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Christian E. Schlekat

Alan W. Decho

University of South Carolina - Columbia, awdecho@mailbox.sc.edu

G. Thomas Chandler

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Dietary assimilation of cadmium associated with bacterial exopolymer sediment coatings by the estuarine amphipod *Leptocheirus plumulosus*: effects of Cd concentration and salinity

Christian E. Schlekert*, Alan W. Decho, G. Thomas Chandler

Department of Environmental Health Sciences, University of South Carolina, Columbia, South Carolina 29208, USA

ABSTRACT: Bacterial extracellular substances (also known as exopolysaccharides, or EPS) may serve as vectors for trophic transfer of metals in benthic systems because these ubiquitous sediment coatings can sorb high concentrations of toxic metals, and because many benthic invertebrates assimilate EPS sediment coatings upon ingestion. We conducted 3 sets of experiments to determine the assimilative bioavailability of EPS-associated Cd to the benthic amphipod *Leptocheirus plumulosus* as a function of Cd concentration and salinity. Bioavailability was measured as *L. plumulosus* Cd assimilation efficiency (AE) from EPS-coated silica (EPS-Si) and from uncoated silica (NC-Si) using modified pulse-chase methods with the gamma-emitting radioisotope ^{109}Cd . Cd AE was significantly greater from NC-Si than from EPS-Si at 7.5‰, but not at 2.5 or 25‰. Overall, Cd AE from EPS-Si was between 15.1 and 21.5%. Because EPS-Si sorbed more Cd than NC-Si, EPS coatings magnified the amount of Cd amphipods accumulated at each salinity by up to a factor of 10. Salinity did not directly affect Cd AE from EPS-Si, but because Cd-EPS partitioning increased with decreasing salinity, amphipods accumulated more Cd from EPS at the lowest Cd-EPS incubation salinity (2.5‰) than at higher salinities (7.5 and 25‰). Finally, Cd concentration in EPS exhibited an inverse relationship with Cd AE at 2.5‰, but not at 25‰. Specifically, Cd AE was 12 times greater at 1 compared with 10 $\mu\text{g Cd pg}^{-1}$ EPS. Together, these results show that estuarine benthos can accumulate Cd from EPS sediment coatings, but that the degree to which this phenomenon occurs is dependent upon seawater salinity and Cd concentration in EPS.

KEY WORDS: Exopolysaccharides · Cadmium · *Leptocheirus plumulosus* · Bioavailability · Salinity

INTRODUCTION

Like many metals, cadmium tends to become concentrated in estuarine sediments (Kennish 1989, Bryan & Langston 1992). Kennish (1989) reported Cd concentration in contaminated estuarine sediments between 1.2 and 860 $\mu\text{g Cd g}^{-1}$ sediment, and Hansen et al. (1996) reported sediment Cd concentrations as high as 38900 $\mu\text{g Cd g}^{-1}$ sediment. Cd was identified as a factor causing toxicity to surrogate benthic invertebrates that were exposed to naturally contaminated estuarine (Hansen et al. 1996, Hall & Alden 1997) and freshwater

(Ankley et al. 1991) sediments in laboratory exposures. However, application of laboratory test results cannot be used to predict toxicity or bioavailability of sediment-associated Cd *in situ* because Cd is subject to remobilization between particulate and dissolved phases in sediments (Bryan & Langston 1992), and because particle-ingesting benthic invertebrates accumulate Cd from different sources using 2 different processes. First, these organisms accumulate dissolved Cd that is present in pore water or in overlying water through permeable membranes (e.g. gills) (Rainbow 1997). Additionally, many benthic invertebrates also accumulate particle-associated Cd through dietary ingestion (Luoma 1989, 1995). The relative importance of these pathways is dictated by interdependent factors, including how Cd partitions between aqueous

*Present address: U.S. Geological Survey, Water Resources Division, MS 465, 345 Middlefield Road, Menlo Park, California 94025, USA. E-mail: cschleka@usgs.gov

and particulate phases, and the rate with which these organisms accumulate metals from each phase.

In estuaries, the relative importance of dissolved versus dietary Cd uptake routes is subject to uncertainty because the degree to which Cd partitions to particles is influenced by seawater salinity, which fluctuates in these environments both spatially and temporally. In oligohaline portions of estuaries, i.e. where salinity is <5‰, Cd partitions preferentially to particles (de Groot 1995). As ionic strength increases (either seaward or in conjunction with daily tidal fluctuations), particle-associated Cd is thought to desorb with subsequent increases in concentrations of Ca^{2+} (which can out-compete Cd^{2+} for cationic binding sites on particles; Paalman et al. 1995) and Cl^- (which forms soluble complexes with Cd^{2+} ; Fisher & Reinfelder 1995). Regardless of the mechanism, increasing salinity seems to cause a general desorption from particles (Fisher & Reinfelder 1995), which should decrease the relative importance of dietary uptake as a means of metal accumulation by particle-ingesting benthic invertebrates. This generalization treats sorptive characteristics of particles as uniform; particles with high metal affinities, however, may not exhibit this salinity-related desorption.

Recent studies examining effects of salinity and Cd concentration on Cd sorption by sediment coatings composed of bacterial extracellular polymeric substances (EPS) showed that Cd affinities of EPS are strong enough that EPS-associated Cd may be important for dietary uptake even at high salinities. EPS coatings, which are produced by sediment-adherent bacteria (Decho 1990, Meyer-Reil 1994) and microalgae (Hoagland et al. 1993), are among the most prevalent phases on the surfaces of oxygenated surficial sediment particles. These polysaccharide-rich polymers are widely distributed in the upper 5 mm of cohesive surficial sediments (Decho & Lopez 1993, Underwood et al. 1995) and in suspended particles (Rogerson & Laybourn-Parry 1992a,b), and exhibit high weight-specific metal-binding capacities (Decho 1990, Ford & Mitchell 1992).

In earlier studies, we examined effects of sediment coatings composed of EPS produced by a cosmopolitan estuarine bacterial isolate on Cd sorption across a broad salinity gradient (i.e. 2.5 to 25‰) (Schlekat 1998, Schlekat et al. 1998). Our results showed that EPS coatings increase the sorptive capacity of particles across this salinity range, and that Cd sorption to EPS coatings decreases with increasing salinity only at high (5 mg l^{-1}) Cd concentrations, which suggests that weaker EPS binding phases become involved in Cd sorption as stronger phases become saturated. In turn, these details suggest the following consequences/hypotheses with regard to the link between Cd bind-

ing strength and bioavailability: (1) because EPS exhibits higher Cd affinity than uncoated particles, Cd sorbed to uncoated particles should exhibit a higher bioavailability than from EPS coatings; (2) Cd sorbed to EPS coatings should be more bioavailable at lower salinities than at higher salinities (e.g. due to competition from Ca^{2+} at higher salinities, which results in increased sorption of Cd to weaker binding phases); and (3) that as EPS becomes saturated with Cd, and as weaker binding phases become involved in the sorptive process, Cd bioavailability should increase.

OBJECTIVES

The specific objectives of this study were to: (1) determine the relative importance of EPS sediment coatings as a Cd uptake vector by benthic particle feeding invertebrates, (2) determine salinity effects on the bioavailability of EPS-associated Cd, and (3) evaluate metal concentration effects on the bioavailability of EPS-sorbed Cd. Cd bioavailability was measured as the efficiency with which the estuarine amphipod, *Leptocheirus plumulosus* assimilated Cd associated with these particles. Assimilation efficiency (AE) was measured using a pulse-chase technique that was modified from methods described by others for copepods (Reinfelder & Fisher 1991) and bivalves (Decho & Luoma 1991). This approach is based on the precept that only a proportion of Cd associated with ingested particles will be solubilized by digestive processes for transport across the gut wall. We used the gamma-emitting radioisotope ^{109}Cd , which allows for sequential, non-destructive quantification of extremely low metal concentrations within living amphipods, to measure this proportion.

MATERIALS AND METHODS

Amphipod source and preparation. *Leptocheirus plumulosus* lives infaunally in fine sediments within estuaries on the east coast of North America (Bousefield 1973). Because *L. plumulosus* is a facultative suspension/surface deposit feeder (DeWitt et al. 1992), it likely encounters EPS particle coatings during its feeding activities. Although *L. plumulosus* is commonly used to test the toxicity of estuarine sediments (McGee et al. 1993, U.S. EPA 1994), little is known about dietary metal uptake by this organism.

Amphipods were cultured in our laboratory according to previously described methods (U.S. EPA 1994). 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 amphipods were held in glass bowls containing SW for at least 4 h, and up to 6 h, prior to introduction into experimental feeding chambers. Feeding experiments were conducted at salinities that ranged from 2.5 to 25‰, and amphipods were acclimated to the test salinity before feeding between 1 and 2 wk.

Bioavailability measurements. Feeding procedure:

All feeding experiments were conducted in 6-well Falcon® polystyrene tissue culture dishes (Becton Dickinson, Franklin Lakes, NJ). Each feeding chamber received 1 amphipod. Before amphipod introduction, 2 wells within each feeding chamber received 10 ml of fine-grained sediment that was covered by 9 ml sea-water (SW). Oxidized surficial sediment (0 to 3 cm) was collected from Bread and Butter Creek, North Inlet, SC, USA (33° 20' N, 79° 10' W), sieved through a 125 µm screen, and frozen at -70°C prior to use. SW was Instant Ocean® that was prepared with deionized water (DI), and 0.2 µm-filtered before use. Approximately 12 h before feeding, amphipods were selected from glass bowls and placed individually into 1 well of the feeding chamber. Amphipods were allowed 30 min to burrow into sediment; those amphipods that did not burrow in this time were replaced.

The feeding experiment was initiated by providing an initial pulse of radiolabeled food to each individual amphipod (Fig. 1). This well was designated the 'hot-feed' well, or HF. In order to stimulate infaunal filter feeding, approximately 100 µl of algae (*Isochrysis* aff. *galbana*, isol. Haines T-iso) was introduced into the feeding chamber near the amphipod's incurrent burrow. Once amphipods began filtering, 6 µl of radiolabeled food was administered near the burrow opening, and the filtration current drew the radiolabeled material into the burrow. For AE measurements, radiolabeled food consisted of either NC-Si or EPS-Si that had been incubated with the radioisotope ¹⁰⁹Cd (see below for particle preparation and radiolabeling procedures).

After ingesting the radiolabeled food, amphipods were removed from the HF well within 5 to 10 min, sieved on a 125 µm screen, and washed thoroughly with clean SW in order to remove any radiolabeled particles that may have adhered to the exoskeleton or setae. Amphipod radioactivity was then measured (see below for details), and this activity represented the quantity of metal associated with the ingested particulate material (referred to hereafter as HF activity).

The amphipod was then placed into the second well of the feeding chamber (the 'cold-feed' [CF] well), which contained fresh, unlabeled sediment. Each amphipod was allowed to feed on the unlabeled sediment until the radiolabeled particulate food had

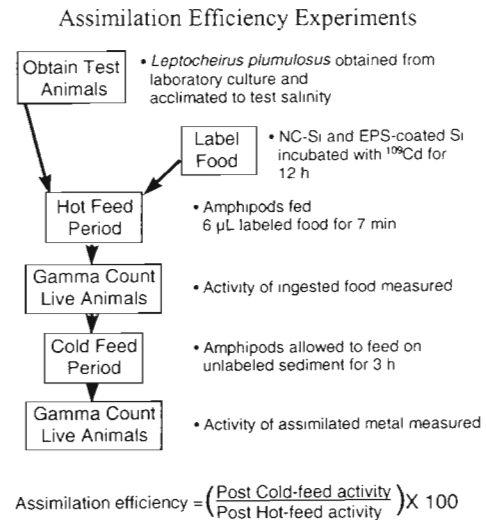


Fig. 1. Flow chart showing method for measuring Cd assimilation efficiency by the amphipod *Leptocheirus plumulosus*

passed through the gut (see below for gut passage time determination). These values represented the concentration of metal that was assimilated by the amphipod (referred to hereafter as CF activity).

Gut passage time: In order to obtain reliable gut passage time estimates for *Leptocheirus plumulosus*, a preliminary experiment employing a modification of the method described by Decho & Luoma (1991) was conducted. A food known to be readily consumed by *L. plumulosus* was prepared by combining 20 µl *Isochrysis galbana* and 20 µl fresh, processed sediment. This was then supplemented with a 50 µl solution of indigestible plastic beads (10 µm diameter) that were impregnated with the radioisotope ⁵⁷Co (ICN). Feeding chambers were prepared by adding sediment and SW in the amounts described above to 6 wells amphipod⁻¹ (1 HF and 5 CF wells per feeding chamber). Twelve amphipods were independently fed 6 µl of the radiolabeled food, and the HF activity of each amphipod was measured after 5 to 10 min. The amphipods were then placed in the first CF chamber to feed on unlabeled sediment. CF activity was measured approximately every 30 to 60 min following HF termination until CF activity was <3% of HF activity. After each CF activity measurement, amphipods were placed into a new CF well. Gut passage time was calculated as the time between initial bead ingestion and the time at which amphipods no longer exhibited a detectable ⁵⁷Co signal (i.e. 120 keV). To determine if gut passage time was related to size, wet weight was determined by blotting amphipods dry and weighing to the nearest 0.1 mg prior to placement into the first well of each feeding chamber.

Assimilation efficiency measurement: Co and Cd radioactivity of each amphipod were determined by measuring the gamma emissions by using the gamma emissions of ^{57}Co (120 keV) and ^{109}Cd (88 keV) with a Packard 3' NaI gamma detector (Packard Instrument Co., Downers Grove, IL). Samples were counted for 2 min unless propagated counting errors exceeded 5%.

Assimilation efficiency (AE) was calculated from the following equation:

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

This approach uses activity measured within the amphipod, and is probably a conservative determination of AE because HF activity values may include radiolabeled particles that adhered to amphipod setae, which can act to overestimate the value of the denominator.

Preparation of experimental particles. In order to determine the potential for EPS sediment coatings to serve as a vector of Cd uptake for benthic invertebrates, we compared the AE of Cd associated with 2 particle types, silica particles (Si) (Selecto Scientific TLC Silica Gel, 8 to 10 μm diameter), which served as a surrogate sediment foundation, and Si that was coated with EPS. We used EPS produced by an estuarine bacterium, NISC1, that was originally isolated from sediments within the North Inlet estuary near Georgetown, SC, USA. Isolation of NISC1, its taxonomic characterization, and compositional analysis of its EPS are described in Schlekert (1998). Detailed coating procedures have been described elsewhere (Schlekert 1998, Schlekert et al. 1998). Briefly, EPS coatings (equivalent to 0.43% organic matter, Schlekert et al. 1998) were prepared by combining 5 mg purified EPS dissolved in 7.5 ml DI with a suspension of 130 mg particulate silica in 7.5 ml SW. After coating, EPS-Si was resuspended in 5 ml fresh SW and washed through a 125 μm sieve in order to remove aggregate material too large for *Leptocheirus plumulosus* to directly ingest. NC-Si was prepared in essentially the same way, except that no

EPS was introduced, i.e. 130 mg particulate silica (dry wt) was added to 7.5 ml SW at 30‰.

Labeling experimental particulate with radioisotopes. Both EPS-Si and NC-Si were prepared for feeding experiments by incubating each particle preparation with ^{109}Cd (as CdCl_2 , obtained from ICN, Watham, MA). With the exception of the last experiment (i.e. that addressing the effects of Cd:EPS ratio on Cd bioavailability; described later), particles were incubated by combining 20 μl of either NC-Si or EPS-Si with 20 μl of SW and 11 μl of a ^{109}Cd solution (radioactivity = 0.11 μCi , quantity = 0.95 μg). After adding the Cd stock solution, the pH of each incubation was adjusted to 8.0 by adding small quantities of dilute NaOH, and the incubations were maintained at room temperature (approx. 20°C) overnight (approx. 12 h). Before initiating all feeding experiments, radiolabeled particles were centrifuged, the aqueous phase removed, the particles resuspended in a small volume of SW, and the activity of each phase measured. To ensure that particles were palatable to amphipods, 10 μl of a concentrated suspension of *Isochrysis galbana* was mixed with the particulate phases of both NC-Si and EPS-Si treatments.

Experimental designs for different objectives.

Effect of EPS coatings on bioavailability of particulate Cd: A series of experiments were conducted to compare Cd AE between EPS-Si and NC-Si at a specific salinity, either 2.5, 7.5, or 25‰. In order to obtain AE data from a sufficient number of individual amphipods, 2 experiments were conducted at each salinity. Within each experiment, 6 amphipods were fed each particle type, for a total of 12 amphipods per particle type within each salinity. A 2-way ANOVA was used to determine effects of experimental unit and particle type on Cd AE within each salinity.

Effect of salinity on Cd AE from EPS-Si: Two experiments were conducted to distinguish between geochemical and physiological effects of salinity on Cd AE from EPS-Si. The first experiment addressed geochem-

Table 1. Components (μl) of ^{109}Cd -labeled particulate food (EPS-coated silica) used to determine effects of the Cd:EPS ratio on Cd assimilation efficiency by the amphipod *Leptocheirus plumulosus*. Separate feeding experiments were conducted at 2.5 and 25‰. SED = processed sediment particles

Sample	Cd (vol./stock concentration)	Constituent volume (μl)			Proc. sed.	Final vol. ^d
		SW (30‰ or 60‰ ^a)	DI (30‰ or 60‰ ^a)	EPS-Si slurry		
0.25 $\mu\text{g Cd g}^{-1}$ EPS	20/28 mg Cd l ⁻¹	8.3 or 42	40 or 10	20	–	88.3 or 92
1.0 $\mu\text{g Cd g}^{-1}$ EPS	20/57 mg Cd l ⁻¹	8.3 or 42	50 or 20	10	–	88.3 or 92
10.0 $\mu\text{g Cd g}^{-1}$ EPS	40/284 mg Cd l ⁻¹	8.3 or 42	30 or 0	10	–	88.3 or 92
SED	20/57 mg Cd l ⁻¹	8.3 or 42	40 or 10	–	20	88.3 or 92

^aFirst and second values in each column represent volumes used for 2.5 and 25‰ incubations, respectively

ical effects and consisted of 2 paired salinity comparisons in which Cd AE from EPS-Si was compared between the following salinity combinations: (1) 2.5 and 7.5‰, and (2) 2.5 and 25‰. Six amphipods were fed at each salinity. In this experiment, particles were incubated with Cd at the same salinity to which amphipods were acclimated. Cd AE was compared between salinities using a 2-sample *t*-test, assuming unequal variance. The second experiment was conducted to determine if amphipods that were acclimated to different salinities (2.5, 7.5, and 25‰) exhibited different Cd AEs when fed EPS-Si that was incubated with Cd at a single, intermediate salinity of 15‰. Four amphipods were fed at each salinity, and Cd AE was compared among test salinities using single factor ANOVA.

Effect of Cd:EPS ratio on Cd bioavailability: Two experiments were conducted to determine the effect of increasing the ratio of Cd to EPS on EPS sediment coatings. Food treatments were prepared by incubating aliquots of EPS-Si with 3×10^9 Cd stock solutions in SW to achieve 3 Cd:EPS ratios, 0.25, 1, and 10 $\mu\text{g Cd } \mu\text{g}^{-1}$ EPS (Table 1). Processed sediment was used as an additional treatment. For this treatment, Bread and Butter Creek sediment was sieved through a 63 μm screen, washed 5 \times with DI, and condensed by autoclaving for 20 min (Chandler 1986). Sediment was incubated with Cd as described in Table 1. Cd:EPS ratio comparisons were conducted at both 2.5 and 25‰. Due to time constraints and in order to obtain data from sufficient amphipods, each experiment was conducted as a series of 3 independent feeding components which were conducted during a single day. Four amphipods were fed each food treatment during each feeding component. Thus, a total of 12 amphipods were fed each food treatment for each experiment.

A nested 2-way ANOVA was performed to determine effects of food treatment and experimental trial on Cd AE. If no significant ($p < 0.05$) interaction was indicated between treatment and experimental trial, mean Cd AE was compared among food treatments using Tukey's test for multiple comparisons. All statistical analyses were conducted using the GLM module of the SAS statistical software package (SAS Institute 1985).

RESULTS

Gut passage time

Eight of the 12 amphipods fed ^{57}Co -labeled particles ingested a sufficient quantity of this food to accurately track gut passage time. Gut passage time, as measured by the time taken for *Leptocheirus plumulosus* to lose

^{57}Co signal after termination of HF, ranged from 35 to 228 min. Mean gut passage time was 95.3 min (SD = 71.7). No significant relation was detected between amphipod weight and gut passage time ($F_{1,7} = 0.9824$, $p = 0.3599$, $r^2 = 0.375$). Based on the high variability in gut passage time (coefficient of variation = 75.2%), we chose a conservative time period of 3 h (180 min, or mean + 1.2 SD) for amphipods to feed on unlabeled sediment in CF wells during Cd AE experiments. Also, amphipods exhibiting 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 final CF measurement.

Bioavailability of Cd associated with EPS sediment coatings

Comparison of Cd AE between NC- and EPS-Si: Sorption of Cd to particulate material

In all experiments conducted to compare Cd AE between NC- and EPS-Si, EPS-coated silica particles sorbed substantially more Cd than uncoated silica regardless of incubation salinity (Table 2). The greatest difference was observed in the second 2.5‰ experiment, where EPS-Si sorbed $>14\times$ than NC-Si (Table 2). The proportion of particulate Cd also varied markedly for EPS-Si between each experiment within each incubation salinity. For example, differences in the proportion of Cd sorbed to EPS-Si differed 1.8, 1.7, and $3.8\times$ between each experiment conducted at 2.5, 7.5, and 25‰, respectively (Table 2).

Table 2. Percent of Cd present in particulate phase for uncoated silica particles (NC-Si) and for silica particles coated with bacterial extracellular polymers (EPS-Si). Aqueous phase was removed after overnight incubation, and total ^{109}Cd activity was measured in both aqueous and particulate phases. Duplicate experiments were conducted at 2.5, 7.5, and 25‰.

Salinity (‰)	Expt	Particle type	Cd in particulate phase (%)
2.5	1	NC-Si	3.9
2.5	1	EPS-Si	28.6
2.5	2	NC-Si	3.6
2.5	2	EPS-Si	51.6
7.5	1	NC-Si	5.5
7.5	1	EPS-Si	51.1
7.5	2	NC-Si	7.0
7.5	2	EPS-Si	30.3
25	1	NC-Si	4.6
25	1	EPS-Si	45.8
25	2	NC-Si	5.7
25	2	EPS-Si	12.2

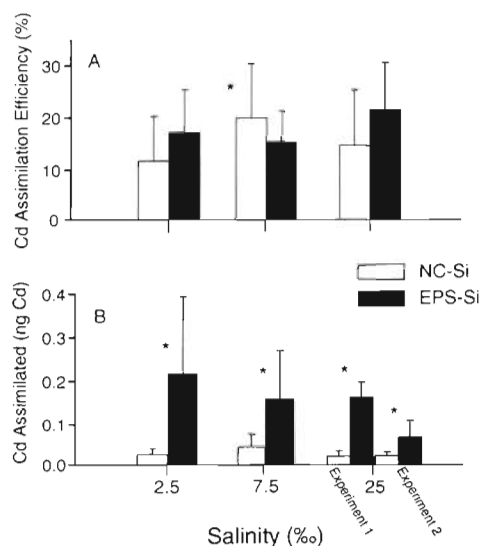


Fig. 2. *Leptocheirus plumulosus*. (A) Mean Cd assimilation efficiency (AE) and (B) mean Cd assimilation as a function of particle type (NC-Si vs EPS-Si). Duplicate experiments were conducted at 2.5, 7.5, and 25‰, and Cd was incubated with each particle type at the feeding salinity. Mean Cd AE was combined for duplicate experiments for all salinities ($n = 10$ to 14), and mean Cd assimilation was combined for duplicate experiments at 2.5 and 7.5‰ ($n = 11$ to 14). Mean Cd assimilation is shown for each experiment conducted at 25‰ ($n = 4$ to 7) because of significant interactive effects. Error bars represent the standard deviation of the mean. *Significant differences between particle types

Comparison of Cd AE between NC- and EPS-Si: Cd AE

No interactive effects between experimental trial (i.e. Expts 1 and 2 within each salinity) and particle type (i.e. EPS-Si and NC-Si) were observed for any of the 3 salinities examined (i.e. 2.5, 7.5, and 25‰) (Table 3). Therefore, mean assimilation efficiencies reported for each salinity represent pooled means from each of the 2 experimental trials.

Among the salinity treatments, mean AE ranged from 21.5% from EPS-Si in the 25‰ experiment to 11.6% from NC-Si in the 2.5‰ experiment (Fig. 2A). Mean Cd AE was significantly greater from NC-Si than from EPS-Si at 7.5‰, but not at 2.5 or 25‰ (Fig. 2A). Since the Cd AE differences between NC-Si and EPS-Si were marginal, it is questionable whether the presence of EPS coatings affected the efficiency with which *Leptocheirus plumulosus* assimilates Cd from Si particles.

Because EPS-Si sorbed more Cd than NC-Si, we hypothesized that amphipods fed EPS-Si assimilated more Cd than those fed NC-Si. This hypothesis was tested by comparing post HF activities (ng Cd) between amphipods fed EPS-Si and those fed NC-Si.

Table 3. Summary of statistical analyses performed on results of Cd assimilation efficiency (AE) experiments between NC- and EPS-Si. Results of experiments were analyzed to compare Cd AE and the quantity of assimilated Cd as a function of particle type (EPS-silica vs NC-silica) and experimental trial within each salinity (2.5, 7.5, and 25‰) using 2-way ANOVA

Salinity (%)	Statistical results (F -value, $p > F$)		
	NC-silica vs EPS-silica	Expt	Interaction
Cd AE			
2.5	2.71, 0.115	3.78, 0.066	0.05, 0.828
7.5	3.05, 0.096	14.13, 0.001	2.50, 0.129
25	2.90, 0.106	0.001, 0.956	0.34, 0.566
Quantity of Cd assimilated			
2.5	15.7, 0.0008	3.06, 0.097	2.39, 0.139
7.5	30.11, 0.0001	24.63, 0.0001	13.91, 0.0012
25	43.56, 0.0001	17.06, 0.0006	12.81, 0.021

Interaction effects between daily feeding trials and particle type were observed for experiments conducted at 7.5 and 25‰ (Table 3). The interaction at 7.5‰ was proportional and did not prevent comparing treatment means. However, the interaction observed at 25‰ was non-proportional, and required that the 2 constituent experiments be analyzed by separate 1-way ANOVAs.

Amphipods assimilated significantly greater quantities of Cd from EPS-Si than from NC-Si regardless of experimental salinity (Fig. 2B). The magnitude of differences between Cd assimilation from EPS-Si and NC-Si appeared to be inversely related to salinity, with the greatest difference occurring at 2.5‰ and the smallest in 1 experiment at 25‰ (Fig. 2B). Qualitatively, mean Cd assimilation per amphipod decreased with increasing salinity from EPS-Si, but not for NC-Si.

Effect of salinity on Cd AE from EPS-Si: Partitioning effects

In separate experiments where Cd was incubated with EPS-Si at each feeding salinity (2.5, 7.5, or 25‰), mean Cd AE was not significantly different between 2.5 and 7.5‰ (Fig. 3A) or between 2.5 and 25‰ (Fig. 3A). EPS sorbed $1.47\times$ more Cd at 2.5 than at 7.5‰, and this difference was reflected in the quantity of Cd assimilated by *Leptocheirus plumulosus* at these salinities. Specifically, amphipods fed at 2.5‰ assimilated significantly more ($1.84\times$) Cd than those fed at 7.5‰ (Fig. 3B). In the experiment comparing 2.5 and 25‰, EPS-Si sorbed $3.23\times$ more Cd at 2.5‰ compared with 25‰. However, the quantity of Cd assimilated by amphipods was variable at 2.5‰ (mean = 0.345 ng Cd

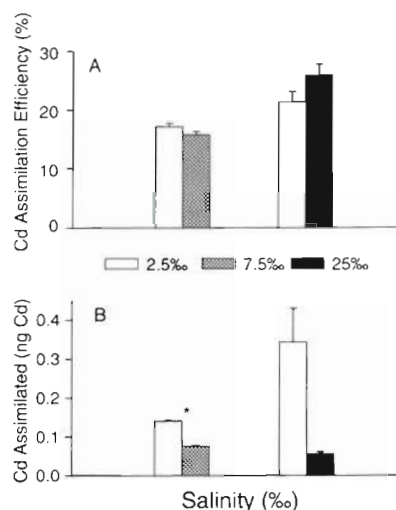


Fig. 3. *Leptocheirus plumulosus*. (A) Mean Cd assimilation efficiency (AE) and (B) mean Cd assimilation from EPS-Si as a function of salinity. Separate comparisons were conducted at 2.5 and 7.5‰ (n = 5 to 6) and at 2.5 and 25‰ (n = 5 to 6). Cd was incubated with EPS-Si at each feeding salinity. Error bars represent the standard deviation of the mean. *Significant differences between salinities

Table 4. Mean percent of Cd (SD in parentheses) in particulate phase for silica particles coated with bacterial extracellular polymers (EPS-Si) and sediment (SED). EPS-Si was incubated with Cd to achieve Cd:EPS ratios of 0.25, 1, and 10 (µg:µg). Aqueous phase was removed after overnight incubation, and total ¹⁰⁹Cd activity was measured in both aqueous and particulate phases

Salinity (%)	Proportion of Cd in particulate phase (SD)			
	EPS-1	EPS-2	EPS-3	SED
2.5	73.3 (7.7)	71.6 (2.3)	86.0 (7.0)	85.7 (1.7)
25.0	41.1 (10.9)	26.9 (3.5)	37.7 (3.2)	45.4 (9.2)

Table 5. Summary of statistical analyses performed on results of experiments examining effects of Cd concentration on Cd assimilation efficiency (AE) by the amphipod *Leptocheirus plumulosus*. Amphipods were fed EPS-Si that had Cd sorbed at the following concentrations: 0.25, 1, and 10 µg Cd µg⁻¹ EPS. Amphipods were also fed processed sediment. Independent experiments were conducted at 2.5 and 25‰, and each experiment was composed of three trials (n = 4 amphipods trial⁻¹). Results of experiments were analyzed to compare Cd AE as a function of treatment and experimental trial within each salinity using 2-way ANOVA

Salinity (‰)	Statistical results (F-value, degrees of freedom, p > F)		
	Food treatment	Experimental trial	Interaction
2.5	18.25, 3, 0.0001	3.54, 2, 0.0394	0.23, 6, 0.9654
25	3.08, 3, 0.0393	3.93, 2, 0.0284	0.06, 6, 0.9992

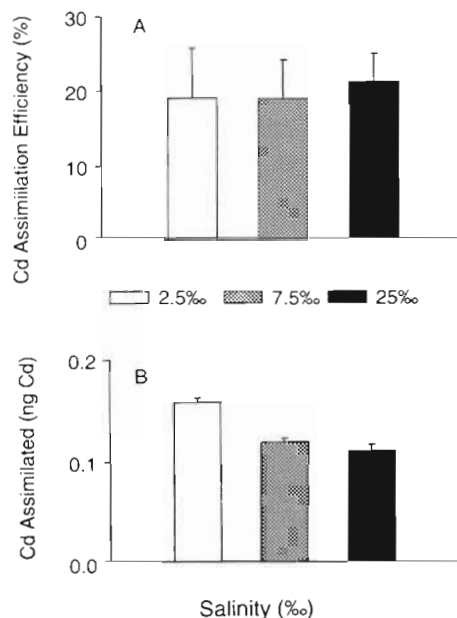


Fig. 4. *Leptocheirus plumulosus*. (A) Mean Cd assimilation efficiency (AE) and (B) mean Cd assimilation from EPS-Si as a function of salinity. Cd AE and assimilation were measured (n = 4) at 2.5, 7.5, and 25‰. Cd was incubated with EPS-Si at 15‰. Error bars represent the standard deviation of the mean

amphipod⁻¹, SD = 0.084, range = 0.123 to 0.861), and mean Cd assimilation at this salinity was not significantly different from that at 25‰ (mean = 0.057 ng Cd amphipod⁻¹, SD = 0.0004, range = 0.035 to 0.079) (Fig. 3B).

Effect of salinity on Cd AE from EPS-Si: Physiological processes

Effects of salinity were compared directly by feeding particles that consisted of EPS-Si incubated with Cd at 1 salinity (15‰) to amphipods that were acclimated at 2.5, 7.5, and 25‰. At 15‰, EPS-Si sorbed 40.4 % of total Cd. Mean Cd AE ranged from 19.0 % at 25‰ to 21.1 % at 2.5‰, but was not significantly different among the 3 test salinities (Fig. 4A). Likewise, the mean quantity of assimilated Cd (ranging from 0.111 ng Cd amphipod⁻¹ at 25‰ to 0.158 ng Cd amphipod⁻¹ at 2.5‰) was not significantly different among salinity treatments (Fig. 4B).

Effect of Cd:EPS ratio on Cd AE from EPS-Si

In general, EPS-Si and processed sediment sorbed more Cd at 2.5 than at 25‰ (Table 4). Mean Cd sorption did not, however, vary as a function of Cd concentration (Table 4).

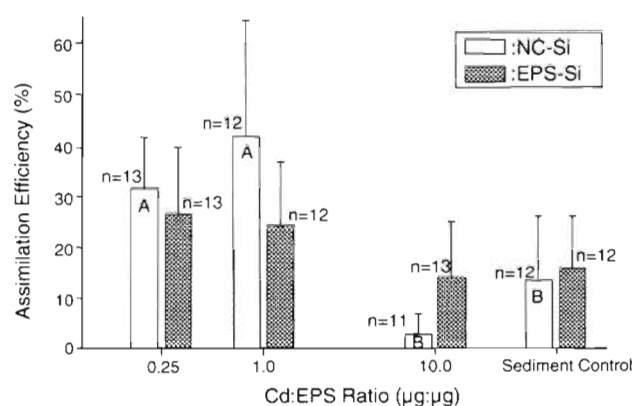


Fig. 5. *Leptocheirus plumulosus*. Mean Cd assimilation efficiency (AE) from EPS-Si as a function of Cd concentration. Three independent trials were conducted at 2.5 and 25‰, and Cd was incubated with EPS-Si and processed sediment at the feeding salinity. Following 2-way ANOVA, Cd AE was compared within 2.5‰ treatment means using Tukey's HSD test. Bars that share the same letter within 2.5‰ are not significantly different at $\alpha = 0.05$.

Results from Cd:EPS ratio experiments indicated no interaction between experimental component and particle type for either experimental salinity (i.e. 2.5 or 25‰, Table 5). Therefore, mean Cd AEs for each particle type were pooled. Mean Cd AE varied significantly among the 4 particle types in experiments conducted at 2.5‰ ($F_{3,6} = 18.25$, $p = 0.0001$) (Fig. 5). Specifically, mean Cd AE from 1 and 0.25 $\mu\text{g Cd g}^{-1}$ EPS together were significantly greater than mean Cd AE measured from processed sediment and 10 $\mu\text{g Cd g}^{-1}$ EPS. That is, *Leptocheirus plumulosus* assimilated Cd with significantly greater efficiency from low Cd:EPS ratios (i.e. 0.25 or 1) than from high Cd:EPS ratios (i.e. 10). At 41.9%, Cd AE from 1 $\mu\text{g Cd g}^{-1}$ EPS was the highest measured in this study. At 25‰, overall ANOVA results indicated that mean Cd AE differed significantly among the 4 particle types ($F_{3,6} = 3.08$, $p = 0.0393$); however, paired comparisons could not discriminate among these differences. Mean Cd AE from processed sediment particles were qualitatively similar between experiments conducted at 2.5 (mean = 13.7 %) and 25‰ (mean = 16.2 %) (Fig. 5).

DISCUSSION

Most aquatic sediments are inhabited by dense communities of microorganisms (including bacteria and microphytobenthos) existing as cells within an exuded EPS matrix attached to particles (Hoagland et al. 1993, Meyer-Reil 1994, Costerton et al. 1995). Previous studies have demonstrated high, salinity-independent Cd affinity by sediment coatings composed of bacterial EPS (Schlekat 1998, Schlekat et al. 1998). The sorption

patterns observed in these studies suggested that the fate of this sorbed Cd to particle-ingesting invertebrates should be influenced by interrelated physico-chemical factors, including seawater salinity and Cd concentration. In the present study, we showed that the efficiency with which the estuarine amphipod *Leptocheirus plumulosus* assimilated Cd sorbed to EPS sediment coatings was approximately 20%. Cd AE was not affected by salinity either directly or indirectly. However, both the presence of EPS coatings and salinity affected the magnitude with which Cd partitioned to particles, and this in turn governed the quantity of Cd available on particles that could be assimilated by amphipods. Finally, and perhaps most interestingly, we demonstrated that, under low salinity conditions, Cd AE by *L. plumulosus* was inversely related to the concentration of Cd sorbed to EPS coatings.

The ability of EPS to serve as a metal uptake source for benthic invertebrates depends in part upon the concentrations of this material in natural sediments. Several studies have measured EPS concentrations in surficial estuarine sediments, and these concentrations are theoretically adequate to sorb substantial quantities of Cd. For example, 2 sites within the Severn Estuary, England, have exhibited EPS concentrations between 2.7 (Underwood & Patterson 1993) and 17.0 (Underwood et al. 1995) mg EPS g^{-1} . Summertime EPS concentrations in a diatom-rich mudflat within the North Inlet, SC, estuary (5.7 mg EPS g^{-1} sediment) were within this range (C.E.S. unpubl. data). Using a specific Cd sorption capacity of 3.3 $\mu\text{g Cd mg}^{-1}$ EPS as calculated by Schlekat et al. (1998), the Severn River EPS concentrations are equivalent to Cd sorption capacities that range from 8.9 to 56.1 $\mu\text{g Cd g}^{-1}$ sediment, which are either equivalent to, or greater than, sediment Cd concentrations expected to elicit adverse effects (e.g. the effects ratio-low [ER-L] concentration for Cd is 9 $\mu\text{g Cd g}^{-1}$, Long et al. 1995). Thus, the prevalence of EPS within estuarine environments and its distribution within the upper 5 mm of sediments presents the opportunity for EPS-Cd associations to occur, and for Cd assimilation to be enhanced for benthic invertebrates feeding upon EPS vectors which are most abundant in surficial sediments.

Effect of EPS coatings

The difference in the quantity of Cd accumulated by *Leptocheirus plumulosus* between NC- and EPS-Si was considerable, and can be explained by differences in Cd partitioning between these 2 particle types. Thus, discussion of Cd accumulation differences between NC- and EPS-Si is a tautological exercise, but it serves to highlight the importance of organic carbon in the

bioavailability of metals associated with oxidized sediments. In anaerobic sediments, amorphous iron sulfides (AVS) are thought to minimize pore water metal concentrations, thereby decreasing metals availability by dissolved uptake routes (DiToro et al. 1989, Ankley et al. 1996). (However, the availability of AVS-complexed metals through dietary ingestion is unknown.) In aerobic, oxidized sediments, AVS concentrations are low, and other sedimentary phases, such as organic carbon coatings, are active in metal binding (Ankley et al. 1996). But organic carbon is represented in sediments by a spectrum of materials and forms, ranging from geochemically amorphous 'humic' substances (which are thought to be recalcitrant) to intact, coordinated EPS molecules that are continuously produced by adherent microorganisms. Results of the present study show that EPS sediment coatings representing 0.43% organic matter can increase by a factor of 10 the quantity of Cd accumulated by particle-ingesting invertebrates. Decho & Luoma (1994) showed that the bivalve *Potamocorbula amurensis* assimilated Cd from humic- and fulvic-acid coatings with lower efficiency than from uncoated particles; this contrast suggests that the effect of organic coatings on metal uptake is not uniform.

Our findings showing enhanced Cd uptake from EPS-coated particles are in agreement with previous studies. For example, Decho & Luoma (1996) measured bioavailability of Cr(III) from EPS-coated beads to 2 bivalves, *Macoma balthica* and *Potamocorbula amurensis*. They observed Cr(III) AE of 35 and 26% for *M. balthica* and *P. amurensis*, respectively. Both bivalves assimilated Cr(III) from EPS coatings more efficiently than from natural sediments (1 and 3% for *M. balthica* and *P. amurensis*, respectively). Also, Harvey & Luoma (1985) showed that amending particles with EPS increased Cd uptake by *M. balthica*. However, these studies were conducted at full marine salinities (32‰), and used EPS produced by the marine bacterium *Alteromonas atlantica*. Results of the present study suggest that estuarine EPS coatings can also facilitate Cd uptake, and that this facilitation is dependent upon prevailing geochemical characteristics, including salinity and metal concentration. Mechanisms for the desorption of Cd from EPS within invertebrate guts are unclear, but may include depressed gut pH and the activity of carbohydrases. Although there is no evidence that amphipod gut pH extends below the range of 6 to 7, carbohydrases are prevalent among invertebrates (Lopez & Levinton 1987).

Effect of salinity

Salinity fluctuations are characteristic of estuarine systems, and these fluctuations can potentially affect dietary

uptake of EPS-associated Cd in 2 ways. First, and more generally, salinity fluctuations can affect osmoregulatory mechanisms which, for some organisms including crustaceans, take place partially in the gut. For example, some estuarine crustaceans respond to lower salinities, which represent osmotically rigorous conditions, by decreasing the water and ionic permeability of the gut to inhibit ionic diffusion across the gut lining (Icely & Nott 1984). Thus, it is possible that at low salinities, the physiological processes operating in the assimilation of nutrients and trace metal ions (both necessary and toxic) across the gut wall may be less than at high salinities. Our results demonstrate that the gut processes transferring Cd across the gut lining were constant across the salinity range tested, which suggests that if *Leptocheirus plumulosus* does respond to lower salinities by decreasing gut ionic permeability, assimilation of Cd²⁺ is not affected. It appears that the digestive physiology of *L. plumulosus* is adapted for assimilating cations at broadly different salinities, and that any effects of salinity fluctuations on Cd accumulation by this estuarine particle-ingesting invertebrate are more likely a result of geochemical factors, e.g. partitioning.

The effect of salinity on the affinity of EPS for Cd did also affect Cd bioaccumulation. Among experiments in which Cd was incubated with particles at different salinities, Cd partitioning was invariably higher at lower incubation salinities. However, Cd AE was not affected by differences in partitioning. At both 2.5 and 25‰, only a fraction of sorbed Cd was available for transport across the gut membrane. But these results show that the same fraction of sorbed Cd is available regardless of salinity, suggesting that at this Cd concentration, the distribution of Cd among different EPS binding sites is similar at these widely disparate salinities.

Effects of metal concentration

Earlier findings which suggested the presence of multiple salinity-responsive Cd binding sites within EPS coatings (Schlekat et al. 1998) raised the hypothesis that bioavailability of EPS-associated Cd should be dependent upon the ratio of Cd to EPS. Our expectation was that, once EPS became saturated with Cd, weaker binding phases would become involved in Cd sorption, and that subsequent Cd bioavailability to particle ingesting invertebrates would be heightened as a result of the greater proportion of Cd associated with these weaker phases. Results conducted to test this hypothesis showed the opposite results, i.e. that Cd AE decreased with increasing Cd concentration. These results were not reproduced at a higher salinity (25‰), indicating that this phenomenon is affected by the way in which EPS responds to changes in salinity.

Applications to sediment quality criteria for metals

These findings present some new possibilities for predicting interactions among metals, surficial sediment phases, and benthic organisms. Some current methods for predicting toxic effects of metal-contaminated sediments normalize metal concentrations to concentrations of specific operative sediment phases, e.g. AVS normalization (DiToro et al. 1989). These normalizations operate under the assumption that metals are not bioavailable until the operative sediment feature, usually AVS, is saturated. They also do not consider dietary uptake. If the operative sediment feature is EPS, our results suggest that dietary uptake is potentially important, and that the magnitude of dietary Cd uptake from EPS cannot be predicted from Cd concentration alone. If our findings (i.e. decreasing Cd bioavailability with increasing Cd concentration) represent a prevalent phenomenon, then the use of sediment metal concentrations (either total or normalized metals) in sediment quality guidelines is of questionable utility for sediments that are moderately to highly contaminated with metals. An alternative approach is to develop critical body residue models that relate toxic effects of metals to measured body burdens within organisms, similar to that which has been developed for non-polar organic compounds (McCarty & MacKay 1993). Because this approach relies on organismal tissue concentrations, it is independent of bulk or normalized sediment metal concentrations. Further studies are needed with field-contaminated bedded sediments where the importance of the metal:EPS relationship is measured relative to that of other important sediment phases, including the AVS:simultaneously extracted metal, percent clay, and total organic carbon.

There is some evidence that an inverse relationship may occur between Cd AE and Cd concentration in organic, polymeric sediment coatings other than EPS. In a separate experiment, we measured AE of Cd that was sorbed to silica particles that were coated with polymers derived from the estuarine cordgrass *Spartina alterniflora*. These experiments were conducted using metal concentrations of 0.55 and 2.02 $\mu\text{g Cd } \mu\text{g}^{-1}$ coating at a salinity of 15‰ (C.E.S. unpubl. data), and Cd AE by *Leptocheirus plumulosus* was measured as described earlier. Cd AE was 15 times less at the higher Cd concentration compared with the low. These findings are in contrast with those of Decho & Luoma (1994), who showed that Cd AE for the bivalve *Potamocorbula amurensis* was consistently higher with increasing metal loadings on humic- and fulvic-acid coatings. Thus, there is some indication that the relationship observed in the present study is restricted to labile polymeric sediment coatings. To determine the

significance of these findings, the bioavailability of Cd associated with natural, surficial sediments needs to be measured.

There are several possible explanations for the observed decreases in Cd AE with increasing Cd concentration. One is that higher Cd concentrations inhibited the physiological processes involved in assimilating free Cd^{2+} across the gut wall of *Leptocheirus plumulosus*. While several mechanisms have been proposed for the uptake of dissolved metals by invertebrates (Simkiss & Taylor 1995, Rainbow 1997), metal uptake systems within the invertebrate gut remain poorly characterized. Ahearn et al. (1994) propose that an electrogenic $\text{Na}^{2+}/\text{H}^{+}$ antiporter system operates within the crustacean gut for Ca^{2+} uptake, among other functions. Significantly, this carrier protein also showed high affinities for Cd^{2+} and Zn^{2+} (Ahearn et al. 1994). Thus, it is possible that the antiporter system, or another carrier protein involved in the transfer of metals across the gut lining of *L. plumulosus*, became saturated with Cd as Cd concentration increased beyond a certain threshold. Another possibility involves the mechanisms involved in EPS-Cd sorption. The high metal affinity exhibited by EPS is a consequence of high concentrations of electronegative hydroxyl, phosphate, pyruvate, succinyl, and carboxyl groups (Decho 1990, Ford & Mitchell 1992, Simkiss 1995). Increasing concentrations of Cd may result in the participation of more functional groups within EPS molecules, and this in turn may lead to conformational compression of the ordinarily 'loosely' structured EPS molecule. This compression may inhibit digestive processes within the gut that act to solubilize Cd. Tighter binding and compression of the EPS matrix by increased metal concentration may also restrict the assimilation of EPS by invertebrates.

SUMMARY

The present study has shown that a common microbial process that occurs in sediments, the production of extracellular polymeric substances by adherent microbes, can bind Cd in a form that is bioavailable to the estuarine amphipod *Leptocheirus plumulosus*. The potential importance of EPS-associated metal uptake is difficult to predict in natural sediments because EPS production is a continual process. However, as microbes grow and produce EPS, metals introduced into a given locale are likely to encounter EPS sediment coating before other metal-binding sediment phases, e.g. AVS. The partitioning of Cd to EPS sediment coatings was a function of salinity, and the efficiency with which *L. plumulosus* assimilated EPS-associated Cd was a function of Cd concentration.

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*Editorial responsibility: Evelyn and Barry Sherr
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