

# The effect of cadmium on fulvic acid adsorption to *Bacillus subtilis*

Paul C. Frost<sup>1</sup>, Patricia A. Maurice\*, Jeremy B. Fein

Department of Civil Engineering and Geological Sciences, University of Notre Dame, 156 Fitzpatrick Hall, Notre Dame, IN 46556-5602, USA

Received 26 September 2002; accepted 13 April 2003

## Abstract

The effects of Cd on the adsorption of an aquatic fulvic acid (FA) to the surface of *Bacillus subtilis* were investigated from pH 2.5 to 7.0, at fixed ionic strength (0.1 M NaClO<sub>4</sub>) and at ambient temperature ( ~ 22 °C). Cd (14 mg/l) had no effect on FA adsorption at pH < 5 but increased FA adsorption at pH > 6. The effects of Cd (0, 14 mg/l) on FA adsorption to *B. subtilis* were further examined as a function of initial FA concentration (0–45 mg C/l) at pH 6.5. FA adsorption isotherms also were measured at pH 6.5 as a function of dissolved Cd concentration (0–14 mg/l) at three initial FA concentrations (4, 8, 22 mg C/l). At all FA concentrations studied at pH 6.5, FA adsorption increased with increasing initial total Cd concentration.

Under all studied conditions, preferential adsorption of high- to intermediate-molecular-weight FA components to *B. subtilis* resulted in a fractionation of the FA pool, with lower-molecular-weight components remaining in solution. At pH > 6, Cd further enhanced the adsorption of high- to intermediate-molecular-weight FA components but did not significantly enhance the adsorption of lower-molecular-weight components. Hence, the overall process of adsorptive fractionation was not altered significantly by the presence of Cd.

Overall, the results of this study (1) demonstrate that FA adsorption to bacterial surfaces can be altered by the presence of a metal cation, and (2) provide further evidence that microbe–metal–ligand interactions may significantly affect the mobility and fate of natural organic matter in the subsurface.

© 2003 Elsevier B.V. All rights reserved.

**Keywords:** Fulvic acid; *Bacillus subtilis*; Cadmium; Fractionation; Adsorption

## 1. Introduction

Aqueous metal cations and organic ligands can adsorb onto bacterial surfaces (see recent reviews by Beveridge et al., 1997; Fein, 2000; Warren and Haack, 2001; Brown and Parks, 2001). Given the high concentration of bacterial biomass in many subsurface

environments (Alexander, 1977; Barnes and Nierzwicki-Bauer, 1997), microbial adsorption has the potential to decrease aqueous concentrations of both metals and organic acids (Fein, 2000), to alter the mobility of metals (Yee and Fein, 2002), and to change the physicochemical characteristics of the dissolved organic matter pool (Maurice et al., in press). However, most of our current understanding of bacterial adsorption is based on binary studies of bacteria with either metal cations or organic ligands, alone. In order to determine the importance of ternary interactions between bacteria, metal cations, and organic ligands, combined systems must be studied directly. Studies of

\* Corresponding author. Fax: +1-219-631-9163.

E-mail addresses: pfrost@nd.edu (P.C. Frost), pmaurice@nd.edu (P.A. Maurice).

<sup>1</sup> Current address: Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556-0369, USA.

bacteria–metal–ligand interactions also may provide insight into processes such as bacterial metabolism of organic matter and bacterially mediated metal precipitation and redox transformations.

Bacterial cell walls contain organic acid functional groups (e.g., carboxyl, hydroxyl, phosphoryl; [Beveridge and Murray, 1980](#)), which become more negatively charged with increasing pH ([Harden and Harris, 1952](#)). Consequently, the adsorption of aqueous metal cations, such as Cd, onto bacterial cell walls increases with increasing pH (e.g., [Mullen et al., 1989](#); [Plette et al., 1996](#); [Fein et al., 1997](#); [He and Tebo, 1998](#)), except when aqueous metal-hydroxide or metal–ligand complexes effectively compete with bacterial surfaces for the available metal. In contrast, because of their net negative charge when deprotonated, adsorption of organic ligands onto bacteria tends to decrease with increasing solution pH ([Daughney and Fein, 1998](#); [Fein and Delea, 1999](#); [Fein et al., 1999](#); [Wightman and Fein, 2001](#); [Maurice et al., in press](#)).

In mixed organic ligand–metal–bacterial systems, the adsorption of metals and organic acids onto bacteria may be increased or decreased by the presence of each other. For example, the organic ligand EDTA significantly decreases the adsorption of Cd by *Bacillus subtilis*, likely through aqueous EDTA–Cd complexation ([Fein and Delea, 1999](#)). Similarly, [Wightman and Fein \(2001\)](#) showed that a humic acid diminished bacterial adsorption of Cd at pH>5.0, apparently due to competitive binding of Cd by aqueous humic acid. Additional mechanisms whereby metals could potentially influence organic–ligand adsorption to bacterial surfaces include, for example, formation of ternary surface complexes, changes in surface charge, and metal-induced coagulation of organic matter. Currently, relatively little is known about how these factors affect mixed bacteria–metal–organic systems.

The objective of this study was to examine how an aquatic fulvic acid (FA) and Cd affect each other's adsorption onto the common soil bacterium, *B. subtilis*. Cd was chosen because it does not form aqueous hydroxide species and remains highly soluble throughout the pH range studied (pH 2.5–7.0). Fulvic acid is a polydisperse mixture of natural organic molecules that are likely to have different binding affinities for the *B. subtilis* surface. [Maurice et al. \(in press\)](#) observed that adsorption of FA to *B. subtilis* was reversible and that high- and intermediate-molecular-weight FA compo-

nents adsorbed preferentially to the bacterial surface. This study investigated the changes in molecular weight distribution of FA upon adsorption to *B. subtilis* in the presence and absence of Cd. This study of FA and Cd adsorption to viable but nonmetabolizing bacteria represents an important first step towards quantifying the complex interactions between bacteria, natural organic matter, and metals in natural environments. In this study, loss of organic matter from solution can be attributed to adsorption alone, with little or no contributions from metabolic processes.

## 2. Methods and materials

### 2.1. Fulvic acid

The FA used in this study was an XAD-8 isolate ([Aiken, 1985](#)) from surface waters collected at McDonalds Branch, a first-order organic-rich stream in the New Jersey coastal plain, USA. See [Maurice et al. \(2002\)](#) for details of this sampling site and of the FA collection, isolation, and preservation. The elemental composition (Huffman Labs, Golden, CO; [Maurice et al., 2002](#)) of the FA (sample S2 S98 XAD8 in [Maurice et al., 2002](#)) is 47.71% C, 4.40% H, 46.94% O, 0.45% N, 0.50% S by weight. The O/C atomic ratio, 0.73, is considerably higher than the average value, ~ 0.60, for aquatic fulvic acids ([Steelink, 1985](#)), suggesting that the FA sample is relatively polar. Additional characteristics of this sample (e.g., <sup>13</sup>C NMR and FTIR characterization) are provided by [Maurice et al. \(2002\)](#). A stock solution of ~ 2400 mg C/l FA was prepared from the freeze-dried XAD-8 isolate in UV-treated (to remove trace organics) deionized water (Millipore). Organic carbon analysis of the FA solution prior to and following filtration, at various dilutions, indicated that this concentrated stock solution did not undergo precipitation.

### 2.2. Bacterium

*B. subtilis* cells were grown and washed as described by [Fein et al. \(2001\)](#). Briefly, cells were grown in a liquid medium containing 30 g/l trypticase soy broth and 5 g/l yeast extract mixture. After growing in 5 ml of the media for 24 h at 32 °C, cells were placed into 1- to 2-l flasks of the sterilized media and allowed 24 h of

additional growth. Cells were harvested by centrifugation (5000 rpm, 8 min) and then washed twice with 0.1 M NaClO<sub>4</sub>. Cells were further washed with HNO<sub>3</sub> at pH 1.5 for 1 h and then five additional times with 0.1 M NaClO<sub>4</sub>. Each wash was terminated by centrifuging the bacteria for 10 min (first three washes) and 2–3 min (last five washes) at 7500 rpm. After the last wash, the *B. subtilis* biomass was centrifuged for 1 h at 7500 rpm and weighed to determine bacterial wet mass. Weighed bacteria were resuspended at a known concentration (4 g wet weight/l) in 0.1 M NaClO<sub>4</sub> prior to running the experiment. Previous work (e.g., Fein et al. 1999) showed that cells remain viable and intact after the wash procedure, although they are nonmetabolizing during the course of the experiments.

### 2.3. Batch adsorption experiments

Four types of batch adsorption experiments with *B. subtilis* were conducted: (1) fixed Cd concentration (14 mg/l) with and without FA (22 mg/l) as a function of pH; (2) fixed FA concentration (22 mg/l) with and without Cd (14 mg/l) as a function of pH; (3) fixed FA concentration (either 4, 8, or 22 mg/l) as a function of Cd concentration (0–18 mg/l) at a fixed pH of ~ 6.5; and (4) fixed Cd concentration (either 0 or 14 mg/l) as a function of FA concentration (0–45 mg/l) at a fixed pH of ~ 6.5.

Batch adsorption experiments were conducted by adding FA and/or Cd to *B. subtilis* (4 g wet weight/l) to the 0.1 M NaClO<sub>4</sub> solution. Following the addition of FA and/or Cd, suspensions were split and placed into 30-ml acid-washed polypropylene tubes. Suspension pH was then adjusted with 0.1 M HCl and/or 0.1 M NaOH. Suspensions were allowed to equilibrate while rotating at 5 rotations per minute for 4 h at 22 °C in the dark. This time period was chosen because Maurice et al. (in press) showed that FA adsorption to *B. subtilis* came to equilibrium within an hour, and was reversible. Batch experiments were terminated after 4 h by centrifugation for 10 min at 7500 rpm. Afterwards, each sample was filtered through a 0.45- $\mu$ m nylon filter and split into three fractions.

### 2.4. Solution analysis

One sample fraction from each vessel was analyzed for aqueous Cd with inductively coupled plasma atom-

ic emission spectrometry (ICPAES) using the primary peak at 214.4 nm. Cadmium standards were made with electrolyte (0.1 M NaClO<sub>4</sub>) by diluting a known concentration of commercially available Cd. Another sample fraction was held at 4 °C for less than 1 week until analysis by high-pressure size exclusion chromatography (HPSEC) for FA molecular weight determination. We used a modified HPSEC method as described by Zhou et al. (2000), with a Waters Protein-Pak 123 silica column on Waters HPSEC instrumentation. The sorbed fraction of FA was determined by subtracting the FA distribution in the postexperimental sample from the FA distribution determined for preexperiment FA. The remaining sample fraction was used to measure FA concentration by visible light absorption at 450 nm with a UV–Vis spectrometer. For this analysis, FA standards were made by diluting the stock FA solution. After equilibration, the aqueous concentrations of Cd and FA were measured and the

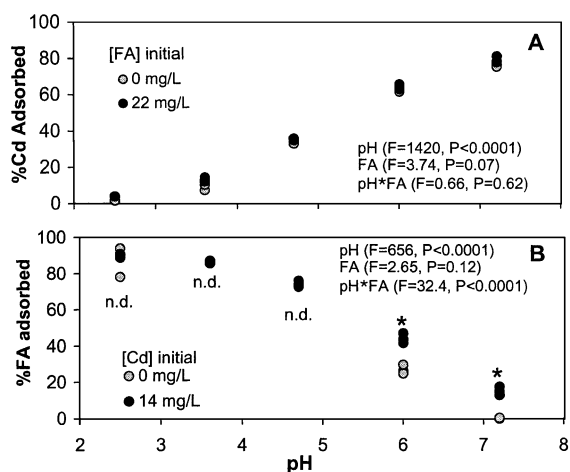


Fig. 1. Cadmium and fulvic acid adsorption to *B. subtilis* in the presence of varying amounts of each other and 4.0 g/l bacteria at different pH. (A) Percentage of Cd adsorbed with and without FA. Initial Cd concentration was 14.0 mg/l. Also given are two-way ANOVA statistics ( $F$  values and  $P$  values), which show a significant effect of pH but not of FA on Cd adsorption. (B) Percentage of FA adsorbed with and without Cd. Initial FA concentration was 22.0 mg/l. Also given are two-way ANOVA statistics ( $F$  values and  $P$  values), which show a significant interaction between pH and Cd on FA adsorption to *B. subtilis*. \* indicates a significant difference (determined with pairwise contrasts) between FA adsorption in the plus and minus Cd levels at that pH whereas n.d. indicates no significant differences were found.

extent of adsorption was determined by difference relative to the initial concentrations.

### 3. Results

Adsorption of Cd onto *B. subtilis* increased with increasing pH (Fig. 1A). However, there was no

effect of FA on the adsorption of Cd by *B. subtilis* under the experimental conditions. In contrast, FA adsorption decreased with increasing pH (Fig. 1B), exhibiting similar behavior to that observed for humic acid adsorption onto the same bacterial surface (Fein et al., 1999; Wightman and Fein, 2001). At low pH (2.5–4.7), Cd did not affect the adsorption of FA by *B. subtilis*. At higher pH (6.0–7.2), Cd

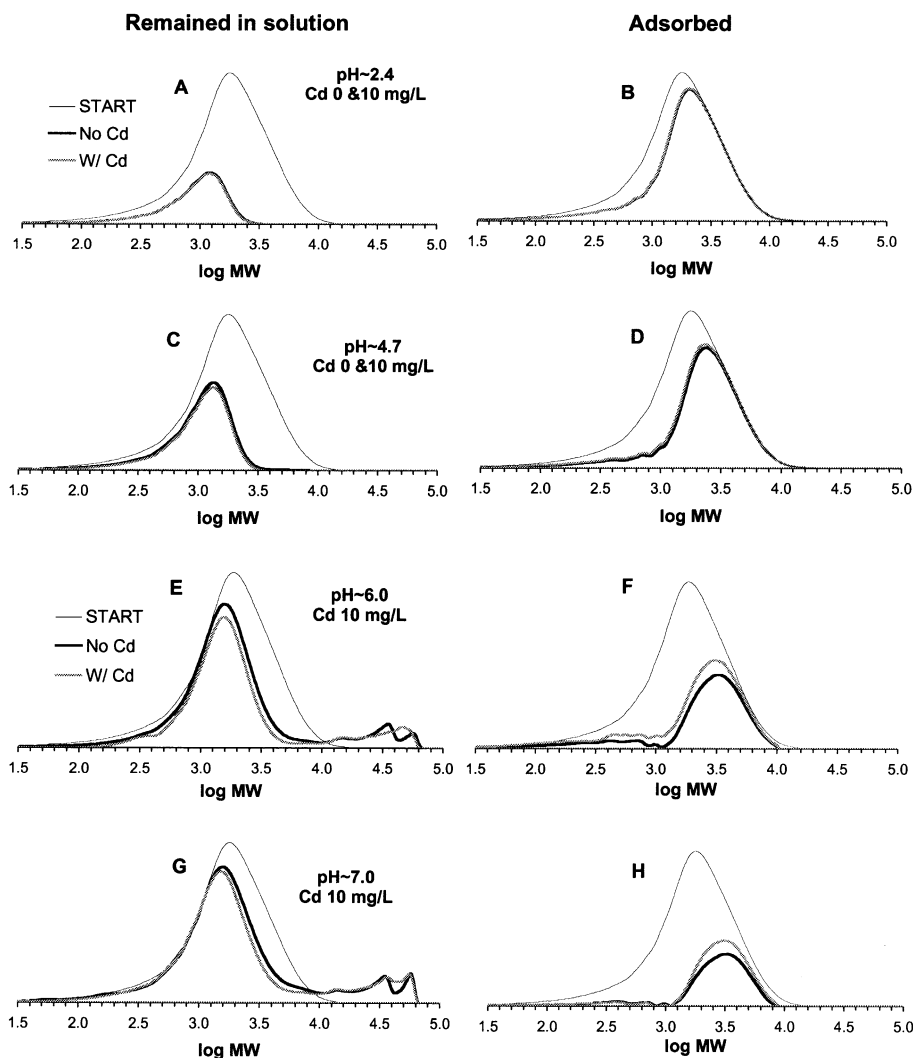


Fig. 2. Molecular weight distributions of fulvic acid remaining in solution (left: plots A, C, E, G) and adsorbed to bacteria (right: plots B, D, F, and H) in the presence and absence of Cd at different pH: 2.5 (A,B); 4.5 (C,D); 6.0 (E,F); 7.0 (G,H). Initial (unreacted) fulvic acid is shown as the highest peak in each plot. For the adsorbed plots (right column), peak height represents the amount of material adsorbed whereas peak morphology shows changes in the relative adsorption of different molecular weight material. These plots show that high-molecular-weight components adsorb preferentially, leaving lower-molecular-weight components in solution. At pH 6 and 7, Cd enhances FA adsorption but this enhanced adsorption is still preferential to higher-molecular-weight components.

significantly increased bacterial adsorption of FA (Fig. 1B).

Bacterial adsorption of FA altered the molecular weight distributions of the organic ligands remaining in solution (Figs. 2 and 3). High- to intermediate-molecular-weight components adsorbed preferentially to the *B. subtilis* surface, with lower-molecular-weight molecules remaining in solution. The molecular weight distributions of FA remaining in solution (Fig. 2A and C) and adsorbed (Fig. 2B and D) to *B. subtilis* were not affected by the presence of Cd at low pH. However, in the Cd-added treatments, less FA remained in solution (Fig. 2E and G) and more adsorbed (Fig. 2F and H) to the bacteria at higher pH values. Despite the increase in adsorption, Cd did not have a statistically significant effect on the average molecular weight of FA remaining in solution (Fig. 3). Fig. 2G and H shows that a small amount of high-molecular-weight material was present in solution following reaction of the FA with the bacteria. This likely results from a small amount of bacterial lysis or exudation over the course of the experiment, and was not included in calculations of average molecular weight.

In Fig. 4, the percentage and total amount (in mg C/l) of FA adsorbed is plotted as a function of initial [Cd], at pH 6.5. The percentage and total amount of

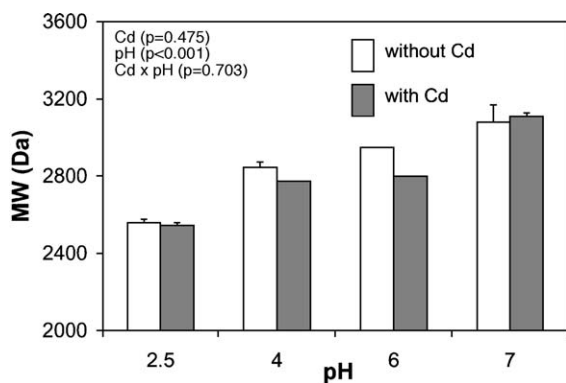


Fig. 3. Average molecular weight ( $M_w$ ) of FA upon adsorption to *B. subtilis* with and without Cd present. Also given are two-way ANOVA statistics ( $F$  values and  $P$  values), which show a significant effect of pH but not of Cd on the  $M_w$  of the adsorbed FA.  $M_w$  was not calculated for the distribution of FA remaining in solution due to presence of the high-molecular-weight material that appeared during the incubation, most probably due to a small amount of cell exudation or lysis.

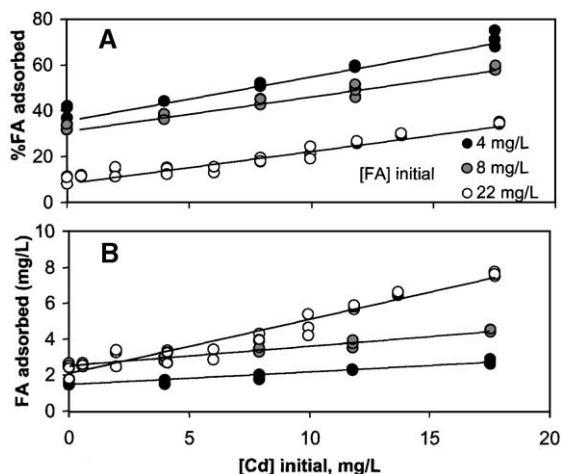


Fig. 4. Fulvic acid adsorption onto *B. subtilis* (4.0 g/l) as a function of increasing starting Cd concentration at pH 6.5. (A) Percentage of FA adsorbed to *B. subtilis*. (B) Total amount of FA adsorbed to *B. subtilis*.

FA adsorbed to *B. subtilis* increased linearly with increasing Cd concentration in the system (Fig. 4;  $R^2 > 0.9$  in all cases), at initial [FA] of 4–22 mg C/l. In Fig. 5, the percentage and total amount (in mg C/l) of FA adsorbed is plotted as a function of initial [FA], at initial [Cd] = 0 and 14 mg Cd/l. With no Cd present in the system, at pH 6.5, there was almost no FA adsorption at any starting FA concentration. With

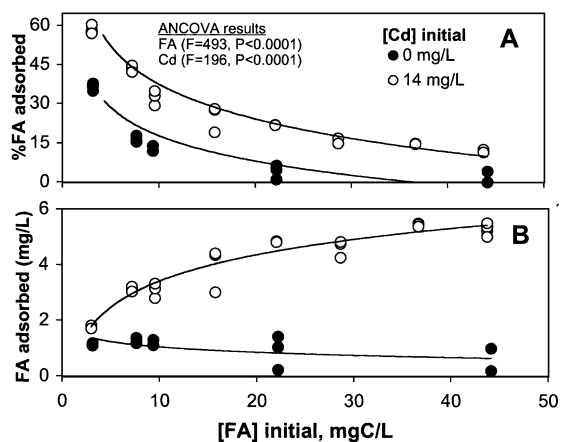


Fig. 5. Fulvic acid adsorption onto *B. subtilis* (4.0 g/l) as a function of increasing FA concentration with (14 mg Cd/l) and without Cd present at pH 6.5. (A) Percentage of FA adsorbed to *B. subtilis*. (B) Total amount adsorbed.

Cd (14 mg/l) present in the system, adsorption increased and attained a plateau at  $\sim 5.5$  mg C/l, which represents 1.4 mg C/g bacteria.

## 4. Discussion

### 4.1. FA effects on Cd adsorption

Most experimental studies of the effects of natural organic matter on metal adsorption involve mineral surfaces (Brown and Parks, 2001). Under some conditions, natural organic matter inhibits metal adsorption due to competition between the dissolved organic ligands and the surface (mineral or bacteria) for the metal. Under other conditions, natural organic matter enhances metal adsorption through a variety of mechanisms including: formation of ternary surface complexes, changes in surface charge, or stereochemical effects that lead to more stable binding to the organic ligand than to the mineral surface.

Under the conditions studied here, FA did not affect the extent of Cd adsorption onto *B. subtilis*. This is consistent with previous results of Davis (1984) who found that Cd adsorption onto the surface of alumina was not affected by the presence of adsorbed natural organic matter. In contrast, Wightman and Fein (2001) observed that the presence of humic acid decreased the adsorption of Cd to *B. subtilis* at pH>4 because the nonadsorbed humic acid in solution competed with the bacterial surface for Cd. When the concentration of bacteria was increased until essentially all of the humic acid adsorbed to the bacteria, Cd adsorption was no longer affected (Wightman and Fein, 2001). The fact that we did not observe a similar competitive effect for FA suggests that Cd associates only weakly or not at all with the FA used herein. Humic acid tends to be considerably higher molecular weight and more aromatic than fulvic acid. The larger humic molecules may be able to generate higher electrostatic fields that promote metal binding (e.g., Cabaniss et al., 2000). Hence, the observed differences in the effects of humic versus fulvic acids are consistent with their likely differences in metal binding affinities, although more detailed metal binding studies at a range of relative concentrations and perhaps using spectroscopic methods are needed.

### 4.2. Cd effects on FA adsorption

The enhanced adsorption of FA in the presence of aqueous Cd at pH>6 could result from: (1) alteration of surface charge (and, hence, a drop in electrostatic repulsion between the NOM and the bacterial surface) due to cationic adsorption, (2) formation of ternary surface complexes, or (3) enhancement of organic matter coagulation. Ternary organic–metal–surface complexes have been reported for mineral surfaces (see Schindler, 1990; Brown and Parks, 2001; Fein, 2002). However, Wightman and Fein (2001) found no evidence for surface humate–Cd–bacteria complexes. As explained above, our experimental results suggest that direct Cd–FA complexation is negligible in solution, indicating that ternary surface complexes are unlikely in this particular system. However, further studies, potentially including spectroscopic verification, are needed.

It is likely that the overall FA adsorption behavior on the bacterial surface is governed by electrostatic and hydrophobic effects. Under low pH conditions, the functional groups on the FA molecules as well as on the bacterial cell walls are protonated and uncharged, and extensive hydrophobic adsorption can occur. Conversely, with increasing pH, these functional groups deprotonate and become negatively charged. Adsorption decreases markedly as electrostatic repulsion overwhelms the hydrophobic effects. However, when present in the system, Cd<sup>2+</sup> adsorbs to negatively charged bacterial surfaces. This cationic adsorption diminishes the net negative charge on the bacterial surface, thus decreasing the electrostatic repulsion between the FA and the bacteria. FA adsorption is thus enhanced without the formation of a FA–Cd–bacteria ternary complex. A similar surface charge effect can lead to enhanced metal adsorption onto mineral surfaces in the presence of organic acid anions (e.g., Venema et al., 1997; Boily and Fein, 1998; Nowack and Stone, 1999; Brown and Parks, 2001). Future work should include spectroscopic analysis in order to examine directly the mechanism responsible for the enhanced FA adsorption.

Although Cd enhanced FA adsorption at near-neutral pH, adsorption remained selective to the intermediate- to high-molecular-weight components. The fact that the molecular weight distribution of adsorbing components did not change is consistent with a gen-

eralized electrostatic effect, although more definitive mechanistic data are needed.

#### 4.3. Implications of the study results

The study results show that FA adsorbs to a bacterial surface, and that the adsorption is increased above pH 6 by the presence of Cd. These observations suggest that studies of organic matter uptake by bacteria need to take into consideration not only metabolism but also adsorption. Ongoing studies by our group (e.g., Young et al., in preparation) show that initial uptake (i.e., within the first hour or two of experiments) of natural organic matter by natural consortia of bacteria results primarily from adsorption to the bacterial surface, as studied in detail herein. This initial adsorption could be mistaken for metabolism, although it is selective to high-molecular-weight components rather than the more easily degraded low-molecular-weight components.

Overall, the study results illustrate the complexity of interactions that govern the movement of metals and organic materials through natural rock–water systems. Accurate predictions of material adsorption by bacteria in these systems will need to be based on knowledge of both the amount of dissolved organic matter and its primary molecular composition. It is clear that more studies are needed in order to characterize fully the interactions between bacteria, organic ligands, and multiple metal species across a wide range of environmental conditions.

#### Acknowledgements

We thank the National Science Foundation, Hydrologic Sciences Division, for partial funding of this research. We thank the Notre Dame Center for Environmental Science and Technology for use of analytical equipment. We thank Dr. George Aiken (U.S. Geological Survey, Boulder) for assistance with XAD-8 isolation of the fulvic acid sample used herein. [EO]

#### References

Aiken, G.R., 1985. Isolation and characterization techniques for aquatic humic substances. In: Aiken, G.R., McKnight, D.M.,

- Wershaw, R.L., MacCarthy, P. (Eds.), *Humic Substances in Soil, Sediment, and Water*. Wiley, New York, pp. 363–385.
- Alexander, M., 1977. *Introduction to Soil Microbiology*. Wiley, New York.
- Barnes, S.M., Nierzwicki-Bauer, S.A., 1997. Microbial diversity in ocean, surface, and subsurface environments. *Rev. Mineral.* 35, 35–79.
- Beveridge, T.J., Murray, R.G.E., 1980. Sites of metal deposition in the cell wall of *Bacillus subtilis*. *J. Bacteriol.* 141, 876–887.
- Beveridge, T.J., Makin, S.A., Kadurugamuwa, J.L., Li, Z.S., 1997. Interactions between biofilms and the environment. *FEMS Microbiol. Rev.* 20, 291–303.
- Boily, J.F., Fein, J.B., 1998. Adsorption of Pb(II) and benzenecarboxylates onto corundum. *Chem. Geol.* 148, 157–175.
- Brown, G.E., Parks, G.A., 2001. Sorption of trace elements on mineral surfaces: modern perspectives from spectroscopic studies, and comments on sorption in the marine environment. *Int. Geol. Rev.* 43, 963–1073.
- Cabaniss, S.E., Zhou, Q., Maurice, P.A., Chin, Y.-P., Aiken, G.R., 2000. A log-normal distribution model for the molecular weight of aquatic fulvic acids. *Environ. Sci. Technol.* 34, 1103–1109.
- Daughney, C.J., Fein, J.B., 1998. The effect of ionic strength on the adsorption of  $H^+$ ,  $Cd^{2+}$ ,  $Pb^{2+}$ , and  $Cu^{2+}$  by *Bacillus subtilis* and *Bacillus licheniformis*: a surface complexation model. *J. Colloid Interface Sci.* 198, 53–77.
- Davis, J.A., 1984. Complexation of trace metals by adsorbed natural organic matter. *Geochim. Cosmochim. Acta* 48, 679–691.
- Fein, J.B., 2000. Quantifying the effects of bacteria on adsorption reactions in water–rock systems. *Chem. Geol.* 169, 265–280.
- Fein, J.B., 2002. The effects of ternary surface complexes on the adsorption of metal cations and organic acids onto mineral surfaces. In: Hellman, R., Wood, S.A. (Eds.), *Water-Rock Interactions, Ore Deposits, and Environmental Geochemistry: A tribute to David A. Crerar*. The Geochemical Society Special Publication, vol. 7, pp. 365–378. St. Louis, MO.
- Fein, J.B., Delea, D., 1999. Experimental study of the effect of EDTA on Cd adsorption by *Bacillus subtilis*: a test of the chemical equilibrium approach. *Chem. Geol.* 161, 375–383.
- Fein, J.B., Daughney, C.J., Yee, N., Davis, T., 1997. A chemical equilibrium model for metal adsorption onto bacterial surfaces. *Geochim. Cosmochim. Acta* 61, 3319–3328.
- Fein, J.B., Boily, J.-F., Guclu, K., Kaulbach, E., 1999. Experimental study of humic acid adsorption onto bacteria and Al-oxide mineral surfaces. *Chem. Geol.* 162, 33–45.
- Fein, J.B., Martin, A.M., Wightman, P.G., 2001. Metal adsorption onto bacterial surfaces: development of a predictive approach. *Geochim. Cosmochim. Acta* 65, 4267–4273.
- Harden, V., Harris, J.O., 1952. The isoelectric point of bacterial cells. *J. Bacteriol.* 65, 198–202.
- He, L.M., Tebo, B.M., 1998. Surface charge properties of and Cu(II) adsorption by spores of the marine *Bacillus* sp. strain SG-1. *Appl. Environ. Microbiol.* 64, 1123–1129.
- Maurice, P.A., Pullin, M.J., Zhou, Q., Namjesnik-Dejanovic, K., Cabaniss, S.E., 2002. A comparison of surface water natural organic matter isolated by reverse osmosis and XAD resin adsorption. *Water Res.* 36, 2357–2371.
- Maurice, P.A., Manecki, M., Fein, J.B., Shaefer, J., 2003. Fractio-

- nation of natural organic matter upon adsorption to the bacterium, *Bacillus subtilis*. Geomicrobiol. J. (in press).
- Mullen, M.D., Wolf, D.C., Ferris, F.G., Beveridge, T.J., Flemming, C.A., Bailey, G.W., 1989. Bacterial sorption of heavy metals. Appl. Environ. Microbiol. 55, 3143–3149.
- Nowack, B., Stone, A.T., 1999. The influence of metal ions on the adsorption of phosphonates onto goethite. Environ. Sci. Technol. 33, 3627–3633.
- Plette, A.C.C., Benedetti, M.F., Van Riemsdijk, W.H., 1996. Competitive binding of protons, calcium, cadmium, zinc, to isolated cell walls of a gram-positive soil bacterium. Environ. Sci. Technol. 30, 1902–1910.
- Schindler, P.W., 1990. Co-adsorption of metal ions and organic ligands: formation of ternary surface complexes. In: Hochella, M.F., White, A.F. (Eds.), Mineral–Water Interface Geochemistry. Reviews in Mineralogy, vol. 23. Mineralogical Society of America, Washington, DC, pp. 281–307.
- Steelink, C., 1985. Implications of elemental characteristics of humic substances. In: Aiken, G.R., McKnight, D.M., Wershaw, R.L., MacCarthy, P. (Eds.), Humic Substances in Soil, Sediment, and Water. Wiley Interscience, New York, pp. 457–476.
- Warren, L.A., Haack, E.A., 2001. Biogeochemical controls on metal behaviour in freshwater environments. Earth-Sci. Rev. 54, 261–320.
- Wightman, P.G., Fein, J.B., 2001. Ternary interactions in a humic acid–Cd–bacteria system. Chem. Geol. 180, 55–65.
- Venema, P., Hiemstra, T., van Riemsdijk, W.H., 1997. Interaction of cadmium with phosphate on goethite. J. Colloid Interface Sci. 192, 94–103.
- Yee, N., Fein, J.B., 2002. Does metal adsorption onto bacterial surfaces inhibit or enhance aqueous metal transport: column and batch reactor experiments on Cd–*Bacillus subtilis*–quartz systems. Chem. Geol. 185, 303–319.
- Zhou, Q., Cabaniss, S.E., Maurice, P.A., 2000. Considerations in the use of high-pressure size exclusion chromatography (HPSEC) for determining molecular weights of aquatic humic substances. Water Res. 34, 3505–3514.