

# Effect of adherent bacteria and bacterial extracellular polymers upon assimilation by *Macoma balthica* of sediment-bound Cd, Zn and Ag

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**ABSTRACT:** Effects of adherent bacteria and bacterial extracellular polymer (exopolymer) upon uptake of particle-bound Cd, Zn and Ag by the deposit-feeding clam *Macoma balthica* were studied in the laboratory. Amorphous iron oxyhydroxide and unaltered and alkaline-extracted sediments were used as model particulates in separate, controlled deposit-feeding experiments. In general, amounts of metal taken up from ingested particles varied dramatically with the nature of the particle surface. Ingestion of contaminated iron oxide particles did not contribute to overall uptake of Cd and Ag in feeding clams, but accounted for 89 to 99% of total Zn uptake. Exopolymer adsorbed on iron oxide particles caused an increase in the biological availability of particle-bound metals in the order  $Ag > Cd > Zn$ , whereas adherent bacteria up to  $3.2 \times 10^{11} \text{ g}^{-1}$  had no effect upon amounts of metal taken up from ingested particulates. At the higher Cd and Ag concentrations employed ( $3.6 \times 10^{-7} \text{ M}$ ), feeding rates declined with increasing amounts of iron oxide-bound exopolymer, suggesting behavioral avoidance due to increased metal availability. Much of the Cd (57%) taken up by clams feeding on unaltered estuarine sediments originated from particulates, even though particle/solute distribution of Cd (86%) was similar to that in experiments with iron oxide particles. Uptake of Cd from alkaline-extracted sediments was insignificant, as it was from unamended iron oxide. However, addition of exopolymer ( $10 \text{ mg g}^{-1}$  sediment) caused a restoration in bioavailability of sediment-bound Cd.

## INTRODUCTION

It is well established that estuarine sediments are highly enriched with trace metals relative to interstitial and overlying waters. However, the extent of metal transfer from concentrated sedimentary reservoirs into deposit-feeding organisms is poorly understood and differs among environments (Luoma & Bryan 1982, Luoma 1983, Vangenechten et al. 1983). Both field and laboratory evidence suggests metal bioavailability is influenced by metal partitioning among sedimentary components (Luoma & Jenne 1977, Luoma & Bryan 1982). For example, in laboratory microcosms, the magnitude of metal uptake by deposit-feeders varies with differences in sediment chemical characteristics (Renfro & Benayoun 1974, Beasley & Fowler 1976, Harrison et al. 1976, Luoma 1977, Phelps 1979, Vangenechten et al. 1983). In a field study comparing 17 English estuaries, availability of Ag, Cd, Co, Pb, and Zn to deposit-feeding bivalves and polychaetes corre-

lated with aspects of sediment chemistry that affected metal partitioning (Luoma & Bryan 1978, 1982). A more thorough understanding of how sediment chemistry affects bioavailability of sediment-bound metals has been hampered by a dearth of data on the contribution of the particulate vector to total metal uptake, since changes in sediment chemistry may alter levels of highly-available solute forms of the metal as well as bioavailability of particle-associated metal.

In this study we examine the effect of several specific chemical associations between metals and sediments upon the availability of particle-bound Zn, Cd, and Ag to a deposit-feeding estuarine clam *Macoma balthica*. Metal uptake by *M. balthica* from particles of amorphous iron oxide, in the presence and absence of particle-bound (adherent) bacteria and bacterial exopolymer was investigated in the laboratory. Iron oxides, which are abundant minerals in estuarine sediment, strongly compete for binding of trace metals. Bacteria and bacterial exopolymer also may bind metals. How-

ever, adherent bacteria appear to be an important food source for *M. balthica*, whereas bacterial exopolymer, used in the process of biological adhesion, may not be a direct source of nutrition for this clam (Harvey & Luoma 1984). Experiments were also conducted with natural sediments; comparing Zn and Cd uptake among unaltered sediments, sediments stripped of organic material, and stripped sediments amended with bacterial exopolymer.

## MATERIALS AND METHODS

*Preparation of modified amorphous iron oxyhydroxide.* Amorphous iron oxyhydroxide was prepared by slow, dropwise addition of carbonate-free NaOH (0.1N) to ferric nitrate solutions and aged for 24 h at pH 8.2 and 25°C. Bacteria or their exopolymer were added prior to addition of the trace metals. Previously-washed *Pseudomonas atlantica* NCMB301 (ATCC #19262), a marine bacterium which readily attaches to solid surfaces (Corpe 1970), were allowed to adhere to iron oxide particles suspended in filtered seawater. Numbers of attaching bacteria were determined using acridine-orange direct counts (Hobbie et al. 1977). Bacterial exopolymer was harvested from <sup>14</sup>C-glucose amended cultures of *P. atlantica* T<sub>6</sub>C using a precipitation and dialysis procedure described by Corpe (1972) and adsorbed onto suspended iron oxide particles in varying amounts. Percent adsorption of exopolymer was determined by the distribution of the <sup>14</sup>C label, using a liquid scintillation counter and standard additions method (<sup>14</sup>C-toluene) for quench factor correction. Both metals and amorphous iron oxyhydroxide (modified and unaltered) were added to the aquaria 24 h before addition of *M. balthica*. Adsorption densities (amounts of adsorbed metal g<sup>-1</sup> adsorbent) of Cd, Zn, and Ag on particles were determined from total metal and solid concentrations and solid/solution distributions of radioisotopes at 24 h. Adsorption density of Ag was also determined at the end of the experiment (7 d), since it was not at equilibrium at 24 h.

*Experimental setup and design.* *Macoma balthica* were collected from a South San Francisco Bay mudflat near Newark, California and acclimated to laboratory conditions for 5 d at 12°C ± 1°C. Salinity was gradually increased during the acclimation from ambient levels in the mudflat to 32‰. Clams of similar size (20 to 21 mm shell length) were used to preclude size-dependent differences in metal uptake observed for this species (Strong & Luoma 1981). Seven acclimated clams were placed in each aquarium (containing 2.5 l of 0.3 μm filtered seawater) and allowed to ingest metal-laden, isotopically-labelled particles. To account for uptake of dissolved metal, 5 additional

clams were kept within a plexiglass dialysis chamber in each aquarium. The chambers were fitted with Nuclepore filters (90 mm dia., 0.4 μm pore size) to exclude particulate material, but allow water exchange (Harvey & Luoma 1985). Feeding and non-feeding clams were removed from their aquaria after 7 d of exposure, placed in beakers of uncontaminated seawater for 24 h, removed from their shells, oven-dried at 90°C, and weighed, before analysis for <sup>109</sup>Cd, <sup>65</sup>Zn, and <sup>110m</sup>Ag tracer.

The uptake of Cd, Zn and Ag by *Macoma balthica* from particles of amorphous iron oxyhydroxide [Fe<sub>2</sub>O<sub>3</sub> · H<sub>2</sub>O(am)] alone and in the presence of adherent bacteria or exopolymer was investigated in four experiments. All aquaria were acid washed and covered, and contained 8.2 × 10<sup>-3</sup> M (1.5 g l<sup>-1</sup>) amorphous iron oxyhydroxide, 2.5 l of filtered seawater (pH = 7.8-8.0) and 12 *M. balthica* (7 feeding and 5 non-feeding). In Experiment 1, 4 aquaria to which similar amounts of Cd and Zn were added (1.8 × 10<sup>-5</sup> and 1.9 × 10<sup>-5</sup> Moles mole<sup>-1</sup> Fe, respectively), were set up to examine the effects of adherent bacteria and exopolymer upon assimilation rates of particle-bound Cd and Zn into clam soft tissue. One aquarium contained 8.0 × 10<sup>10</sup> adherent bacteria g<sup>-1</sup> amorphous iron oxyhydroxide, the second 3.2 × 10<sup>11</sup> bacteria g<sup>-1</sup>, and the third 27 mg exopolymer g<sup>-1</sup>. The fourth aquarium had no bacteria or exopolymer added to the amorphous iron oxyhydroxide. The lowest bacterial density was approximately twice that observed on fine-grained sediment in South San Francisco Bay (Dagovitz & Luoma unpubl.).

The effect of varying amounts of adsorbed exopolymer upon the uptake of Cd and Zn associated with iron oxide particles was examined in Experiments 2 and 3, which consisted of 8 aquaria. Adsorption densities of exopolymer on iron oxide particles ranged from 0 to 39.9 mg g<sup>-1</sup>. Although the range in abundance of bacterial exopolymer in estuarine sediments has not been quantitatively determined, it is presumably lower than that used in these experiments. Because the overall binding constant describing adsorption of Cd and Zn onto amorphous iron oxide can vary with the ratio of metal/Fe (Benjamin & Leckie 1981), 2 concentrations of Cd and Zn were used. Adsorption densities of Cd and Zn were 2 × 10<sup>-5</sup> Moles Cd and Zn per Mole total Fe (Fe<sub>T</sub>) in 4 aquaria (Experiment 2) and 2 × 10<sup>-6</sup> in the other 4 (Experiment 3).

In Experiment 4, four aquaria were used to assess the effect of adsorbed exopolymer upon uptake of iron oxide-bound Cd and Ag. Amounts of adsorbed exopolymer were varied from 0 to 38.7 mg g<sup>-1</sup>. Silver and Cd were added to each aquarium at 2.3 × 10<sup>-5</sup> Moles per Mole Fe<sub>T</sub>, and 2.5 × 10<sup>-5</sup> Moles per Mole Fe<sub>T</sub>, respectively.

Two additional series (Experiments 5 and 6) were conducted with natural oxidized sediments from San Francisco Bay. Natural sediments, which were either unaltered, chemically stripped of organic material, or stripped and recoated with bacterial exopolymer, were employed in metal-uptake experiments, with the recognition that precise metal partitioning among sediment components could not be defined (Luoma & Davis 1983). In Experiment 5, sediments were stripped of organic matter with 6 sequential, 1-h leachings with 0.1N NaOH. Stripped sediments ( $6 \text{ gl}^{-1}$  seawater) were placed in each of 2 aquaria, one labelled with  $6 \times 10^{-7}\text{M}$  total Cd and Zn, the other with  $6 \times 10^{-8}\text{M}$  (calculated). It is recognized that the lower metal concentrations are first approximations since contributions by background contamination may have been significant. In Experiment 6, uptake of Cd and Zn from unaltered, oxidized sediments and NaOH-extracted sediments ( $4.2 \text{ g l}^{-1}$  seawater) to which exopolymer had been added ( $10 \text{ mg g}^{-1}$  sediment) was monitored. Total concentrations for both Cd and Zn in Experiment 6 were  $2 \times 10^{-6}\text{M}$ . Five replicate aquaria for each of the 2 sediment types were employed for analysis of variance.

*Determination of metal uptake.* Amounts of metal taken up from ingestion of particles were determined from computed differences (where statistically significant at  $p \leq 0.10$ ) in levels of radioisotope between feeding and non-feeding (filter chamber) clams. Metal concentrations were calculated from soft tissue dry weights, specific activities for  $^{109}\text{Cd}$ ,  $^{65}\text{Zn}$ , and  $^{110\text{m}}\text{Ag}$ , and counting efficiencies. All gamma emitters were used strictly as tracers, the majority of the added metal in each system being in the form of stable isotopes. Transfer factors (TF) for metal uptake from particles to clam soft tissue were computed as:

$$\text{TF} = \frac{(M_f/W_f) - (M_n/W_n)}{(M_p/W_p)} \quad (1)$$

where  $M_f$  and  $M_n$  = amounts of metal (Moles) taken up into soft tissue during the time course of the experiment by feeding and non-feeding clams, respectively;  $M_p$  = amount of metal sorbed onto particles (Moles);  $W_p$ ,  $W_f$ , and  $W_n$  = dry weights of particles, feeding clam soft tissue, and non-feeding clam soft tissue, respectively. Amounts of metals taken up were computed as averages for the 7 feeding clams and 5 non-feeding clams (controls) in each aquarium. Differences in particle ingestion rates by the organisms were estimated by weighing the feces recovered from the 7 feeding clams during the 24-h purge period prior to dissection. This method would not necessarily detect differences in the turnover rate of food in a full digestive tract. Levels of  $^{109}\text{Cd}$ ,  $^{65}\text{Zn}$ ,  $^{110\text{m}}\text{Ag}$  tracer were determined on a gamma emissions counter (model

Gamma 8000, Beckman Instruments Co.), employing a NaI crystal, 3 energy windows, and background subtraction. Counts of  $^{109}\text{Cd}$  (88 KeV) were separated from those of  $^{65}\text{Zn}$  (1.11 MeV) or  $^{110\text{m}}\text{Ag}$  (0.44–2.46 MeV) in the same sample. Since a portion of the total emissions of both  $^{65}\text{Zn}$  and  $^{110}\text{Ag}$  were detectable in the lower-energy window designated for  $^{109}\text{Cd}$ , corrections for overlap in the lower portion of the energy range (10 to 150 KeV) had to be applied to determine separate activities for these isotopes.

## RESULTS

### Cadmium

Uptake of Cd from solution was the only assimilation pathway detectable when *Macoma balthica* were fed labelled particles of amorphous iron oxide (Table 1). Cadmium uptake in feeding clams was not significantly different ( $p \leq 0.10$ ) from that in non-feeding (control) clams housed in filter chambers within the same aquaria in any of the 3 different experiments employing unmodified iron oxide particles. Similar results were observed when iron oxide particles were modified with adherent bacteria. At  $10^{-7}\text{M}$  total Cd, binding onto amorphous iron oxide ranged from 83 to 85% in the 3 different experiments and was 85% when bacteria were present at  $8 \times 10^{10}$  cells  $\text{g}^{-1}$  on the iron oxide particles. A suppression of Cd adsorption (to only 65% of Cd added) occurred when abundance of particle-bound bacteria was increased by a factor of 4. The reduced binding could have resulted either from a reduction in the overall binding constant of bacterially-modified iron oxide or increased concentrations of dissolved bacterial metabolites capable of complexing Cd in solution. However, the reduced binding was not accompanied by any detectable change in Cd uptake from ingested particles by *M. balthica*, although there was an overall 2-fold increase in Cd uptake by both feeding and non-feeding clams, due to increased levels of Cd in solution.

Addition of bacterial exopolymer to iron oxide particles had no effect on the extent of Cd adsorption onto particles (Table 1), but small increases in Cd availability from ingested particles were observed. Cadmium uptake was significantly greater ( $p \leq 0.10$ ) in feeding clams than in the clams in chambers in 5 of the 7 aquaria containing exopolymer-amended iron oxide and  $3.6 \times 10^{-7}\text{M}$   $\text{Cd}_T$  (Experiments 1, 2 & 4; Table 1) and in all 3 aquaria employing exopolymer-amended iron oxide and  $3.6 \times 10^{-8}\text{M}$   $\text{Cd}_T$  (Experiment 3; Fig. 1). In Experiment 3, amounts of Cd taken up from ingested particles correlated strongly with exopolymer concentrations (Fig. 1). However, the solute vector remained

Table 1. *Macoma balthica*. Cadmium uptake ( $\pm 90\%$  C.I.) in microcosm experiments with unamended, bacteria-amended, and exopolymer-amended amorphous iron oxide particles at  $3.6 \times 10^{-7} \text{M Cd}_T$ . For each system, Cd uptake by feeding ( $n = 7$ ) and non-feeding clams ( $n = 5$ ), the percentage deriving from ingested particles, and corresponding transfer factors are presented

Cd (% adsorbed)	Exopolymer $\text{mg l}^{-1}$ (% adsorbed)	Bacteria cells $\text{g}^{-1}$ (% adsorbed)	Cd uptake by clams $\text{nMoles g}^{-1}$		Transfer factor
			Feeding (% from particles alone)	Non-feeding	
83 <sup>1</sup>	—	—	$3.78 \pm 0.48$ (ns)	$3.06 \pm 0.78$	ns
84 <sup>2</sup>	—	—	$5.38 \pm 0.59$ (ns)	$4.76 \pm 1.89$	ns
87 <sup>3</sup>	—	—	$9.65 \pm 1.54$ (ns)	$7.77 \pm 1.63$	ns
85 <sup>1</sup>	—	$8.0 \times 10^{10}$ (>99)	$2.97 \pm 0.69$ (ns)	$3.44 \pm 0.65$	ns
67 <sup>1</sup>	—	$3.2 \times 10^{11}$ (>99)	$6.72 \pm 0.93$ (ns)	$6.64 \pm 2.15$	ns
82 <sup>2</sup>	11 (>99)	—	$5.50 \pm 0.74$ (ns)	$9.96 \pm 1.96$	ns
88 <sup>3</sup>	12 (>99)	—	$5.26 \pm 0.29$ (24)	$4.01 \pm 0.71$	.006
89 <sup>3</sup>	30 (>99)	—	$7.74 \pm 1.03$ (31)	$5.34 \pm 0.69$	.011
87 <sup>2</sup>	34 (88)	—	$4.80 \pm 0.66$ (ns)	$4.15 \pm 0.74$	ns
84 <sup>1</sup>	40 (nd)	—	$4.92 \pm 1.14$ (41)	$2.91 \pm 0.64$	.010
87 <sup>3</sup>	68 (88)	—	$5.79 \pm 0.76$ (23)	$4.53 \pm 1.52$	.006
83 <sup>2</sup>	81 (48)	—	$6.91 \pm 1.37$ (34)	$4.57 \pm 0.71$	.012

ns = Not significantly greater than for non-feeding sample population (controls) at  $p \leq 0.10$ . nd = Not determined.  
<sup>1</sup> Experiment 1. <sup>2</sup> Experiment 2. <sup>3</sup> Experiment 4. Results for Experiment 3 given in Fig. 1

the greatest contributor to Cd uptake, as was the case for experiments employing  $3.6 \times 10^{-7} \text{M Cd}_T$ . Mean Cd uptake in unfed clams was 59 to 81% of that in feeding clams in all aquaria containing exopolymer-amended iron oxide.

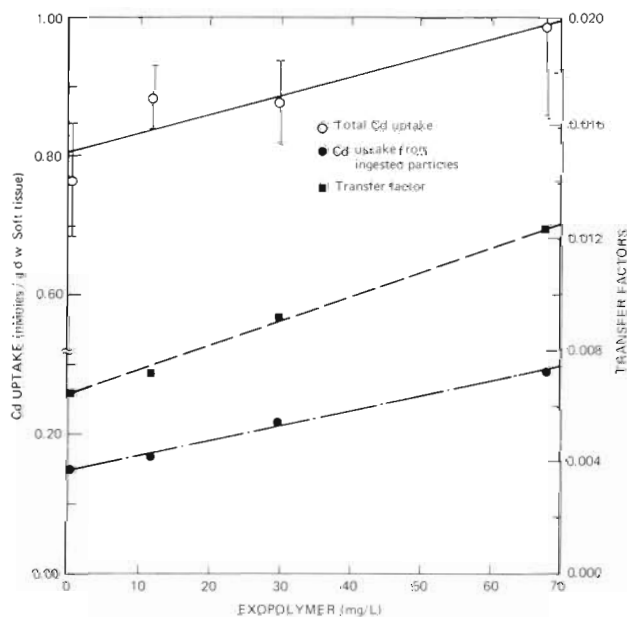


Fig. 1. Total Cd uptake, calculated Cd uptake from ingested particles into *Macoma balthica* soft tissue, and corresponding transfer factors as a function of total bacterial exopolymer concentration in Experiment 3. Calculated  $\text{Cd}_T$  is  $3.6 \times 10^{-8} \text{M}$ . Error bars: standard error for total Cd uptake among 7 feeding clams. Particles are amorphous iron oxyhydroxide. Coefficient of determination,  $r^2$ , for Cd uptake from ingested particles is 0.98;  $p \leq 0.01$

At  $3.6 \times 10^{-7} \text{M Cd}_T$  (Experiments 1, 2 & 4), apparent feeding rate was inversely related to levels of adsorbed exopolymer, judging from the quantity of fecal material purged by clams at the end of the experiment. In Experiment 2, twice as much fecal material was collected from clams ingesting unamended iron oxide ( $1.29 \text{ mg clam}^{-1}$ ) as from clams ingesting iron oxide amended with  $26 \text{ mg/g}$  exopolymer ( $0.60 \text{ mg clam}^{-1}$ ). In Experiment 4, the presence of  $40 \text{ mg}$  exopolymer per  $\text{g}$  of iron oxide resulted in an 8-fold decrease in the quantity of recovered feces from  $2.43 \text{ mg clam}^{-1}$  to  $0.29 \text{ mg clam}^{-1}$ . When differences in feeding rate are taken into account by normalizing Cd uptake from particles to fecal deposition, Cd availability to *Macoma balthica* from exopolymer-coated iron oxides is substantially enhanced as compared to the amorphous iron oxides alone. At  $3.6 \times 10^{-8} \text{M Cd}_T$  (Experiment 3), fecal deposition actually increased slightly with increased addition of exopolymer, from  $1.71 \text{ mg clam}^{-1}$  when no polymer was present to  $2.83 \text{ mg clam}^{-1}$  at  $68 \text{ mg l}^{-1}$  exopolymer. This suggests that exopolymer itself did not cause the feeding rate differences observed in Experiments 2 and 4.

When *Macoma balthica* were exposed to Cd-labeled natural sediments, differences in Cd uptake between fed and unfed clams were highly significant ( $p \leq 0.001$ ) in each of the 5 replicate experiments (Experiment 6; Table 2). Ingested particulates accounted for more than half of the Cd uptake in feeding clams when natural sediments were the food source. In contrast, when organic materials were removed from the sediments by NaOH extraction, the

results were similar to those observed when amorphous iron oxide particles were employed. At neither  $6 \times 10^{-7}$  M  $\text{Cd}_T$  nor at  $6 \times 10^{-8}$  M  $\text{Cd}_T$  was uptake by feeding clams significantly different from uptake by clams held in filter chambers. When bacterial exopolymer was added to the NaOH-extracted sediment ( $10 \text{ mg g}^{-1}$ ), Cd availability returned to that characteristic of unaltered sediments (Table 2). In all 5 replicate

rather than a preference of *Macoma balthica* for particle-bound relative to dissolved forms of zinc. Essentially all of the Zn added to aquaria containing iron oxide or iron oxide amended with bacterial exopolymer adsorbed onto particles. Little or no Zn was detectable in solution at 24 h, except in the aquarium containing the highest abundance of adherent bacteria ( $1.6 \times 10^{11}$  cells  $\text{g}^{-1}$  iron oxide, Experiment 1). In this

Table 2. *Macoma balthica*. Cadmium and zinc uptake in microcosm experiments with natural, alkaline-extracted, and exopolymer-amended sediments. For each type of sediment, Cd and Zn fractional adsorption, uptake by feeding and non-feeding clams, percentages deriving from ingested sediments, and corresponding transfer factors are given

Sediment type	Cd and Zn concentration (M)	(% adsorbed)		Cd uptake by clams nMoles $\text{g}^{-1}$		Zn uptake by clams nMoles $\text{g}^{-1}$		Transfer factor	
		$\text{Cd}_T$	$\text{Zn}_T$	Feeding (% from particles)	Non-feeding	Feeding (% from particles)	Non-feeding	Cd	Zn
Natural <sup>1</sup> (unaltered)	$2.1 \times 10^{-6}$	86	>99	$32.06 \pm 1.85$ (57)	$13.86 \pm 1.26$	$36.26 \pm 1.04$ (86)	$4.95 \pm 0.22$	.036	.063
Extracted <sup>2</sup> (0.1N NaOH)	$6.0 \times 10^{-7}$	63	99	$9.19 \pm 1.23$ (ns)	$8.58 \pm 0.70$	$2.24 \pm 0.59$ (88)	$0.17 \pm 0.07$	ns	.021
Extracted <sup>2</sup> (0.1N NaOH)	$6.0 \times 10^{-8}$	66	>99	$0.80 \pm 0.09$ (ns)	$0.67 \pm 0.05$	$0.26 \pm 0.11$ (97)	$0.01 \pm 0.00$	ns	.025
Extracted/ exopolymer amended <sup>1</sup>	$2.1 \times 10^{-6}$	86	>99	$35.67 \pm 2.87$ (65)	$12.37 \pm 0.75$	$36.24 \pm 2.73$ (90)	$3.80 \pm 0.37$	.047	.065

ns = no significant difference between feeding and non-feeding sample population at  $p \leq 0.10$   
<sup>1</sup> Values are means  $\pm 90\%$  C.I. for 5 replicate experiments (Experiment 6)  
<sup>2</sup> Values are means  $\pm 90\%$  C.I. for sample population in single system (Experiment 5)

experiments, clams ingesting sediments contained significantly ( $p \leq 0.001$ ) more Cd than unfed clams. Cadmium uptake in clams fed unaltered sediments were not significantly different ( $p \leq 0.50$ ) from that in clams fed NaOH-extracted sediments amended with exopolymer. The extent of Cd-binding by the natural sediment and by stripped sediments amended with exopolymer was similar (86% of  $\text{Cd}_T$ ), but sediments stripped with NaOH bound smaller fractions of total cadmium (63 to 66%). The apparent reduction in Cd-binding of these sediments was accompanied by a reduction in the bioavailability of Cd associated with these particles.

### Zinc

In contrast to Cd, a majority of the Zn uptake in feeding clams originated directly from ingestion of Zn-enriched particles. The solute vector accounted for less than 10% of Zn uptake in feeding clams in nine of the 12 experiments employing amorphous iron oxide, and was not detected in 4 cases (Table 3). Small contributions by the solute vector to total zinc uptake appeared to be due to the extremely low levels of dissolved Zn,

the solute vector contribution to total Zn uptake in feeding clams was substantially greater (23%), even though solute Zn was only 1% of that bound to bacterially-modified iron oxide particles. This suggests that availability of dissolved forms of zinc can be relatively high. No correlation ( $p \geq 0.10$ ) was observed between amounts of Zn taken up by *M. balthica* from ingested particles and the amount of exopolymer sorbed onto amorphous iron oxyhydroxide, even at  $3.1 \times 10^{-8}$  M added Zn (Experiment 3), where a strong correlation was observed for Cd. However, transfer factors in the latter experiment were 2-fold higher in the 3 aquaria containing adsorbed exopolymer.

Uptake of Zn in clams feeding on natural, alkaline-extracted and exopolymer-amended sediments largely resulted from uptake of this metal from ingested particulates (Table 2). At  $2.1 \times 10^{-6}$  M  $\text{Zn}_T$  (Experiment 6), Zn uptake from ingested natural sediments and NaOH-extracted, exopolymer-amended sediments were similar ( $36.3 \pm 1.0$  vs  $36.2 \pm 2.7$  nMoles  $\text{g}^{-1}$  dry wt soft tissue). Fractions of total Zn uptake deriving from ingestion of natural sediments were also similar to those for unamended, alkaline-extracted sediments.

Table 3. *Macoma balthica*. Zinc uptake ( $\pm 90\%$  C.I.) in microcosm experiments with unamended, bacteria-amended, and exopolymer-amended iron oxide particles. For each system, Zn uptake by feeding ( $n = 7$ ) and non-feeding clams ( $n = 5$ ), percentage deriving from ingested particles, and corresponding transfer factors are presented

Zn [M] (% adsorbed)	Exopolymer mg l <sup>-1</sup> (% adsorbed)	Bacteria cells g <sup>-1</sup> (% adsorbed)	Zn uptake by clams nMoles g <sup>-1</sup>		Transfer factor
			Feeding (% from particles)	Non-feeding	
3.1 × 10 <sup>-7</sup> (>99) <sup>1</sup>	-	-	2.39 ± 0.31 (89)	0.26 ± 0.03	.010
(>99) <sup>2</sup>	-	-	3.29 ± 0.58 (>99)	0.00 ± 0.06	.016
(>99) <sup>1</sup>	-	8.0 × 10 <sup>10</sup> (>99)	1.60 ± 0.42 (82)	0.29 ± 0.05	.006
(99) <sup>1</sup>	-	1.6 × 10 <sup>10</sup> (>99)	2.56 ± 0.38 (77)	0.58 ± 0.14	.010
(>99) <sup>2</sup>	11 (>99)	-	3.50 ± 0.64 (>99)	0.00 ± 0.03	.017
(>99) <sup>2</sup>	34 (88)	-	3.16 ± 0.81 (>99)	0.00 ± 0.01	.016
(>99) <sup>1</sup>	40 (nd)	-	2.86 ± 0.45 (89)	0.32 ± 0.06	.013
(>99) <sup>2</sup>	81 (48)	-	2.50 ± 0.46 (>99)	0.00 ± 0.05	.012
3.1 × 10 <sup>-8</sup> (>99) <sup>3</sup>	-	-	0.48 ± 0.14 (95)	0.03 ± 0.01	.012
(>99) <sup>3</sup>	12 (nd)	-	0.88 ± 0.15 (97)	0.03 ± 0.01	.042
(>99) <sup>3</sup>	30 (nd)	-	1.14 ± 0.18 (97)	0.04 ± 0.01	.054
(>99) <sup>3</sup>	68 (nd)	-	0.97 ± 0.35 (97)	0.03 ± 0.01	.046

<sup>1</sup> Experiment 1; <sup>2</sup> Experiment 2; <sup>3</sup> Experiment 3

However, transfer factors for Zn uptake for the latter sediment type were approximately 3-fold lower than for natural or exopolymer-amended sediments.

### Silver

The only experiment with Ag (Experiment 4) compared uptake of this metal from iron oxides at different concentrations of adsorbed exopolymer. Adsorption of Ag onto amorphous iron oxyhydroxide was slow relative to adsorption of either Cd or Zn. After 24h, only 2 to 7% of the Ag was adsorbed, whereas 39 to 52% had sorbed onto iron oxide particles after 7 d (Table 4). The percent binding did not differ consistently with polymer-coating. However, concentrations of Ag in soft tissue originating from ingestion of Ag-contaminated iron oxide particles increased steadily with increasing

levels of adsorbed exopolymer (Table 4; Fig. 2). Considerable Ag uptake from particle ingestion was evident at the higher exopolymer concentrations, despite the small proportion of Ag that was adsorbed.

At the lower exopolymer concentrations (0 and 12 mg l<sup>-1</sup>), Ag uptake was higher in non-feeding clams than clams ingesting Ag-laden iron oxide particles. Concentrations of Ag were 10- to 100-fold higher in recovered feces than in uningested particles. This suggests that particles in the digestive tract of feeding clams scavenged Ag, thus reducing either the availability or the net uptake of Ag from solution as compared to that for unfed clams. Calculation of Ag availability from particles was made by subtracting the solute vector from total metal uptake and thus appears to represent a conservative estimate of Ag uptake from ingested particulates. Exopolymer at the 2 higher concentrations must have greatly enhanced the availabil-

Table 4. *Macoma balthica*. Silver uptake ( $\pm 90\%$  C.I.) in microcosm experiments with unamended and exopolymer-amended amorphous iron oxide particles. For each system, 24h and 7d fractional adsorption of Ag, Ag uptake in feeding ( $n = 7$ ) and non-feeding clams ( $n = 5$ ), and Ag concentration in purged and uningested particles are presented

Exopolymer mg l <sup>-1</sup> (% adsorbed)	% Ag adsorbed		Feces mg clam <sup>-1</sup>	Ag uptake by clams nMoles g <sup>-1</sup>		Transfer factor <sup>1</sup>	Ag in particles nMoles g <sup>-1</sup>	
	24h	7d		Feeding	Non-feeding		Feces	Sediment
0	7.6	47	2.43	53.1 ± 7.29	72.7 ± 18.0	-0.72	230	9.3
12 (>99)	7.3	52	1.43	43.1 ± 5.88	56.1 ± 7.89	-0.44	268	21.6
30 (>99)	1.7	39	0.57	64.2 ± 8.54	46.9 ± 6.24	0.86	508	25.0
68 (88)	3.4	47	0.29	70.1 ± 17.6	36.3 ± 7.00	1.34	829	8.4

<sup>1</sup> Transfer factors were computed using average Ag adsorption densities between Days 1 and 7

ity of the Ag from ingested particles to overcome the intestinal scavenging by Fe oxide. Transfer factors for Ag at the 2 higher exopolymer concentrations were approximately two orders of magnitude higher than transfer factors for Cd and Zn in similar experiments.

The quantity of ingested particles recovered from feeding clams during the 24h purge varied inversely with concentrations of adsorbed exopolymer (Table 4). Feeding rate apparently declined with increasing polymer abundance and increasing metal availability, similar to results where higher Cd concentrations had been employed. When differences in apparent feeding rate are taken into account, enhancement in the availability of particle-bound Ag due to the presence of exopolymer is even greater than is suggested in Fig. 2.

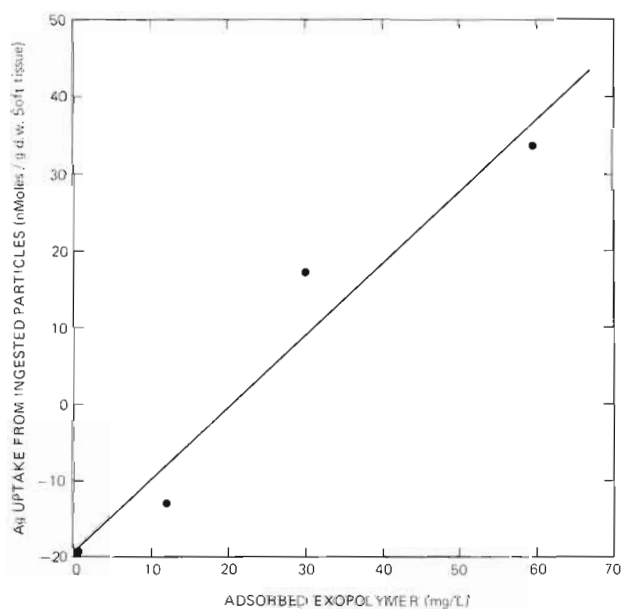


Fig. 2. Calculated Ag uptake from ingested particles into *Macoma balthica* soft tissue as a function of adsorbed bacterial exopolymer concentration. Uptake of Ag from the solute vector has been subtracted out

## CONCLUSIONS

These experiments lead to the somewhat surprising conclusion that alteration of inorganic particles with bacterial exopolymer enhances the availability of particle-bound Ag, Cd, and perhaps Zn to the deposit-feeder *Macoma balthica*. The effect was most evident with Ag. The positive effect of exopolymer on Cd and Zn availability was more evident in natural sediments. When organic materials were removed from the natural sediment with NaOH, availability of particle-bound metals declined. However, Cd and Zn availability to *M. balthica* returned to the level characteristic of

unaltered sediment when alkaline-stripped sediments were amended with exopolymer (to a level of 0.5 % total organic carbon). Adsorbed bacterial exopolymer and NaOH-extractable organics had a similar effect on availability of Cd. Thus, it seems the organic moiety of the sediment has a substantial effect upon determining the biological availability of this metal. The inconsistency in Zn results between the iron oxide experiments and the experiments with natural sediments suggested the effect of the organic moiety on Zn availability might be negated by an abundance of iron oxide which strongly binds this metal.

The mechanism of enhanced bioavailability of particle-bound metal by the presence of adherent exopolymer was not clear. Luoma & Jenne (1977) demonstrated that substantial differences in binding intensity among different types of laboratory-prepared particles correlated with changes in availability of particle-bound Ag, Co, and Zn. In the present experiments, adsorbed exopolymer had little effect on the binding efficiency of Cd, since Cd adsorption was not changed by exopolymer addition. However, changes in distribution coefficients cannot always be related directly to changes in metal-binding intensity (Davis 1984). This also suggests that very little Cd was bound to the adherent exopolymer itself. Thus, changes in overall metal-binding intensity do not appear to be the only mechanism involved in alterations of Cd uptake from particulates observed in these experiments. The effect of binding efficiency on Zn availability could not be determined in these experiments, since essentially all of the added Zn had adsorbed onto particles at 24h. Vangenechten et al. (1983) also observed differences between 2 sediments in adsorbate availability ( $^{241}\text{Am}$ ) to a deposit-feeder that were not consistent with differences in solute/particle distribution of the metal.

Changes in feeding rate did not appear to cause enhancement of metal availability by exopolymer. Clams feeding on exopolymer-amended sediments at the highest Cd and Ag concentrations actually appeared to ingest fewer particles than clams feeding on uncoated iron oxides. Decreases in the quantity of feces deposited with increases in abundance of adsorbed exopolymer may reflect behavioral avoidance of increasingly available Cd and Ag, since such decreases were not observed in the experiment employing 10-fold lower Cd $_{T}$ . Reduced feeding rates have been observed in oysters exposed to Cd-labelled bacteria (Ward 1982) and behavioral avoidance of abiotic particulates was also observed when *Macoma inquinata* was exposed to oil-contaminated sediments (Crecelius et al. 1980). If behavioral avoidance can be invoked to explain apparent feeding rate differences in our experiments, then decreased fecal deposition among clams exposed to high Cd or Ag and adsorbed

exopolymer concentrations further substantiate enhanced availability of these metals in the presence of adherent bacterial exopolymer. Although adherent exopolymer does not appear to be assimilated by the clams within the time frame of these experiments (Harvey & Luoma 1984), its presence may have stimulated secretion of enzymes capable of competing directly with ingested iron oxide particles for the adsorbed metal, or its metal-binding properties may have been altered within the clam.

Binding to iron oxide appeared to reduce the availability of Cd and Ag in these laboratory experiments. Such results are consistent with field observations. In a field comparison of different estuarine environments, Luoma & Bryon (1982) found a negative relation between concentrations of Cd and Ag in the deposit-feeding clam *Scrobicularia plana* and concentrations of extractable (amorphous) Fe in oxidized sediments. They suggested such a relation was indicative of enhanced metal partitioning to strong metal-binding sites of low biological availability on amorphous iron oxide. This suggests, as do the results of our experiments, that Cd and Ag availability to deposit feeders should be reduced in natural, oxidized sediments rich in Fe.

In contrast to results with exopolymer, modifying iron oxide particles with adherent bacteria did not enhance Cd or Zn availability to *Macoma balthica*. Both metals are accumulated from ingestion of suspended bacteria alone (Harvey & Luoma 1985). Thus, it is possible that the metals remained partitioned largely onto amorphous iron oxides when both iron oxides and bacteria were present. Also, the bioavailability of Cd associated with adherent bacteria may be low relative to Cd associated with unamended amorphous iron oxide (Luoma & Jenne 1976).

The strong importance of the solute vector in Cd uptake (compared to other metals) evidenced in all our experiments is supported by similar observations in nature (Luoma & Bryan 1982). The generally weak binding of Cd to particles may be the cause. Binding efficiency was important in determining the total amounts of Cd and Zn taken up by clams in the experimental studies, whether or not it was related to differences in the bioavailability of metals from ingested particles. Where metal binding was less efficient, more metal was taken up by feeding clams due to increased uptake from solution. Therefore, where particles bind metals less efficiently in the natural environment, increased metal burdens in organisms might be expected and the solute vector of uptake may be predicted to increase in importance, provided that availability of particle-associated metals and metal speciation in solution are not substantially altered.

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