Modeling bacterial metal toxicity using a surface complexation approach

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A B S T R A C T

In this study, a detailed understanding of the metal binding reactions on bacterial cell envelopes was used to create an advanced biotic ligand model based on mechanistic adsorption reactions. A surface complexation model (SCM) was used to relate metal toxicity to the speciation and concentration of the metal adsorbed to the bacterial surface. Batch Bacillus subtilis growth measurements were used as a measure of Cd toxicity and to validate and calibrate this approach. Bacterial growth was measured by optical density (O.D.) in the presence of a constant concentration of Cd at either 1 or 2 ppm. EDTA was added to control the bacterial adsorption of Cd, using EDTA/Cd molar ratios of 0, 0.25, 0.5, 1 and 2. The toxic effect of Cd was quantified by measuring growth inhibition in terms of a parameter defined as the growth factor which is the experimental O.D. at a specific sampling time divided by the O.D. of the corresponding Cd-free control at the same sampling time. In all cases, a minimal growth medium was used to limit system components, allowing the precise calculation of aqueous and surface speciation of Cd for each experimental condition. These calculations used previously determined site-specific binding constants, acidity constants and site concentrations for the B. subtilis cell envelope functional groups. Cd toxicity increased with decreasing EDTA concentrations. A strong correlation between the total concentration of adsorbed Cd and the calculated growth factor was observed, with a dramatic increase in the growth factor when adsorption was below 0.31 μmol/g. The results suggest that biotic ligand models that incorporate surface complexation modeling yield flexible and accurate bioavailability models that can dramatically improve our understanding and ability to predict toxicity and other reactions linked to metal bioavailability in complex geologic systems.

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1. Introduction

Although early studies of metal bioavailability documented a relationship between metal toxicity to organisms and the total concentration of the metal in solution (e.g., Anderson, 1948; Bringmann and Kuhn, 1959; Warmick and Bell, 1969), more recent research indicates that not all dissolved metal species are bioavailable. The presence of metal-complexing ligands, such as dissolved organic matter or EDTA has been shown to exert a mitigating effect on metal toxicity (Zitko et al., 1973). Subsequent studies noted that the free ion metal concentration in solution correlates better to metal toxicity than does the total metal concentration (e.g., Anderson et al., 1978; Sunda and Gillespie, 1979; Allen et al., 1980) and the free-ion activity model (FIAM) of metal toxicity was developed based on these observations (e.g., Morel, 1983). In FIAMs, it is implicitly assumed that the mechanism of metal bioavailability to organisms is the adsorption of free metal ions to the organism through an equilibrium or pseudo-equilibrium reaction. Toxicity is related to the free metal ion activity rather than to the bound concentration of the metal as the free ion activity is more readily calculated than the bound metal concentration using available thermodynamic data and is a property that can be directly measured for some metals.

Although the FIAM is successful at describing metal bioavailability in some systems (e.g., Anderson et al., 1978; Sunda and Gillespie, 1979; Allen et al., 1980), the approach does not account for the effect of competitive cations or pH on metal bioavailability and toxicity. The addition of competitive cations or a change in system pH does not necessarily change the free ion concentration of the metal in question, but may dramatically alter the extent of the metal adsorbed onto the organism thereby reducing the bioavailability and hence the toxic effect of the metal. For example, the FIAM approach was found to fail at low pH values by over-estimating the predicted toxicity of metals, indicating a strong pH effect on metal bioavailability that is not accounted for by the FIAM (Parent and Campbell, 1994; Roy and Campbell, 1995). Some FIAM models were developed to account for H⁺ competition on the reactive site, and these models have had success in predicting toxicity of divalent metals in simple synthetic waters (Hare and Tessier, 1996). However, these FIAMs only account for H⁺ competition, and do not explicitly account for the adsorption of other cations or complexes onto the reactive site. In experiments which varied total Al in conjunction with fulvic acid concentrations in order to maintain a constant Al³⁺ free ion concentration, Parent et al. (1996) found that algal growth does not correlate well to the aqueous Al³⁺ activity. The FIAM fails...
because it is too simplistic; the model fails to account for the actual speciation of the metal on the organism of interest or the competitive adsorption of other aqueous species on the reactive site or sites (Playle and Dixon, 1993).

Biotic ligand models (BLMs) take a different approach to quantifying metal bioavailability. The BLM approach relates metal toxicity to the extent that the metal is adsorbed onto a hypothetical generic reactive adsorption site (termed the ‘biotic ligand’) on an organism (Di Toro et al., 2001; Santore et al., 2001; Paquin et al., 2002; Slaveykova and Wilkinson, 2005). The BLM, in quantifying the adsorption of the metal to the reactive site, can account for the adsorption of other cations (Ca^{2+}, Mg^{2+}, Na^+ and H^+) onto the reactive site, and for the effect of complexing aqueous ligands which can reduce the extent of metal adsorption. Due to the finite number of adsorption sites, the adsorption of other cations reduces the number of sites available for metal adsorption and decreases the toxicity of the metal to the organism. Several studies involving a range of organisms have successfully applied the BLM to account for metal toxicity in laboratory experiments (Meyer et al., 1999; Santore et al., 2001; De Schamphelaere and Janssen, 2002; Paquin et al., 2002).

Despite the success of the BLM in accounting for metal toxicity in some systems, there are severe limitations to BLMs as they are currently formulated. BLMs quantify metal adsorption at a single generic reactive site using parameters from empirical (non-mechanistic) adsorption models such as the Ks, Freundlich or Langmuir isotherms (Reid and McDonald, 1991; Playle and Dixon, 1993). Empirical models cannot account for changes in solution conditions because they are not based on balanced chemical reactions (Bethke and Brady, 2000; Koretsky, 2000), and hence BLMs fail when applied to systems with pH, ionic strength, or solute/sorbent concentration values different than those used to calibrate the model. Furthermore, bacterial cell envelopes contain multiple types of metal binding sites (Beveridge and Murry, 1980; Fein et al., 1997), including carboxyl, phosphoryl, amino, and sulfhydryl sites (Kelly et al., 2002; Boyanov et al., 2003; Guine et al., 2006; Mishra et al., 2009; Mishra et al., 2010). Each type of site has its own site concentration, proton and metal adsorption behaviors, and hence describing metal binding onto bacterial cell envelopes with a single generic biotic ligand site ignores the complexity of the binding environment within the cell envelope. Another potential problem with the BLM is that the approach accounts only for adsorption of free metal ions, but many metals undergo hydrolysis reactions or aqueous complexation reactions under environmentally-relevant pH conditions. The free metal ion can represent only a small percentage of the dissolved metal budget, but the aqueous metal hydrolysis products and other mixed ligand metal complexes can also adsorb onto bacteria and be bioavailable (Haas et al., 2001; Kenney and Fein, 2011; Sheng et al., 2011).

Over the past 15 years, our understanding of metal binding onto bacterial cells has advanced dramatically. Chemically-complex, site-specific models of metal binding onto bacterial cells have been developed (e.g., Fein et al., 1997; Cox et al., 1999; Haas et al., 2001; Ngwenya et al., 2003), so it is now possible to develop an advanced biotic ligand model (ABLIM) approach to account for metal toxicity to bacteria, quantifying metal binding with mechanistic adsorption reactions based on surface complexation modeling (SCM). Instead of the simplified single biotic ligand of the BLM approach, in this study we test an approach that uses surface complexation modeling to account for the effects of pH, competing cations, and aqueous metal speciation and complexation on metal toxicity. The SCM approach has been used to relate metal bioavailability as measured by the extent or rate of metal chemotaxis and enzymatic metal reduction to the extent and speciation of metal adsorbed onto bacterial cells (e.g., Borrok et al., 2005; Sheng et al., 2011). In this study, the detailed understanding of the metal binding reactions on bacterial cell envelopes is used to create an advanced biotic ligand model that relates metal toxicity to the speciation and concentration of the metal adsorbed to the bacterial surface. Batch growth experiments are used to measure Cd toxicity to Bacillus subtilis in order to validate and calibrate this approach, varying the extent of Cd adsorption through the addition of different concentrations of EDTA, a strong Cd chelator, to the experiments. Because the stability constants for the important aqueous and bacterial surface complexes involving Cd and the other metals that are present are known from previous work, we test whether a relationship exists between the calculated concentration of Cd on the bacterial cell envelope (the bioavailable Cd) and the observed Cd toxicity.

2. Materials and methods

2.1. Bacteria

B. subtilis, a common Gram-positive aerobic soil bacterium, was used in this study due to its well characterized cell envelope for which site concentrations, pKa values and stability constants for metal-bacteria complexes including those involving Cd are known (Boyanov et al., 2003; Fein et al., 2005; Wightman and Fein, 2005; Alessi and Fein, 2010; Alessi et al., 2010; Ginn et al., 2010; Mishra et al., 2010). A pure culture of B. subtilis ATCC 168 was obtained from American Type Culture Collection, Manassas VA, and stored at —80 °C in glycerol. Bacteria cells for the experiments were grown and maintained for up to 14 days on trypticase soy agar with 5% yeast extract at 20 °C.

2.1.1. Growth medium

A minimal growth medium was selected for batch growth experiments to limit the number of cations and anions in the system, to simplify the aqueous speciation and adsorption calculations. The medium was prepared as described in Fry et al. (2000) with the exception of antifoam B which was not included. The medium consisted of: 1 g of NH₄Cl, 0.04 g of trytophan, 1.0 g of KH₂PO₄, 2.72 g of KH₂PO₄, 0.284 g of Na₂SO₄, 0.17 g of NaNO₃, 0.15 g of KCl, 25 mg of MgCl₂ - 6H₂O, 2.16 mg of FeCl₃ - 6H₂O, 15 mg of MnCl₂ - 4H₂O, and 22 mg of CaCl₂ - 6H₂O per liter of ultrapure 18 MΩ water. Glucose was added as the primary carbon source (5 g/L). The growth medium was filter sterilized using a Nalgene 0.22 µm nylon filtration membrane. Cd was added from a prepared stock solution of Cd(ClO₄)₂ to achieve either 1 ppm or 2 ppm in the growth medium. EDTA was added from a stock solution of disodium EDTA, to achieve EDTA: Cd molar ratios of 0.025, 0.5, 1.0 or 2.0. The initial pH of the medium was measured to be approximately 7.0.

2.1.2. Growth experiments

For each experiment, bacteria from a trypticase soy agar slant were transferred to an autoclaved 250 mL starter flask containing 25 mL of sterile growth medium. This starter bacterial suspension was then cultured for 24 h at 32 °C on a platform rotating at 100 rpm. Each run consisted of 15 experiments in autoclaved flasks that were filled with treated medium (containing both Cd and EDTA) and 15 controls in autoclaved flasks that were filled with medium containing the same concentration of EDTA, but with no Cd. 250 µL of the bacterial starter suspension was added to each of the 30 flasks. All flasks were placed in an incubator at 32 °C on a platform rotating at 100 rpm. At 0, 8, 24, and 32 and 3 h, 3 treated flasks and 3 control flasks were removed for sampling. A cuvette was filled with the suspension from each flask and used to measure optical density (OD) as a measurement of cell density, using a Varian Cary 300 Bio UV–Visible spectrophotometer at 610 nm. An average O.D. reading for each 3 flask set was recorded for each time point during the run. Each 30-flask run was repeated three times.

2.1.3. Optical density–cell density conversion

Previous studies report bacterial binding site concentration in units of moles of sites per gram of wet bacteria (e.g., Fein et al., 2005). In order to calculate site concentrations in the experiments, optical density measurements needed to be converted to cell density values in units of grams per liter. To calibrate this conversion, cells were grown in 25 mL of growth medium at 32 °C for 24 h. The cells were then transferred to 1
L of medium and incubated at 32 °C in an orbital shaker rotating at 100 rpm for 24 h. A bacterial pellet was collected by centrifugation of the liquid culture at 7500 rpm for 5 min. The pellet was resuspended in 10 mL of 0.1 M NaClO₄ and centrifuged at 7500 rpm for 5 min. This rinse procedure was repeated five times. The decanted pellet was centrifuged at 7500 rpm for 60 min to remove all excess liquid for the determination of the bacterial wet weight. Bacteria were then resuspended to achieve cell concentrations ranging from 0.004 to 0.08 g (wet mass)/L. The O.D. values of these suspensions were then measured using the UV–vis at 610 nm and were used to determine the correlation between optical density and cell density (0.73 × Absorbance = wet weight of bacteria [g/L] with an R value of 0.99). O.D. absorption values varied from 0.001 to 0.06.

3. Results

3.1. Toxicity measurements

Bacterial growth was measured for each experiment and control as a function of the optical density at each sampling point for the duration of the experiment (the O.D. data can be found in Tables 1 and 2 of the Supplemental Information). The toxic response of the bacteria to Cd was measured as the growth inhibition relative to a Cd-free control, and is reported using a growth factor, calculated as the experimental O.D. divided by the O.D. of the corresponding Cd-free control at each time point. A growth factor of less than 1 indicates growth inhibition caused by Cd toxicity, and a growth factor of 1 indicates growth that was uninhibited by the presence of Cd. Fig. 1 shows a representative growth curve for one of the runs from the 1 ppm 1:1 EDTA:Cd experiment and its corresponding Cd-free control. The O.D. for theCd-bearing experiment shown in Fig. 1 at 24 h was 0.167 while the O.D. for the control was 0.211, resulting in a growth factor of 0.791. The diminished growth in the Cd-bearing experiment indicates a small but measureable toxic response of B. subtilis to 1 ppm Cd under the experimental conditions.

The effect of the EDTA treatments on the growth factor as a function of time is shown for the 1 ppm and 2 ppm Cd experiments in Fig. 2A and B, respectively. Time zero is defined as the time at which bacterial cells were first added to the experimental media. Therefore, because no growth occurred in the experiments prior to the first sampling, the experimental systems should and do show no significant difference in growth between the Cd-free controls and any of the EDTA treatment systems. The calculated growth factors at time 0 are all within 2 standard deviation units of a value of 1. After 8 h, all experiments had growth factors below 1 with 1:1 and 2:1 EDTA:Cd experiments exhibiting the highest growth factors. After 24 h in both the 1 ppm Cd and 2 ppm Cd experiments, the growth factors in experiments with

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**Fig. 1.** A representative growth curve showing Vis absorption values for an experiment with a 1:1 Cd:EDTA ratio and with 1 ppm Cd showing the 1:1 Cd:EDTA (open squares ♦) and the corresponding control (filled diamonds □).

**Fig. 2.** Bacterial growth represented as the growth factor as a function of experiment duration for the different EDTA treatments for the (A) 1 ppm Cd experiments and the (B) and 2 ppm experiments. Error bars represent 1 standard deviation from the mean values.

**Fig. 3.** The 24 hour growth factor as a function of the EDTA concentration for the 1 ppm Cd experiments (open circles ○) and for the 2 ppm Cd experiments (filled squares ■). Error bars represent 1 standard deviation from the mean values.
4. Discussion

The extent of Cd adsorption onto the B. subtilis cell envelope functional groups was calculated for all initial experimental conditions using a surface complexation modeling approach in order to determine if the observed toxicity effect is related to the concentration of Cd adsorbed onto the cell envelopes. The advantage of multi-site surface complexation modeling relative to modeling a single generic site (or a biotic ligand) is that the surface complexation approach more closely approximates the complexities of the binding environment within bacterial cell envelopes. Cell envelopes contain a number of functional group types that are capable of binding a wide range of metals. Carboxyl, phosphonyl, hydroxyl, amino, and sulfhydryl functional groups have been documented within bacterial cell envelopes (Beveridge and Murry, 1976; Beveridge and Murry, 1980; Boyanov et al., 2003; Mishra et al., 2010). Each of these types of binding sites is proton-active, yielding a cell envelope with fairly continuous buffering capacity over a pH range of at least pH 3 to 9.5. This buffering capacity is due to proton adsorption onto proton-active binding sites, and a number of modeling approaches have been invoked to account for the adsorption behavior (e.g., Plette et al., 1995; Fein et al., 1997; Cox et al., 1999; Yee et al., 2004; Fein et al., 2005). Surface complexation modeling ascribes the buffering behaviour of the cells to protonation/deprotonation reactions involving cell envelope organic acid functional groups:

\[ R - A_i(H)^0 \rightleftharpoons R - A_i^- + H^+ \]  

where \( R \) represents the bacterial cell envelope macromolecule to which each functional group type, \( A_i \), is attached. The acidity constant for each functional group type can be expressed as:

\[ K_a = \frac{[R - A_i^-][a_{H^+}]}{[R - A_i(H)^0]} \]  

where \( K_a \) represents the acidity constant of site \( R - A_i \), \( a_{H^+} \) represents the activity of the subscripted species, and brackets represent concentration in units of moles per liter. In this study, we use a non-electrostatic model of the protonation behavior, following the approach of Fein et al. (2005) using four discrete binding site types, each exhibiting individual acidity constant values and site concentrations. The acidity constants and site concentration values that were used in the calculations to characterize the sites on B. subtilis are listed in Table 3 in the Supplemental Information.

Metal adsorption onto deprotonated surface sites within the B. subtilis cell envelopes can be described by the following general reaction:

\[ R - A_i^- + M^{m+} \rightleftharpoons R - A_i(M)^{m-1} \]  

in which \( M \) represents a generic metal cation with a charge of \( m^+ \), and \( R - A_i(M)^{m-1} \) is the metal–bacterial surface complex. The extent of metal adsorption onto each bacterial cell envelope functional group type is quantified by the following mass action equation:

\[ K_{ads} = \frac{[R - A_i(M)^{m-1}]}{[R - A_i^-][a_{M^{m+}}]} \]  

where \( K_{ads} \) represents the stability constant for reaction 3. Experimental studies of metal adsorption onto bacterial surfaces have produced stability constants for species- and site-specific metal–bacteria surface complexes (e.g., Fein et al., 1997; Daughney et al., 1998; Fowle and Fein, 2000; Haas et al., 2001; Yee and Fein, 2001; Ngwenya et al., 2003). Metal adsorption reactions are rapid, reversible, and strongly pH dependent (e.g., Fowle and Fein, 1999, 2000), with increasing cell envelope adsorption with increasing pH. These results are consistent with metal cation adsorption onto deprotonated surface functional groups, and with proton competition for these sites under low pH conditions. In addition, equilibrium constants for metal–bacterial surface complexes that are derived from single metal, single bacterial species experiments can be used to successfully predict the extent of adsorption in more complex multi-component systems, successfully accounting for the competitive adsorption between metal cations (Delea and Fein, 1999; Fowle and Fein, 1999; Yee and Fein, 2003; Alessi and Fein, 2010).

The extent of bacterial Cd adsorption under each experimental condition was calculated using the mass action and mass balance equations listed in Tables 2 and 3 in the Supplemental Information Martell and Smith (2001) and Jenson (2003). Although the aqueous speciation of Cd is calculated to be dominated by aqueous Cd complexes, we modeled adsorption as only Cd\(^{2+} \) binding onto the bacterial sites. The stability constants that have been determined for Cd binding onto bacteria only involve the adsorption of Cd\(^{2+} \). Furthermore, the dominant aqueous Cd complexes that form under the experimental conditions are Cd-EDTA\(^{-2} \) and Cd(HPO\(_4\))\(^{3+} \), neither of which are likely to adsorb significantly onto the negatively-charged bacteria. The following cations are present in the experimental media: Na\(^+ \), K\(^+ \), Fe\(^{3+} \), Mg\(^{2+} \), Ca\(^{2+} \), Mn\(^{2+} \), and Cd\(^{2+} \). The extent of adsorption of Cd\(^{2+} \) and Fe\(^{3+} \) onto bacterial sites under our initial experimental conditions was calculated using available stability constants from Alessi and Fein (2010) and Wightman and Fein (2005), respectively. Monovalent cation adsorption was calculated using the universal binding constant described by Alessi et al. (2010), and the binding constants for the remaining divalent cations were calculated using the linear free energy relationship with acetate defined by Ginn et al. (2010). The anions in the media include Cl\(^- \), PO\(_4\)^{3-} \), SO\(_4\)^{2-} \), NO\(_3\) \(^- \) and tryptophan, which is an amino acid with an amine and a carboxyl group available for protonation and metal complexation. Because the experiments were conducted open the atmosphere, the aqueous carbonate species within the system were assumed to be in equilibrium with atmospheric CO\(_2\). The bacterial surface and aqueous speciation reactions are shown in Tables 1 and 2, respectively, of the Supplemental Information, and the mass balance constraints considered in these calculations are listed in Table 4 of the Supplemental Information. A non-commercial Fortran program which includes a Newton–Raphson iteration technique was used to solve the series of non-linear mass action and mass balance equations and to calculate the equilibrium concentrations of all aqueous and adsorbed species in the experimental system. The site concentration for bacterial binding sites was calculated using the previously determined site concentrations for B. subtilis by Fein et al. (2005). pH was fixed in the calculations to the experimental value of 7.0. Activity coefficients were calculated using the extended Debye–Hückel equation, using the approach and parameters of Helgeson et al. (1981). The \( b \) and \( v \) values for the dominant electrolyte in the system, NH\(_4\)Cl, were used for these calculations.

The calculated distribution of aqueous Cd under the range of experimental conditions is shown in Fig. 4. The figure depicts the concentration of the Cd(EDTA\(^{-2} \) complex along with the concentration of the three most-concentrated Cd species, which under the experimental conditions are Cd-HPO\(_4\), Cd\(^{2+} \), and CdCl\(^+\). Cd(EDTA\(^{-2} \) is relatively unimportant in the total Cd budget for the 0.25:1 EDTA:Cd ratio condition, but increases in importance with increasing EDTA concentration to 19, 63, and 95% of the total aqueous Cd budget in the 0.5:1, the 1:1, and the 2:1 EDTA:Cd systems, respectively for the 1 ppm system; and represents 34, 78, and 98% of the aqueous Cd budget for the same EDTA:Cd ratio in the 2 ppm system. The effects of the EDTA treatments on the calculated concentration of adsorbed Cd are shown in Fig. 5A and B. These calculations are conducted for the initial bacterial concentrations in each system prior to growth during the experiment, and normalized to the average initial mass of bacteria used under each condition considered. The initial concentrations of adsorbed Cd in the 0.1 treatments for the 1 ppm and 2 ppm experiments were 0.38 and 0.73 µmol/g,
Cd concentration and the observed growth factor, supporting the 2 ppm Cd experiments yield similar relationships between the adsorbed factor to values near 1.0 for the highest EDTA:Cd ratios studied. The 1 and decreases below 0.31 1 ppm and 2 ppm Cd experiments, as the concentration of adsorbed Cd cell envelope and the bacterial growth factor (Fig. 6). For both the between the calculated concentration of Cd adsorbed onto the bacterial 

described in our study. For example, Fig. 7 shows the predicted growth factors for B. subtilis as a function of goethite concentration at pH 7 in a system containing 1 ppm Cd, 7 g/L of B. subtilis and at an ionic strength of 0.05 M. At low concentrations of goethite, the extent of Cd adsorption onto the bacteria exceeds 0.31 μmol/g. As shown in Fig. 6 from our experiments, this level of adsorbed Cd is toxic to the cells and no growth would occur in the system. However, increasing the concentration of goethite enables the mineral surface to effectively compete with the bacteria for the available Cd, and the bioavailability of the Cd decreases dramatically. Above a concentration of approximately 2.0 g/L, the goethite adsorbs enough Cd to reduce the concentration of Cd that adsorbs

respectively, and the 0.25:1 EDTA:Cd experiments exhibited similar initial adsorbed Cd concentrations (adsorption data for each run can be found in Tables 1 and 2 of the Supplemental Information). For the experiments with EDTA:Cd ratios above 0.25:1, the concentration of adsorbed Cd decreases with increasing EDTA in the system due to the increased importance of the Cd-EDTA aqueous complex. The lowest adsorbed Cd concentrations were in the 2:1 EDTA:Cd experiments, with the calculated adsorbed Cd concentration being over an order of magnitude lower than the values calculated for the 0:1 experiments. As Fig. 5 demonstrates, due to the low concentration of bacteria in these experiments, the concentration of Cd adsorbed onto the bacterial cells is only a small component of the total Cd in the system, with significantly less than 1% of the total Cd present as adsorbed Cd. Adsorbed Cd species represent approximately 0.12 and 0.25% of the total concentration of available bacterial sites under the initial conditions of the 0:1 EDTA:Cd experiments for the 1 ppm and 2 ppm treatments respectively.

Adsortion of Cd is clearly not the only process involved in the bioavailability and toxicity of Cd to the cells, and other processes such as Cd internalization may be involved. However, adsorption is a necessary step in making the metal bioavailable, and is likely the initial step preceding these other secondary processes. Our results suggest that even low concentrations of Cd adsorbed onto the cell can result in a toxic response growth inhibition. At adsorbed Cd concentrations of 0.31 μmol/g and above, our experiments demonstrate growth factors near zero (Fig. 6), indicating a strong toxic response to the adsorbed Cd. Below a concentration of adsorbed Cd of 0.31 μmol/g, an inverse relationship is observed between the calculated concentration of Cd adsorbed onto the bacterial cell envelope and the bacterial growth factor (Fig. 6). For both the 1 ppm and 2 ppm Cd experiments, as the concentration of adsorbed Cd decreases below 0.31 μmol/g, we observed a dramatic increase in growth factor to values near 1.0 for the highest EDTA:Cd ratios studied. The 1 and 2 ppm Cd experiments yield similar relationships between the adsorbed Cd concentration and the observed growth factor, supporting the underlying assumption in our model that the concentration of Cd adsorbed onto the bacterial cells is a controlling factor in Cd bioavailability under the experimental conditions and can be used to predict toxicity.

The effects of pH, ionic strength, competing cations, metal-binding ligands, competing adsorption surfaces, and ternary complexation can all be estimated using a SCM approach by calculating the extent of metal adsorption and relating it to a growth factor using the approach described in our study. For example, Fig. 7 shows the predicted growth factors for B. subtilis as a function of goethite concentration at pH 7 in a system containing 1 ppm Cd, 7 g/L of B. subtilis and at an ionic strength of 0.05 M. At low concentrations of goethite, the extent of Cd adsorption onto the bacteria exceeds 0.31 μmol/g. As shown in Fig. 6 from our experiments, this level of adsorbed Cd is toxic to the cells and no growth would occur in the system. However, increasing the concentration of goethite enables the mineral surface to effectively compete with the bacteria for the available Cd, and the bioavailability of the Cd decreases dramatically. Above a concentration of approximately 2.0 g/L, the goethite adsorbs enough Cd to reduce the concentration of Cd that adsorbs

![Fig. 4. Calculated aqueous Cd speciation for (A) the 1 ppm Cd experiments and (B) the 2 ppm Cd experiments. Only species that account for more than 0.03% of the total aqueous Cd are shown.](image)

![Fig. 5. Calculated adsorbed Cd concentrations as a function of EDTA:Cd ratio for the (A) 1 ppm Cd experiments and (B) 2 ppm Cd experiments.](image)

![Fig. 6. Cd toxicity reported as the growth factor as a function of the concentration of Cd adsorbed onto the bacteria after 24 h of growth. The 1 ppm Cd experiments are represented as hollow circles ○ and 2 ppm Cd experiments by filled squares ■.](image)
Fig. 7. The effect of goethite on the predicted growth factor for Bacillus subtilis at pH 7 in a system containing 1 ppm Cd, 7.7 g of B. subtilis and at an ionic strength of 0.03 M. These calculations used values for the Cd-goethite binding constants from Morsad (1999). Mass action equation shown in Table 5 of the Supplemental Information.

onto the bacteria to below 0.31 μmol/g, allowing bacterial growth, and an increasing growth factor is predicted for increasing goethite concentrations above 2.0 g/L (Fig. 7).

5. Conclusions

The SCM approach that we describe in this study represents an advancement of the traditional biotic ligand model to a more mechanistic one that can account for the heterogeneous nature of the bacterial cell envelope binding environment. Like the biotic ligand model, the SCM approach assumes that the adsorbed metal represents the bioavailable component. The model relates metal toxicity and bioavailability to the concentration of metal adsorbed onto an organism, thereby accounting for the effects of competitive adsorption and metal speciation in solution on the toxicity of the metal to the bacteria. However, biotic ligand models typically involve a single binding site on an organism and relate toxicity to the extent of metal binding that occurs on that single generic site. Because the SCM approach accounts for the multiple binding site types that exist within cell envelopes and for the differences between these binding sites, toxicity models based on surface complexation modeling should be more flexible and accurate than those based on more simplistic biotic ligand models.

With a SCM approach, it becomes possible to examine differences in bioavailability of different bacterial surface complexes, further improving the accuracy of these bioavailability models. For example, Sheng et al. (2011) found that enzymatic U(VI) reduction by Shewanella oneidensis is not related to the total adsorbed U(VI) concentration, but rather to the concentration of only certain bacteria-bound uranium species. Similarly, Hu et al. (2013) noted a dependence of Hg(II) bioavailability on the speciation of bacterially adsorbed Hg. Toxicity and other adsorption-related metabolic processes may involve only certain metal–bacterial site complexes, and hence accurate bioavailability models require the mechanistic understanding that the SCM approach enables. The approach outlined and tested in this study paves the way for the application of surface complexation modeling to be used to quantitatively model adsorption-related processes, and enables the prediction of metal bioavailability under conditions other than those directly studied in the laboratory.

Acknowledgments

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Appendix A. Supplementary data

A PDF document containing experimental details of metal analysis, a table of raw experimental O.D. data and calculated adsorbed Cd, a table of bacterial site binding constants, a table of aqueous complexation reactions considered in Cd experiments and a table of mass action constraints for speciation calculations. Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.chemgeo.2014.03.010.

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