

ADSORPTION OF Pb AND Cd ONTO METAL OXIDES AND ORGANIC MATERIAL IN NATURAL SURFACE COATINGS AS DETERMINED BY SELECTIVE EXTRACTIONS: NEW EVIDENCE FOR THE IMPORTANCE OF Mn AND Fe OXIDES

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Abstract—Surface coatings (biofilms and associated minerals) were collected on glass slides in the oxic surface waters of Cayuga Lake (New York State, U.S.A.) and were used to evaluate the relative contributions of Fe, Mn and Al oxides and organic material to total observed Pb and Cd adsorption by the surface coating materials. Several alternative selective extraction techniques were evaluated with respect to both selectivity and alteration of the residual unextracted material. Pb and Cd adsorption was measured under controlled laboratory conditions (mineral salts solution with defined metal speciation, ionic strength 0.05 M, 25°C and pH 6.0) before and after extractions to determine by difference the adsorptive properties of the extracted component(s). Hydroxylamine hydrochloride (0.01 M NH₂OH·HCl + 0.01 M HNO₃) was used to selectively remove Mn oxides, sodium dithionite (0.3 M Na₂S₂O₄) was used to remove Mn and Fe oxides, and 10% oxalic acid was used to remove metal oxides and organic materials. Several other extractants were evaluated, but preliminary experiments indicated that they were not suitable for these experiments because of undesirable alterations of the residual, unextracted material. The selected extraction methods removed target components with efficiencies between 71 and 83%, but significant amounts of metal oxides and organic materials other than the target components were also removed by the extractants (up to 39%). Nonlinear regression analysis of the observed Pb and Cd adsorption based on the assumption of additive Langmuir adsorption isotherms was used to estimate the relative contributions of each surface coating constituent to total Pb and Cd binding of the biofilms. Adsorption of Cd to the lake biofilms was dominated by Fe oxides, with lesser roles attributed to adsorption by Mn and Al oxides and organic material. Adsorption of Pb was dominated by Mn oxides, with lesser roles indicated for adsorption to Fe oxides and organic material, and the estimated contribution of Al oxides to Pb adsorption was insignificant. The fitted Pb adsorption isotherm for Fe oxides was in excellent agreement with those obtained through direct experiments and reported in independent investigations. The estimated Pb distribution between surface coating components also agreed well with that previously predicted by an additive adsorption model based on Pb adsorption isotherms for laboratory surrogates for Mn, Fe and Al oxides and defined biological components.

Key words—selective extraction, adsorption, lead, cadmium, iron oxide, manganese oxide

INTRODUCTION

The toxicity and bioaccumulation potential of heavy metals has prompted great interest in developing models to describe their transport and fate in aquatic environments. Development of meaningful models for trace metal phase distribution requires

an understanding of trace metal adsorption onto solid phases and associated biofilms, which is a key factor influencing the residence time, bioavailability and effects of toxic metals on organisms in aquatic ecosystems (Krauskopf, 1956; Jenne, 1968; Turekian, 1977; Vuceta and Morgan, 1978; Murray, 1987; Santschi *et al.*, 1997). In addition to the well established effects of solution chemistry (e.g. pH, ionic strength, metal speciation), trace metal adsorption is expected to be governed by the com-

position of the solid phase, particularly the content of metal oxides and organic materials. Studies have been undertaken to quantify the relative roles of these components in controlling the adsorption of transition metals to surfaces in natural lake waters (Sigg, 1985), lake sediments (Tessier and Campbell, 1987) and biofilms (Nelson *et al.*, 1995). However, there remains some uncertainty about the roles of metal oxides vs organic materials in controlling the adsorption of trace metals to natural heterogeneous materials. Indeed, some researchers report that metal oxides are the single most important determinant of trace metal adsorption (Krauskopf, 1956; Jenne, 1968), while others report that organic materials exert a stronger effect (Balistrieri and Murray, 1983; Salim, 1983; Sigg, 1985). Additionally, interactions between constituents could alter the metal adsorption properties of these constituents in a heterogeneous matrix (Davis and Leckie, 1978; Balistrieri and Murray, 1982; Tipping and Cooke, 1982; Honeyman and Santschi, 1988). The purpose of the research presented here is to use a new selective extraction approach to carefully elucidate the relative roles of metal oxides and organic materials. The resulting information is expected to facilitate the development of trace metal adsorption and transport models.

The use of selective extractants is a useful approach for determining the relative significance of the mineral and organic components in controlling trace metal adsorption. Extractants have previously been used to dissolve metal oxide or organic components in sediments and soils along with the trace metals associated with these components (Lindsay and Norvell, 1978; Tessier *et al.*, 1979; Lion *et al.*, 1982; Bauer and Kheboian, 1986; Martin *et al.*, 1987; Tessier and Campbell, 1987; Luoma, 1989; Campbell and Tessier, 1991; Young *et al.*, 1992; Young and Harvey, 1992). While useful for estimating trace metal bioavailability, selective extraction methods are difficult to use for accurately quantifying trace metals associated with specific biogeochemical phases because the extracted phases are operationally defined and are subject to experimental limitations such as removal of additional materials besides the target component during extraction. For example, when hydroxylamine hydrochloride ($\text{NH}_2\text{OH}\cdot\text{HCl} + \text{HNO}_3$) is used to extract Mn oxides, the extraction reagent is likely to also extract some fraction of other components, such as other metal oxides and organic materials. Another limitation is that extractants can potentially desorb trace metals from other components that were not extracted, which would lead to an overestimation of trace metal associated with the target component. For example, when $\text{NH}_2\text{OH}\cdot\text{HCl} + \text{HNO}_3$ is used to extract Mn oxides and associated trace metals, the extraction reagent may also desorb trace metals from other surface components, such as Fe oxides. Yet another possi-

bility is that metals extracted from one solid phase may re-adsorb to unextracted materials, which would lead to an underestimation of the importance of the extracted component.

In the present work, trace metal adsorption was measured for residues before and after selective extraction to avoid problems associated with desorption and/or re-adsorption of metals from other components. By determining metal adsorption isotherms for composite surface coatings before and after extraction, the adsorptive role of the removed component(s) was revealed by difference. The selectivities of the extractants were determined by measuring Fe, Mn and Al concentrations and chemical oxygen demand (COD) before and after each extraction. Since standard extractants were found to remove significant quantities of non-target components, non-linear regression analysis of the adsorption isotherm data was used to determine the adsorptive contribution of each surface phase. This approach does not identify phase associations of contaminant metals already present on natural materials collected from the field because it relies on measuring adsorption of trace metals from defined solutions before and after extraction. Instead, this method provides an alternative means for estimating the reactive roles of metal oxide and organic phases in controlling trace metal adsorption in freshwater environments. In this way, this work contributes to the mechanistic understanding of trace metal associations with adsorptive components of the heterogeneous surfaces in natural aquatic environments.

For the experiments described here, natural biofilms that developed on glass slides in oxic lake waters were used to represent typical lake surface coating materials. It is expected that these surface coatings may also be representative of the materials contained in suspended particulate material (SPM) given their morphological and compositional similarities. Indeed, the biofilms in this study were likely formed in part by the deposition of SPM onto the glass slides. Pb and Cd adsorption to the collected biofilms was measured before and after selective extractions under conditions of controlled temperature, pH and solution chemistry. Extraction efficiency and selectivity were evaluated by analyzing for Fe, Mn, Al and COD concentrations before and after extractions with conventional extractants. In addition, several modifications of conventional extractants were tested as well as a novel extractant based on the use of Ti(III) as a reductant. Pb and Cd adsorption to each surface component was estimated through a non-linear regression analysis, and the results for Pb were compared to independent predictions based on representative laboratory surrogate materials for the oxide and organic phases.

MATERIALS AND METHODS

Development and characterization of natural biofilms

Cayuga Lake in central New York State (U.S.A.) was chosen as the field site for collection of biofilms because of prior biofilm characterization by the researchers at this site (Nelson, 1997; Nelson *et al.*, 1999b). Biofilms developed on glass microscope slides (5.1×7.6 cm) held in polypropylene racks (Fluoroware, Chaska, MN, U.S.A.) that were submerged in the lake at a depth of approximately 30 cm for a period of 4 weeks. Several sets of biofilms were collected between January and March 1998, while the lake water temperature was approximately 4°C . A similar collection method was reported by Tessier for collection of sediments on Teflon[®] sheets (Tessier *et al.*, 1996). Prior to placement in the lake, glass slides and racks were precleaned with detergent, soaked for 24 h in soap solution, acid washed for 24 h in 6:1 (v/v) $\text{H}_2\text{O}:\text{HNO}_3$ (trace metal grade, Fisher Scientific, Pittsburgh, PA), and then rinsed in distilled-deionized water (ddH_2O), followed by a second 24-h acid wash and a final rinse in ddH_2O .

After retrieval from the lake, glass slides with attached biofilms were transported within 1 h to the laboratory (submerged in lake water) for microscopic examination, chemical characterization and measurement of Pb and Cd binding. Biofilms were consistent from slide to slide (Fe, Mn and Al concentrations varied by less than 5%), allowing the use of different slides for characterizations and for measurement of Pb and Cd binding.

Organic material in the biofilms was quantified by measuring chemical oxygen demand (COD) using a modification of Standard Method # 5220 B (APHA, 1995). The COD, reported here in units of $\text{mg O}_2/\text{L}$, is approximately equivalent to 2.7 times the organic carbon content in mg C/L (assuming an oxidation state of zero for all organic carbon in the biofilm and 100% efficiency of oxidation to CO_2). For the COD analysis, the slides with attached biofilms were broken into small pieces and placed in 250-mL Erlenmeyer flasks. To each flask was added 50 mL ddH_2O , 0.3 g HgSO_4 , 5 mL sulfuric acid reagent (w/ Ag_2SO_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 the remaining $\text{Cr}_2\text{O}_7^{2-}$ was titrated with standardized 0.025 M ferrous ammonium sulfate.

Total extractable metal concentrations (Fe, Mn, Al) in the biofilms were determined by extracting with 50 mL of 10% HNO_3 (trace metal grade, Fisher Scientific, Pittsburgh, PA, U.S.A.) for 24 h. Acid extracts were analyzed by graphite furnace atomic absorption spectrometry (GFAAS) using a Perkin Elmer (Norwalk, CT, U.S.A.) Analyst 100 equipped with a HGA 800 graphite furnace and an AS-72 autosampler.

Selective extraction techniques

Each biofilm-coated slide was extracted in 50 mL of extraction reagent in 150 mm plastic petri dishes using several extraction techniques. Our initial experiments with a previously reported hydroxylamine extraction method for selective removal of Mn and Fe oxides (0.04 M $\text{NH}_2\text{OH}\cdot\text{HCl}$, 25% acetic acid, 6 h at 95°C) (Tessier *et al.*, 1979; Young *et al.*, 1992; Young and Harvey, 1992) suggested that the high temperature altered adsorption characteristics of the remaining organic material. There was also evidence that the acetic acid increased the organic content (COD) of the extracted biofilms. The added COD was likely the result of acetate binding to the biofilm and could be expected to alter trace metal adsorption. Thus, we modified this extraction procedure by reducing the temperature to 25°C , reducing the $\text{NH}_2\text{OH}\cdot\text{HCl}$ concentration to 0.01 M, eliminating the acetic acid and reducing the extraction time to 30 min.

Preliminary experiments with a previously reported sodium dithionite reagent to extract Fe oxides [0.3 M $\text{Na}_2\text{S}_2\text{O}_4$ with a citrate buffer (0.175 M Na-citrate + 0.025 citric acid)] (Anderson and Jenne, 1970; Tessier *et al.*, 1979) indicated that this reagent caused the organic content of the biofilms to increase by a factor of two as measured by COD. Similar to the difficulty with acetic acid extraction noted above, this increase was presumably caused by citrate binding to the biofilm which would interfere with accurate subsequent measurement of Pb and Cd adsorption. Thus, we eliminated the citrate buffer from the reagent, and pH was controlled at 6.0 by manual addition of dilute HNO_3 or NaOH solutions. The final modified extraction procedure used 50 mL of 0.3 M $\text{Na}_2\text{S}_2\text{O}_4$ for 40 min at pH 6.0. This extractant was prepared just before use to avoid any reduction of $\text{S}_2\text{O}_4^{2-}$.

Extraction with 10% oxalic acid for 60 h (Ramsay *et al.*, 1988) was employed to remove organic materials from biofilms, but also removed most of the metal oxides (see the Results Section).

Several extraction reagents based on Ti(III) as a reductant were evaluated for use in selectively removing Fe oxides. Hudson and Morel (1989) employed a Ti(III) reagent containing 0.05 M Ti, 0.05 M EDTA and 0.05 M citrate, and the extraction was carried out for 15 min at room temperature. The Ti(III) solutions used in this procedure are unstable without EDTA and citrate buffer. Since residual EDTA or citrate could influence Pb and Cd adsorption, the use of this reagent was discontinued in subsequent experiments.

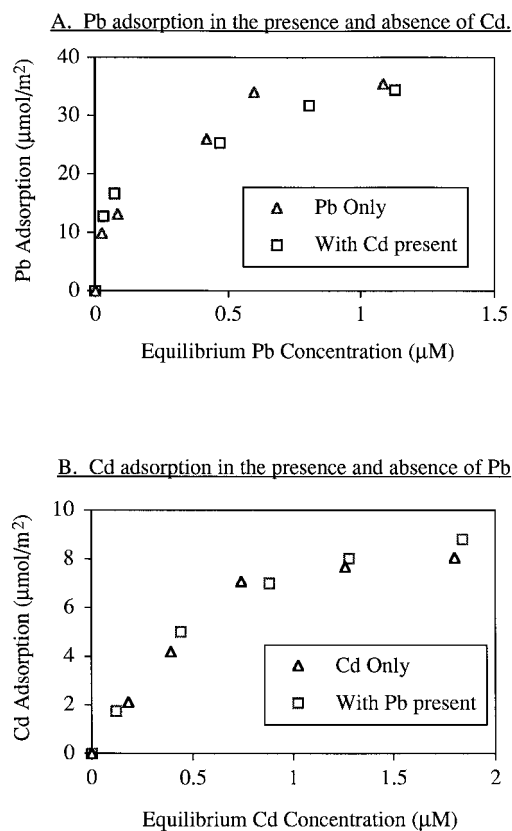


Fig. 1. Test for adsorption interference between Cd and Pb. A. Pb adsorption to biofilms in the presence and absence of Cd. B. Cd adsorption in the presence and absence of Pb. For adsorption from mixtures, the initial levels (μM) of Cd and Pb were equal.

Table 1. Composition and Pb/Cd speciation of MMS solution used in metal adsorption experiments

	Component or species	Concentration (μM)	Concentration (mg/l)
MMS medium ^a	CaCl ₂ ·2H ₂ O	200	30
	MgSO ₄ ·7H ₂ O	140	35
	(NH ₄) ₂ SO ₄	910	120
	KNO ₃	150	15
	NaHCO ₃	10	0.84
	KH ₂ PO ₄	5	0.70
Pb speciation ^b	Pb ²⁺	89%	
	PbSO ₄	9%	
	PbOH ⁺	1%	
Cd speciation ^b	Cd ²⁺	89%	
	Cd Cl ⁺	1.5%	
	Cd SO ₄	4.6%	
	Cd NO ₃ ⁺	4.9%	

^aIonic strength adjusted to 0.05 M w/NaNO₃; pH adjusted to 6.0 before autoclaving.

^bPb and Cd speciation calculated by MINEQL for a total Pb/Cd concentration of 1.0 μM .

Measurement of Pb and Cd adsorption to natural biofilms

Pb and Cd adsorption isotherms were obtained for extracted and unextracted biofilms by measuring Pb and Cd adsorption from solutions with defined metal speciation and initial Pb and Cd concentrations ranging from 0.2 to 2.0 μM . The equilibration solutions were prepared by dilution of 1000 mg/L PbNO₃ and CdNO₃ reference solutions (Fisher Scientific, Pittsburgh, PA, U.S.A.) using a minimal mineral salts (MMS) solution with ionic strength adjusted to 0.05 M with NaNO₃ (Table 1). Pb and Cd speciation in the defined solutions was calculated using MINEQL (Westall *et al.*, 1976). The calculations showed that because of low inorganic ligand concentrations, free Pb²⁺ or Cd²⁺ ions would comprise 89% of the total dissolved metal (Table 1). Three slides from each treatment were placed in polypropylene racks and submerged into each of five 800-mL solutions with five different Pb and Cd concentrations. These solutions were contained in 2-L water-jacketed beakers to maintain a constant temperature of $25 \pm 1^\circ\text{C}$. The solutions were stirred continuously with magnetic stirrers for 24 h while maintaining the pH at 6.0 ± 0.1 using pH controllers (Cole Parmer, Vernon Hills, IL) to regulate the addition of 0.01 M HNO₃ and NaOH. After equilibration, slides with biofilms were removed from the Pb and Cd solutions, rinsed for 1 s in metal-free MMS solution, and extracted into 50 mL of 10% HNO₃ (trace metal grade) for 24 h in 150 mm plastic petri dishes. Pb and Cd in extracts were measured using GFAAS as described above. The coefficient of variation for the GFAAS analyses was less than 5%.

Preliminary experiments with Pb and Cd adsorption measured together and separately showed that Cd did not interfere with Pb adsorption to the biofilms and vice versa under the conditions of these experiments (Fig. 1). This permitted the simultaneous measurement of Pb and Cd adsorption in subsequent experiments.

Statistical analyses

As described above, none of the selective extractions removed only one component from the biofilms without also partially removing at least one of the other components as well. Accurate determination of Pb and Cd associated with each individual component (by difference before and after extraction) thus required consideration of contributions from the partial fractions of the other components removed from the slides. Tessier *et al.* (Tessier *et al.*, 1996) recently addressed this problem by using simultaneous solution of two equations for Fe and Mn contributions to trace metal binding. Because our work included additional variables (i.e. Fe and Mn, as well as Al oxides

and organic materials) Pb and Cd adsorption to each component was estimated with non-linear regression analyses of all of the isotherm data including unaltered biofilms and biofilms after each of the three extractions. The model used for the regression analysis considered total adsorption by the biofilm at a given Pb or Cd concentration (Γ_{total} , $\mu\text{mol Pb}$ or Cd/m^2) to be the sum of contributions from four constituents (Fe, Mn and Al oxides and COD):

$$\Gamma_{\text{total}} = C_{\text{Fe}} \cdot \Gamma_{\text{Fe}} + C_{\text{Mn}} \cdot \Gamma_{\text{Mn}} + C_{\text{Al}} \cdot \Gamma_{\text{Al}} + C_{\text{COD}} \cdot \Gamma_{\text{COD}}, \quad (1)$$

where C_{Fe} , C_{Mn} , C_{Al} and C_{COD} are the surface concentrations of each component ($\mu\text{mol Fe}$, Mn or Al/m^2 and $\text{mg COD}/\text{m}^2$) and the Γ terms are adsorption on a per quantity of material basis (e.g. $\mu\text{mol Pb}/\mu\text{mol Fe}$). Γ for each component was expressed as a Langmuir adsorption isotherm:

$$\Gamma_i = \frac{\Gamma_i^{\text{max}} K_i [\text{M}^{2+}]}{1 + K_i [\text{M}^{2+}]}, \quad (2)$$

where Γ_i is the adsorption of M^{2+} by component i per unit surface area, Γ_i^{max} is the maximum adsorption of M^{2+} by component i , K_i is the Langmuir equilibrium coefficient and $[\text{M}^{2+}]$ is the concentration of free Pb or Cd metal ions. The predicted metal adsorption to bare glass slides at each metal concentration was subtracted from the observed metal adsorption. Adsorption to each component is expressed per unit nominal surface area of the glass slides containing the biofilm, not the total surface area of the adsorbing phase.

The nonlinear regression was performed using SAS software (SAS Version 6.12, SAS Institute, Cary, NC). The regression minimized the error associated with a total of eight variables (four values of Γ_i^{max} and four values of K_i). The data set consisted of adsorption data for the unextracted biofilms plus biofilms extracted with each of the three extractants, with triplicate samples at five metal concentrations, for a total of 60 observations. The regression was initialized with estimates for each Γ_i^{max} based on the assumption that all components adsorbed equal surface concentrations of metal. If the algorithm did not initially converge when all eight variables were regressed, the regression was performed iteratively for the four values of Γ_i^{max} and the four values of K_i until convergence on both Γ_i^{max} and K_i was obtained.

Table 2. . Assessment of removal of organic material and metal oxides from natural coatings by selective extractions

Extractant	Organic material ^a		Fe oxide ^b		Mn oxide ^b		Al oxide ^b	
	Surf. Conc. (mg COD/m ²)	Removal (%)	Surf. Conc. (μmol Fe/m ²)	Removal (%)	Surf. Conc. (μmol Mn/m ²)	Removal (%)	Surf. Conc. (μmol Al/m ²)	Removal (%)
None (total acid extractable)	484 ± 29	0	353 ± 19	0	21.5 ± 0.3	0	767 ± 26	0
0.01 M NH ₂ OH·HCl + 0.01 M HNO ₃ 30 min	330 ± 54	32	302 ± 25	14	6.3 ± 0.7	71	470 ± 54	39
0.3 M Na ₂ S ₂ O ₄ 40 min	470 ± 25	3	60.2 ± 1.4	83	1.7 ± 0.14	92	134 ± 7.2	83
10% Oxalic Acid 60 h	88 ± 3	82	15.4 ± 2.5	96	< 0.6 (bd ³)	100	29.6 ± 4.8	96

^aMean ($n = 3$) ± one standard deviation.

^bMean ($n = 15$) ± one standard deviation.

Determination of adsorption isotherms for laboratory surrogate materials

For comparison to results of the selective extraction experiments, Pb adsorption isotherms were determined for pure laboratory surrogate materials representing the Fe, Mn and Al oxides and organic materials in the natural biofilms. Fe oxyhydroxide was prepared by precipitation of Fe(III) by addition of NaOH to a 0.1 M Fe(NO₃)₃ solution to reach a pH of 8.0 (Matijevic and Scheiner, 1978). The resulting colloidal suspension exhibited an X-ray diffraction pattern that suggested an amorphous structure. Biogenic Mn oxides were prepared via biologically catalyzed oxidation of Mn(II) by the bacterium *Leptothrix discophora* SS-1 (Nelson *et al.*, 1999a). A fresh abiotic Mn(IV) oxide was prepared by oxidation of Mn(II) with KMnO₄ and NaOH at 90°C (Murray, 1974). Al oxide was obtained commercially as γ-Al₂O₃ (Alfa Products, Danvers, MS, U.S.A.) (Nelson *et al.*, 1999b). Pb adsorption to the laboratory oxides was determined by equilibrating suspensions of the oxides with Pb solutions prepared in MMS and maintained at pH 6.0 and 25°C for 24 h. Pb adsorption was determined by measuring Pb concentrations (GFAAS) before and after centrifuging at 12,900 rpm for 30 min.

Surrogates for the biological components of the natural biofilms were laboratory biofilms of pure cultures of the bacteria *Burkholderia cepacia* strain 17616 and *L. discophora* strain SS-1. Biofilms were grown on glass slides in a biofilm reactor (Nelson *et al.*, 1996) and Pb adsorption was measured using the same method as that described above for the lake biofilms.

RESULTS AND DISCUSSION

Biofilms that developed on glass slides after four weeks in Cayuga Lake consisted of assemblages of microorganisms in a biofilm matrix and associated mineral deposits. The biofilms contained large numbers of diatoms, green and red algae, bacterial cells, filamentous cyanobacteria and filamentous bacteria resembling iron-depositing bacteria such as *Leptothrix* spp. (Ghiorse, 1984). The biological composition of Cayuga Lake biofilms is described more extensively elsewhere (Nelson, 1997; Nelson *et al.*, 1999b). Microscopic observation after staining with Prussian Blue and Leukoberbelin Blue revealed strong associations between Fe and Mn mineral deposits and organic materials. From the present investigation it was not possible to determine if the Fe and Mn oxides were formed by oxidation in the biofilm or if these oxides were formed in the water column and then deposited onto the biofilm sur-

faces. The total organic material in the biofilms exerted a chemical oxygen demand (COD) of 484 ± 29 mg/m² (Table 2). Surface concentrations of metal oxides decreased in the order Al > Fe > Mn (Table 2).

The extractant reagents employed were intended to selectively remove specific adsorbing phases without removing other components. Hydroxylamine hydrochloride (NH₂OH·HCl) was used to extract easily reducible Mn oxides, sodium dithionite (Na₂S₂O₄) to extract Mn and Fe oxides, and oxalic acid to extract metal oxides and organic material. As noted in the Methods Section above, use of other extractants resulted in unacceptable alterations of the residual biofilms. Each extractant removed additional components besides the target materials. NH₂OH·HCl removed 71% of the biofilm Mn, but also removed 14% of the Fe, 39% of the Al and 32% of the organic material (Table 2). Na₂S₂O₄ removed 83 and 92% of the Fe and Mn, respectively, but also removed 83% of the Al. Very little (3%) of the organic material was removed by the Na₂S₂O₄ extractant. Oxalic acid removed 82% of the organic material, but also removed nearly all of the Fe, Mn and Al (Table 2).

Pb and Cd adsorption to the unextracted biofilms at pH 6.0 and 25°C followed Langmuir adsorption isotherms, and Pb adsorption was almost an order of magnitude greater than that of Cd (Figs 2 and 3). Extraction with NH₂OH·HCl and Na₂S₂O₄ significantly reduced both Pb and Cd adsorption, and adsorption to biofilms extracted with oxalic acid was only slightly greater than that of bare glass (Figs 2 and 3).

Relative contributions of metal oxide and organic phases to total observed Pb and Cd adsorption by the biofilms were estimated using nonlinear regression analysis of biofilm composition data and adsorption data for extracted and unextracted biofilms. This analysis provided estimates of Langmuir parameters (I_{max} and K) for each of the components for both Pb and Cd adsorption (Table 3). These parameters were then used to construct adsorption isotherms for the original unextracted biofilms showing estimated adsorption to each of the components considered in the model. For Pb,

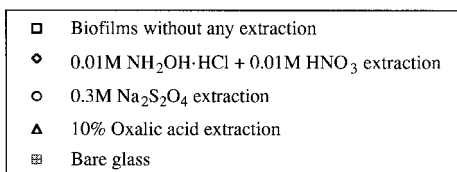
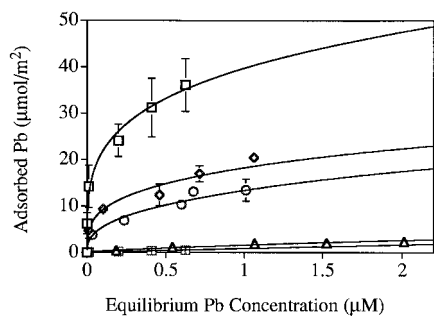


Fig. 2. Pb adsorption to Cayuga Lake biofilms before and after several selective extraction treatments. Error bars indicate \pm one standard deviation.

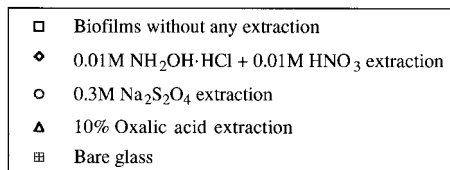
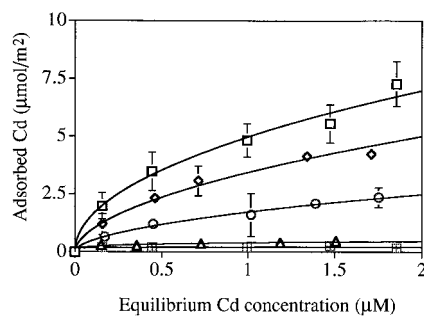


Fig. 3. Cd adsorption to Cayuga Lake biofilms before and after several selective extraction treatments. Error bars indicate \pm one standard deviation.

the regression analysis indicates that the greatest contribution to total Pb adsorption was from Mn oxides, followed by lesser contributions from Fe oxides and organic material (Fig. 4). The estimated contribution to Pb adsorption by Al oxides was negligible (Table 3, $\Gamma_{Al}^{max} = 0.00 \pm 0.0035$ mol Pb/

mol Al). For Cd, the regression analysis indicated that Fe oxides exerted the greatest influence on Cd binding, followed by lesser contributions from Al oxides, Mn oxides and organic material (Fig. 5). However, at low Cd concentrations ($< 0.1 \mu\text{M}$), the estimated contribution of Mn was much greater

Table 3. Estimated Langmuir parameters for Pb and Cd adsorption to organic material and metal oxides in Cayuga Lake biofilms based on a nonlinear regression analysis of adsorption after selective extractions

Parameter	Lead		Cadmium	
	Estimate	Asymptotic Std. Error	Estimate	Asymptotic Std. Error
Γ_{maxFe} (mol/mol Fe)	0.0363	0.0092	0.0099	0.0017
Γ_{maxMn} (mol/mol Mn)	0.833	0.054	0.0402	0.013
Γ_{maxAl} (mol/mol Al)	0.000	0.0035	0.0076	0.0035
Γ_{maxCOD} (mol/mg COD)	0.0245	0.0023	0.0052	0.00067
K_{Fe} (L/ μmol)	1.97	0.59	1.75	0.53
K_{Mn} (L/ μmol)	319	81.4	22.3	48
K_{Al} (L/ μmol)	—	—	0.13	0.046
K_{COD} (L/ μmol)	3.8	0.97	0.65	0.15

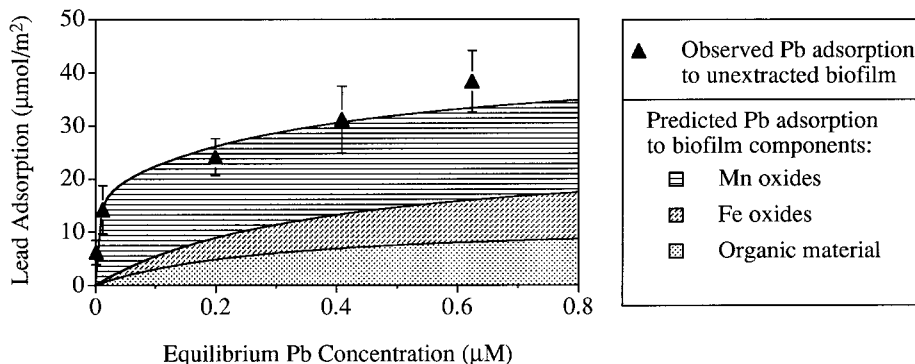


Fig. 4. Estimated Pb adsorption to metal oxide and organic components of unextracted Cayuga Lake biofilms based on non-linear regression analysis of Pb adsorption isotherm data for extracted and unextracted biofilms. Error bars indicate \pm one standard deviation.

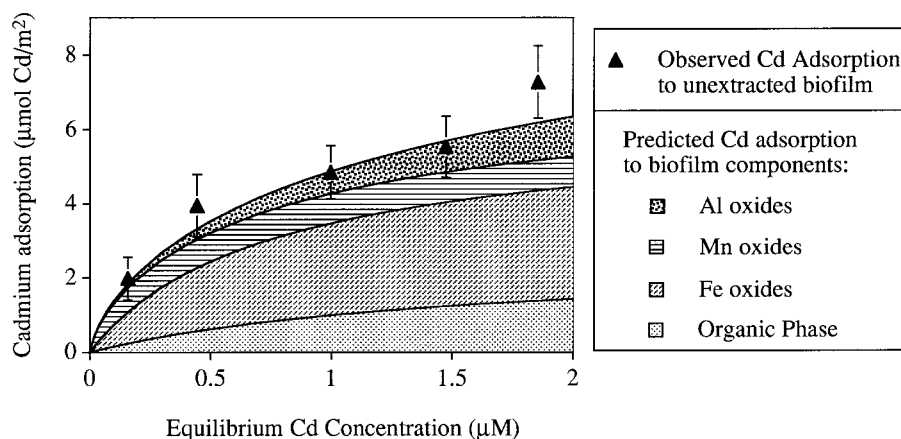


Fig. 5. Estimated Cd adsorption to metal oxide and organic components of unextracted Cayuga Lake biofilms based on non-linear regression analysis of Cd adsorption isotherm data for extracted and unextracted biofilms. Error bars indicate \pm one standard deviation.

than that of organic material and Al oxides and similar to that of Fe (Fig. 5). Errors associated with estimated adsorption to each phase are depicted in Fig. 6, which shows Pb and Cd adsorption at a single adsorbate concentration ($0.5 \mu\text{M}$). The standard errors depicted in Fig. 6 were determined by considering the propagated errors from both T_{max} and K estimations. These calculations indicate that the higher adsorption of Pb by Mn is statistically significant. The estimated Pb adsorption by Fe was not significantly different from that of organic material.

The distribution of Pb between biofilm components estimated by the non-linear regression analysis is similar to that estimated for Cayuga Lake biofilms using an adsorption additivity model (Nelson *et al.*, 1999b). In the additivity model, total adsorption was predicted from the sum of contributions of individual components that were determined using Pb adsorption isotherms for pure laboratory surrogate materials selected to represent

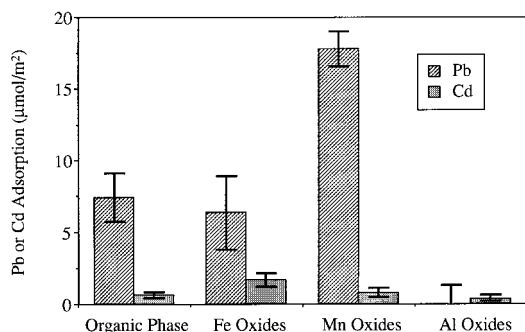


Fig. 6. Estimated Pb and Cd adsorption to metal oxide and organic components of Cayuga Lake biofilms for dissolved metal concentrations of $0.5 \mu\text{M}$. Error bars indicate asymptotic standard error of the mean for the non-linear regression analysis.

natural Fe, Mn, and Al oxides and organic material. When the adsorption capacity of laboratory-derived biogenic Mn oxides was used as the surrogate for natural Mn oxides (Nelson *et al.*, 1999a), the additivity model predicted a strong role of Mn oxides (Nelson *et al.*, 1999b) similar to that observed in the present work. The additivity model used Pb adsorption to pure cultures of microorganisms to estimate Pb adsorption to the organic phase of the biofilms, and resulted in a lower estimation of the role of organic material than in the present work. The low concentration of Pb associated with Al oxides predicted by the selective extractions also agrees with predictions based on laboratory adsorption isotherms. Based on previously measured Pb adsorption to $\gamma\text{Al}_2\text{O}_3$ (Nelson, 1997; Nelson *et al.*, 1999b), the expected T_{max} for Al oxide in the unextracted Cayuga Lake biofilms would be $1.2 \mu\text{mol Pb/m}^2$, which is much lower than Pb adsorption measured for the other oxide and organic phases.

The regression analysis provides Langmuir adsorption isotherms for each of the biofilm components investigated, and these can be compared to Pb adsorption isotherms for representative laboratory materials determined under the same conditions (MMS solution matrix at 25°C , pH 6.0, ionic strength 0.05 M). The regression-derived Pb adsorption isotherm for the Fe oxide component of the biofilms was very similar to Pb adsorption to amorphous Fe oxyhydroxide previously measured in our lab (Nelson *et al.*, 1995) as well as to that estimated using a model described by Benjamin and Leckie (1981) (Fig. 7). The excellent agreement of the Pb adsorptive behavior of Fe oxides obtained from these distinctly different approaches suggests that the isotherm parameters have a predictive utility. The agreement of the regression results for the Pb isotherm to those independently attained by other methods also suggests the extraction approach

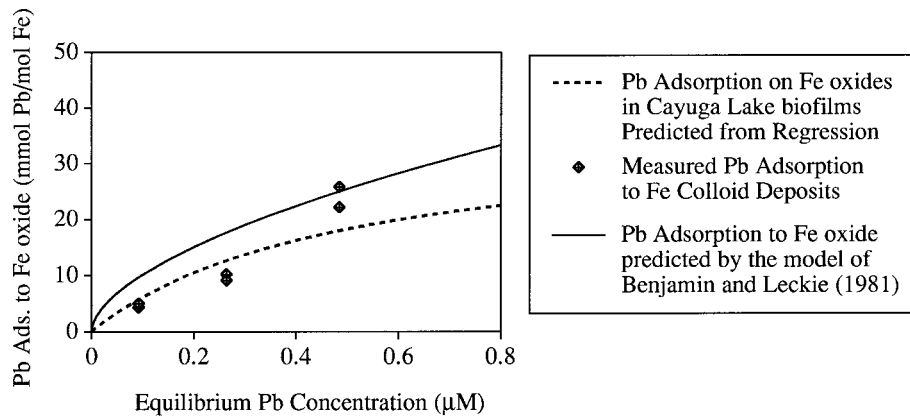


Fig. 7. Comparison of Pb adsorption to Fe oxide predicted by the non-linear regression analysis of extracted biofilm data to that measured for Fe colloids and that reported by Benjamin and Leckie (1981). Temperature = 25°C, pH 6.0. Adsorption to Fe colloid data after Nelson *et al.* (1995).

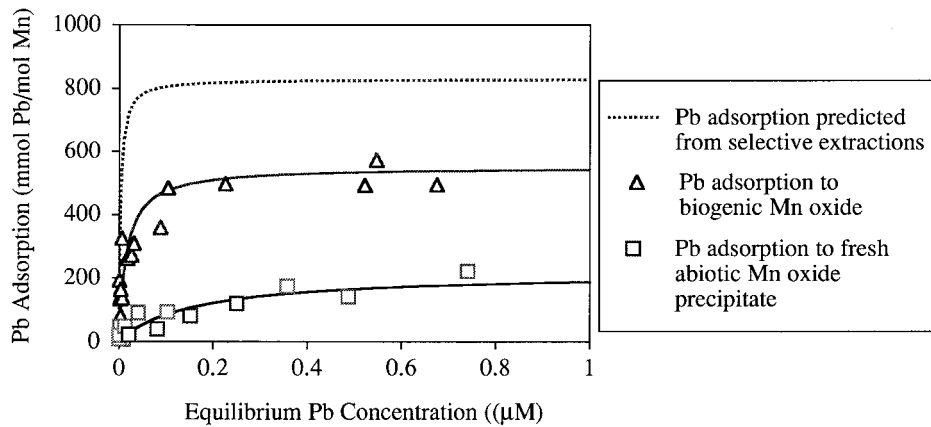


Fig. 8. Comparison of Pb adsorption to Mn oxide predicted by the non-linear regression analysis of extracted biofilm data to that measured for biogenic Mn oxide and a fresh abiotically precipitated Mn oxide. Data for adsorption to biogenic Mn oxide after Nelson *et al.* (1999a).

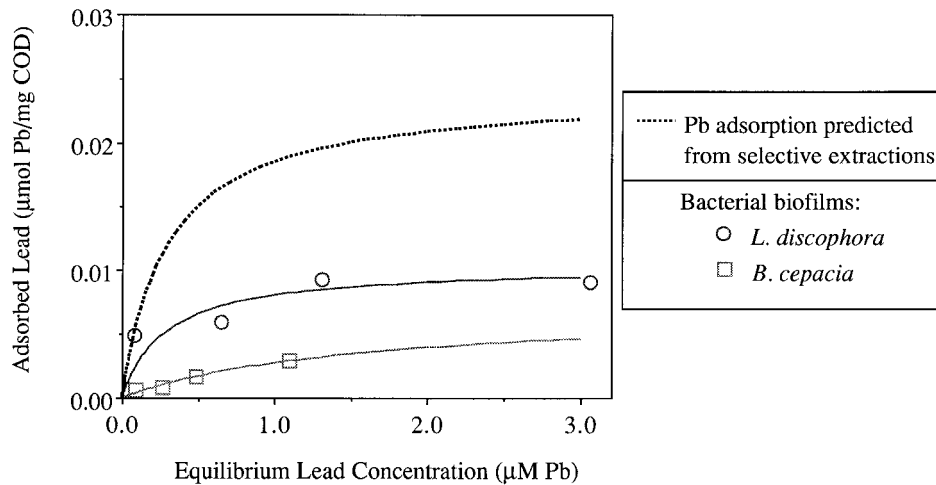


Fig. 9. Comparison of Pb adsorption to organic material predicted by the non-linear regression analysis of extracted biofilm data to that measured for bacterial biofilms. Adsorption to *L. discophora* and *B. cepacia* biofilms after Nelson *et al.* (1999b).

used in this study can yield realistic estimates of the behavior of adsorptive phases in nature.

Regression analysis of selective extraction data indicated greater Pb adsorption to Mn oxides (approx. 2×) than that observed for laboratory produced biogenic and abiotic Mn oxides (Fig. 8). Similarly, the selective extraction technique suggests greater (approx. 2×) Pb adsorption by organic materials than that observed for laboratory biofilms produced by two different species of bacteria (Fig. 9). The possible overestimation of Pb adsorption to the Mn oxide and organic phases may have resulted from performing the regression analysis with consideration of only four adsorbing components (Mn, Fe, Al and organic materials). While there could be additional adsorbing phases and/or adsorption mechanisms influencing Pb and Cd adsorption in the surface coatings, the regression analysis was forced to converge for only the four components. Thus, any adsorption to other components not considered would be included with adsorption attributed to these four components. This could lead to overestimation of metal adsorption to one or more components. Alternatively, the laboratory surrogates for Mn oxide and organic matter may adsorb less Pb than their naturally occurring counterparts. However, the excellent agreement of the results for Pb isotherms on Fe oxides with estimations previously made using laboratory adsorption isotherms and the reasonable (approx. 2×) agreement with other surrogate biofilm components suggests that the contribution of other adsorptive components is likely to have been small.

CONCLUSIONS

The selective extraction method presented here is unique because of the measurement of trace metal adsorption before and after extraction. This approach avoids the possibility of desorption of trace metals from components other than the target component(s) being extracted. The selective extractions removed target components with efficiencies between 71 and 83%, but significant amounts of metal oxides and organic materials other than the target components were also removed by the extractants (up to 39%). Because of this, the amount of Pb and Cd adsorption associated with each phase could not be determined by a simple calculation, and a nonlinear regression analysis was used to estimate relative contributions of each surface constituent. This analysis suggested a very strong role of Mn oxides in controlling Pb adsorption to the lake biofilms and lesser but significant roles of Fe oxides and organic material. Adsorption of Cd to the lake biofilms was dominated by Fe oxides, with lesser roles of Mn and Al oxides and organic material. The results for Pb agree with previous results of a model based on Pb adsorption to laboratory surrogate materials for Mn, Fe and Al oxides and

defined organic constituents. This agreement suggests that the extraction method presented here provides a reliable estimate of the relative contributions of each component to total trace metal adsorption.

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