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# Enrichment and association of lead and bacteria at particulate surfaces in a salt-marsh surface layer

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## ABSTRACT

The particle-laden surface layer ( $\sim 150\text{-}370\ \mu\text{m}$ ) and subsurface waters of a South San Francisco Bay salt marsh were sampled over two tidal cycles and analyzed for particle numbers and particulate-associated and total concentrations of lead and bacteria. Laboratory studies examined the ability of a bacterial isolate from the surface layer and a bacterial "film-former" to sorb Pb at environmentally significant concentrations in seawater. Degrees by which Pb concentrated in the surface layer relative to the subsurface strongly correlated with enrichments of surface layer bacteria (bacterioneuston). A significant fraction of the bacterioneuston and surface layer Pb were associated with particles. Data suggest that particle-bound bacterioneuston may interact with Pb at particulate surfaces in this microenvironment.

## 1. Introduction

Elevated Pb concentrations in marine surface water microlayers relative to subsurface waters have been reported by a number of investigators (Duce *et al.*, 1972; Lion *et al.*, 1979; Szekiolda *et al.*, 1972), although degrees of enrichment varied with location and type of sampling device employed (Lion and Leckie, 1981). In general, much of the Pb enriched in the surface layer appears to be particulate-associated (Lion *et al.*, 1979; 1982) and reported surface concentration factors (ratio of the surface layer concentration/subsurface concentration) for total Pb are higher for estuaries than for other coastal environments (Lion and Leckie, 1981).

Since elevated concentrations of microorganisms in marine surface water microlayers have also been documented (Harvey and Young, 1980a; Marumo *et al.*, 1971; Sieburth *et al.*, 1976) it has been suggested that this microenvironment may serve as an important site for incorporation of toxic metal into marine food chains (Piotrowicz *et al.*, 1972). Although bioavailability of Pb in estuaries depends upon complex biogeochemical factors, such as its chemical speciation (Luoma and

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Bryan, 1978) significant enrichments of both particulate lead (Lion *et al.*, 1979) and particle-bound bacteria (Harvey and Young, 1980a,b) in the surface layer of the Palo Alto salt marsh (Palo Alto, CA) may facilitate other bacterial-Pb interactions at particle surfaces in this microenvironment. For example, particle-bound bacterioneuston may directly influence the distribution of Pb in the salt-marsh surface layer by sorbing Pb or, indirectly, by altering the Pb-adsorptive capabilities of particulates to which they attach (Harvey, 1981).

The primary objective of these investigations was to examine the likelihood of such interactions, based upon correlations of available field data. The major hypothesis was that, in the particle-laden surface layer of the Palo Alto salt marsh, Pb enrichment correlates strongly with bacterial enrichment and significant portions of Pb and bacteria associate with the same particulates. Since Pb associated with particulate surfaces may be distributed between bacteria and nonbacterial surface components and since available techniques for removal of bacteria from these surfaces (Corpe, 1974; Scheraga *et al.*, 1979) would necessitate physiochemical alterations of the whole surface, the precise distribution of Pb among the various surface components cannot be accurately determined. Hence, the specific contribution of particle-bound bacterioneuston to total and particulate Pb enrichments was not determined in this study. However, degrees of enrichment and of particulate association for both surface layer bacteria and Pb in the same set of samples were monitored over time. In addition, the abilities of selected marine bacteria, including an isolate from the salt-marsh surface layer, to sorb Pb at environmentally significant concentrations were examined in the laboratory.

## 2. Methods

*a. Sample collection.* Surface and subsurface waters of the Palo Alto salt marsh were sampled over two tidal periods (10-15-78 and 2-11-79) and analyzed for levels of particle-bound and free bacteria, dissolved and particulate lead, and particle number. The sampling site for these investigations was located near the confluence of the main tidal creek with South San Francisco Bay. All samples were taken from an inflatable rubber raft to reduce the possibility of Pb contamination (Lion, 1980). Surface samples were taken using the Garrett (1965) technique with circular teflon screens (18 mesh, 38 cm dia.). Subsurface samples were obtained by opening acid-washed sampling containers ~ 0.3 m below the surface and closing at depth.

One-liter, acid-washed polyethylene containers were used to transport samples to the laboratory. Containers and sampling apparatus were cleaned by sequential soakings in the following progressively cleaner 10% (w/v) nitric acid solutions: reagent grade HNO<sub>3</sub> (Baker) in deionized water, redistilled HNO<sub>3</sub> (G. Frederick Smith) in permanganate-distilled water, and ultrapure HNO<sub>3</sub> (BDH Aristar grade) in

evaporative distilled water. The high-purity water used in the last acid bath and final rinsings was obtained from a subboiling quartz still. This cleaning procedure and sample preparations for lead analysis were performed in a laboratory clean room, equipped with laminar flow hoods, double entry doors, and epoxy-sealed walls and floor. Plastic caps, gloves, and boots were worn inside the clean room to lessen possible introduction of particulate contaminants (Lion, 1980).

*b. Numbers of bacteria and particles.* Subsamples to be used in the determination of particle-bound and total concentrations of bacteria and numbers of nonbacterial particles (2.5 to 101  $\mu\text{m}$  dia. size range) were fixed in the field with glutaraldehyde (0.25% final concentration) and stored at 4°C until final analysis. Particle-bound and total bacterial counts were determined to within  $\pm 10\%$  or better at the 90% confidence level using an acridine orange epifluorescence procedure (Hobbie *et al.*, 1977). Since bacteria on the undersides of particles are not accounted for with this procedure, a correction factor of 2 was applied to the number of observed particle-bound bacteria. Particles were enumerated using a model TA II counter (Coulter Electronics, Inc., Hialeah, FL). These analytical procedures are discussed by Harvey and Young (1980a) and Lion *et al.* (1979), respectively.

*c. Determination of particulate and dissolved lead.* Particulate and dissolved lead concentrations were determined for each sample by flameless atomic absorption (FAA) analyses. Two preparative procedures were employed, an acid-digestion technique modified from the method of Eggimann and Betzer (1976) for particulate ( $> 0.4 \mu\text{m}$ ) Pb and a chelation-extraction procedure modified from that used by Kinrade and Van Loon (1974) for 0.4  $\mu\text{m}$  filterable (dissolved) Pb.

Chelation-extraction was used to remove and concentrate dissolved lead from estuarine samples. The chelation step involved addition of a 5-ml, 1% w/v mixture of ammonium pyrrolidindithiocarbamate (APDC) and diethylammonium-diethyldithiocarbamate (DDDC) to 100 ml of sample which had been buffered at pH 5.5 with trisodium citrate. After 20 min, the APDC/DDDC-chelated Pb was extracted from solution with 10 ml of methylisobutylketone (MIBK) and the extracted aqueous phase discarded after one hour equilibration. The Pb was then back-extracted from the remaining MIBK solution by addition of 3 ml 6N  $\text{HNO}_3$  (BDH Aristar grade). The acid back-extract was recovered after one hour equilibration and used for FAA Pb determinations.

Particulate-associated Pb was removed from each sample by 0.4- $\mu\text{m}$  filtration. The Nuclepore filter and filter residue were then digested for 1 hr at 100°C in a sealed teflon bomb containing 500  $\mu\text{l}$  concentrated HCl followed by addition of 1500  $\mu\text{l}$  concentrated  $\text{HNO}_3$  and one hour additional heated digestion (both acids BDH Aristar grade). The recovered acid solutions were then diluted to 10.0 ml with evaporative distilled water and analyzed for Pb by FAA. These preparative techniques are delineated in detail by Lion and Leckie (1982).

Table 1. Lead in the surface layer and subsurface waters of the Palo Alto Salt Marsh.

Sample	Total concentration (nM)		Surface concentration factor ( $S_1/S_2$ )*	Percent particle-bound (surface)†
	Surface	Subsurface		
10-15-78 tidal study				
1	232	9.32	24.9	98.9
2	207	5.36	38.7	99.4
3	7.73	4.05	1.91	92.8
4	27.0	3.37	7.56	98.8
5	6.23	2.57	2.39	83.7
6	6.09	3.56	1.71	89.7
7	15.1	11.5	2.56	98.4
8	10.4	11.3	0.93	96.8
9	41.6	8.94	4.66	99.0
Mean $\pm$ std. dev.	$61.4 \pm 90.3$	$6.71 \pm 3.57$	$9.48 \pm 13.3$	$95.3 \pm 5.16$
2-11-79 tidal study				
1	204	9.57	21.4	88.5
2	123	5.85	21.1	99.5
3	150	3.07	48.7	99.6
4	43.6	2.70	16.2	93.5
5	5.46	2.20	2.48	73.7
6	20.1	2.43	9.29	94.9
7	8.21	5.12	1.60	90.5
8	8.94	6.91	1.29	94.5
9	10.4	4.72	2.20	95.6
Mean $\pm$ std. dev.	$63.8 \pm 75.4$	$4.78 \pm 2.46$	$13.7 \pm 15.5$	$91.6 \pm 7.96$

\* Abbreviations:  $S_1$  = surface;  $S_2$  = subsurface.

† Calculated for the surface microlayer as  $\left( \frac{\text{conc. particulate Pb}}{\text{conc. total Pb}} \right) \times 100$ .

d. *Adsorption of Pb onto bacteria.* An unidentified chromagenic bacterium (RO3), isolated from the surface layer of the Palo Alto salt marsh and the NCMB 301 strain of the marine film-forming bacterium, *Pseudomonas atlantica*, were used as model estuarine bacterioneuston in laboratory experiments with Pb.

Adsorption experiments were performed at 25°C in a controlled environmental chamber (Lab-Line Instruments, Inc., Melrose Park, CA), modified to lessen the possibility of significant Pb and particulate contamination. Exposed metal surfaces were painted with white epoxy paint or covered with plastic to prevent corrosion and/or contamination by heavy metals. Filters installed in recirculating air ducts and plastic sheeting surrounding the experiments themselves lessened contamination by airborne particulates.

Experimental apparatus consisted of Pyrex reaction vessels, polyethylene covers, and heat-shielded magnetic stirrers. Reaction vessels made of Pyrex and coated with

Table 2. Bacteria in the surface layer and subsurface waters of the Palo Alto Salt Marsh.

Sample	Total concentration ( $\times 10^6/\text{ml}$ )*		Surface concentration factor ( $S_1/S_2$ )†	Percent particle-bound (surface)‡
	Surface	Subsurface		
10-15-78 tidal study				
1	74	15.4	4.8	98
2	67	13.2	5.0	95
3	10.8	9.0	1.2	39
4	10.6	7.7	1.4	31
5	10.1	8.1	1.3	36
6	4.7	7.8	0.60	21
7	7.5	7.6	0.99	45
8	4.9	9.1	0.54	22
9	23	9.6	2.3	93
Mean $\pm$ std. dev.	$24 \pm 27$	$9.7 \pm 2.7$	$2.0 \pm 1.7$	$53 \pm 32$
2-11-79 tidal study				
1	48.6	5.46	8.89	92.5
2	24.3	6.89	3.53	88.3
3	15.9	3.38	4.70	80.2
4	8.29	3.72	2.23	34.7
5	5.75	3.12	1.84	21.4
6	10.8	3.39	3.18	81.0
7	4.71	4.17	1.13	48.5
8	8.26	4.06	2.03	71.0
9	9.41	5.16	1.82	71.8
Mean $\pm$ std. dev.	$15.1 \pm 13.9$	$4.37 \pm 1.24$	$3.37 \pm 2.33$	$65.5 \pm 24$

\* Determined by acridine orange epifluorescence. Analysis of individual samples made to within  $\pm 10\%$  at the 90% confidence interval. Bacteria numbers were corrected for undercounts of cells attached to undersides of particles.

† Abbreviations:  $S_1$  = surface microlayer;  $S_2$  = subsurface.

‡ Calculated for the surface microlayer as  $\left( \frac{\text{no. particle-bound bacteria}}{\text{total no. bacteria}} \right) \times 100$ .

dichlorodimethyl silane (Eastman #9650) reduced losses of solution Pb to container walls to a greater extent than vessels made of polypropylene, polyethylene, or teflon; losses of Pb-210 from artificial seawater solutions containing  $10^{-8}\text{M}$  Pb were insignificant at 24 hr. Apertures in the polyethylene covers facilitated introduction of reagents, probes, and pipets.

Experimental procedures involved adding cells and Pb spiked with  $\text{Pb}^{210}$  to filtered artificial seawater (pH 7.6) and monitoring fractional Pb adsorption with changes in time. Incubated samples were assayed for fractional Pb adsorption by comparing amounts of  $\text{Pb}^{210}$  in unfiltered 1.00-ml aliquots to that retained on 25 mm (dia),  $0.22 \mu\text{m}$  (pore size) filters after rinsing at pH 7.0. Amounts of Pb sorbing onto the filters themselves were accounted for by running control systems which

had no bacteria. Detailed descriptions for preparation of artificial seawater and washed bacterial suspensions and the analytical procedure used to count  $Pb^{210}$  are given elsewhere (Harvey, 1981).

### 3. Results and discussion

Lead and bacterial concentrations for surface layer and subsurface waters of the Palo Alto salt marsh are summarized in Tables 1 and 2, respectively. Surface concentrations for total Pb, calculated as the surface microlayer concentration/subsurface concentration (Table 1), indicate considerable surface enrichment for this metal. The average enrichment of 9.5 and 14 for the 10-15-78 and 2-11-79 studies, respectively, are significantly higher than those reported by other investigators for coastal surface layers where screen samplers were employed (Piotrowicz *et al.*, 1972; Szekielda *et al.*, 1972). This may be explained, in part, by the location of this salt marsh within a large urban area which results in significant atmospheric fallout of particulate Pb (Lion *et al.*, 1979), and, possibly, by entrapment at the air/seawater interface of sedimentary particles from adjacent mudflats, particularly during initial periods of incoming tides (Lion, 1980). Corresponding data for total-count bacteria (Table 2) exhibit many of the same characteristics as for Pb, i.e., higher, but more variable concentrations in the surface layer relative to the subsurface. The large variation in these bacterioneuston counts during tidal cycles may be due, in part, to temporal changes in surface film "compression" caused by the combined effects of tide and wind (Harvey *et al.*, 1983). Surface concentration factors for bacteria were generally lower than those for Pb, however, indicating that there may be significant amounts of microlayer Pb not directly associated with bacterioneuston.

Correspondence between surface concentration factors for total Pb and bacteria during the 10-15-78 tidal cycle is depicted in Figure 1. From this graph, there appear to be similar trends in surface layer enrichments for Pb and bacteria. For instance, the surface/subsurface ratios for both Pb and bacteria are significantly higher in the first two samples compared with the latter seven. The correlation between surface layer enrichments of Pb and bacteria [correlation coefficient ( $r^2$ ) = 0.89] may result, in part, from dependence of both factors upon relative abundance of surface layer particulates. For example, strong correlations were also observed between Pb and particulate enrichment ( $r^2$  = 0.98) and between bacteria and particle enrichment ( $r^2$  = 0.73) at the salt-marsh surface layer for the 10-15-78 sample set.

Only a weak correlation between surface concentration factors for total Pb and bacteria was observed for the 2-11-79 tidal cycle. This appears to reflect a similarly poor correlation between surface enrichments of bacteria and particles and appears to have resulted from analytical difficulties in counting particle-bound bacteria by epifluorescence due to a large number of brightly fluorescing diatoms in the first

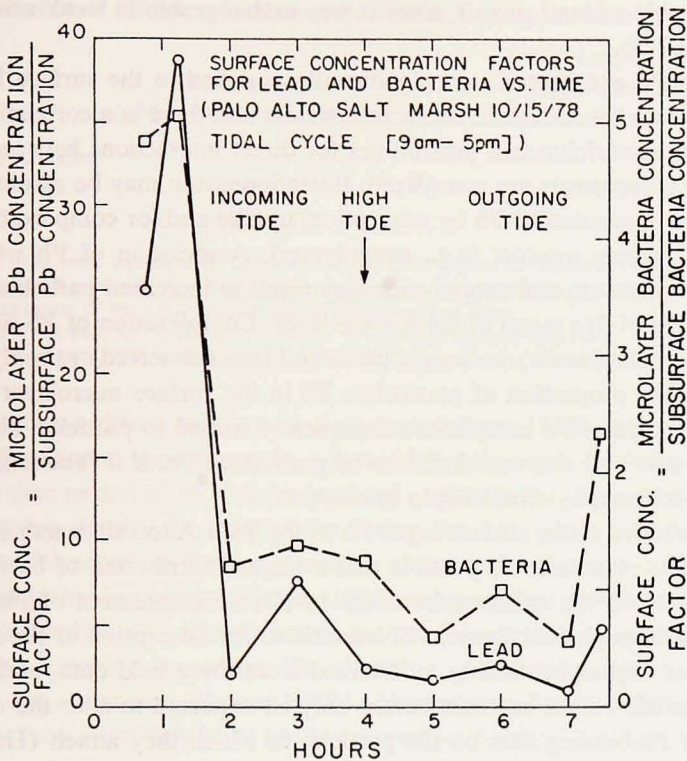


Figure 1. Surface layer concentration factors for total Pb and bacteria over the 10-15-78 tidal cycle. Solid line corresponds to scale on left, broken line to scale on right;  $n = 9$  samples.

samples (Harvey *et al.*, 1983). However, strong correlations between surface concentration factors for bacteria and particles were observed in previous investigations at this site (Harvey, 1981; Harvey and Young, 1980a,b) and significant correlations between surface layer enrichments for total Pb and bacteria would be expected, if diatom-bound bacteria had been enumerated.

The degree of particulate association for total Pb and bacteria in the surface layer is illustrated in Tables 1 and 2. A majority of bacterioneuston (53 and 65% (average) of total) were associated with surface layer particles during the two tidal cycles, as was observed in a previous study (Harvey and Young, 1980b). During the same sampling periods, an average of 92 and 95% of surface layer Pb was associated with particulate material. The high degree of Pb-particulate association in the estuarine salt marsh was also demonstrated in earlier investigations (Lion *et al.*, 1979), and may help explain the apparent dependence of Pb enrichment upon particulate enrichment in the surface layer of this salt marsh. Much of the particulate Pb in the Palo Alto salt marsh appears to be adsorbed or associated



with acid-soluble mineral phases, since it was exchangeable in weak acid (pH  $\sim$  2) (Lion *et al.*, 1979).

Since both Pb and bacteria are significantly enriched in the surface layer of the Palo Alto salt marsh relative to subsurface waters and there is a correlation between Pb and bacterial enrichments, possibilities for direct interactions between these two surface layer components are considered. Bacterioneuston may be assumed to influence chemical speciation of Pb by adsorption/uptake and/or complexation with an extracellular organic product (e.g., exopolymer). Association of Pb with particle-bound bacterioneuston and exopolymer may result in increased particulate fractions and enrichment of this metal in the surface layer. Complexation of Pb with surfaces of unattached (planktonic) bacterioneuston and free (dissolved) exopolymer would also increase the proportion of particulate Pb in the surface microlayer if the bacteria-Pb and polymer-Pb complexes subsequently sorbed to particles, although free exopolymer may also decrease fractions of particulate Pb, if it functions as a competing organic ligand by remaining in solution.

Since a majority of the surface layer Pb in the Palo Alto salt marsh is associated with particulate material, it is possible that a major contribution of bacterioneuston to Pb speciation in this microenvironment involves enhancement of Pb adsorption to particles. Although contributions of bacteria to the adsorption of Pb onto surface layer particles cannot be directly ascertained from these field data, enriched populations of particle-bound bacterioneuston may be expected to alter the number and selectivity of Pb-binding sites on the particles to which they attach (Harvey *et al.*, 1982). Since extracellular polymer isolated from film-forming, marine bacteria have also been shown to bind Pb (Corpe, 1975; Harvey, 1981) exopolymer associated with particle-bound bacterioneuston may further increase surface layer concentrations of particulate Pb by providing additional binding sites on these surfaces. These possibilities were examined in the laboratory and are discussed elsewhere (Harvey, 1981; Harvey *et al.*, 1982).

The ability of a bacterial isolate from the surface layer of the Palo Alto salt marsh and *Pseudomonas atlantica* NCMB 301 to sorb Pb at environmentally significant ( $10^{-7}$ - $10^{-8}$ M) concentrations in artificial seawater is illustrated in Table 3. The kinetics of Pb adsorption onto these bacteria appear to involve two distinct phases; i.e., a rapid removal of Pb from solution within the first few minutes, followed by a slow but constant removal over many hours (Harvey and Leckie, 1982). Amounts of Pb adsorbed within the first 15 min account for significant fractions of the total Pb added and corresponding Pb-adsorption densities were similar for the same ratios of total Pb/total bacterial surface area. For example, the  $\sim$  1.6-fold difference between amounts of Pb sorbing onto isolate RO3 and *P. atlantica* within a 15-min period (15.5 vs 9.46 pMoles/cm<sup>2</sup> cell surface, respectively) appears to correlate with the  $\sim$  1.7-fold difference in respective adsorbate/adsorbent ratios (44.1 vs 25.5 pMoles Pb/cm<sup>2</sup> cell surface). In contrast, amounts of Pb adsorbing

Table 3. Adsorption of Pb per unit of bacterial surface for two species of marine bacteria.\*

Bacterium	Lead added (pMoles/cm <sup>2</sup> cell surface)	Lead adsorbed (pMoles/cm <sup>2</sup> cell surface)	
		First 15 min.	15 to 60 min.
<i>Pseudomonas atlantica</i>			
NCMB 301	25.5†	9.46	2.64
Isolate RO3	44.1‡	15.5	0.65

\* Since cell sizes were significantly different for the two species of bacteria examined, i.e.,  $4.67 \pm 1.29$  vs  $8.09 \pm 1.54 \mu\text{m}^2/\text{cell}$  for *P. atlantica* and isolate RO3, respectively, Pb-adsorption densities are expressed as moles of Pb adsorbed per unit bacterial surface area.

† Based on  $10^{-7}\text{M Pb}_T$ ,  $8.4 \times 10^7$  cells/ml,  $4.67 \pm 1.29 \mu\text{m}^2$  surface area/cell.

‡ Based on  $10^{-8}\text{M Pb}_T$ ,  $2.8 \times 10^9$  cells/ml,  $8.09 \pm 1.54 \mu\text{m}^2$  surface area/cell.

onto *P. atlantica* within a subsequent time period from 15-60 min (2.64 pMoles/cm<sup>2</sup> cell surface) were approximately 4 times higher than that for isolate RO3 within the same time period (0.68 pMoles/cm<sup>2</sup> cell surface). The higher rate of increase in Pb-adsorption density for *P. atlantica* during the slow phase of adsorption may be related to differences in surface structure, since copious amounts of exopolymer associated with this bacterium (Corpe, 1970) may provide substantial numbers of additional Pb-binding sites after the more kinetically available sites have been filled. Although the behavior governing Pb adsorption onto gram-negative bacteria in seawater is quite complex (Harvey, 1981; Harvey and Leckie, 1982), it appears that these surfaces have significant binding capacities for this metal.

The effect of particulate enrichments upon potential bacterioneuston contributions to surface layer Pb concentrations in the Palo Alto salt marsh is illustrated in Table 4, based upon cell Pb levels calculated from data listed in Table 3 and 10-

Table 4. Effect of particulate enrichments upon potential (calculated) contributions by bacterioneuston to surface layer Pb levels in the Palo Alto Salt Marsh.

Measured particulate enrichment*	Measured surface layer concentrations†		Calculated bacterial contribution to surface layer Pb ( $\times 10^{-8}\text{M}$ )	
	Bacteria	Lead ( $\times 10^{-8}\text{M}$ )	At $4.42 \times 10^{-10}$ Moles Pb/cell‡ (% of total)	At $1.25 \times 10^{-10}$ Moles Pb/cell§ (% of total)
1.8	$4.7 \times 10^9/\text{ml}$	0.61	0.21 (34)	0.59 (96)
21.7	$7.4 \times 10^7/\text{ml}$	23.2	3.27 (14)	9.25 (40)

\* High and low values from 10/15/78 tidal cycle study.

† Bacterial numbers were corrected for undercounts of cells attached to undersides of particles.

‡ Based on Pb-adsorption density for *pseudomonas atlantica* (calculated from data in Table 3).

§ Based on Pb-adsorption density for bacterial isolate RO3 (calculated from data in Table 3).

15-78 field measurements. For a surface concentration factor for particulates of 1.8 and assumed Pb-adsorption densities of 0.44 and  $1.24 \times 10^{-18}$  M Pb/cell, potential bacterioneuston contributions to observed total Pb levels in the salt marsh surface layer were 34 and 96%, respectively. However, for an increase in surface layer enrichment of particulate matter from 1.8 to 21.7, there are predicted declines in the fractions of surface-layer total Pb associated with bacterioneuston from 34 to 14% and from 96 to 40%, respectively. Such a decrease seems plausible, since nonbacterial particulate surfaces would be expected to compete directly with bacterial surfaces for dissolved Pb. These calculations are only first approximations, since Pb-adsorption-density data derived from laboratory investigations may not be directly applicable to the estuarine environment. However, based upon this research, it is clear that bacterioneuston may have the potential to influence Pb distribution in this microenvironment.

In addition, the presence of significant quantities of both bacteria and Pb on surface layer particles in the Palo Alto salt marsh suggests that these particulate surfaces may function as sites for Pb incorporation into estuarine food chains. Correlations between levels of acid-extractable Pb on sedimentary particulates and in tissues of estuarine clams have been demonstrated by Luoma and Bryan (1978), although fractions of particle-bound Pb associated with adherent bacteria and exopolymer were unknown. The influence of adherent bacterioneuston and extracellular polymers upon bioavailability of Pb to higher animals in the salt marsh has not been examined and is worthy of further study.

*Acknowledgments.* We thank S. Altmann, R. Appleton, V. Gallington, K. Hayes, B. Honeyman, M. Perry, and J. Scott of Stanford University for their help with field studies and D. Cain of the U.S. Geological Survey for review of the final manuscript.

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