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Bioavailability of particle-associated silver, cadmium, and zinc to the estuarine amphipod *Leptocheirus plumulosus* through dietary ingestion

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Abstract

We conducted experiments to determine effects of particle type on assimilatory metal bioavailability to *Leptocheirus plumulosus*, an infaunal, estuarine amphipod that is commonly used in sediment toxicity tests. The following particles were used to represent natural food items encountered by this surface-deposit and suspension-feeding amphipod: bacterial exopolymeric sediment coatings, polymeric coatings made from *Spartina alterniflora* extract, amorphous iron oxide coatings, the diatom *Phaeodactylum tricornutum*, the chlorophyte *Dunaliella tertiolecta*, processed estuarine sediment, and fresh estuarine sediment. Bioavailability of the gamma-emitting radioisotopes ^{110m}Ag, ^{109}Cd, and ^{65}Zn was measured as the efficiency with which *L. plumulosus* assimilated metals from particles using pulse-chase methods. Ag and Cd assimilation efficiencies were highest from bacterial exopolymeric coatings. Zn assimilation efficiency exhibited considerable interexperimental variation; the highest Zn assimilation efficiencies were measured from phytoplankton and processed sediment. In general, Ag and Cd assimilation efficiencies from phytoplankton were low and not related to the proportion of metal associated with cell cytosol or cytoplasm, a phenomenon reported for other particle-ingesting invertebrates. Amphipod digestive processes explain differences in Ag and Cd assimilation efficiencies between exopolymeric coatings and phytoplankton. Results highlight the importance of labile polymeric organic carbon sediment coatings in dietary metals uptake by this benthic invertebrate, rather than recalcitrant organic carbon, mineralogical features such as iron oxides, or phytoplankton.

Estuarine benthic invertebrates accumulate metals from their surrounding environment through two pathways: dissolved metals are accumulated through permeable membranes (Rainbow 1997), whereas particle-associated metals are assimilated after dietary ingestion (Luoma 1995). The relative importance of these two pathways depends on many factors (Luoma 1989, 1995; Wood et al. 1997). Dietary uptake, which has been poorly studied relative to dissolved uptake, is important because sediments (Turekian 1977; Luoma and Davis 1983; Tessier and Campbell 1987; U.S. EPA 1997) and suspended particles (Broman et al. 1994) often show high metal concentrations, and because some benthic invertebrates consume these particles in quantities equal to or greater than their body weight per day (Lopez and Levinton 1987).

A number of studies have quantified dietary metal uptake, and the factors affecting this process, for fish (Reinfelder and Fisher 1992) and a variety of marine and estuarine invertebrates including copepods (Reinfelder and Fisher 1991; Wang et al 1996*b*; Wang and Fisher 1998) and bivalves (Decho and Luoma 1991; Luoma et al. 1992; Decho and Luoma 1994, 1996; Wang and Fisher 1996; Gagnon and Fisher 1997; Reinfelder et al. 1997). The extent to which these animals accumulate metals from particulate food is dependent upon metal geochemistry, animal particle selection, and animal digestive processes. For example, dissolved forms of some metals (e.g., class B and some borderline metals) penetrate algal cell walls and concentrate within the cytoplasm (Fisher 1986); cytoplasmic metals are, in turn, assimilated by suspension-feeding invertebrates with correspondingly high efficiencies (Reinfelder and Fisher 1991; Fisher and Reinfelder 1995). Additionally, several studies have shown that bivalves exhibit flexible digestive processes in which particle retention time is positively related to particle nutritional value (Decho and Luoma 1991, 1996; Wang and Fisher 1996; Gagnon and Fisher 1997). Metals associated with highly nutritive particles (e.g., phytoplankton cells or organic sediment coatings) are, as a consequence of longer retention times, assimilated by bivalves at higher efficiencies than metals associated with particles representing low nutritive quality (e.g., iron oxides) (Decho and Luoma 1994; Gagnon and Fisher 1997).

The broad application of results from these studies to sediment quality management is limited because the only organisms that have been examined with direct regard to uptake of sediment-associated metals are epifaunal and infaunal bivalves (Luoma et al. 1992; Gagnon and Fisher 1997). Many benthic invertebrates are underrepresented in these studies, so understanding flux rates of metals from ingested particles to benthic biomass is limited (Luoma et al. 1992). Determining the dietary bioavailability of metals associated with sediment and suspended particles has other applications, including modeling metal fate in estuaries and evaluation of the effectiveness of laboratory sediment bioassays as predictors of toxicity under field conditions.

The objective of the present study was to determine how metal bioavailability varies with particle type for the amphipod *Leptocheirus plumulosus.* This is the first study to

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Table 1. Design of assimilation efficiency experiments conducted with *L. plumulosus.* Assimilation efficiencies of Ag, Cd, and Zn were measured from three major categories of particle types (coated silica particles, phytoplankton cells, and sediment particles) in five experiments. Within each experiment, assimilation efficiencies for different metal-particle type combinations were measured. Each of these metal-particle type combinations (shown below) was measured separately using individual amphipods as the experimental unit. Experiment 1 was repeated three times to obtain data from a sufficient number of amphipods for statistical purposes. Metal assimilation efficiency was measured from processed sediment (a homogenous source of thoroughly washed and autoclaved estuarine sediment stored at 4°C) to assess interexperimental variability.

	Particles tested					
Experiment	Coated silica particles	Phytoplankton	Natural and processed sediments	Metal incubation duration (h)	Number of trials per experiment	Number of amphipods tested for each particle type
1	EPS-Si*	*Dt*†, *Pt*‡	PS§	12–16	3	4 trial^{-1} (total of 12 amphipods metal-particle combination^{-1})
2	*Sp*-Si‖	—	PS§	12–16	1	5
3	AIO-Si¶	—	PS§	12–16	1	5
4	—	*Dt*†, *Pt*‡	PS§	96	1	5–7
5	—	—	MF#, SP**, PS§	12–16	1	6

* Silica coated with bacterial exopolymers.
† *D. tertiolecta.*
‡ *P. tricornutum.*
§ Processed sediment.
‖ Silica coated with *S. alterniflora* extract.
¶ Silica coated with amorphous iron oxide.
# Natural sediment from mud flat.
** Natural sediment from *S. alterniflora* marsh.

quantify dietary metal bioavailability to infaunal amphipods. *L. plumulosus* is a euryhaline, infaunal amphipod that reaches densities of $>2.5 \times 10^4$ individuals m^{-2} in estuaries along the east coast of North America (Bousefield 1973; Holland et al. 1988). Because *L. plumulosus* is a facultative suspension and surface-deposit feeder (DeWitt et al. 1992), it likely ingests a wide range of estuarine particles. Despite the fact that *L. plumulosus* is a standard species in acute (Schlekat et al. 1992; U.S. EPA 1994) and ''chronic'' (McGee et al. 1993) sediment toxicity tests, very little is known about how efficiently it accumulates metals through dietary uptake.

The metals we chose were silver (Ag), cadmium (Cd), and zinc (Zn). Cadmium and zinc sediment concentrations are often elevated in estuarine sediments (Long et al. 1995). Dietary uptake patterns may differ between these two metals because Cd has no known biological function, whereas concentrations of Zn, a necessary micronutrient, can be regulated by many crustaceans (Langston and Spence 1995). Silver, in its dissolved free ion form (Ag^+), is among the most toxic metals to aquatic organisms (Luoma et al. 1995), but the bioavailability of particle-associated Ag to benthic organisms other than bivalves is poorly studied (Fisher and Wang 1998). Bioavailability of Ag, Cd, and Zn was determined from three classes of particles that vary geochemically and nutritionally: sediment coatings, phytoplankton cells, and natural estuarine sediments. Sediment coatings consisted of silica particles that were coated with either bacterial extracellular polymeric substances, extract from the estuarine cord grass *Spartina alterniflora,* or amorphous iron oxides. Two species of phytoplankton were examined, the diatom *Phaeodactylum tricornutum* and the chlorophyte *Dunaliella tertiolecta.* Estuarine sediments included both freshly collected sediment and processed sediment.

Materials and Methods

Amphipod source and preparation—Amphipods were cultured according to previously described methods (U.S. EPA 1994) in 15‰ artificial seawater (Instant Ocean® diluted with deionized water), which was the salinity used for feeding experiments. For each feeding experiment, amphipods between 0.6 and 0.9 mm in length were retrieved from culture chambers by pouring culture sediment over a sieve series that consisted of 1.0- and 0.5-mm mesh screens. These animals were held in glass bowls containing 15‰ artificial seawater for at least 4 h, and up to 6 h, prior to introduction into experimental feeding chambers.

Experimental design—Five independent experiments were conducted to measure Ag, Cd, and Zn assimilation efficiency from three types of particles: silica coated with bacterial exopolymers, *Spartina* extract, and amorphous iron oxides; the phytoplankters *D. tertiolecta* and *P. tricornutum;* and natural and processed sediments (Table 1). Assimilation efficiency was determined using the gamma-emitting radioisotopes ^{110m}Ag (specific activity $= 5.0 \times 10^3$ kBq μmol^{-1}), ^{109}Cd (specific activity $= 2.8 \times 10^3$ kBq μmol^{-1}), and ^{65}Zn (specific activity $= 5.5 \times 10^3$ kBq μmol^{-1}). Particles were labeled with radioisotopes for 12–16 h with the exception of experiment 4, in which algal cells were incubated with radioisotopes for 4 d. Suspensions of coated silica particles and sediments were labeled by adding 18.5–37 kBq of each

radioisotope (which corresponded to 7.4 nmol Ag, 6.6 nmol Cd, and 6.7 nmol Zn). Suspensions were composed of approximately 100 μg of each particle in 40 μl seawater. Short algae incubations were prepared by adding 18.5–37 kBq of each metal with 0.5 ml of each species. Metal concentrations on particles were within concentration ranges reported for contaminated estuarine sediments (Knutson et al. 1987; Luoma 1990). The salinity of each incubation was 15‰, pH was adjusted to 8.0 by adding small quantities of dilute NaOH, and the incubations were maintained at room temperature (~20°C).

Particle preparation

Bacterial exopolymeric coatings—Bacterial exopolymers were coated onto silica particles (TLC Silica Gel, 8–10 μm diameter, Selecto Scientific) according to methods described by Schlekat et al. (1998). Briefly, exopolymers were extracted from an estuarine bacterial isolate that belongs to the enteric group of the proteobacteria (Schlekat 1998). Exopolymers from this isolate are composed glucose, galactose, and glucuronic acid in the ratio 5 : 2 : 1 (Schlekat 1998). Sediment coatings were achieved by combining exopolymers dissolved in deionized water with a suspension of silica particles in seawater and raising the pH from 5–6 to 8. Coatings represent approximately 0.43% organic matter (Schlekat et al. 1998).

Spartina *coatings*—The salt marsh cord grass *S. alterniflora* contributes significantly to particulate organic matter within eastern U.S. estuaries (Fogel et al. 1989). Plants were collected from a salt marsh near Charleston, South Carolina, and cleaned with seawater and deionized water to obtain organic matter for coatings. Leaves (approximately 25 g wet weight) were added to 500 ml deionized water and homogenized in a blender. Fifty milliliters of 1 N NaOH was added to achieve a concentration of 0.1 N NaOH, and the cord grass suspension was then heated at 60°C while stirring for 2 h. The resulting solution was poured through a 63-μm screen. Equal volumes of *S. alterniflora* extract and silica in 30‰ seawater were combined, which prompted aggregation of polymeric material around silica particles. Coated silica particles were then centrifuged and rinsed with seawater until the supernatant remained clear.

Amorphous iron oxide coatings—Amorphous iron oxide coatings were prepared according to methods described by Decho and Luoma (1994). Briefly, 1 g silica (wet weight) was added to a glass scintillation vial that contained 1.5 ml 2M $FeNO_3$. This solution was then dried at 100°C for 24 h and then rinsed with deionized water. To ensure that the iron oxides remained in an amorphous state, particles were used within 2 d after preparation. According to Decho and Luoma (1994), this procedure results in amorphous iron oxide coatings of $1 \pm 0.2 \times 10^{-2}$ g Fe g^{-1} particle.

Microscopic examination of all sediment coatings revealed that silica particles were richly coated and that little or no polymeric material or amorphous iron oxide remained in solution.

Phytoplankton—Both phytoplankton species were obtained from stationary phase laboratory cultures. Experiment 1 measured bioavailability of metals associated with algal cells for short (i.e., hours) periods. In preparation for radiolabeling in this experiment, 5 ml of algae was centrifuged, rinsed 3 times with sterile seawater (15‰), and resuspended in 2 ml sterile seawater, which resulted in cell concentrations between 14 and 27 $\times 10^6$ cells ml^{-1} for *D. tertiolecta* and 150 and 250 $\times 10^6$ cells ml^{-1} for *P. tricornutum.* Cells were then radiolabeled according to procedures described for silica-coated particles and sediments.

Experiment 4 measured bioavailability of metals associated with algal cells for longer (i.e., days) periods and followed procedures described by Fisher et al. (1991). Assimilation efficiencies were determined in separate experiments for each metal and each algal species, so a total of six incubations were performed. Phytoplankton (10 ml) were initially added to 100-ml growth medium and grown for 4 d under 24 h light and 20°C. Cells were centrifuged, rinsed with and resuspended in sterile seawater (30‰), and passed through a 63-μm screen. Cell concentrations were then measured with a Coulter counter. Appropriate volumes of *P. tricornutum* cells were added to 100-ml sterile seawater for a final concentration of 1.5×10^6 cells ml^{-1}. For *D. tertiolecta,* final cell concentrations varied for each experiment as follows (cell concentrations in cells ml^{-1}): Ag = 6.1×10^5; Cd = 5.4×10^5; Zn = 6.4×10^5. Radioisotopes and carrier were added to these cell suspensions to achieve the following concentrations: Ag = 0.22 μmol L^{-1} (1,110 kBq L^{-1}); Cd = 0.40 μmol L^{-1} (1,110 kBq L^{-1}); Zn = 0.20 μmol L^{-1} (1,110 kBq L^{-1}). Incubations were maintained at 20–24°C under 24 h light. Gentle aeration was provided via a 1-ml pipette to keep cells suspended. After 4 d, each incubation was separated into two aliquots, one for assimilation efficiency experiments and one for subcellular fractionation (Table 2). For assimilation efficiency experiments, cells were centrifuged and then resuspended in 0.5-ml sterile seawater prior to feeding. For subcellular fractionation, three 1.5-μl subsamples were subjected to a sequential fractionation procedure following methods described by Reinfelder and Fisher (1991) and summarized in Table 2. Assimilation efficiencies from these algae by *L. plumulosus* were compared to the proportion of metal in algal cytoplasm and cytosol (as defined in Table 2).

Natural sediments—Natural sediments (upper 5 mm) were collected at low tide from two sites within the pristine North Inlet estuary near Georgetown, South Carolina (33°20′N, 79°10′W). The first site was a diatom-dominated mud flat that exhibited cohesive surficial sediments. The second site was located in the upper marsh within a stand of *S. alterniflora.* Three replicate samples were collected from each site, returned to the laboratory, and incubated with metals within 12 h. Assimilation efficiency was measured on two amphipods per replicate the following day. For each sediment replicate, microbial exopolymer concentrations were measured following techniques described by Underwood et al. (1995), total organic carbon was measured following methods described by Hagopian (1998), and acid volatile

Table 2. Fractionation approach for determining distribution of Ag, Cd, and Zn within cells of *D. tertiolecta* and *P. tricornutum* following the sequential centrifugation method described by Reinfelder and Fisher (1991). Cytosolic metals are defined as those in the supernatant, whereas cytoplasmic metals are defined as the summation of metals in the supernatant and Pellets 2 and 3 (Lee and Luoma 1998).

Fractionation step	Centrifugation speed (g)	Centrifugation duration (min)	Corresponding cell fraction
Pellet 1	750	5	Cell wall material, plasmalemmae, nuclei
Pellet 2	2,000	15	Mitochondria, lysosomes, peroxisomes
Pellet 3	10,000	15	Same as above
Supernatant	NA*	NA*	Cytosol, endoplasmic reticula, ribosomes, and Golgi complexes

* NA: Not applicable. Supernatant from the final (i.e., 10,000 × *g*) centrifugation.

sulfide concentrations were measured following methods described by Allen et al. (1991).

Processed sediment—Sediment collected from Bread and Butter Creek within the North Inlet estuary was processed according to methods described by Chandler (1986) to obtain a source of sediment that exhibited consistent physical and geochemical properties over time. Metal assimilation efficiency from processed sediment was measured within each experiment as a means of determining interexperiment variability. Briefly, sediment processing consisted of sieving sediment through a 63-μm screen, washing 5 times with deionized water, condensing by centrifugation, autoclaving for 1 h, and storing at 4°C until use.

Assimilation efficiency measurement—Before initiating all feeding experiments, radiolabeled particles were centrifuged, the aqueous phase removed, the particles resuspended in a small volume of seawater, and the activity of each phase measured. To ensure that coated silica particles were palatable to amphipods, 10 μl of a concentrated suspension of *Isochrysis galbana* was mixed with the particles immediately before feeding.

Assimilation efficiency was measured using a modified pulse-chase approach described by Schlekat et al. (1999). Briefly, feeding chambers were prepared by filling two wells of a multiwell polystyrene tissue culture dish with 10 ml fine-grained sediment (collected from Bread and Butter Creek and sieved through a 125-μm screen) that were covered by 9 ml of 0.2-μm–filtered seawater. One amphipod was placed in the first well of each chamber and fed 6 μl of radiolabeled food. After 5–10 min, the amphipod was retrieved from sediment by washing the well's contents over a 125-μm screen. The radioactivity of each amphipod was then determined by measuring the gamma emissions of ^{110m}Ag (658 keV), ^{109}Cd (88 keV), and ^{65}Zn (1116 keV) with a Packard 3 in. NaI gamma detector (Packard Instrument Co.). Samples were counted for 2 min unless propagated counting errors exceeded 5%. Counts of each element were corrected for the detector's counting efficiency.

Exhibition of radioactivity by amphipods after they had been removed from sediment and thoroughly rinsed with seawater verified that they had ingested a bolus of radiolabeled food. We discarded the small proportion (~5%) of amphipods that exhibited no activity after drawing radioactive food into their burrows; the absence of activity in these specimens shows that amphipods did not accumulate measurable quantities of metals that may have desorbed from food particles during the feeding process.

The initial activity represented the quantity of metal associated with the ingested radioactive food and is referred to hereafter as ''hot feed'' (HF) activity. The animal was then placed into the second well (the ''cold feed'' [CF] well), which contained unlabeled sediment. After 3 h, amphipods were removed from the sediment for a second radioactivity measurement. This activity represented the concentration of metal assimilated by the animal, and is referred to hereafter as cold feed (CF) activity. Schlekat et al. (1999) showed that the mean gut passage time for *L. plumulosus* was 95 min, and in the present study the 3-h feeding period was sufficient for most amphipods to clear their guts. However, amphipods that exhibited aberrantly high CF activities (which could have resulted from radiolabeled food remaining in the gut) were returned to fresh unlabeled sediment and allowed to feed for an additional hour (for a total of 240 min) before the final assimilation efficiency (AE) measurement.

AE was calculated from the following equation:

$$\%\,\mathrm{AE} = [(\mathrm{CF\,activity})_{\mathrm{amphipod}}/(\mathrm{HF\,activity})_{\mathrm{amphipod}}] \times 100. \quad (1)$$

Cell digestion assessment—The ability of *L. plumulosus* to rupture ingested phytoplankton cells was qualitatively assessed by placing two groups of five starved amphipods (i.e., animals were allowed to purge their digestive tracts) in 0.5 L suspensions of *D. tertiolecta* and *P. tricornutum* (1×10^6 cells ml^{-1}) for 30 min. Amphipods were removed from algal suspensions, rinsed over a 0.5-mm screen to remove adherent algal cells, and placed in clean seawater. Fecal pellets were collected by pipette, rinsed over a 0.63-μm screen, and visualized under a microscope.

Statistical analysis—All data were tested for normality and homoscedasticity before mean comparisons were conducted. Experiment 1 was conducted as a series of three independent feeding trials, each of which was conducted during a single day (Table 1). A nested two-way analysis of variance (ANOVA) was performed to determine effects of food treatment and experimental trial on metal assimilation efficiency (SAS Institute 1985). If no significant ($P < 0.05$) interaction was indicated between treatment and experimental trial, mean metal assimilation efficiency was compared among food treatments using Tukey's test for multiple comparisons.

For experiments 2 and 3, mean assimilation efficiency be-

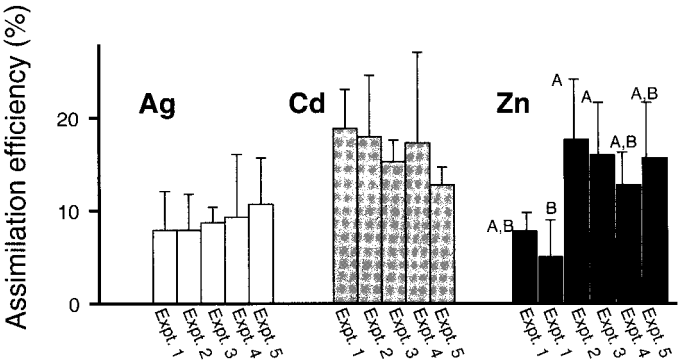

Fig. 1. Summary of mean Ag, Cd, and Zn assimilation efficiency (%) from processed sediment by the amphipod *L. plumulosus* for experiments 1–5. Error bars represent one standard deviation from the mean. Bars sharing the same letter were not significantly different following Tukey's honest significant difference test for unequal *n*.

tween Si coatings and processed sediment was compared using a *t*-test that assumed unequal variance. In experiments 4 and 5, mean assimilation efficiency was compared among treatments using a one-way ANOVA followed by Tukey's honest significant difference (HSD) test.

Results

Variability among experiments—A comparison of all five experiments showed mean assimilation efficiencies from processed sediment were not significantly different for Ag ($F_{4,26} = 0.294$, $p = 0.880$) or Cd ($F_{4,26} = 1.121$, $p = 0.368$), with mean assimilation efficiencies ranging from 7.8 to 10.7% for Ag and from 12.8 to 18.9% for Cd (Fig. 1). The interexperimental agreement for Ag and Cd shows that the amphipod assimilation process was consistent throughout all experiments for these elements and indicates that interexperimental comparisons of assimilation efficiencies are warranted. Mean Zn assimilation efficiencies were significantly different among experiments ($F_{4,28} = 6.841$, $p < 0.001$), showing that internal regulation of this element may have affected its assimilation efficiency. Uptake of Zn is highly regulated by crustaceans (Mason and Jenkins 1995), so comparisons of Zn assimilation efficiencies by *L. plumulosus* among different experiments should exclude results of trial 2 from experiment 1 because Zn assimilation efficiency from processed sediment in this trial (AE = 5.0%) was significantly lower than in experiment 2 (AE = 17.7%) and experiment 3 (AE = 16.0%) (Fig. 1).

Experiment 1—All metals exhibited higher sorption to processed sediment than to other particles (Table 3). Greater than 90% of Ag and Zn sorbed to processed sediment. Bacterial exopolymeric coatings sorbed the next highest amounts of metals, and the two phytoplankton species took up the least (Table 3).

No significant interactive effects were detected among Ag and Cd experiments, so the data in the three feeding trials for each metal were pooled. *L. plumulosus* assimilated Ag from bacterial exopolymeric coatings (mean AE = 26.9%) with significantly greater efficiency ($p < 0.0125$) than from *P. tricornutum* (mean AE = 12.8%), processed sediment (mean AE = 7.9%), and *D. tertiolecta* (mean AE = 4.3%) (Fig. 2).

Cadmium assimilation efficiency by *L. plumulosus* was significantly different among all four particle types tested. *L. plumulosus* assimilated Cd associated with bacterial coatings (mean AE = 26.8%) more efficiently than from processed sediment (mean AE = 18.9%), *P. tricornutum* (mean AE = 10.6%), and *D. tertiolecta* (mean AE = 2.9%) (Fig. 2).

In one Zn trial, animals did not readily ingest the radiolabeled food. Statistical results showed significant interactive effects between the remaining two trials (for trial *x* particle type interaction, F = 8.83, $p = 0.0003$). Therefore, the effect of particle type on Zn assimilation efficiency within each trial was analyzed separately. In the first trial, Zn assimilation efficiency from *D. tertiolecta* (mean AE = 32.6%) was significantly higher than from the other three particles. Zinc assimilation efficiency from *P. tricornutum* (mean AE = 15.1%), processed sediment (mean AE = 7.8%), and bacterial exopolymeric coatings (AE = 5.4%) were not significantly different (Fig. 2). In the second trial, Zn assimilation

Table 3. Percent of metal associated with particulate phase for particles used in assimilation efficiency experiments. Values for experiment 1 represent the mean of three independent trials, and standard deviations are in parentheses.

Experiment	Particle type	Ag	Cd	Zn
1	*D. tertiolecta*	53.6 (6.1)	37.3 (5.7)	29.7 (19.3)
1	*P. tricornutum*	61.3 (14.8)	42.5 (20.8)	39.2 (17.1)
1	Bacterial exopolymer coatings	77.4 (7.6)	66.4 (6.8)	90.6 (5.6)
1	Processed sediment	93.9 (2.7)	80.3 (4.7)	99.1 (0.7)
2	*S. alterniflora* coatings	89.9	97.5	99.1
2	Processed sediment	96.9	85.7	99.4
3	Amorphous iron oxide coatings	97.8	99.4	99.8
3	Processed sediment	94.4	86.4	98.6
4	*D. tertiolecta*	94.6	94.9	96.0
4	*P. tricornutum*	84.4	90.1	91.9
4	Processed sediment	92.5	60.8	98.1
5	Natural sediment: Mudflat	87.2	75.9	89.4
5	Natural sediment: *S. alterniflora* stand	91.1	90.7	94.2
5	Processed sediment	93.4	84.7	98.2

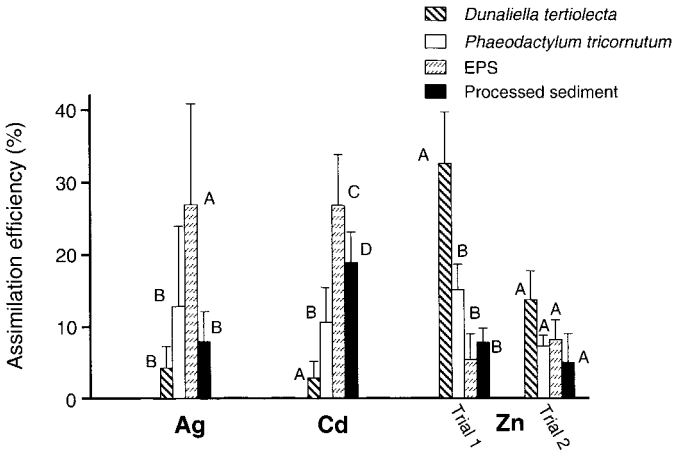

Fig. 2. Mean Ag, Cd, and Zn assimilation efficiency (%) by the amphipod *L. plumulosus* from the phytoplankters *D. tertiolecta* and *P. tricornutum*, silica coated with bacterial exopolymers, and processed sediment (experiment 1). Error bars represent one standard deviation from the mean. Bars sharing the same letter were not significantly different following Tukey's honest significant difference test.

efficiency from both phytoplankton species decreased by a factor of 2–2.3 from the first trial (Fig. 2). Here, Zn assimilation efficiency was not significantly different among the four particles (Fig. 2).

Experiments 2 and 3—Metal sorption and assimilation efficiency patterns between *Spartina* and amorphous iron oxide coatings were similar. Both particles sorbed >89% of all three metals (Table 3). For all metals, mean assimilation efficiencies from these two coatings were <13% (Fig. 3). Comparisons among experiments 1–3 reveal differences among the three sediment coatings used in this study, i.e., bacterial exopolymers, *Spartina* extract, and amorphous iron oxide. Amphipods assimilation efficiency of Cd from bacterial exopolymers was 3.4 and 5.4 times greater than from *Spartina* and amorphous iron oxide coatings, respectively. Similarly, Ag assimilation efficiency from bacterial exopolymers was 2.7 and 2.4 times greater from *Spartina* and amorphous iron oxide coatings, respectively. Zn assimilation efficiency from all sediment coatings was similar, ranging from 5 to 8% for amorphous iron oxides and *Spartina* extract, respectively.

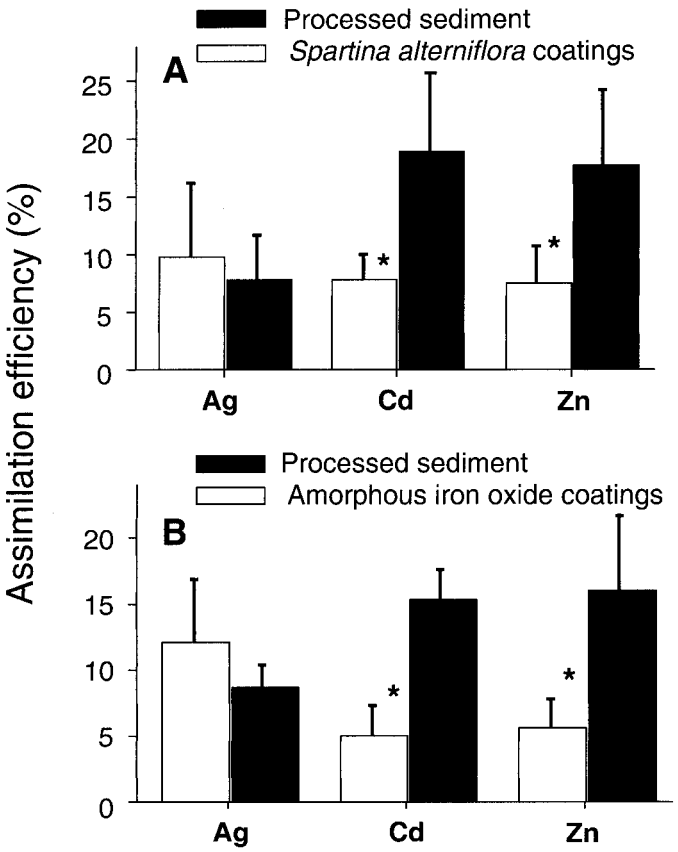

Fig. 3. Mean Ag, Cd, and Zn assimilation efficiency (%) by the amphipod *L. plumulosus* from (A) silica coated with *S. alterniflora* extract and processed sediment (experiment 2) and (B) silica coated with amorphous iron oxide and processed sediment (experiment 3). Error bars represent one standard deviation from the mean. Asterisks represent significant assimilation efficiency differences between the particles based on *t*-test results.

Experiment 4—Fractionation results from 4-d metal incubations showed that >40% of Ag was distributed in cell cytoplasm for both phytoplankton species, and that the majority of this was present in the pellet 2 fraction (Table 4). Zn showed the highest cytosolic proportion for both species. Cytosolic and cytoplasmic proportions of Cd and Zn were 2.2 to 5.5 times higher in *P. tricornutum* than in *D. tertiolecta* (Table 4).

Table 4. Mean percent (standard deviation in parentheses) of Ag, Cd, and Zn within cells of two marine phytoplankton species, *D. tertiolecta* and *P. tricornutum.* Cells were subjected to a sequential fractionation procedure as described by Reinfelder and Fisher (1991). Metals in supernatant are considered cytosolic, and cytoplasmic metals are defined as the sum of metals in the supernatant and pellets 2 and 3.

	D. tertiolecta			*P. tricornutum*		
	Ag	Cd	Zn	Ag	Cd	Zn
Pellet 1	49.1 (3.9)	89.6 (2.7)	73.7 (5.1)	58.3 (4.6)	66.1 (1.0)	41.8 (1.2)
Pellet 2	38.8 (2.9)	4.4 (0.6)	13.0 (0.9)	31.8 (3.6)	11.2 (0.9)	14.5 (0.2)
Pellet 3	2.1 (0.4)	1.8 (0.6)	1.0 (0.1)	3.5 (0.2)	1.0 (0.1)	2.7 (0.1)
Supernatant	10.0 (0.7)	4.2 (1.2)	4.2 (0.5)	6.5 (0.6)	21.8 (0.4)	41.0 (1.0)
Cytoplasm	50.9	10.4	26.4	41.8	34.0	58.2

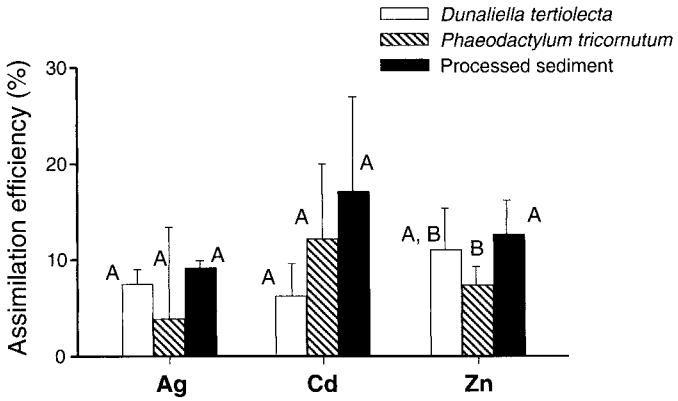

Fig. 4. Mean Ag, Cd, and Zn assimilation efficiency (%) by the amphipod *L. plumulosus* from the phytoplankters *D. tertiolecta* and *P. tricornutum,* and processed sediment (experiment 4). Error bars represent one standard deviation from the mean. Bars sharing the same letter were not significantly different following Tukey's multiple comparison test.

Mean metal assimilation efficiencies from 4-d incubations ranged from 6.2% (Cd) to 11.2% (Zn) for *D. tertiolecta* and from 4.0 (Ag) to 12.3% (Cd) for *P. tricornutum* (Fig. 4). Comparing results from experiments 1 and 4 shows both metal- and algae-specific differences as a result of incubation duration. First, Ag and Cd assimilation efficiencies from *D. tricornutum* were uniformly <10% in both experiments (Figs. 2 and 4). Second, amphipods assimilated Ag from *P. tricornutum* 3.3 times more efficiently in short versus long incubations. Finally, Zn assimilation efficiencies from both algal species were highest when algae was incubated for shorter incubation periods. These differences were more pronounced for *D. tertiolecta,* where Zn assimilation efficiency in 12–16-h incubations was up to 3 times greater than from 4-d incubations (Figs. 2 and 4).

Comparisons between assimilation efficiency and algal cell fractionation results indicate that amphipods did not uniformly assimilate metals within algal cell cytoplasm or cytosol (Fig. 5). Assimilation efficiency increased with increasing proportions of cytosolic metal in *D. tertiolecta,* but the correlation was not significant ($r = 0.885$, $p = 0.308$). There was no relationship between cytosolic metal and assimilation efficiency for *P. tricornutum* ($r = 0.399$, $p = 0.738$) (Fig. 5). For both algal species, the proportion of cytoplasmic metal was always greater than the proportion of metal assimilated by *L. plumulosus,* and correlations between these parameters were weak and not significant (*D. tertiolecta:* $r = 0.154$, $p = 0.902$; *P. tricornutum:* $r = 0.360$, $p = 0.766$) (Fig. 5).

Experiment 5—Characteristics of the two natural sediments were similar with the exception of acid volatile sulfide concentration, which was 5 times higher in sediments collected from mud flat sediments (mean = 0.11 μmol g^{-1} dry sediment ± 0.04) compared with *S. alterniflora* sediments (mean = 0.02 μmol g^{-1} dry sediment ± 0.01). Total organic carbon ranged from 6.9% (SD = 2.5) for the mud flat site to 6.1% (SD = 1.5) for the cord grass site, and microbial exopolymer concentrations for both sites were approximately 1 mg g^{-1} dry sediment. Mean assimilation efficiency was not significantly different among the two natural sediments and processed sediments for Ag ($F_{2,5} = 1.26$, $p = 0.317$), Cd ($F_{2,5} = 1.75$, $p = 0.212$), or Zn ($F_{2,5} = 1.54$, $p = 0.252$). Assimilation efficiencies for Ag ranged from 10.7% (SD = 5.0) from processed sediment to 21.1% (SD = 5.0) from *S. alterniflora* sediments. For Cd, mean assimilation efficiencies ranged from 9.8% (SD = 5.4) from mud flat sediment to 15.5% (SD = 4.7) from *S. alterniflora* sediments. For Zn, mean assimilation efficiencies ranged from 11.1% (SD = 1.6) for mud flat sediments to 16.3% (SD = 3.6) from *S. alterniflora* sediments for Zn.

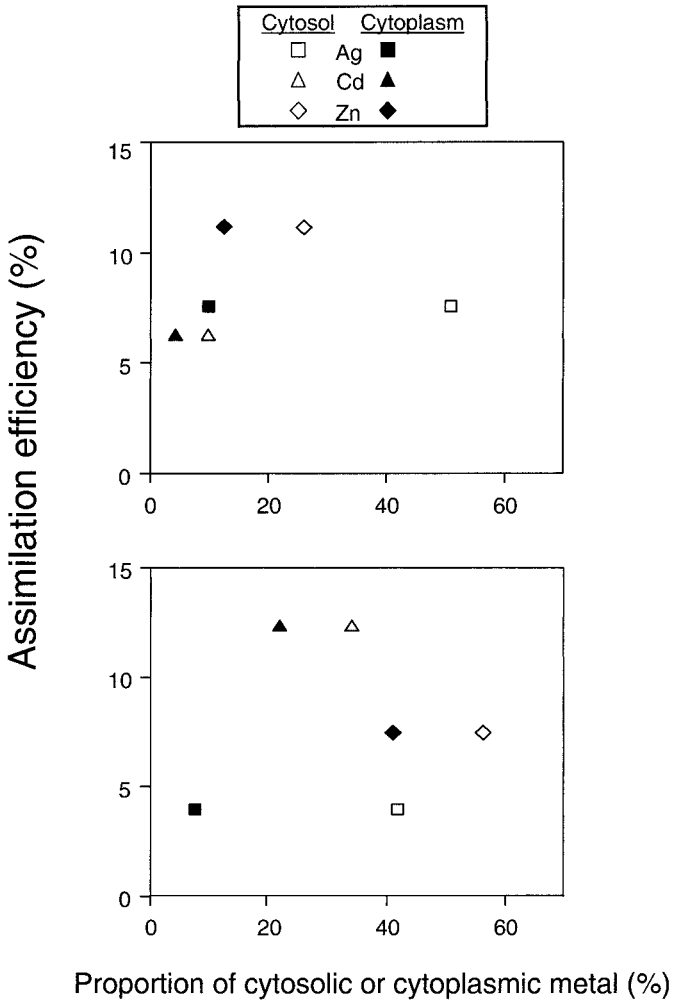

Fig. 5. Relationship between the distribution of metals within cytosol and cytoplasm of *D. tertiolecta* and *P. tricornutum* and the efficiency with which metals were assimilated from these algae by *L. plumulosus* (experiment 4).

Cell digestion assessment—Microscopic examination of fecal pellets from animals fed both *D. tertiolecta* and *P. tricornutum* showed both intact, living cells and incompletely digested cells (that is, ruptured *D. tertiolecta* cells and empty *P. tricornutum* frustules). The integrity of intact cells was confirmed by fluorescence of chloroplasts within cells when visualized under ultraviolet light. This indicates that

only a portion of ingested algal cells are ruptured in the stomach and that many are simply egested.

Discussion

This study represents the first attempt to quantify dietary metal assimilation from ecologically and environmentally relevant particles to an infaunal estuarine amphipod. Our results show that bioavailability patterns observed for bivalves and copepods may not describe those of all marine and estuarine particle feeders. For example, ranges of metal assimilation efficiencies for all particles in this study (Ag 4–26%, Cd 3–26%, Zn 5–33%) were generally below those reported for the mussel *Mytilus edulis* feeding on phytoplankton and natural seston (Wang et al. 1996*a*). In particular, amphipod metal assimilation efficiencies from phytoplankton showed the greatest divergence from previous studies conducted with bivalves (Wang et al. 1996*a*) and copepods (Reinfelder and Fisher 1991). Patterns of results from this study show that Ag and Cd associated with bacterial exopolymer sediment coatings, which are presumably labile, were assimilated with higher efficiency than those associated with recalcitrant particles, including amorphous iron oxide coatings. This pattern is in agreement with previous studies that have measured metal assimilation efficiencies by bivalves from diverse particle coatings (Harvey and Luoma 1985; Gagnon and Fisher 1997) in which particle-incubation periods were similar (within 8–12 h) to those used in the present study. The particle-specific differences observed in the present study are important because they show that metal bioavailability was not related to how particles are classified on a biogeochemical basis (e.g., organic carbon vs. mineral grains), but rather on the basis of how the amphipod digestive system processes particles and thereby liberates particle-associated metals.

Metal assimilation from sediment coatings—Our results showing relatively high Ag and Cd assimilation efficiencies from bacterial exopolymeric coatings agree with previous studies conducted with bivalves (Harvey and Luoma 1985; Decho and Luoma 1996). These observations of enhanced bioavailability for metals associated with bacterial exopolymeric coatings support Simkiss' (1995) empirical contention that these sediment phases can act as vectors of metal uptake to benthic invertebrates in nature. This contention was based on several factors, including the ubiquity of bacterial exopolymeric coatings in estuarine and marine sediments (Decho 1990; Meyer-Reil 1994; Underwood et al. 1995) and in suspended particulate matter (Rogerson and Layborn-Parry 1992). Also, many well-characterized forms of exopolymers exhibit high metal-binding affinities (Ford and Mitchell 1992; Schlekat 1998; Schlekat et al. 1998). Finally, many marine and estuarine benthic invertebrates efficiently use these bacterial polysaccharides as a carbon source (Baird and Thistle 1986; Decho and Moriarty 1990; Bernhard and Bowser 1992). Although sorption of Cd to bacterial exopolymeric coatings has been shown to be inversely related to pH (Schlekat et al. 1998), there is no evidence that amphipod gut pH extends below the range of 6–7, and so the role of pH in the relatively high Cd assimilation efficiency from exopolymeric coatings is doubtful. Carbohydrases, however, are prevalent among invertebrates (Lopez and Levinton 1987); these enzymes can presumably degrade bacterial exopolymers, thus solubilizing metals for assimilation. This process does not, however, explain low Zn assimilation efficiency from bacterial exopolymeric coatings.

The apparent importance of bacterial exopolymers in metal uptake is consequential to oxidized surficial sediments because of the importance of organic carbon in metal sorption within these regions (Ankley et al. 1996). The mediating effect of organic carbon on metal bioavailability is considered to be uniform, yet many different forms of organic carbon can occur in sediments. *Spartina*-derived coatings should be comprised of the structural polymers cellulose, lignocellulose, and lignin of this plant, which are recalcitrant to invertebrates (Mann 1988), whereas many studies have demonstrated invertebrate use of bacterial exopolymers (Baird and Thistle 1986; Decho and Moriarty 1990; Bernhard and Bowser 1992). Thus, Ag and Cd assimilation efficiency appears to be linked to the nutritional quality of these organic coatings. This concept is supported by results of Decho and Luoma (1994), who showed that the bivalve *Potamocorbula amurensis* assimilated Cd from recalcitrant organic coatings (humic and fulvic acids) with lower efficiency than from uncoated particles. Explanations for the observed differences include longer gut residence time for more nutritious particles; alternatively, bacterial polymers may be more susceptible to amphipod digestive processes than *Spartina*-derived particles. Because particulate organic matter within estuarine systems is composed of mixtures of labile microbial and recalcitrant plant material (Fogel et al. 1989), future studies on metal bioavailability from well-characterized mixed-polymeric sediment coatings and from natural suspended particulate organic matter are needed to determine if metal bioavailability from organic material shifts as the composition of organic material changes with respect to its source and to geochemical aging, and among different invertebrate classes.

Our results showing low assimilation efficiencies for Cd and Zn from recalcitrant amorphous iron oxide coatings support a relationship between bioavailability of metals associated with particles and particle nutritional quality. Previous studies have shown that the bivalves *Macoma balthica* and *P. amurensis* (Decho and Luoma 1994) and *M. edulis* (Gagnon and Fisher 1997) also assimilate metals associated with these coatings with low efficiencies relative to organic coatings. Iron oxide sediment coatings have been thought to affect metal bioavailability within surficial oxidized sediments (Luoma 1989; Decho and Luoma 1994; Ankley et al. 1996), and our results support the contention that metals within this dominant sediment phase are relatively unavailable through dietary ingestion.

Metal assimilation from phytoplankton—Our a priori expectations regarding metal assimilation from phytoplankton were not fully met. First, the difference in Zn assimilation efficiency from *D. tertiolecta* between short and long incubations was unexpected. Most previous studies determining bioavailability of algae associated metals have used 4–8-d incubations (Reinfelder and Fisher 1991; Wang and Fisher 1996). Our results show that metals associated with algal

cells for shorter time periods (hours), which could occur through the introduction of metal pulses via storm-related urban runoff, may be more bioavailable than longer metal algae associations (days). Second, the absence of a strong relationship between metal assimilation efficiency and proportions of metal in algal cell cytosol and cytoplasm runs counter to previous findings with other invertebrates. Reinfelder and Fisher (1991) demonstrated that calanoid copepods efficiently assimilated cytosolic proportions of many metals from the diatom *Thallasiosira pseudonana.* Because Fisher et al. (1991) observed similar metal assimilation efficiencies by copepods from the prymnesiophyte *I. galbana,* Reinfelder and Fisher (1991) concluded that this relationship for calanoid copepods is independent of the algal species. Wang and Fisher (1996) showed that cytoplasmic metals were important to how efficiently the bivalve *M. edulis* assimilated metals, but that this importance was strongly metal specific and was tempered by the response of *M. edulis* to differences in the nutrition of different algal species. Although we found some suggestion that *L. plumulosus* assimilated cytoplasmic metals from *D. tertiolecta,* amphipods clearly did not assimilate this fraction of ingested *P. tricornutum* cells.

Reinfelder and Fisher (1991) suggested that their results were due to rupturing of cells by copepods during digestion, which facilitated the release of metals associated with cytoplasmic proteins through desorption or enzymatic protein degradation. The absence of this relationship with *L. plumulosus* may reflect differences between its digestive physiology and that of other particle-ingesting invertebrates. Icely and Nott (1984) described the digestive physiology of *Corophium volutator,* a comember with *L. plumulosus* of the superfamily Corophioidea. They suggested that food particles are subject to rapid, size-selective processing within the stomach, which separates soluble material from indigestible particles. Dissolved digestate (<0.06 μm) is directed into ventral caeca where solubilized materials, including nutrients (Icely and Nott 1984) and copper (Icely and Nott 1980), have been shown to be absorbed. In order for metals within algal cell cytoplasm to be assimilated, therefore, they must first be ruptured and solubilized within the stomach; intact cells, because of their size (i.e., >1 μm), will be selectively transported to the midgut for egestion. The incomplete algal processing observed for *L. plumulosus* in this study can explain why metal assimilation efficiency from algal cells was low and why it was not strongly related to cytosolic or cytoplasmic metal.

Our algal fractionation results differ from previous studies using the same technique. Specifically, cytoplasmic Ag proportions in this study were substantially higher than previously recorded (Reinfelder and Fisher 1991; Wang and Fisher 1996; Wang et al. 1996*a*), perhaps because of incomplete separation of algal cell wall material within pellet 1. Many previous studies show Ag to be concentrated in cell walls (Reinfelder and Fisher 1991; Wang and Fisher 1996; Wang et al. 1996*a*), but if our observation of high cytoplasmic Ag is valid, then the relative importance of dietary Ag uptake for organisms that efficiently assimilate and retain cytoplasmic metals, e.g., *M. edulis* (Wang and Fisher 1996), would increase. High cytoplasmic Ag should be inconsequential to amphipods, which appear to assimilate metals only from the proportion of cells that are digested (this study), and to copepods, which exhibit faster metal efflux rates when metals are accumulated via dietary ingestion than when dissolved metals are accumulated through permeable membranes (Wang and Fisher 1998).

Metal assimilation from natural sediments—Metal assimilation efficiencies from natural sediments were generally between 10 and 20%. The impact of these efficiencies becomes clear when it is considered that *L. plumulosus* can ingest up to 3 times its body weight in sediment per day (Schlekat unpubl. data). In laboratory toxicity tests conducted with *L. plumulosus,* organisms are supplied with abundant artificial diets (DeWitt et al. 1992) upon which they may feed with preference over test sediments. Galay Burgos and Rainbow (1998) recently demonstrated that the amphipod *C. volutator* progressively accumulates Cd, Co, and Zn from sediments amended with metal-contaminated sewage sludge when no additional food was added. Thus, organisms in laboratory toxicity bioassays may not accumulate as much metal as do organisms living among metal-contaminated sediments or those experiencing continuous metal introduction via sedimentation of phytoplankton or other suspended material.

The influence of particle type on assimilatory metal bioavailability indicates that dietary metals uptake for *L. plumulosus* may exhibit temporal variability. Amphipods feeding on sediment or flocculent material coated with bacterial exopolymers assimilated metals with a higher efficiency than those feeding on phytoplankton. In the field, such a seasonally based, dichotomous diet is thought to occur with *L. plumulosus,* as well as other estuarine surface-deposit–feeding invertebrates, with the importance of suspension feeding restricted to spring phytoplankton blooms (Marsh and Tenore 1990). Metal introduction to sediments often exhibits a temporal relationship with periods of primary productivity (Shaw et al. 1994; Luoma et al. 1998), which would suggest that metal flux from particulate matter to benthic biomass would be maximized under these conditions. However, *L. plumulosus* assimilated metals most efficiently from labile organic carbon sediment coatings, on which it presumably subsists during periods of low primary productivity. Further studies are needed to examine temporal changes in metal body burdens of *L. plumulosus* and other particle-feeding invertebrates.

In summary, *L. plumulosus* assimilated Ag, Cd, and Zn from particles that are pertinent to this amphipod's role as a surficial deposit or suspension feeder. Metal assimilation efficiency was highly particle dependent. Ag and Cd showed similar patterns across particle types, and assimilation of Zn exhibited the most interexperimental variability. Importantly, our results showed that metal assimilation efficiencies from two types of organic carbon were substantially different; that metal assimilation efficiency from iron oxide coatings were minimal; and, in contrast to assimilation patterns exhibited by bivalves and copepods, amphipod metal assimilation efficiency from phytoplankton was low and unrelated to the proportion of metal associated with algal cell cytosol or cytoplasm. These results highlight the importance of dietary metals uptake by benthic invertebrates and indicate the need

to further examine the role of organic carbon in the fate of metals in estuarine systems.

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