

## Lead binding to metal oxide and organic phases of natural aquatic biofilms

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### Abstract

The role of the composition of surface-coating materials in controlling trace metal adsorption in aquatic environments was investigated using natural biofilms that developed on glass slides in three New York State lakes and a water-supply well. Adsorption isotherms were obtained for Pb binding to each of the biofilms in solutions with defined Pb speciation at 25°C and pH 6.0, with Pb concentrations ranging from 0.2 to 2.0  $\mu\text{M}$ . Adsorption isotherms for Pb binding to laboratory-derived metal oxides and surrogate organic materials were determined under the same conditions. These isotherms, combined with characterization of natural biofilm composition, were used to estimate the relative contributions of the organic and metal oxide surface-coating constituents by assuming additivity of adsorption to discrete adsorbing phases. Cells of a diatom (*Navicula peliculosa*), a green alga (*Chlorella vulgaris*), the bacterium *Leptothrix discophora*, and extracellular polymer of the bacterium *Burkholderia cepacia* were tested as laboratory analogs for the organic phase of the biofilms. Amorphous Fe oxyhydroxide,  $\gamma\text{Al}_2\text{O}_3$ , and a laboratory-derived biogenic Mn oxyhydroxide were used as laboratory surrogates for biofilm minerals. The sum total of predicted Pb binding to the defined surrogates accounted for at least 90% of the total observed Pb binding in the three lake biofilms and 60% of that observed for the well biofilms. For the lake biofilms, Pb adsorption to Fe and Mn oxides was significantly greater than that estimated for organic materials. The use of biogenic Mn oxide as a model component resulted in an estimated Pb adsorption to Mn oxyhydroxides in the lake biofilms up to four times greater than that estimated for Fe oxyhydroxide. Estimated Pb binding by Al oxide was negligible for all four biofilms. These results suggest that Fe and biogenic Mn oxides exert the greatest influence on Pb adsorption in oxic freshwater environments at pH 6.0.

The cycling of transition metals in aquatic environments is controlled by adsorptive scavenging, which in turn is controlled by the composition of the aqueous phase and the reactive organic and inorganic components at surfaces (Krauskopf 1956; Jenne 1968; Turekian 1977; Vuceta and Morgan 1978; Shlokovitz and Copland 1982; Murray 1987). The presence of surface coatings such as adherent microorganisms and oxide mineral deposits, the presence of dissolved and adsorbed ligands, the concentration and speciation of the trace metal in the aqueous phase, the concentration of competing trace metals, and solution variables such as pH and salinity all can influence metal adsorption (Muller and Sigg 1990; Giusti et al. 1993). Much progress has been made recently in developing models to

describe trace metal adsorption to heterogeneous particulates (Buffle et al. 1990; Davis and Kent 1990; Dzombak and Morel 1990; Schindler 1991; Westall et al. 1995). However, because of the chemical and biological complexity of natural waters, the relative roles of the biological vs. mineral surface components in the adsorption of transition metals have yet to be fully quantified and defined. Some researchers have reported that metal oxides are the single most important environmental determinant of transition metal adsorption (Krauskopf 1956; Jenne 1968), while others have reported a more important role for organic materials because of their high active surface area (Balistrieri and Murray 1983; Salim 1983; Sigg 1985). Selective extraction methods, in which metal bound to extracted phases is quantified, have been used to estimate the relative roles of the reactive components of sediments in adsorbing trace metals (Tessier et al. 1979; Lion et al. 1982; Luoma 1989; Campbell and Tessier 1991; Davies 1992; Weimin et al. 1992; Coetzee et al. 1995; Martin et al. 1996). However, adsorbing phases identified with these methods are operationally defined by the extractants employed. Alternative means are needed to quantify and verify the relative contributions of the constituents of natural biofilms and sediments to trace metal adsorption in aquatic environments.

Another approach to investigating the complex interactions governing trace metal cycling is to construct model

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### Acknowledgments

We thank Raja Krishnan for help with surface-coating analyses, Paul Koster van Groos and Elizabeth Costello for help with measuring Pb adsorption by Mn and Fe oxides, Cameron Willkens for repair of vital instruments, Ed Mills for access to sampling sites at Shakleton Point Biological Field Station, John Livingston for sampling access at Green Lakes State Park, and Linda Westlake for the provision of a dock for sampling Cayuga Lake.

This research was supported by NSF grants BES-97067715 and CHE-9708093 and through a STAR fellowship from the U.S. EPA (Y.N.).

laboratory systems that allow the development of mechanistic mathematical models to describe the observations made under controlled conditions (Hsieh et al. 1994a,b; Nelson et al. 1995, 1996). In such systems, it has been shown that total Pb adsorption to thin composite surface coatings composed of laboratory-generated colloidal Fe oxyhydroxide deposited with a bacterial biofilm can be accurately determined by adding the adsorption isotherms for each constituent under comparable solution conditions (pH, ionic strength, and metal speciation) (Nelson et al. 1995). These laboratory results suggest that the relative contributions to metal binding of metal oxides and organic phases in more complex natural biofilms could also be successfully estimated based on the composition of the biofilm by using adsorption isotherms for laboratory surrogates of individual biofilm constituents. This approach is similar to that proposed by Oakley et al. (1981), in which Cu and Cd adsorption to aquatic sediments was modeled by summing the adsorption to discrete geochemical phases. This additive approach has also been used in developing rigorous surface complexation models for trace metal adsorption (Luoma and Davis 1983; Sigg 1987; Davis and Kent 1990; Dzombak and Morel 1990; Smith and Jenne 1991; Wang and Chen 1997; Radovanovic and Koelmans 1998).

To test the adsorption additivity model on natural biofilms and to estimate the relative contributions of adsorption by metal oxide and organic phases, aquatic biofilms were collected on glass slides in four different freshwater environments (three lakes and a water-supply well). Biofilms that developed on the slides were characterized in terms of their organic and mineral composition, and their Pb adsorption was measured in the laboratory under conditions with controlled solution chemistry. To test the assumption of additivity of adsorption to the discrete model phases, metal oxide and organic phases present in the biofilms were quantified, and their relative contributions to total Pb binding were estimated based on Pb adsorption isotherms for model analogs of these materials. Laboratory analogs included cells and extracellular polymer of several microorganisms selected to represent the organic phase and amorphous Fe oxyhydroxide,  $\gamma\text{Al}_2\text{O}_3$ , and biogenic Mn oxides to represent the mineral phases. Pb adsorption to these surrogate materials was measured under the same conditions as used for measuring Pb adsorption to the natural biofilms, so that effects of pH, temperature, and solution chemistry (ionic strength and Pb speciation) could be controlled.

## Methods

*Collection and characterization of natural biofilms*—Glass microscope slides ( $5.1 \times 7.6$  cm) were placed in polypropylene racks (Fluoroware) and submerged at each of four aquatic field sites (see below) in the fall of 1996. A similar method has been used for collecting freshwater sediments on Teflon sheets (Belzile et al. 1989). Before placement, the glass slides were washed with detergent, acid washed two times with 10%  $\text{HNO}_3$  (glass distilled, GFS Chemicals) for 24 h, and rinsed with distilled, deionized water (dd  $\text{H}_2\text{O}$ ). In each case, a visible biofilm was present on the glass slides

after 3 weeks of exposure. Three of the field sites were freshwater lakes in central New York State that represented a range of trophic conditions. Green Lake is a small meromictic lake with  $\text{CaCO}_3$  stromatolites and was chosen to represent oligotrophic lakes. Cayuga Lake is a large, deep lake with mesotrophic conditions, and Oneida Lake is a large, shallow, and highly productive lake with significant annual Mn deposition (Aguilar and Neilson 1994). For the lakes, the racks of slides were submerged at a depth of approximately 30 cm, and the water temperature was 10–15°C. A water-supply well (60 m deep) with high Fe content was also selected to represent conditions of low organic content and high Fe. Racks of slides were placed directly in the well below the water level, and the water temperature was ca. 10°C.

After exposure in the field, the slides with attached biofilms were transported within 2 h to the laboratory (submerged in water from the field site) for microscopic examination, chemical characterization, and measurement of Pb binding. Biofilms were consistent from slide to slide (trace metal concentrations varied by <5%), allowing the use of different slides for each characterization and for measurement of Pb binding. Microscopic analyses were used to identify classes of adherent microorganisms by acridine orange (0.01%) epifluorescence and phase contrast microscopy. To visualize the associations of metal oxyhydroxides with other materials in the biofilms, Fe minerals were stained using the Prussian Blue (PB) spot-test reagent specific for Fe(III) oxides, and Mn oxyhydroxides were stained with acidified Leukoberbelin Blue (LBB) reagent specific for Mn(III) and Mn(IV) oxides, as described by Ghiorse and Hirsch (1979). Total organic material on the slides was determined by measuring chemical oxygen demand (COD) using a modification of Standard Method 5220 B (APHA 1995). For this analysis, the slides were broken into small pieces and placed in 250-ml Erlenmeyer flasks. To each flask were added 50 ml dd  $\text{H}_2\text{O}$ , 0.3 g  $\text{HgSO}_4$ , 5 ml sulfuric acid reagent (w/ $\text{Ag}_2\text{SO}_4$ ), 25 ml of 0.00417 M  $\text{K}_2\text{Cr}_2\text{O}_7$ , and an additional 70 ml of sulfuric acid reagent. These solutions were refluxed for 2 h, cooled, and titrated with standardized 0.025 M ferrous ammonium sulfate. Chlorophyll *a* (Chl *a*) was determined after removing biofilms by rubbing slides with 2.5-cm glass-fiber filters, extracting chlorophyll from the filters with acetone/aqueous  $\text{MgCO}_3$  solution, and measuring absorbance with a Hewlett-Packard model 8452 UV/Vis diode array spectrophotometer (Standard Method 10200H [APHA 1995]). Elemental composition of the acid-soluble components of the biofilms was determined by extraction into 25 ml of 10%  $\text{HNO}_3$  (glass distilled) for 24 h, and analysis of the extracts, by using inductively coupled plasma (ICP) spectroscopy.

*Measurement of Pb adsorption to natural biofilms*—Pb adsorption isotherms were obtained for the biofilms by measuring Pb adsorption from chemically defined solutions with a range of Pb concentrations. Sets of three slides from each aquatic environment were placed into each of four different Pb solutions (0.2, 0.5, 1.0, and 2.0  $\mu\text{M}$  Pb) and equilibrated for 24 h at 25°C, with pH maintained at 6.0 using pH controllers (Cole Parmer) to control the addition of 0.01 N  $\text{HNO}_3$  and NaOH. The Pb solutions were prepared in a min-

Table 1. Composition and lead speciation of MMS medium used for growth of bacterial cultures and in Pb adsorption experiments.

	Component or species	Concentration ( $\mu\text{M}$ )
MMS medium:*	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	200
	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	140
	$(\text{NH}_4)_2\text{SO}_4$	910
	$\text{KNO}_3$	150
	$\text{NaHCO}_3$	10
	$\text{KH}_2\text{PO}_4$	5
Pb speciation:†	$\text{Pb}^{2+}$	0.89
	$\text{PbSO}_4$	0.09
	$\text{PbOH}^+$	0.01

\* Ionic strength of MMS was adjusted to 0.05 M w/ $\text{NaNO}_3$ ; pH adjusted to 6.0 before autoclaving.

† Lead speciation calculated by MINEQL for a total lead concentration of 1.0  $\mu\text{M}$ .

imal mineral salts (MMS) solution (Table 1). Pb speciation in the defined solutions was calculated using MINEQL (Weinstall et al. 1976), and, because of low ligand concentrations, free  $\text{Pb}^{2+}$  concentrations comprised 89% of the total Pb (Table 1). After equilibration, slides with biofilms were removed from the Pb solutions, rinsed twice for 1 s in Pb-free MMS solution, and extracted into 25 ml of 10% glass distilled  $\text{HNO}_3$  for 24 h. Pb in extracts was measured using graphite furnace atomic absorption spectrophotometry (GFAAS), with a model 460 atomic absorption spectrophotometer (Perkin-Elmer) equipped with a model HGA-2100 graphite furnace (Perkin-Elmer). The coefficient of variation for the GFAAS analyses was <2%.

After extraction of natural biofilms with 10%  $\text{HNO}_3$  for elemental analysis, residual material remained on the slides. Because components of this material could contribute to Pb binding of the composite biofilm surface coatings, Pb adsorption to these residual deposits was also measured. The COD of the residue was measured as described above, and this analysis indicated that >80% of the organic phase was removed by the acid extraction, so that the residual material was mostly inorganic in nature.

*Measurement of Pb adsorption to model biofilm components*—Pb adsorption isotherms were determined for model microorganisms and oxide minerals selected as laboratory surrogates to represent constituents of the natural biofilms. Phototrophic organisms were represented by the diatom *Navicula peliculosa* (UTEX 668) and the green alga *Chlorella vulgaris* (UTEX 259). Pure cultures of these organisms were obtained from the UTEX Algal Culture Collection (Austin, Texas) and grown at 23°C under fluorescent light in modified Bristol's solution (Bold 1949) with no trace metals added (Table 2). Cells were harvested after 8 d by centrifuging (8,000  $\times$  g, 10 min) and were rinsed twice by resuspending in MMS followed by centrifugation. The cells were resuspended in MMS, and the suspensions were used for measuring Pb adsorption. Suspended solids concentrations (dry weight [DW]), determined with Standard Method 2540 D [APHA 1995]) were 160 and 220 mg liter<sup>-1</sup>, and the Chl a content of the cells was 0.37 and 0.59% for *C. vulgaris* and

Table 2. Modified Bristol's medium for growth of *C. vulgaris* and *N. peliculosa*.

Component	Concentration (mg liter <sup>-1</sup> )
$\text{NaNO}_3$	250
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	25
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	75
$\text{K}_2\text{HPO}_4$	7.5
$\text{KH}_2\text{PO}_4$	17.5
$\text{NaCl}$	25

*N. peliculosa*, respectively. Pb adsorption was measured by equilibrating cell suspensions in MMS with a range of Pb concentrations (0.25–2.0  $\mu\text{M}$ ) in Teflon centrifuge tubes. The pH was adjusted to 6.0, and the solutions were placed on a rotary mixer to achieve 12 inversions min<sup>-1</sup> at 25°C for 24 h. Eight-milliliter aliquots were then filtered through 0.45- $\mu\text{m}$  polyvinylidene difluoride (PVDF) membranes (Millipore). Filters were placed in 20-ml polypropylene vials, and 20 ml of 1%  $\text{HNO}_3$  was added to extract Pb. The filtrate was filtered through a second membrane filter and also analyzed to serve as a blank to account for any adsorption of dissolved Pb to the membrane filters. Pb concentrations in unfiltered solutions and in acid extracts of filters were determined using GFAAS.

Estimation of Pb binding to bacteria was based on Pb adsorption isotherms for the bacteria *Burkholderia cepacia* and *Leptothrix discophora*. *B. cepacia* (formerly *Pseudomonas cepacia* [Buchanan and Gibbons 1984]), strain 17616, was originally obtained from T. Lessie at the University of Massachusetts. *L. discophora* SS-1 (ATCC 43182) was originally isolated from a wetland near Ithaca, New York (Ghiorse and Chapnick 1983). *B. cepacia* was grown on MMS (Table 1) with 79 mg liter<sup>-1</sup> pyruvate added, and *L. discophora* was grown in MMS with 240 mg liter<sup>-1</sup> pyruvate and 2  $\mu\text{g}$  liter<sup>-1</sup> vitamin B12 added. Cell suspensions were equilibrated in the MMS solutions, with Pb concentrations varying from 0.1 to 2  $\mu\text{M}$  at pH 6.0 and 25°C. Pb adsorption to the cells was determined by filtration as described above.

Pb binding to the extracellular polymer of *B. cepacia* was measured using a dialysis method (Nelson et al. 1996). Exopolymer was obtained by growing *B. cepacia* in shake flasks and recovering dissolved exopolymer released from the cell suspension. The suspension was filtered with 0.45- $\mu\text{m}$  PVDF membranes to remove cells, the filtrate was dialyzed against distilled water in 6–8,000 molecular-weight cutoff (MWCO) membranes (6–8,000 MWCO, Spectrapor, Spectrum Medical Industries) to remove low-molecular-weight impurities, and the purified product was obtained by lyophilization. To measure Pb adsorption, reconstituted solutions of freeze-dried polymer (60 mg liter<sup>-1</sup>, 20 ml) were placed inside prerinsed dialysis tubing and equilibrated with solutions containing a range of Pb concentrations on the outside of the membranes. Both the Pb and exopolymer solutions were prepared in a modified MMS solution (Table 1) with all anions replaced by nitrate to facilitate observation of Pb binding to the exopolymer. Each solution was adjusted to pH 6.0 and equilibrated in 250-ml flasks on a shaker at 25°C for 24 h. Pb concentrations inside and outside the di-

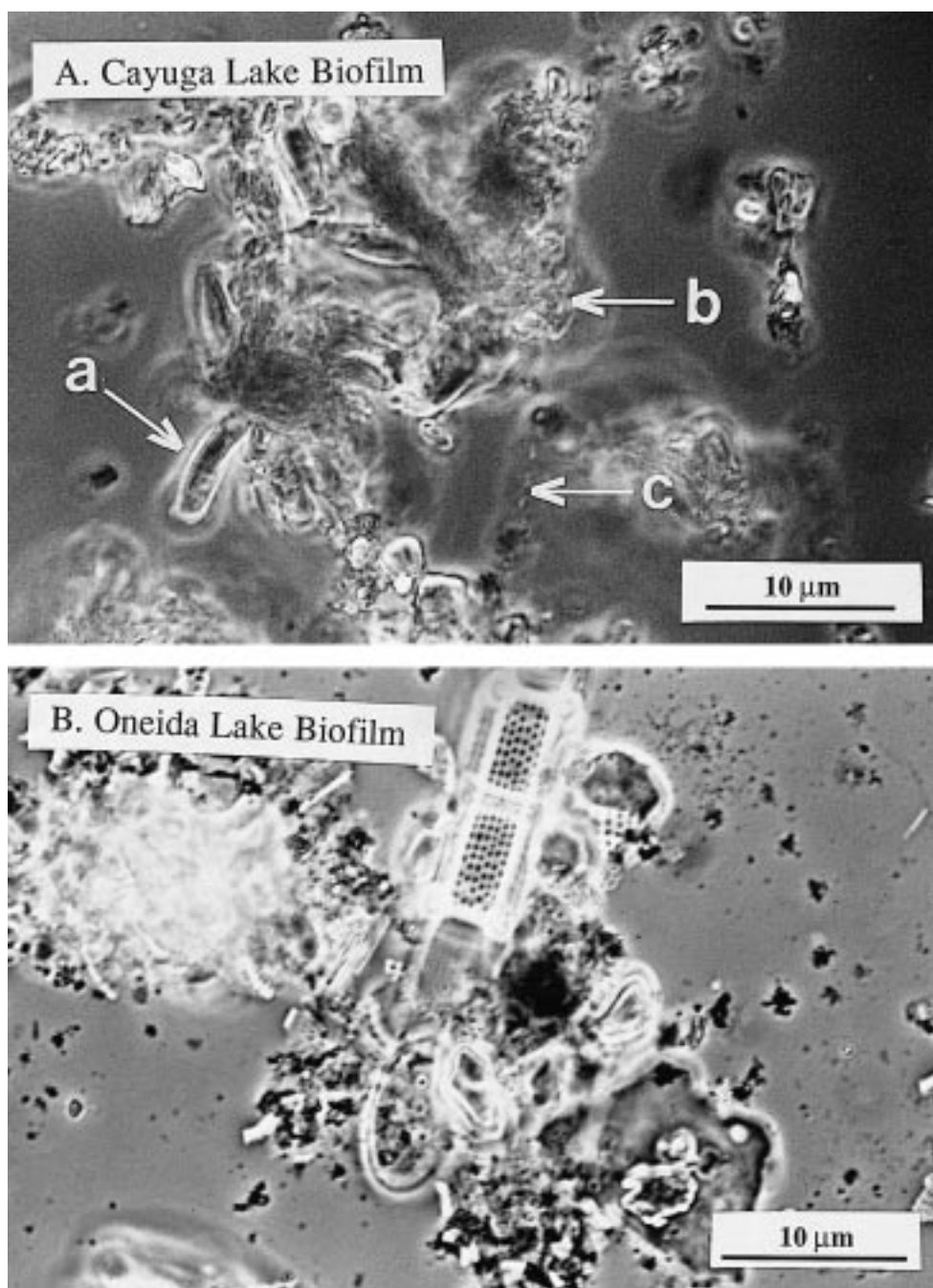


Fig. 1. Phase-contrast photomicrographs of surface coatings collected on glass slides from (A) Cayuga Lake—markers show diatoms (a), mineral deposits (b), and filamentous organisms (c); (B) Oneida Lake; (C) Green Lake—markers show *Caulobacter* spp. (d); (D) water-supply well—markers show Fe oxide deposits (e); and Fe bacteria (f).

alysis membrane were measured by GFAAS, and Pb binding by the exopolymer was calculated by difference.

Pb binding to bare glass slides and Fe oxyhydroxide was determined at 25°C and pH 6.0 by measuring Pb adsorption to glass slides with and without Fe oxyhydroxide deposits, using a method described previously (Nelson et al. 1995). Colloidal Fe oxyhydroxide was prepared by addition of 3 M NaOH to an acidified 0.1-M solution of  $\text{Fe}(\text{NO}_3)_3$  at 25°C

until pH 8.0 was attained. Pb adsorption to Al oxides was determined by equilibrating suspensions of  $\gamma\text{Al}_2\text{O}_3$  (Alfa Products) with Pb solutions in MMS in Teflon centrifuge tubes with initial Pb concentrations ranging from 0.2 to 2.0  $\mu\text{M}$ .

Pb adsorption was determined for several different Mn oxides: two commercially available Mn oxides (Fisher Scientific and ICN), a fresh  $\delta\text{MnO}_2$  precipitate, and a biogenic

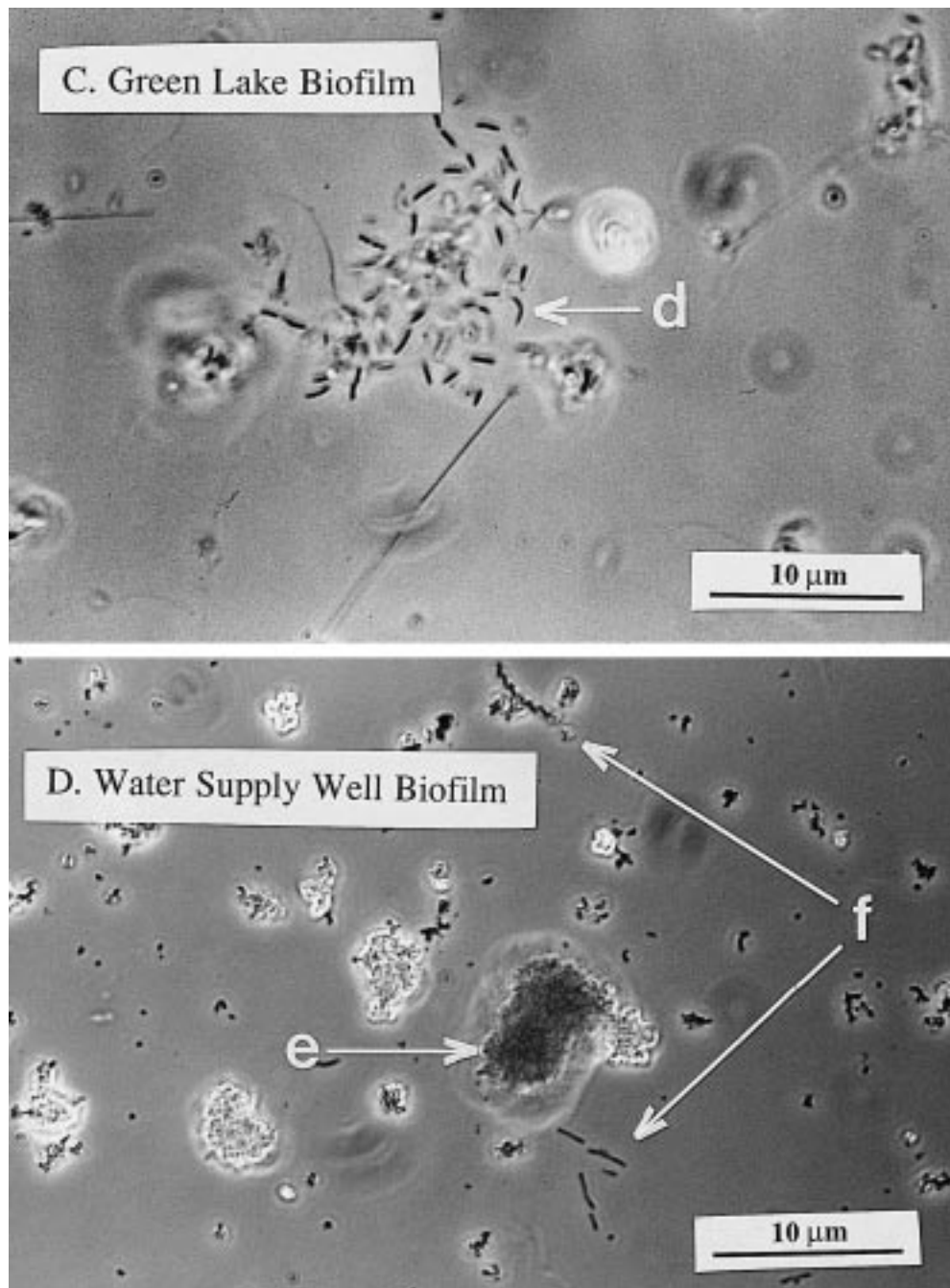


Fig. 1. Continued.

Mn oxyhydroxide. The fresh Mn oxyhydroxide precipitate was prepared by reacting  $\text{MnO}_4^-$  with  $\text{Mn}^{2+}$  using a method described previously (Murray 1974). Biogenic Mn oxyhydroxides were prepared by adding  $50 \mu\text{M}$   $\text{MnSO}_4$  to a broth of *L. discophora* SS-1 grown to stationary phase ( $65 \text{ mg DW liter}^{-1}$ ) in MMS medium with  $2 \mu\text{g liter}^{-1}$  vitamin B12 and  $0.1 \mu\text{M}$   $\text{FeSO}_4$  (Nelson et al. unpubl.). The biogenic Mn oxide was used for adsorption experiments ca. 24 h after addition of  $\text{MnSO}_4$  to the broth. Complete Mn(II) oxidation was verified by centrifuging at  $13,400 \times g$  and measuring supernatant Mn(II) concentration by AAS. Biological oxi-

ation was confirmed by the absence of Mn oxidation in azide-inhibited controls. Suspensions of the metal oxides and Pb were equilibrated for 24 h at  $25^\circ\text{C}$  and pH 6.0 in 1-liter pH and temperature-controlled reactors. After equilibration, the suspensions were centrifuged at  $13,400 \times g$  for 30 min. Pb concentrations were measured for centrifuged and uncentrifuged samples (acidified with glass distilled  $\text{HNO}_3$ ) using GFAAS, and adsorbed Pb was calculated by difference. Pb adsorption to *L. discophora* cells without Mn was negligible compared to that of the Mn oxide deposits (Nelson et al. unpubl.).

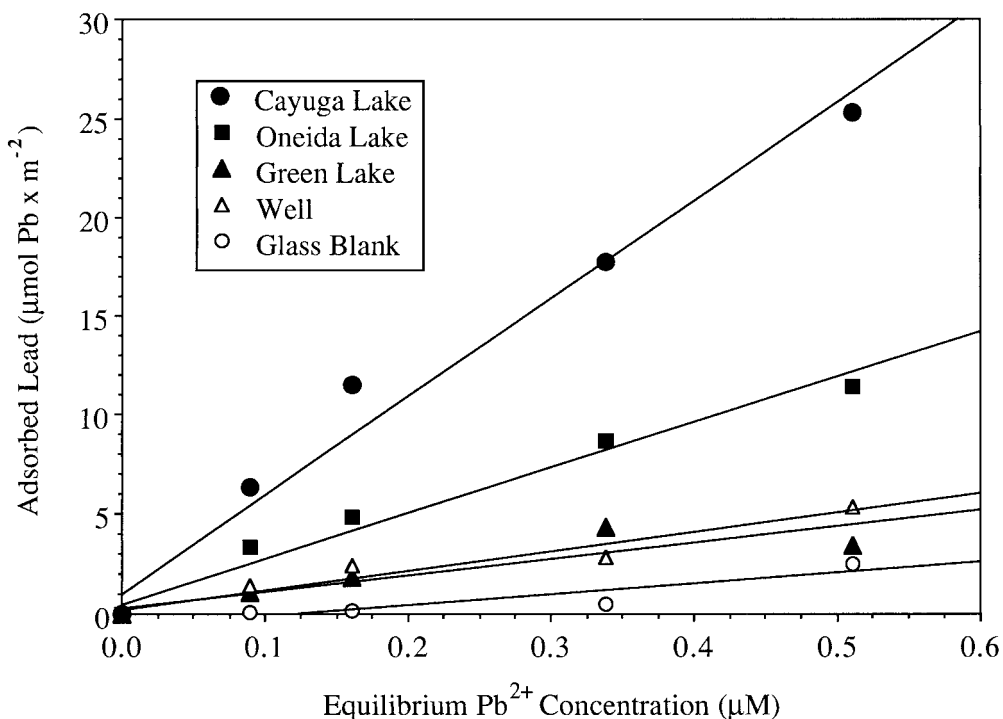


Fig. 2. Pb adsorption to surface coatings from three lakes and a well at pH 6.0 and  $T = 25^{\circ}\text{C}$ .

Crystal structures of the Fe and Mn oxides were determined using x-ray diffraction (XRD) with a theta-theta diffractometer model PAD X (Scintag) using Cu radiation at  $\lambda = 0.15406$  nm. XRD analysis of the colloidal Fe oxide indicated an amorphous structure. The XRD patterns of the commercial Mn oxides matched that of pyrolusite, and the XRD pattern of the fresh abiotic Mn oxide precipitate sug-

gested a mixture of ramsdellite and an unidentified  $\text{MnO}_2$  crystalline structure. The XRD pattern of the biogenic Mn oxide was difficult to interpret, but it suggested a poorly crystalline or amorphous structure. Specific surface areas of the metal oxides were measured using a Quantasorb Sorption System (Syosset) to measure  $\text{N}_2$  adsorption and desorption from a mixture of 30%  $\text{N}_2$  and 70% He. Surface areas were

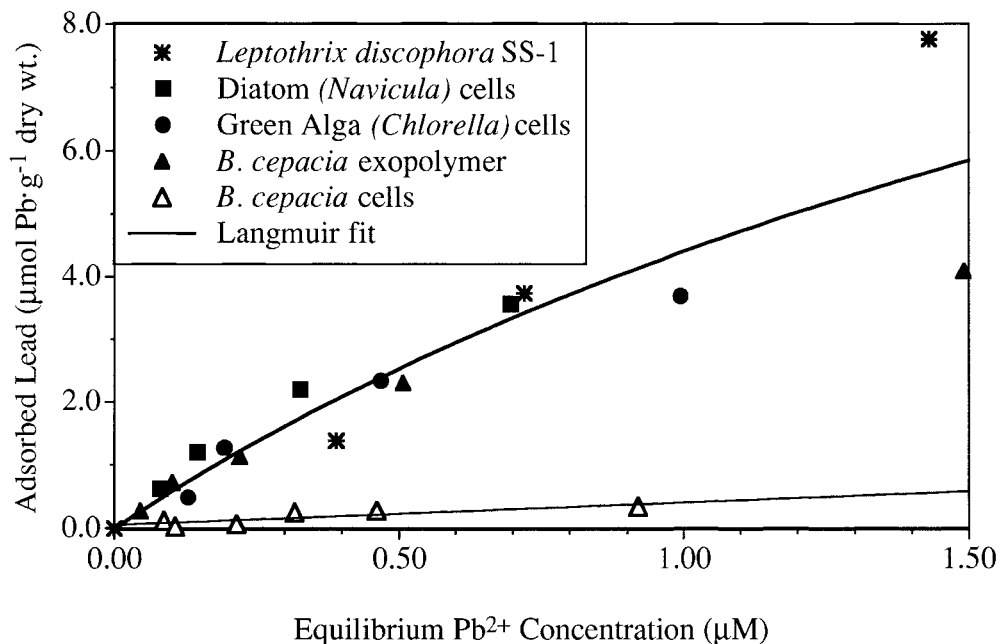


Fig. 3. Pb adsorption to suspended cells of *N. peliculosa*, *C. vulgaris*, and *L. discophora* and extracellular polymer of the bacterium *B. cepacia* at pH 6.0 and  $25^{\circ}\text{C}$ .

Table 3. Total organic content of biofilms on the basis of COD and Chl *a*.

Biofilm source*	Cayuga Lake	Oneida Lake	Green Lake	Well
COD (meq m <sup>-2</sup> )	31	10	5.5	1.4
Chl <i>a</i> (mg·m <sup>-2</sup> )	0.48	0.26	0.11	0.13

\* Nominal surface area of glass slides is used to express surface concentrations.

calculated using a single-point Brunauer, Emmett, and Teller (BET) method (Brunauer et al. 1938).

## Results

**Natural biofilm characterization**—Natural biofilms that developed on slides in all four aquatic environments consisted of assemblages of microorganisms in a biofilm matrix associated with mineral deposits. The mineral deposits were similar in appearance to floc particles, suggesting their development could be partly attributed to deposition of flocculated suspended particulate material (SPM). Cayuga Lake biofilms (Fig. 1A) contained large numbers of diatoms, green and red algae, bacterial cells, and filamentous microorganisms. Fragments of diatoms dominated the biofilms obtained on the glass slides in Oneida Lake (Fig. 1B). Biofilms collected in Green Lake were sparsely colonized by stalked bacteria with a morphology consistent with *Caulobacter* spp., indicative of oligotrophic conditions (Fig. 1C). Biofilms from the water-supply well contained Fe mineral deposits and bacteria similar in morphology to Fe-oxidizing bacteria (Ghiorse 1984; Emerson and Revsbech 1994) (Fig. 1D).

The total organic material accumulated on the glass slides in the three lakes after 3 weeks varied from 5.5 to 31 meq COD m<sup>-2</sup> of nominal slide surface area, and the total for the well biofilms was only 1.4 meq COD m<sup>-2</sup> (Table 3). Chl *a* concentrations were two to four times higher for Cayuga and Oneida Lakes than for the more oligotrophic Green Lake and the well (Table 3). The Cayuga Lake biofilms, which had the highest COD content, also exhibited the highest surface concentrations of Fe, Mn, and Al (Table 4). Fe concentrations were more than one order of magnitude greater than Mn concentrations in the biofilms from Cayuga and Green Lakes. Fe concentrations were about five times greater than Mn concentrations in biofilms from Oneida Lake, which is known for its deposits of Fe/Mn in nodules (Aguilar and Neilson 1994). PB and LBB reagents were used to identify Fe and Mn oxides (respectively) by microscopy. These reagents revealed strong associations between organic matrix materials and Fe and Mn oxide mineral deposits. Particularly in the Oneida Lake biofilms, haloes of Mn oxide surrounding diatoms and other microorganisms were observed. Green Lake biofilms were white in appearance, presumably due to Ca and Mg carbonate deposits that accumulate extensively in this lake (Thompson et al. 1990; Schultze et al. 1992). COD, Fe, Mn, and Al concentrations were very low in the Green Lake biofilms. Biofilm surface coatings obtained in

Table 4. Elemental analysis of HNO<sub>3</sub> extracts of the natural biofilms. BD, below detection.

Element	Concentration (μmol m <sup>-2</sup> )*			
	Cayuga Lake	Oneida Lake	Green Lake	Well
P	38.8	12.9	BD	BD
Ca	714	154	1090	37.1
Mg	241	101	121	15.1
Mn	15.8	13.0	1.1	BD
Fe	319	59.6	16.0	39.0
Zn	1.4	2.7	0.86	0.29
Al	327	34.8	2.4	BD
S	44.1	18.2	77.8	5.0
Si	502	218	110	128

\* Cu, Zn, Mo, V, As, Na, Co, Cd, Cr, Ni, Pb, Se, Y, Ag, Hg, and Sr were below detection limits for all surface coatings. Nominal surface area of glass slides is used to express surface concentrations.

the water-supply well were high in Fe, while Mn and Al were below detection (Table 4).

**Pb adsorption by natural biofilms**—Pb adsorption to the natural biofilms was measured from defined solutions (MMS medium, see Table 2) under controlled laboratory conditions at pH 6.0 and 25°C. This approach was used to avoid the uncertainties associated with using water from the field sites, particularly the presence of undefined organic ligands and competing trace metals. MINEQL calculations indicated that, under the conditions employed, only a small fraction of the Pb was complexed with inorganic ligands in the MMS, and the free aquo Pb<sup>2+</sup> concentration was 89% of the total dissolved Pb concentration (Table 1). Pb adsorption to the biofilms is reported per unit nominal surface area of the slides used for collection of the biofilms (micromoles of Pb per square meter). Pb adsorption for all four biofilms followed nearly linear adsorption isotherms over a Pb<sup>2+</sup> concentration range of 0–0.5 μM at pH 6.0 (Fig. 2) and followed the order of Cayuga L. > Oneida L. > Green L. = water-supply well > glass blank. Pb adsorption increased both with increasing COD content and with increasing Fe content for all of the biofilms (Table 4; Fig. 2).

**Pb adsorption to representative biofilm constituents**—Since diatoms and green algae were abundant phototrophic organisms observed in the natural aquatic biofilms, Pb adsorption isotherms were obtained in the laboratory for pure cultures of *N. peliculosa*, a common freshwater diatom, and *C. vulgaris*, a common green alga, which were selected to represent these organisms. Pb adsorption isotherms for cultures of suspended cells of these organisms were very similar to each other (on a DW basis) and followed a Langmuir adsorption model (Fig. 3) of the form  $\Gamma = \Gamma_{\max} K[\text{Pb}^{2+}] / (1 + K[\text{Pb}^{2+}])^{-1}$ , where  $\Gamma$  = Pb adsorption (μmol Pb g<sup>-1</sup> DW),  $\Gamma_{\max}$  = maximum Pb adsorption, and  $K$  = the Langmuir equilibrium constant (Stumm and Morgan 1981).

Pb adsorption by suspended cells of *L. discophora* and extracellular polymer of *B. cepacia* was nearly identical to that observed for the phototrophs on a weight basis (Fig. 3).

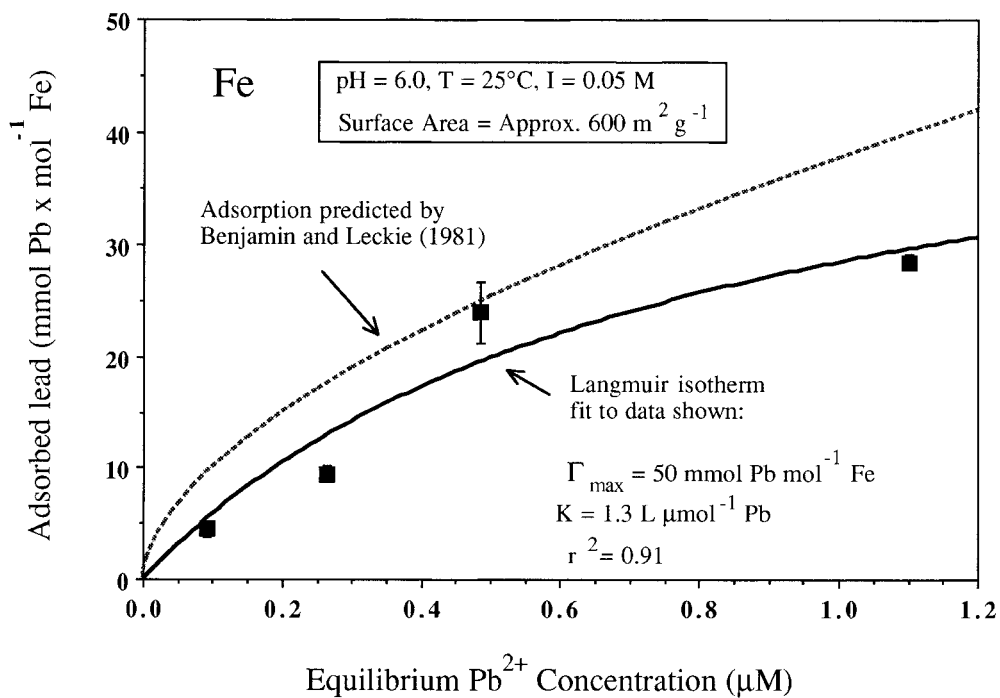


Fig. 4. Pb adsorption isotherm for amorphous Fe oxide deposits at pH 6.0 and  $T = 25^\circ\text{C}$ .

These bacteria were selected to represent adherent bacteria in the biofilms because of previous characterization by the authors (Ghiorse 1984; Nelson et al. 1995, 1996). Suspended cells of *B. cepacia* adsorbed significantly less Pb than its concentrated polymer on a DW basis (Fig. 3). However, our prior investigation of *B. cepacia* biofilms indicated that ex-

tracellular polymer constituted up to 80% of the total COD of 3-week-old biofilms (Nelson et al. 1996), and previously measured Pb adsorption to *B. cepacia* biofilms (Nelson et al. 1995) was similar to that observed for *L. discophora* cells and the phototrophs. These results clearly show that *B. cepacia* exopolymer, not the cells, is responsible for Pb binding

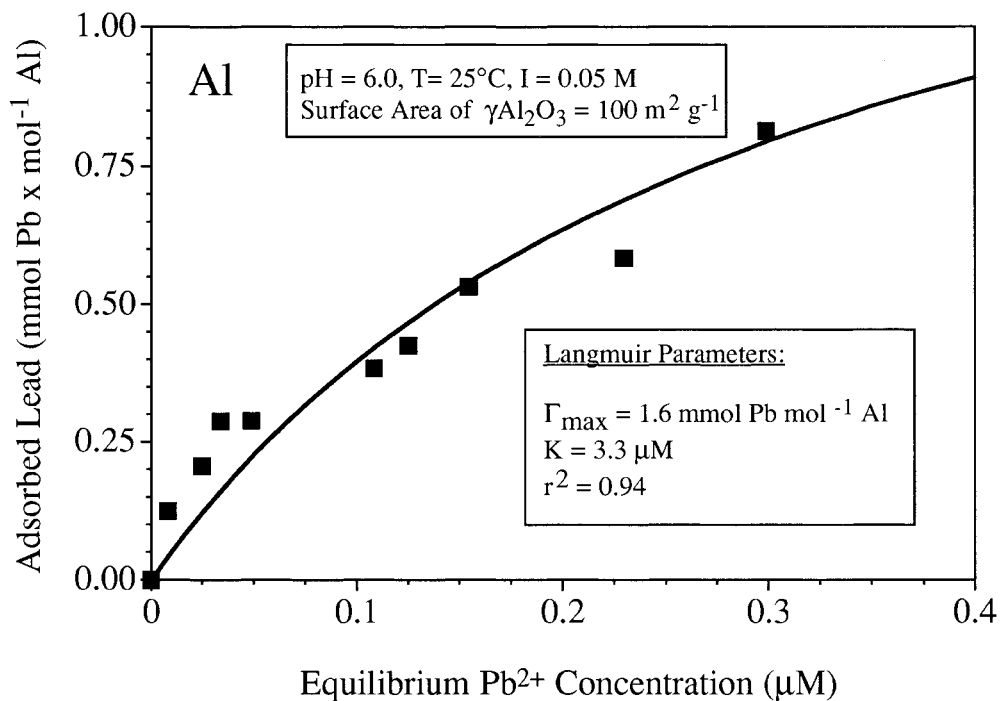


Fig. 5. Pb adsorption to  $\gamma\text{Al}_2\text{O}_3$  (100 m<sup>2</sup> g<sup>-1</sup>) at pH 6.0 and  $T = 25^\circ\text{C}$ .

Table 5. Langmuir parameters for Pb adsorption to representative biofilm constituents, as determined under controlled laboratory conditions.\*

Inorganic constituents	$\Gamma_{\max}$ ( $\mu\text{mol Pb } \mu\text{mol}^{-1}$ )	K (liters $\text{mmol}^{-1}$ $\text{Pb}^{2+}$ )	$r^2$	Biological constituents	$\Gamma_{\max}$ ( $\mu\text{mol Pb eq}^{-1}$ COD)	K (liters $\mu\text{mol}^{-1}$ $\text{Pb}^{2+}$ )	$r^2$
amFe(III) oxide ( $600 \text{ m}^2 \text{ g}^{-1}$ ) <sup>†</sup>	50	1.32	0.91	Combined isotherms for all cells and <i>B. cepacia</i> polymer	70	2.1	0.90
Al oxide ( $\gamma\text{Al}_2\text{O}_3$ , $100 \text{ m}^2 \text{ g}^{-1}$ ) <sup>‡</sup>	1.6	3.3	0.94				
Biogenic Mn oxide ( $224 \text{ m}^2 \text{ g}^{-1}$ ) <sup>‡</sup>	550	62	0.83				

\* Determined by nonlinear, least-squares fit of data to the following relationship:  $\Gamma = \Gamma_{\max} K[M] / (1 + K[M])$ , where  $\Gamma$  = adsorbed metal concentration,  $\Gamma_{\max}$  = maximum adsorbed metal concentration, K = Langmuir equilibrium constant, and [M] = metal ion concentration.

<sup>†</sup> Amorphous Fe oxide surface area estimated by Davis and Leckie (1978).

<sup>‡</sup> Al and Mn specific surface area determined by BET; Mn oxide prepared in the laboratory.

by *B. cepacia* biofilms. Prediction of Pb adsorption to the organic phase of the biofilms was therefore based on average Langmuir adsorption isotherm parameters for *N. peliculosa*, *C. vulgaris*, and *L. discophora* cells and *B. cepacia* exopolymer (which were all similar). A laboratory measurement of COD and DW of *B. cepacia* was used to convert Pb adsorption to a COD basis ( $0.029 \text{ meq COD mg}^{-1} \text{ DW}$ ). Langmuir parameters for the combined isotherm are shown in Table 5.

Pb adsorption isotherms obtained for Fe, Mn, and Al oxides under controlled laboratory conditions were used to estimate the relative contributions of Pb adsorption to each of these phases to the total observed Pb adsorption. For amorphous Fe oxyhydroxide deposits on glass slides, Pb adsorption followed a Langmuir adsorption isotherm (from Nelson et al. 1995) and was remarkably similar to that predicted for amorphous Fe oxyhydroxide at pH 6.0 based on a published model (Benjamin and Leckie 1981) (Fig. 4). Pb adsorption to  $\gamma\text{Al}_2\text{O}_3$  (specific BET surface area =  $100 \text{ m}^2 \text{ g}^{-1}$ ) likewise fit the Langmuir model (Fig. 5; Table 5). Pb adsorption to several candidate Mn oxyhydroxide surrogates displayed the expected dependence on specific surface area (Nelson et al. 1999a) (Fig. 6). Pb adsorption to freshly precipitated  $\delta\text{MnO}_2$  (mixed crystalline structure) was several orders of magnitude greater than that observed for two commercially available Mn oxide minerals (pyrolusite), and Pb adsorption of

the biogenic Mn oxyhydroxide was several times greater than that of the fresh  $\delta\text{MnO}_2$  precipitate (Fig. 6). The isotherm for the biogenic Mn oxyhydroxide (Fig. 6; Table 5) was used for estimating Pb adsorption to the Mn oxyhydroxide component of the biofilms (*see below*) because Mn minerals in these biofilms are expected to have been biologically oxidized and thus, to exhibit a high surface area (Sigg 1987; Nealson et al. 1988; Tebo et al. 1997).

*Estimated contributions of biofilm constituents to total Pb adsorption*—Estimated contributions to Pb adsorption of each constituent phase were calculated based on the analyzed compositions of the biofilms shown in Tables 3 and 4 and the Langmuir parameters for Pb adsorption by each component (Table 5). Pb adsorption to the biofilms was modeled as the sum of adsorption of five constituent phases: (1) inorganic residue on the glass slides after acid extraction of trace elements, (2) the organic phase, as represented by the microorganisms listed above, (3) amorphous Fe oxyhydroxide, (4) biogenic Mn oxyhydroxide, and (5) Al oxide. The model considers Pb adsorption to follow Langmuir adsorption isotherms and to be additive for the discreet biogeochemical phases chosen.

$$\Gamma_{\text{Pb}} = \sum_{i=1}^n \frac{C_i \Gamma_i^{\max} K_i [\text{Pb}^{2+}]}{1 + K_i [\text{Pb}^{2+}]}, \quad (1)$$

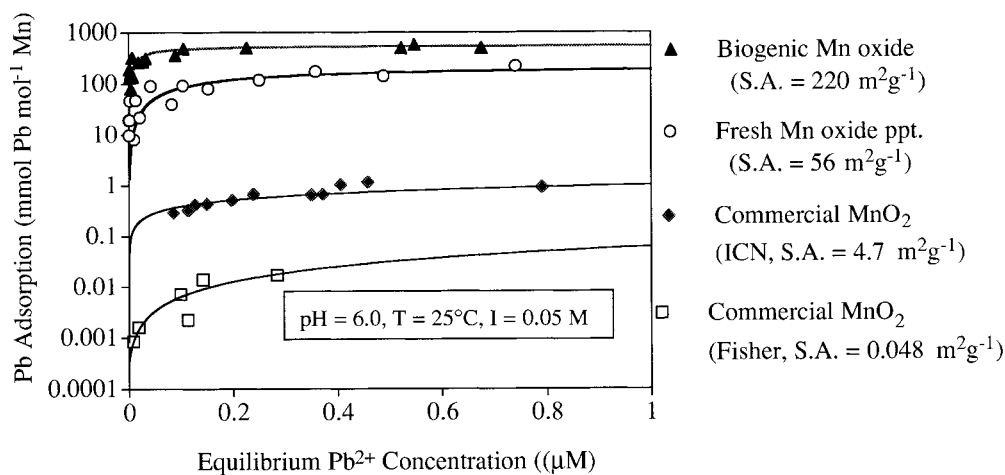


Fig. 6. Pb adsorption to model Mn oxides and biogenic Mn oxides at pH 6.0 and  $T = 25^\circ\text{C}$ .

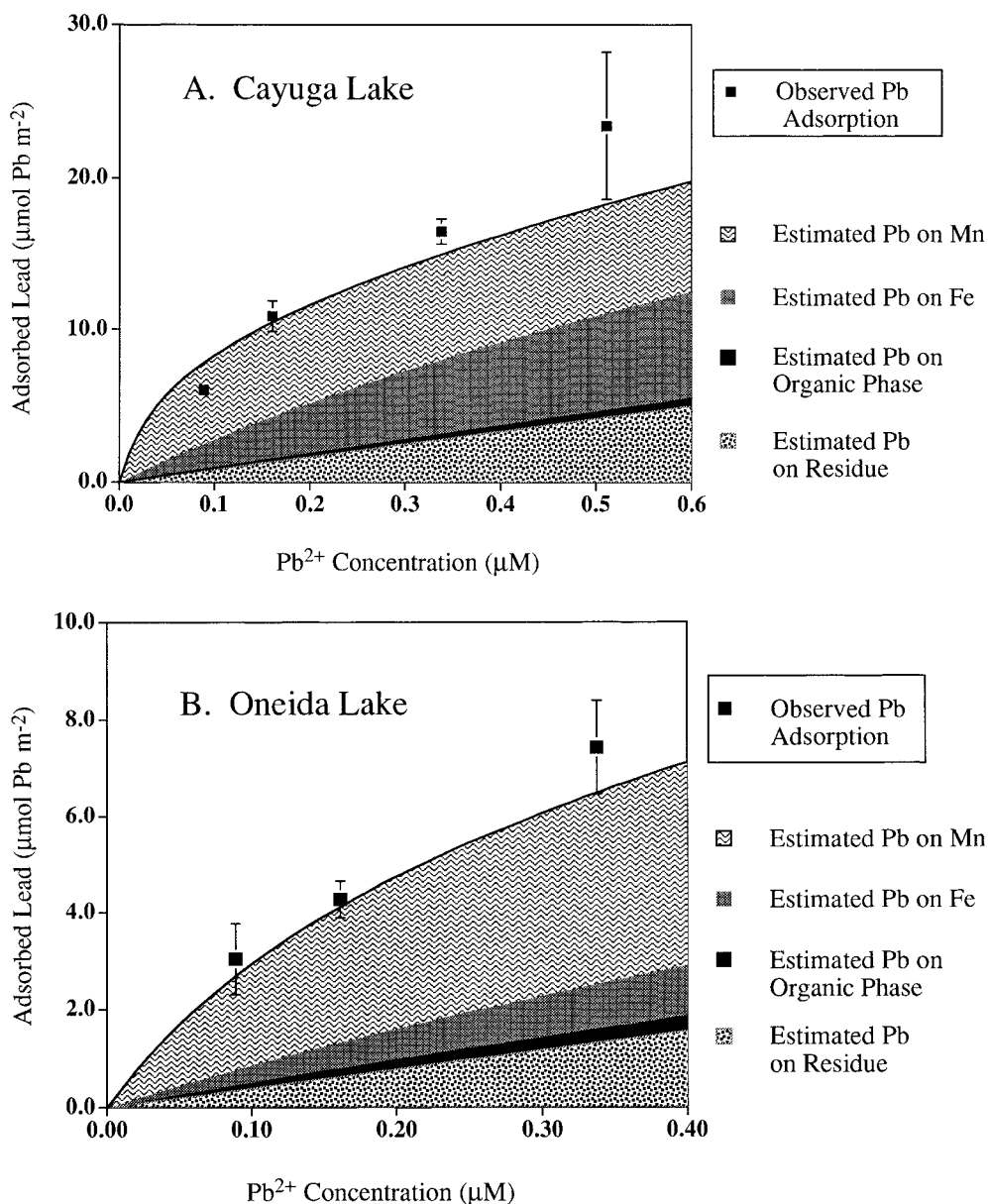


Fig. 7. Observed and predicted Pb adsorption to natural biofilms based on additivity of Pb adsorption to discrete phases. (A) Cayuga Lake, (B) Oneida Lake, (C) Green Lake, and (D) Water-supply well.

where  $\Gamma_{\text{pb}}$  is the total Pb adsorption by the biofilm (micromoles per square meter) at concentration  $[\text{Pb}^{2+}]$ ,  $C_i$  is the surface concentration of phase  $i$ , and  $\Gamma_i^{\text{max}}$  and  $K_i$  are the associated Langmuir parameters. Note that adsorption is expressed per unit nominal surface area of the glass slides containing the biofilm, not the total surface area of the adsorbing phase. The values of  $C_i$  are also expressed on the basis of nominal biofilm surface area. For example, the units on  $C_i$  for the metal oxides are  $\mu\text{mol metal m}^{-2}$ , and  $C_i$  units for the organic material are  $\text{meq COD m}^{-2}$ . The implicit assumption in the use of this additive model is that all of the component material present in the biofilms is available for Pb binding.

For each of the biofilms, the estimated Pb adsorption to

each phase and the sum of Pb adsorption to these phases were compared to the total observed Pb adsorption under controlled laboratory conditions (adsorption to bare glass subtracted) (Fig. 7). Estimation of Pb adsorption, calculated by summing the estimated contributions from the constituents listed above, accounted for at least 90% of the total observed Pb adsorption in the lake biofilms (Fig. 7A–C) and about 60% of the total observed Pb adsorption in the water-supply well (Fig. 7D).

For all of the lake biofilms, estimated Pb adsorption by Fe and Mn oxyhydroxides was significantly greater than that predicted for organic materials (Fig. 7A–C). Estimated adsorption to Mn oxyhydroxides in all of the lake biofilms was equal to or greater than that estimated for Fe oxyhydroxide,

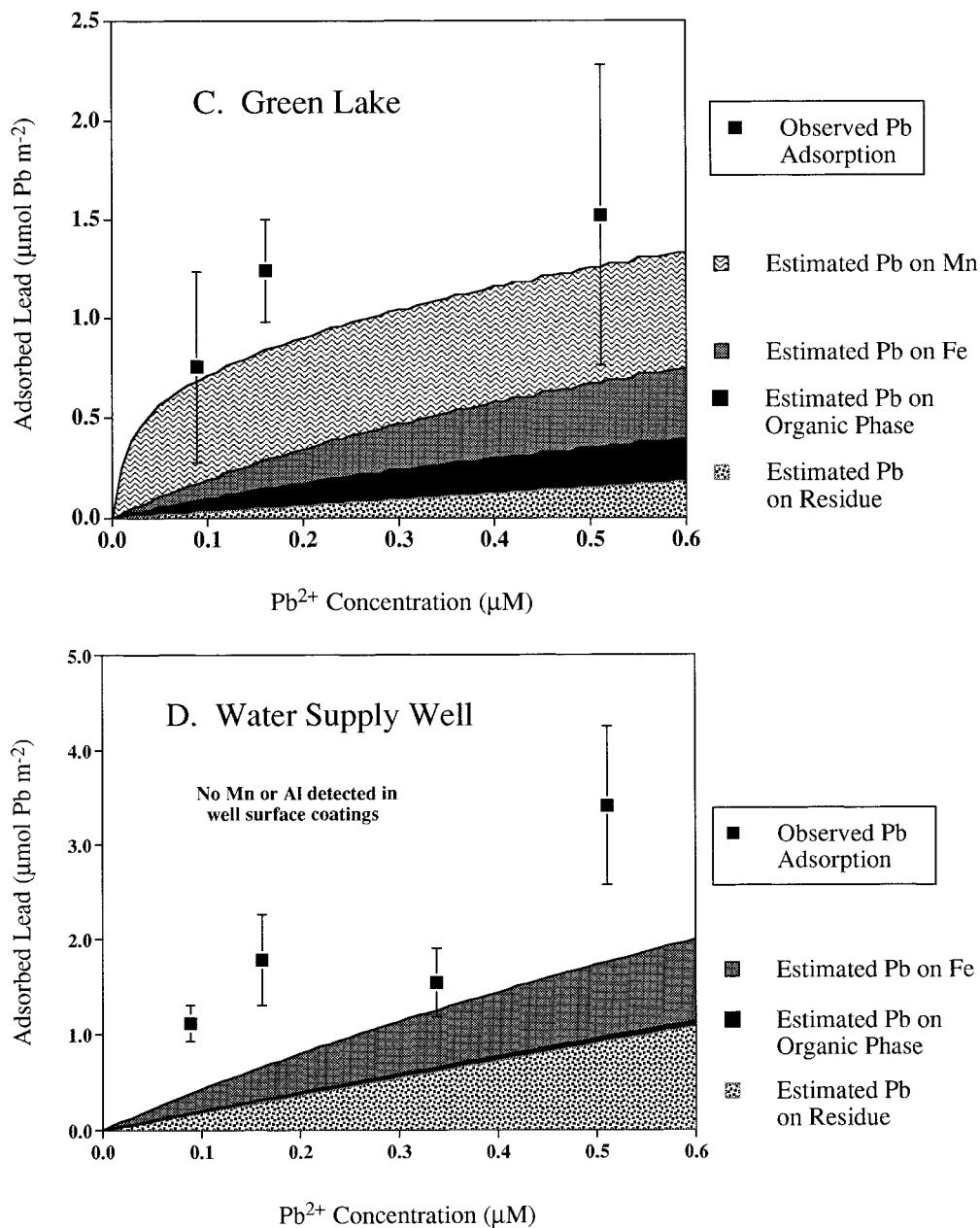


Fig. 7. Continued.

and in Oneida Lake biofilms, Pb adsorption by the Mn oxyhydroxide phase was four times greater than that predicted for Fe (Fig. 7B). No Mn was detected in the well biofilms, and estimated Pb adsorption to Fe oxyhydroxide was much greater than that estimated for the organic phase in these biofilms (Fig. 7D). Estimated Pb adsorption to acid-soluble Al oxide accounted for <2% of the total Pb adsorption for all of the biofilms.

The adsorption of Pb to residues on glass slides after acid extraction for elemental analysis (insoluble minerals) also contributed to the total estimated Pb adsorption of biofilms from all four sources (Fig. 7). Since >80% of the organic materials were removed from this residual phase during acid extraction and the total estimated contribution of organic

materials to Pb binding was estimated to be small, the contribution to total Pb binding of the residue is presumed to arise from adsorption by acid-insoluble inorganic materials (such as clay minerals and silicates).

## Discussion

A significant finding of this work was that the estimated contribution of Fe and Mn oxyhydroxides to Pb binding by natural biofilm surface coatings, under the chemical conditions examined, was much greater than that of organic constituents. This result confirms earlier assertions of the role of metal oxides in controlling transition metal adsorption (Krauskopf 1956; Jenne 1968; Turekian 1977) and is con-

sistent with results of selective extractions that indicate that Pb is associated predominantly with Fe/Mn oxyhydroxide phases of sediments (Luoma and Bryan 1978; Lion et al. 1982; Belokon 1989; Allen et al. 1990; Borovec 1994). The results of this research are based on measured Pb binding to four representative microorganisms that adsorbed Pb similarly, but it is possible that other organisms could bind significantly more Pb than the selected organisms, and this could alter the conclusions. However, it should be noted that the close approximation of the additive model to the observed data would suggest that any significant increase in the Pb adsorption of a surface component would have to be accompanied by a decrease in adsorption or adsorption site masking of one or more other components. The present research was also restricted to Pb as a model transition metal, and other transition metals may interact more strongly with organic phases than Pb. For example, selective extraction of estuarine sediments previously indicated that 70% of Pb was associated with metal oxyhydroxides, while Cd was predominantly associated with organic phases (Lion et al. 1982). Alterations in pH may also influence the relative roles of adsorbing phases. For example, increasing pH would favor ionization of carboxyl functional groups on organic materials and surface hydroxyl groups on oxides.

Even though Fe was much more abundant than Mn in the lake biofilms (Table 4), estimated Pb adsorption of Mn oxyhydroxides was equal to or greater than that of Fe oxyhydroxides (Fig. 7A–C). Also, because the adsorption isotherm for biogenic Mn oxyhydroxide is steep at low Pb concentrations (reflected by high  $K$ ), the estimated contributions of Mn oxyhydroxides at low  $[Pb^{2+}]$  are particularly significant (Fig. 7A–C). Since  $[Pb^{2+}]$  is often low in natural waters, conditions favoring a dominant role of Mn oxyhydroxides are likely to prevail. These results contrast with those of Tessier et al. (1996), who estimated (using selective extractions) that Fe oxyhydroxides in lake sediments bound five times more Pb than did Mn oxyhydroxides. However, recent work using a new selective extraction approach suggests much greater adsorption of Pb by Mn oxides than by Fe oxides in Cayuga Lake biofilms (Dong et al. 2000; Nelson et al. 1999a). These similar results obtained using a completely independent approach add considerable validity to the results described here for the additive approach. The high estimations of Pb binding to the Mn phase in biofilms in the present work result from the use of Pb adsorption to amorphous biogenic Mn oxyhydroxides to predict Pb adsorption to the Mn phase in the surface coatings. If Pb binding to abiotic Mn oxyhydroxides is used to estimate Pb binding to the Mn phase, the estimated contribution of Mn oxyhydroxides to total Pb binding is significantly reduced (Nelson 1997; Nelson et al. unpubl.), and as a consequence, a significant fraction of the observed Pb adsorption would not be accounted for by the additive model. The use of biogenic Mn oxides for the additive estimation is expected to be more realistic because Mn oxyhydroxides are likely to be of biological origin in lakes with circumneutral pH (Stumm and Morgan 1981; Ghiorse 1984; Boogerd and DeVrind 1987; Nealson et al. 1988; Ehrlich et al. 1991; Johnson et al. 1995; Moffett 1997). Indeed, several researchers have previously alluded to the amorphous nature and potentially high surface

area of biogenic Mn oxyhydroxides in contributing to trace metal adsorption by SPM (Sigg 1987; Mandernack et al. 1995; Wehrli et al. 1995; Tebo et al. 1997). Further evidence for the importance of Mn oxyhydroxides is provided by field studies of SPM in an urban river, where Mn oxyhydroxides appear to contribute more to total surface area than Fe oxyhydroxides (Warren 1994). Additionally, redox cycles of Mn in aquatic environments occur at such a rate that suspended Mn oxyhydroxides in the water column are likely to be freshly oxidized (Sunda and Huntsman 1987; Wehrli et al. 1995) and thus, most likely amorphous. Because of this cycling, it is possible that Pb could be coprecipitated during *in situ* formation of Mn oxyhydroxides, which could lead to even greater Pb binding by Mn oxyhydroxides than that reported here. Greater trace metal scavenging by Mn oxides may also occur deeper in the water column, where relatively higher concentrations of Mn have been observed (Sigg 1987).

Total Pb adsorption by the water-supply well biofilms was underpredicted by the additivity model. It is likely that the Fe oxyhydroxide deposits in these biofilms are freshly formed from Fe(II) in the groundwater, possibly via biologically induced oxidation. These fresh Fe oxyhydroxides might be expected to adsorb Pb more strongly than the aged Fe oxyhydroxide colloids used in this study as the laboratory analog. Further experiments are currently being pursued to compare trace metal adsorption by Fe oxyhydroxides prepared by slow precipitation (e.g., Deng 1997) and by enzymatically catalyzed biological deposition.

Although the results of this research suggest that the contribution to Pb adsorption by direct adsorption by Fe and Mn oxyhydroxides is much greater than direct Pb adsorption by biogenic materials, it is important to consider that microorganisms can indirectly, but significantly, affect Pb binding through their interactions with metal oxides. For example, biofilms could alter the deposition of metal oxides through either passive or active biological processes (Ghiorse 1984; Lion et al. 1988; Lo et al. 1996). Biological catalysis of Mn oxidation (as described here) is an example of an active deposition process, and similarly, biological oxidation of Fe could result in increased trace metal adsorption by Fe oxides. Additionally, biological materials deposited on metal oxide surfaces can alter the adsorption properties of metal oxides by changing their surface charge or by occupying adsorption sites on the oxide surface (Hunter and Liss 1979; Balistrieri and Murray 1982; Davis 1982; Tipping and Cooke 1982; Honeyman and Santschi 1988; Tessier et al. 1996). Similarly, organic ligands produced by microorganisms can influence trace metal adsorption to metal oxides by forming ternary complexes between the adsorbing trace metal and the metal oxide surface (Davis 1982).

## Conclusions

The model presented here appears to be a viable means of estimating relative contributions of biofilm constituents to total metal binding by natural aquatic biofilms. These results suggest that the lake biofilms can be accurately depicted as being comprised of distinct adsorbing phases and that site

masking of adsorption sites in these biofilms can be considered minimal. This additive method is expected to easily extend to analysis of metal adsorption to suspended particulates and other surfaces in oxic aquatic environments because the composition of these materials is similar to that of the biofilms characterized in this study. By considering Pb adsorption to discrete biogeochemical phases, the additive model accurately predicted total Pb adsorption to biofilms from the three lakes and slightly underpredicted Pb adsorption to biofilms from a water-supply well. Estimated Pb adsorption to Fe and Mn oxyhydroxides was much greater than that to the organic phase, and estimated Pb adsorption by acid-soluble Al oxide was negligible. Even though Fe concentrations were much greater than Mn concentrations in the biofilms, estimated Pb adsorption by the Mn oxides in the lake biofilms was equal to or greater than that of the Fe oxides, suggesting that Mn oxides are highly reactive and exert a significant impact on trace metal cycling in aquatic environments. Significant Pb adsorption was also observed for the undefined inorganic residue remaining on the slides after acid extraction, suggesting that insoluble clay minerals may also contribute to total Pb binding. Although Fe and Mn oxyhydroxides are implicated as the dominant phase controlling Pb adsorption, the biological interactions that affect Fe and Mn oxidation/reduction warrant further investigation because of their potential influence on the deposition and surface characteristics of Fe/Mn coatings and subsequent trace metal binding. Further verification of these results awaits measurements of Pb adsorption to additional biological materials and to biologically deposited Fe oxyhydroxide. The approach used in this work could be extended to other transition metals, such as Cd or Cu, that may interact with the model constituents differently than Pb.

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*Received: 3 November 1998*

*Accepted: 26 May 1999*

*Amended: 14 June 1999*