mytilus edulis						
organic-rich	22 ± 2.8	20 ± 1.1	9 ± 2	5 ± 1	12.5 ± 3.1	21 ± 4
organic-poor	8.9 ± 2.7*	9.5 ± 5.2*	12 ± 4.5	10 ± 1.8*	24 ± 2.7*	28.6 ± 2.8*
LIS Sediment (NS) and LIS Sediment Augmented with Iron Oxide or 50% Clay						
Mytilus edulis						
NS	19 ± 1.2^{a}	20.6 ± 1.1^{a}	14.5 ± 2.2^{a}	nd	15 ± 2^{a}	30 ± 4^{a}
NS + Fe	23 ± 3^{b}	21 ± 2^{a}	16.2 ± 3.8^{a}	nd	12 ± 2^{a}	36 ± 4.2^{a}
NS + clay	17 ± 2.6^{a}	16 ± 1.5^{b}	8.3 ± 0.8^{b}	nd	12.7 ± 1.5^{a}	28 ± 2.2^{b}
Synthetic Particles: Bacteria, *Spartina* Leachate (Humic), CaCO$_3$, Iron Oxide, Ashed Sediment						
Macoma balthica						
iron oxide	35 ± 2.2	22.6 ± 1.7	30 ± 4.4	nd	nd	nd
ashed sediment	29 ± 4.3	51.7 ± 3.7*	29.2 ± 2.8	nd	nd	nd
Mytilus edulis						
bacteria	38 ± 9.7^{a}	15 ± 3.4^{a}	20 ± 2^{a}	6 ± 2^{a}	nd	41 ± 2^{a}
humic	21 ± 2.8^{b}	14 ± 6^{a}	13 ± 2^{b}	4.5 ± 2^{b}	nd	30 ± 2^{b}
CaCO$_3$	40 ± 12^{a}	40 ± 7.4^{b}	21 ± 2^{ac}	8 ± 2^{a}	nd	33 ± 2^{ab}
iron oxide	32 ± 9.5^{a}	40.5 ± 8.7^{b}	26 ± 7.1^{ac}	nd	nd	nd
ashed sediment	69 ± 12^{c}	47 ± 7.6^{b}	33 ± 7.4^{c}	nd	nd	nd

^{a} AE was calculated from the 72-h activity (% radioisotope remaining in soft tissue between 2 and 4 d was log transformed and 3 d % AE was calculated for each individual). Letters (a, b, c) or an asterisk (*) denote that AEs between treatments for a specific metal were significantly different within each experiment ($P < 0.05$). nd, not determined.

among metals. The AEs from the anoxic sediment ranged from about 5% for Cr and Ag to over 30% for Cd and Zn. The AEs of all trace elements associated with anoxic sediment (Table 1) were 1.5–7 times higher in *M. edulis* than metals bound to oxic sediment, except for Ag.

Oxic Sediment: Influence of Sediment Contact Time. Depuration patterns of metals in *M. edulis* show that metals in contact with sediment for longer time periods are generally less bioavailable. A representative depuration pattern (for Co) is shown in Figure 1. This effect is manifested by greater fecal elimination of metal within the first 3 h of depuration after pulse feeding, as time after labeling increases, presumably reflecting lower assimilation in the early stages of digestion. The exposure time of metals in the sediments correlated with a progressive decrease in AE by *M. edulis* for Co, Cd, Zn, and possibly Se, but exposure time did not affect Ag AE (Figure 2). The AE of Co decreased from 17.5% in sediment labeled for 1 d to 5.1% in sediment labeled for 35 d, Cd decreased from 21.7% to 8.5%, and Zn decreased from 41.7% to 23.4%. Se was unaffected by exposure time (23% to 26.7%) until 35 d when there was a sharp drop to 12.7%.

Sequential chemical extractions of radiolabeled sediment of varying ages showed that the labile fraction (operationally defined here as the first three extractions: rinse, exchangeable, weak acid) declined for all metals over time (Figures 2 and 3). While the decrease in metal extracted in the labile fraction coincided with a decrease of metal AE in the bivalves, the total amount of metal in the labile fraction did not correlate well with AE. Between metals, 1.5 times more Cd than Zn was extracted from the labile fraction, but the AE of Zn was twice that of Cd. Furthermore, the rates of decrease in metal AE over time in *M. edulis* and the rates of decrease in the percent of the same metal associated with the labile fraction were significantly different ($P < 0.01$), although the differences were not pronounced (rates determined by linear regressions on log-transformed data, significance calculated using a *t*-test). More than 95% of Cd was removed from the sediment by the first three extractions. A total of 60–80% of the Co and Zn was extracted by weak acid. Extracted Se was evenly distributed throughout all fractions with 20–40% in the rinse, which decreased with sediment aging, and 10–23% in the strong acid/oxidant phase, which increased with aging. A total of 60–70% of the Ag was extracted in the weak acid with 19–32% in the reducible phase.

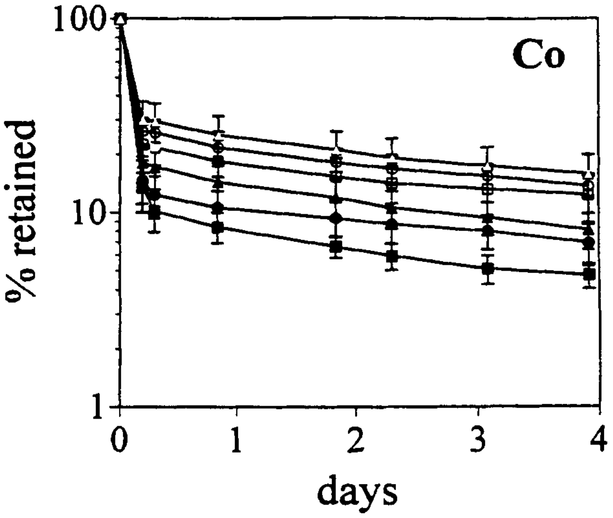

FIGURE 1. Retention of Co in *M. edulis* soft tissues over a 4-d depuration period following pulse feeding of radiolabeled sediment aged: (△) 1, (○) 2, (□) 9, (▲) 14, (●) 24, and (■) 35 d. Error bars denote 1 SD.

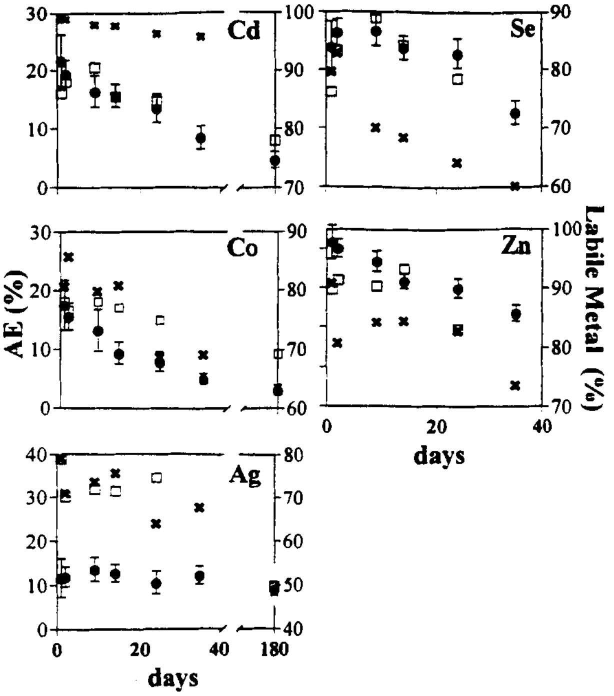

FIGURE 2. Influence of sediment exposure time to metal on assimilation efficiancies (AEs) of ingested metals in *M. edulis* (●) and *M. balthica* (□). Also shown are % metal in the labile fraction (X) (defined as the rinse + exchangeable + weak acid extrectable fractions) as a function of sediment exposure time (see also Figure 3). The relationships of AEs in *M. edulis* vs metal lability were analyzed by linear regression on log-transformed data, and a *t*-test was used to compare the slopes. All pairs of slopes (for a specific metal) were significantly different from each other ($P < 0.01$). AE, assimilation efficiency; LM, labile metals; d, days. Cd: AE = 1.33−0.011 (± 0.001) d; LM = 2.00−0.0004 (± 0.00003) d. Co: AE = 1.23−0.015 (± 0.001) d; LM = 1.9−0.0027 (± 0.0006) d. Se: AE = 1.45−0.0076 (± 0.0025) d; LM = 1.9−0.0038 (± 0.0006) d. Zn: AE = 1.62−0.007 (± 0.0005) d; LM = 1.94−0.0017 (± 0.0007) d. Ag: AE = 1.09−0.0007 (± 0.001) d; LM = 1.88−0.0018 (± 0.0009) d.

Oxic Sediment: Influence of Sediment Geochemistry. The effects of organic matter on AE was not consistent among the elements studied here. In *M. edulis*, the Co AE was not significantly different between organic-rich and -poor sediments, whereas Ag and Cd AEs were 2−3 times higher from the organic-rich sediment and Cr, Se, and Zn AEs were 1.5−2 times greater from organic-poor sediment. Augmenting the organic-rich sediments with iron oxides or clays had little effect on the AE in *M. edulis*. The AEs from oxidized LIS natural sediment (NS) were comparable with AEs from the same sediment augmented with iron oxide or with montmorillonite clay (Table 1). The low AEs for Cd, Co, and Zn from sediment augmented with montmorillonite were statistically significant ($P < 0.05$), but the effect was small.

Desorption of each metal at pH 8 and pH 5 was similar among the augmented and unaugmented LIS sediments. Desporption was greater at pH 5 than at pH 8, as expected. The presence of surfactants did not significantly affect desorption of Cd, Co, Se, and Zn. Less Ag was desorbed at both pH values in the presence of the surfactant (Figure 4), and Se and Ag exhibited re-adsorption to particles after 48 h at pH 8 (data not shown). No statistically significant relationship was observed when the mean AE (among particle types) for the five metals was compared to mean desorption for the metals. But there was a distinct correspondence (among metals) between AE in *M. edulis* and metal desorption from particles after 3 h (especially at pH 5) if Zn was excluded from the relationship. There was less correspondence of AE and desorption at pH 8, without Zn, and no correspondence if surfactants were present.

Synthetic Particles. The effect of organic matter on AE in pure phase synthetic particles was not fully consistent with the results from whole sediments. For example, AEs of Ag and Cd from organic-rich LIS sediments exceeded AEs from organic-poor SFB whole sediments. Inorganic synthetic particles all showed AEs for Ag in excess of any values from whole sediments. Although an elevated Ag AE was measured for organic-enriched bacteria-coated beads, the lowest AE for Ag was from organic-rich humic-coated beads. Thus, the nature of the organic matter was important for Ag. The AEs for Cd were 3 times lower from the organic-coated particles than from inorganic particles. It is notable that the AEs of all metals in the humic treatment were lower than all AEs from uncoated inorganic particles. In both synthetic and whole sediments, the AE of Cr in *M. edulis* was low (4−8%) (Table 1).

Comparisons between Species. Fewer experiments were conducted with *M. balthica* than with *M. edulis*. Metal AEs, in general, were comparable between the species where data was available, but there were instances where important differences occurred between species. For natural oxic sediments, the greatest difference in AEs between the two bivalves was noted for Ag, for which values were 2−3 times greater in *M. balthica*. Detectable assimilation of all metals from anoxic sediments was observed in both bivalves. Both species assimilated less Ag from anoxic than from oxic sediments. But AEs for Cd and Co were reduced 2-fold in *M. balthica* in the anoxic treatment as compared to oxic sediments; while the AE for Cd increased in anoxic conditions in *M. edulis* (Table 1). In comparing oxic sediments with varying organic carbon contents, only Cd and Se showed similar trends in both species. There was no significant effect of exposure time (radiolabeling length) on the AE of any metal in *M. balthica* (Figure 2), in contrast with *M. edulis*, for which AEs decreased by 50−80% from 1 to 35 d ($P < 0.05$). But after 180 d of exposure time, *M. balthica* AEs were significantly reduced ($P < 0.05$) for Cd, Co, and Ag as compared to the first 25 d (no Cr, Zn, or Se was used in the 180-d treatments).

Discussion

Many studies show that total concentrations are not adequate to explain the availability to biota of metals from sediments (*2, 14, 18, 22*). Recent proposals suggest that metal bioavailability should be considered in development of criteria for management of metal-contaminated sediments and propose some generic protocols to account for geochemical influences on bioavailability (*14*). However, the comparative importance of geochemistry, metal-specific influences, and species-specific influences on bioavailability has not been quantified. AE offers a tool for quantification of these influences on metal uptake from food. An important generalization from our experiments is that AE differs more widely among metals than among geochemical conditions for a single metal. Different sediment-bound trace elements were assimilated to varying degrees, ranging from 1% for Cr to 41% for Zn. Moreover, the change in bioavailability elicited by changing geochemistry often differed among metals. Differences in geochemical conditions (like sulfide abundance) can greatly affect pore water metal concentrations (*10, 14, 16, 17*). Geochemical influences on dietary uptake may be less extreme. Furthermore, correction factors that are universal among metals and among species (like AVS or TOC normalization) (*17, 20*) may not be applicable for dietary metal uptake.

In general, AEs from natural sediments in both bivalve species were less than or equal to AEs from phytoplankton or natural seston (*8, 26, 41*), although some exceptions were

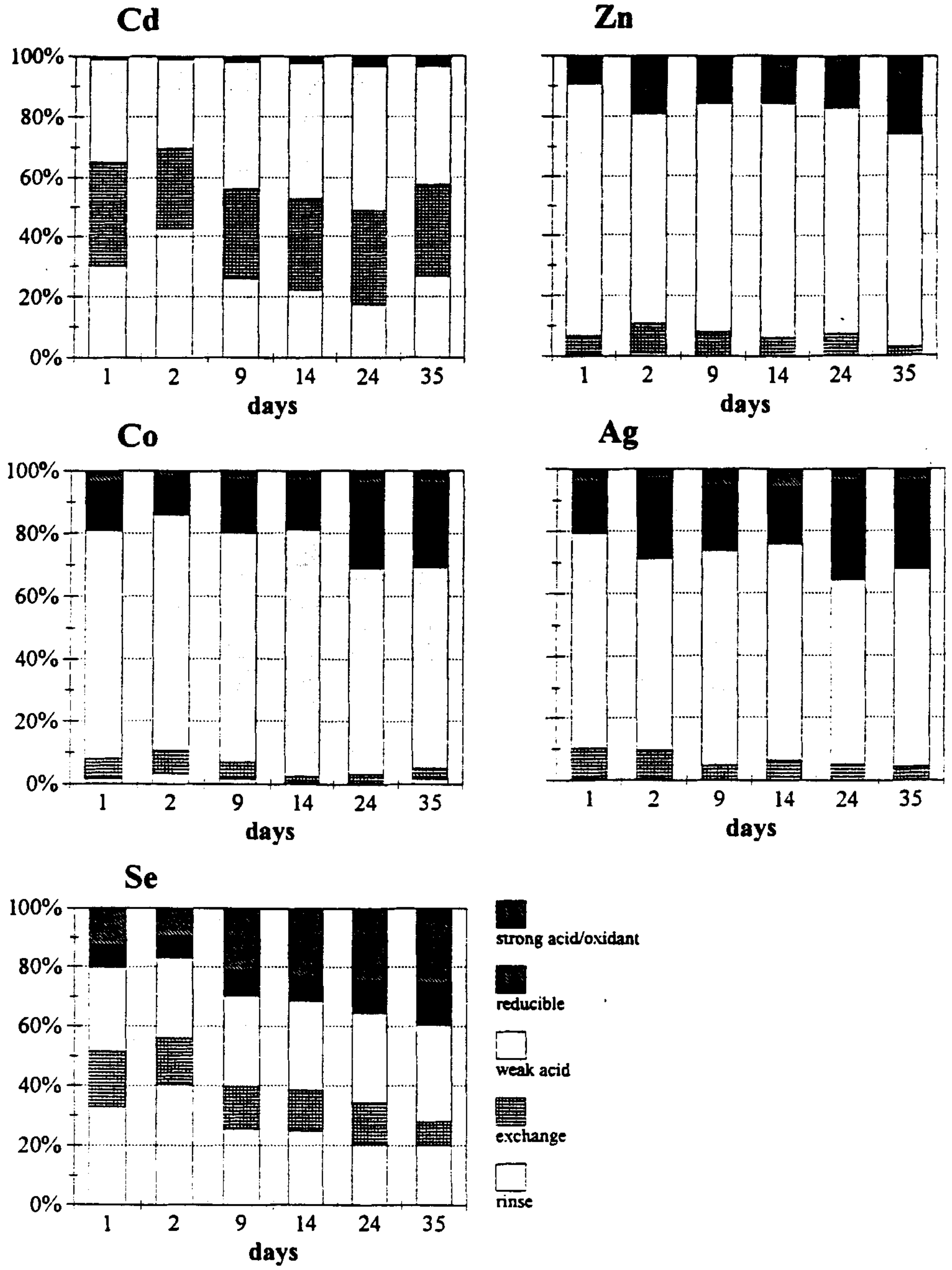

FIGURE 3. Distribution of radioisotopes among operationally defined fractions of radiolabeled sediment following different exposure times.

noted. Inter-experimental variability in AEs for a given sediment type was typically greater than AE variability when individual phytoplankton species serve as the food source (*26*). Although AEs for Cr were generally low, Cr AE had the largest proportionate variability between particle/sediment types. For the other elements, variability generally decreased with Ag > Cd > Co > Se > Zn.

Anoxic and Oxic Sediment. The geochemistry of metals in anoxic sediments is obviously very different from the geochemistry of metals in oxic sediments. Metal partitioning and behavior may be most complex in oxidized sediments. Trace metal associations in oxic sediments can change with the delivery of fresh organic material, the accumulation of iron oxides as they precipitate along the oxic/anoxic interface, and the bioturbation of sediments that can remove surface bacterial coatings and expose new surfaces sites for adsorption of metals. Generally, the most important trace metal binding substrates in oxidized estuarine sediments are iron-(III) oxyhydroxides, organic matter, and, to a lesser extent, manganese(IV) oxides (*42, 43*). Metal binding affinity is typically an order of magnitude lower for silicates (*44*), clays probably act more as a substrate for oxide and organic coatings, whereas $CaCO_3$, which has a relatively low binding affinity for most metals, tends to dilute trace metal concentrations in sediments (*44–47*).

Anoxic conditions, presumably accompanied by the formation of metal sulfides, do not appear to eliminate metal uptake from diet by benthic invertebrates. Metals were less assimilable from anoxic than from oxic sediments in *M. balthica*, but metals in anoxic sediment were still bioavailable with AE values of Cd, Co, and Ag ranging from 10% to 15%. Higher Cd and Co AEs in *M. edulis* from anoxic sediment than from oxic sediment were an unexpected result given the strong binding of metals to sulfides and the slow reoxidation (on the order of days for some metal sulfides; *12, 34*). In an earlier study, metal AEs from anoxic sediments in the deposit-feeding polychaete, *N. succinea*, were lower than from oxic sediments, especially for Cd and Co. But all metals

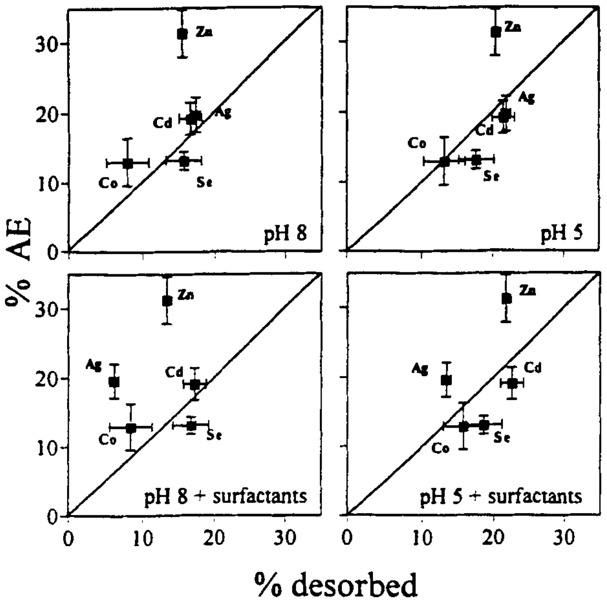

FIGURE 4. Relationship between assimilation efficiency (AE) of ingested metal in *M. edulis* and desorption of sediment-bound metals over a 3-h period from sediment particles resuspended into pH 8 and pH 5 filtered seawater with and without surfactant. Line represents 1:1 relationship of AE with desorption. Error bars represent 1 SD for mean values (■) of three sediment types (natural sediment, natural sediment with 4% iron oxides, and natural sediment augmented with 50% montmorillonite clay).

(Ag, Cd, Co, and Zn) were still assimilated with moderately high efficiencies (5–35%) from anoxic sediment (*13*).

The mechanstic reasons why there were higher metal AEs from anoxic sediment than from oxic sediments in *M. edulis* and not in *M. balthica* are not completely clear. One possibility is that processes within the gut might enhance dissolution and increase assimilation more in some species than in others. Recent work in our laboratory has provided two lines of evidence (differences in iron reduction, measured using ferrozine, and reduction of tetrazoleum indicator salts) to support the idea that *M. edulis* does, in fact, have a significantly more reducing gut than *M. balthica* (*48*). Differing gut reducing potentials might inhibit or enhance remobilization of trace metals into the dissolved phase and facilitate increased uptake. AgS has the lowest solubility of the sulfides of the metals studied here (*49*) and the slowest reoxidation rate (*50*). The very low solubility of AgS may explain the low AE in both species from anoxic sediment, and the slow reoxidation rate may allow differences to develop between *M. edulis* and *M. balthica*. The high AE of Cd and Zn from anoxic sediments (compared to Ag and Co) in *M. edulis* may also reflect weak acid extractability (*49*). Acid solubility alone is unlikely to be an adequate predictor of the bioavailability of metals in the gut however. Other factors such as gut pH, enzymatic activity, surfactant concentration, particle processing time and intensity, and abrasional mixing may also affect the fate of ingested metals and result in some of the more complicated differences found in our results. Future experiments must take into account the complexities of gut physiology. For example, they might consider desorption of metals from particles into digestive juices (*23*) at the same E_h found in the digestive tract of different animal species.

Sediment Aging. Physical location of metals, or the depth to which metals have penetrated and are occluded in sedimentary particles, may vary and play a role in controlling bioavailability. Trace metals may be loosely sorbed to particle surfaces, coprecipitated with iron/manganese oxides and/or trapped under multiple layers of inorganic and organic matter of various densities (fluffy flocs to multicoated authigenic precipitates).

The age of the metal–sediment association had as pronounced an effect on metal AE in *M. edulis* as any other geochemical factor. The decreased metal bioavailability with time might be explained by a progressive redistribution of the added metals into more resistant phases in the sediment over time. Sequential extractions of the radiolabeled sediment showed a progressive redistribution of all trace elements into more resistant phases. Organic contaminants also show a decreased availability to benthic organisms over days to months as they are aged in sediments (*51, 52*). Recent work (*53*) suggests that changes in absorptive and diffusive characteristics of different organic contaminants may control the decrease (smaller organic molecules diffuse into less available sites faster than large molecules). Over time, some trace elements may eventually become lodged into sedimentary mesopores, a mechanism that Mayer (*54*) used to explain the loss of labile organic matter in sediment, although this process is probably unimportant for our short-term study. Drawing on studies from the soil science literature, loss of Cd and Zn was measured over a 6-yr period in plants grown on soil following termination of contaminated sludge mulching (*55*). Cd concentrations in plants decreased 4–5-fold, and Zn decreased by only 25%, indicating a progressive conversion of metals to less bioavailable forms and decreased availability that occurred at different rates for the two metals, similar to our results. Furthermore, extractions of Zn and Cd from the soil were also found to be unreliable predictors of metal availability over time.

The effect of labeling time on bioavailability was observed progressively in *M. edulis*, but only after 35 days of aging in *M. balthica*. So biologically specific factors influenced the importance of the age of the metal–sediment association. *M. balthica* holds a larger proportion of its food in its gut for a longer period of time than *M. edulis* (e.g., initial gut transit time is 6 h vs 2.5 h, respectively) (compare refs *25* and *26*). Longer gut residence time enhances assimilation of at least some forms of particle-bound metal (*7, 26*) presumably by subjecting sediment-bound metals to more intensive digestion. This could diminish the effect of exposure time in *M. balthica*. An earlier study showed longer gut retention and significantly higher AEs for Ag, Am, Cd, and Co (but not Se and Zn) from ingested phytoplankton cells in *M. balthica* than in *M. edulis* (*41*).

Constant Ag and Se AEs in *M. edulis*, regardless of sediment contact time, suggest either a different rate at which these elements redistribute within the sediment or a binding preference for a sediment component, perhaps an organic phase, that retains a fairly constant bioavailability for *M. edulis*. Ag and Se behavior in the desorption experiments differed from the behavior of other metals in that these elements re-adsorbed to sediment particles in the presence of surfactant after 48 h. The surfactant concentration in this treatment was intended to mimic the surfactancy of *M. balthica* gut juice; *M. edulis* gut juice has a relatively low surfactancy (Griscom, unpublished). Re-adsorption of initially desorbed metals may minimize assimilation in animals, consistent with earlier findings for Cd and Hg in marine polychaetes (*56, 57*). This process could reduce AEs for Ag; however, 90% of the sediment volume initially ingested by *M. edulis* or *M. balthica* is egested within 6 or 12 h, before the re-adsorption of Se occurs in our treatments. In addition, desorption experiments have shown that the gut juice of different animal species vary considerably in their ability to extract sediment-bound metals, with some animals showing no greater desorption than that found in seawater (L. Mayer, personal communication).

In our radiolabeling experiments, sediments were not exposed to certain conditions that sediments in the field would encounter, such as bioturbation or redox oscillations. It is unknown how these processes would affect the aging effect reported here, but it is clear that bioavailability or toxicity studies involving metals and sediments need to consider the age of metal–sediment contact as well as more obvious geochemical and biological factors when evaluating metal bioavailability.

Influence of Organic Matter. The maximum differences in metal AEs from organic-rich LIS and organic-poor SFB sediments were about 2-fold, and the direction of the influence of TOC on bioavailability (positive or negative) was not consistent among metals or among species. Many characteristics other than TOC were similar between these sediments; however, because the organic matter was not characterized in these sediments, it is difficult to draw a general conclusion regarding the influence of organic matter on AE. This is partly because TOC measurements do not differentiate between humic, algal, or bacterial matter, each of which can affect assimilation. For example, Hg and Ag accumulation decreased inversely with TOC of some sediments (*58*). In laboratory experiments, Ag accumulation by *M. balthica* from marsh grass detritus was lower than Ag accumulated from a variety of other particle types (iron oxide, $CaCO_3$, manganese oxide) suggesting a strong binding affinity (and lower bioavailability) of Ag for some types of organic matter (*59*). On the other hand, Harvey and Luoma (*60*) showed that Ag uptake was enhanced in the presence of organic bacterial exudates, and Bryan and Langston (*61*) observed that elevated concentration factors of Ag and Cd occurred in *M. balthica* living in sediments near sewage outfalls, possibly high in bacterial exudates. In a study that directly considered metal uptake from food, the abundance of benthic algae, another living component, also increased rather than decreased AEs of Cd and Zn in *M. balthica* from sediment (*8*).

In general, the presence of refractory humic matter in sediments has been thought to decrease bioavailability of trace metals (*33*). Typically high binding coefficients and high concentrations of ligands in humic matter efficiently sequester metals (*33, 62*), and it is possible that humic matter may exert a primary control on metal binding. However, in *M. edulis* in this study, the AEs of Ag, Cd, Co, Cr, and Zn bound to humic-coated beads were within the same range of natural sediment AEs. A 2-fold increase of Ag and Co AEs from bacteria-coated beads as compared to humic-coated beads in *M. edulis* was consistent with (but lower than) the 5–10-fold increase in Cr AE in *M. balthica* fed humic-coated beads and free-living bacteria (*7, 33*). Slight or no differences in Cd, Cr, or Zn AEs from humic- and bacteria-coated beads suggest that bacteria associated with beads may not undergo intensive digestion unlike the case with free-living bacteria (*7, 25*) (at least in some bivalves), although all bacteria were presumably removed from the bead surfaces during digestion (*63*). It is notable, however, that experiments with single components yielded some results that were inconsistent with results from experiments with natural sediments; perhaps caution should be employed in directly comparing the two approaches.

It is apparent that geochemical factors alone are not responsible for affecting metal bioavailability from sediments, although for animals with relatively short gut transit times or metal desorption under conditions similar to those prevalent in the gut may have some predictive value for estimating AEs. Several studies (*2, 4, 23, 33*) point out the significant effect of biological factors as particle selection, flexible digestion, redox status of the gut, reproductive state, and gut fluid geochemistry. These studies suggest that sediment geochemistry and biological processing should be considered together to evaluate the complexities of the many geochemical influences on bioavailability.

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Literature Cited

(1) Campbell, P. G. C.; Lewis, A. G.; Chapman, P. M.; Crowder, A. A.; Fletcher, W. K.; Imber, B.; Luoma, S. N.; Stokes, P. M.; Winfrey, M. *Biologically Available Metals in Sediments*; National Research Council of Canada: Ottawa, Canada, 1988; Publ. 27694.

(2) Luoma, S. N. *Hydrobiologia* **1989**, *176*, 379–401.

(3) Luoma, S. N.; Johns, C.; Fisher, N. S.; Steinberg, N. S.; Oremland, R. S.; Reinfelder, J. R. *Environ. Sci. Technol.* **1992**, *26*, 485–491.

(4) Wang, W.-X.; Fisher, N. S.; Luoma, S. N. *Mar. Ecol. Prog. Ser.* **1996**, *140*, 91–113.

(5) Fisher, N. S.; Teyssié, J.-L.; Fowler, S. W.; Wang, W.-X. *Environ. Sci. Technol.* **1996**, *30*, 3232–3242.

(6) Phillips, D. J. H. *Quantitative Aquatic Biological Indicators*; Applied Science Publishers: London, 1980.

(7) Decho, A. W.; Luoma, S. N. *Limnol. Oceanogr.* **1996**, *41*, 568–572.

(8) Lee, B.-G.; Luoma, S. N. *Limnol. Oceanogr.* **1998**, *43*, 1455–1466.

(9) Gagnon, C.; Fisher, N. S. *Can. J. Fish. Aquat. Sci.* **1997**, *54*, 147–156.

(10) Pesch, C. E.; Hanson, D. J.; Boothman, W. S.; Berry, W. J.; Mahony, J. D. *Environ. Toxicol. Chem.* **1995**, *14*, 129–141.

(11) Davies-Colley, R. J.; Nelson, P. O.; Williamson, K. J. *Mar. Chem.* **1985**, *16*, 173–186.

(12) Morse, J. W. *Mar. Chem.* **1994**, *46*, 1–6.

(13) Wang, W.-X.; Stupakoff, I.; Fisher, N. S. *Mar. Ecol. Prog. Ser.* **1999**, *178*, 281–293.

(14) DiToro, D. M.; Mahoney, J. D.; Hansen, D. J.; Scott, K. J.; Carlson, A. R.; Ankley, G. T. *Environ. Sci. Technol.* **1992**, *26*, 96–101.

(15) Casas, A. M.; Crecelius, E. A. *Environ. Toxicol. Chem.* **1994**, *13*, 529–536.

(16) Berry, W. J.; Hansen, D. J.; Mahoney, J. D.; Robson, D. L.; DiToro, D. M.; Shipley, B. P.; Rogers, B.; Corbin, J. M.; Boothman, W. S. *Environ. Toxicol. Chem.* **1996**, *15*, 2067–2079.

(17) Hansen, D. J.; Berry, W. J.; Mahoney, J. D.; Boothman, W. S.; DiToro, D. M.; Robson, D. L.; Ankley, D.; Ma, D.; Yan, Q.; Pesch, C. E. *Environ. Toxicol. Chem.* **1996**, *15*, 2080–2094.

(18) Luoma, S. N.; Fisher, S. N. In *Ecological Risk Assessment of Contaminated Sediments*; Ingersoll, C. G., Dillon, T., Biddinger, G. R., Eds.; SETAC: Pensacola, FL, 1997; pp 211–237.

(19) Tessier, A.; Campbell, P. G. C.; Auclair, J. C.; Bisson, M. *Can. J. Fish. Aquat. Sci.* **1984**, *41*, 1463–1472.

(20) Ankley, G. T.; DiToro, D. M.; Hansen, D. J.; Berry, W. J. *Environ. Toxicol. Chem.* **1996**, *15*, 2056–2066.

(21) Tessier, A.; Campbell, P. G. C.; Bisson, M. *Anal. Chem.* **1979**, *51*, 844–851.

(22) Tessier, A.; Campbell, P. G. C. *Hydrobiologia* **1987**, *149*, 43–52.

(23) Mayer, L. M.; Chen, Z.; Findlay, R. H.; Fang, J.; Sampson, S.; Self, R. F. L.; Jumars, P. A.; Quetel, C.; Donard, O. F. X. *Environ. Sci. Technol.* **1996**, *30*, 2641–2645.

(24) Peterson, G. S.; Ankley, G. T.; Leonard, E. N. *Environ. Toxicol. Chem.* **1996**, *15*, 2147–2155.

(25) Decho, A. W.; Luoma, S. N. *Mar. Ecol. Prog. Ser.* **1991**, *71*, 303–314.

(26) Wang, W.-X.; Fisher, N. S. *Limnol. Oceanogr.* **1996**, *41*, 197–207.

(27) Thomson, E. A.; Luoma, S. N.; Cain, D. J.; Johansson, C. E. *Water Air Soil Pollut.* **1980**, *14*, 215–233.

(28) Luoma, S. N.; Cain, D. J.; Brown, C.; Hornberger, M.; Bouse, R. *Near field receiving water monitoring of trace metals in clams (Macoma balthica) and sediments near the Palo Alto and San Jose/Sunnyvale water quality control plants in South San*

Francisco Bay, California, 1996; U.S. Geological Survey Open File Report 97-585; U.S. Geological Survey: Reston, VA, 1997; 91 pp.

(29) Knutson, A. B. Sediment as a source of trace metals to the hard clam, *Mercenaria mercenaria*. M.S. Thesis, State University of New York, Stony Brook, 1984, p 111.

(30) Cochran, J. K.; Hirschberg, D. J.; Wang, J.; Dere, C. *Estuarine Coastal Shelf Sci.* **1998**, *46*, 503–522.

(31) Ellery, W. N.; Schleyer, M. H. *Mar. Ecol. Prog. Ser.* **1984**, *15*, 247–250.

(32) Taylor, G. T.; Gulnick, J. D. *Can. J. Microbiol.* **1996**, *42*, 911–918.

(33) Decho, A. W.; Luoma, S. N. *Mar. Ecol. Prog. Ser.* **1994**, *108*, 133–145.

(34) DiToro, D. M.; Mahoney, J. D.; Gonzalez, A. M. *Environ. Toxicol. Chem.* **1996**, *15*, 2156–2167.

(35) Simpson, S. L.; Apte, S. C.; Batley, G. E. *Environ. Sci. Technol.* **1998**, *32*, 620–625.

(36) Fisher, N. S.; Bjerregaard, P.; Fowler, S. W. *Limnol. Oceanogr.* **1983**, *28*, 432–447.

(37) Wang, W.-X.; Fisher, N. S.; Luoma, S. N. *Mar. Ecol. Prog. Ser.* **1995**, *129*, 165–176.

(38) Owen, G. *Adv. Comp. Physiol. Biochem.* **1974**, *5*, 1–35.

(39) Mayer, L. M.; Schick, L. L.; Self, R. F. L.; Jumars, P. A.; Findlay, R. H.; Chen, Z.; Sampson, S. *J. Mar. Res.* **1997**, *55*, 785–812.

(40) Fisher, N. S.; Teyssié, J.-L. *Mar. Ecol. Prog. Ser.* **1986**, *28*, 197–207.

(41) Reinfelder J. R.; Wang W.-X.; Luoma S. N.; Fisher, N. S. *Mar. Biol.* **1997**, *129*, 443–452.

(42) Jenne, E. A. *Adv. Chem. Ser.* **1968**, *73*, 337–387.

(43) Lion, L. W.; Altmann, R. S.; Leckie, J. O. *Environ. Sci. Technol.* **1982**, *16*, 660–666.

(44) Luoma, S. N.; Davis, J. A. *Mar. Chem.* **1983**, *12*, 159–181.

(45) Luoma, S. N.; Bryan, G. W. *Sci. Total Environ.* **1981**, *17*, 165–196.

(46) Jenne, E. A.; Zachara, J. M. In *Fate and Effects of Sediment-bound Chemicals in Aquatic Systems*; Dickson, K. L., Maki, A. W., Brungs, W. A., Eds.; Pergamon Press: New York, 1984; pp 83–98.

(47) Tessier, A. In *Environmental Particles: Environmental, Analytical and Physical Chemistry Series*; Buffle, J., Van Leeuwen, H. P., Eds.; Lewis: Boca Raton, FL, 1992; pp 425–456.

(48) Griscom, S. B.; Fisher, N. S.; Aller, R. C. *Trans. ASLO Abstracts*, Santa Fe, NM, 1999.

(49) Cooper, D. C.; Morse, J. W. *Environ. Sci. Technol.* **1998**, *32*, 327–330.

(50) DiToro, D. M.; Mahony, J. D.; Hansen, D. J.; Berry, W. J. *Environ. Toxicol. Chem.* **1996**, *15*, 2168–2186.

(51) Landrum, P. F. *Environ. Sci. Technol.* **1989**, *23*, 588–595.

(52) Hatzinger, P. B.; Alexander, M. *Environ. Sci. Technol.* **1995**, *31*, 603–611.

(53) Lamoureux, E. M.; Brownawell, B. J. *Environ. Toxicol. Chem.* **1999**, *18*, 1733–1741.

(54) Mayer, L. M. *Geochim. Cosmochim. Acta* **1994**, *58*, 1271–1284.

(55) Bidwell, A. M.; Dowdy, R. H. *J. Environ. Qual.* **1987**, *16*, 438–442

(56) Chen, Z.; Mayer, L. M. *Mar. Ecol. Prog. Ser.* **1999**, *176*, 139–151.

(57) Lawrence, R. L.; McAloon, K. M.; Mason, R. P.; Mayer, L. M. *Environ. Sci. Technol.* **1999**, *33*, 1871–1876.

(58) Langston, W. J. *J. Mar. Biol. Assoc. U.K.* **1982**, *62*, 667–684.

(59) Luoma, S. N.; Jenne, E. A. In *Biological Implications of Metal in the Environment*; Drucker, H., Wildung, R. E., Eds.; ERDA Symposium Series 42; Energy Research and Development Administration: Washington, DC, 1977; pp 213–230.

(60) Harvey, R. W.; Luoma, S. N. *Mar. Ecol. Prog. Ser.* **1985**, *22*, 281–289.

(61) Bryan, G. W.; Langston, W. J. *Environ. Pollut.* **1992**, *76*, 89–131.

(62) Rashid, M. A. *Geochemistry of Marine Humic Compounds*; Springer-Verlag: New York, 1985.

(63) Plante, C. J.; Mayer, L. M. *Mar. Ecol. Prog. Ser.* **1994**, *109*, 183–194.

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