



Surface complexation modeling of Cd(II) sorption to montmorillonite, bacteria, and their composite Ning Wang^{1,2,*}, Huihui Du^{1,2,*}, Qiaoyun Huang^{1,2,**}, Peng Cai^{1,2}, Xingmin Rong² and Wenli Chen^{1,**} ¹ State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, China. ² Key Laboratory of Arable Land Conservation (Middle and Lower Reaches of Yangtze River), Ministry of Agriculture, College of Resources and Environment, Huazhong Agricultural University, Wuhan 430070, China. *These authors contributed equally to this work. **Corresponding author: Q. Huang, E-mail: <u>qyhuang@mail.hzau.edu.cn</u>; W. Chen, wlchen@mail.hzau.edu.cn; Tel: +86-27-87671033; Fax: +86-27-87280670





28 Abstract

29	Surface complexation modeling (SCM) has emerged as a powerful tool for
30	simulating heavy metal adsorption processes on the surface of soil solid components
31	under different geochemical conditions. The component additivity (CA) approach is
32	one of the strategies that have been widely used in multi-component systems. In this
33	study, potentiometric titration, isothermal adsorption, zeta potential measurement, and
34	extended X-ray absorption fine structure (EXAFS) spectra analysis were conducted to
35	investigate Cd adsorption on 2:1 clay mineral montmorillonite, on Gram-positive
36	bacteria Bacillus subtilis, and their mineral-organic composite. We developed constant
37	capacitance models (CCM) of Cd adsorption on montmorillonite, bacterial cells, and
38	mineral-organic composite. The adsorption behavior of Cd on the surface of the
39	composite was well explained by CA-SCM. Some deviations were observed from the
40	model simulations at $pH < 5$, where the values predicted by the model were lower
41	than the experimental results. The Cd complexes of X_2Cd , $SOCd^+$, $R\text{-}COOCd^+$, and
42	$\ensuremath{\text{R-POCd}^{\scriptscriptstyle +}}\xspace$ were the predominant species on the composite surface over the pH range
43	of 3 to 8. The distribution ratio of the adsorbed Cd between montmorillonite and
44	bacterial fractions in the composite as predicted by CA-SCM closely coincided with
45	the estimated value of EXAFS at pH 6. The model could be useful for the prediction
46	of heavy metals distribution at the interface of multicomponents and their risk
47	evaluation in soils and associated environments.

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50 1. Introduction

51 Anthropogenic activity and industrialization release large quantities of heavy 52 metals into soils (Fomina and Gadd, 2014; He and Chen, 2014). The interactions between soil solid components and heavy metals in complex natural systems should 53 54 be understood in order to predict their transport and fate. Surface complexation 55 modeling (SCM) is a powerful tool for describing adsorption processes and 56 mechanisms under different geochemical conditions (Wang and Giammar, 2013; Dong and Wan, 2014). SCM has been adopted to simulate the adsorption of heavy 57 metals on the surface of soil solid components such as kaolinite (Angove et al., 1997), 58 59 montmorillonite (Bradbury et al., 2005; Ikhsan et al., 2005; Akafia et al., 2011; 60 Benedicto et al., 2014; Soltermann et al., 2014), magnetite (Kim et al., 2012), manganese oxide (Wang et al., 2013), fulvic and humic acid (Christl et al., 2001; 61 Milne et al., 2003; Koopal et al., 2005), natural organic matter (Koopal et al., 2005; 62 63 Gustafsson et al., 2014), and bacteria (Fein et al., 2001; Daughney et al., 2001; Borrok 64 et al., 2004; Kenney and Fein, 2011; Borrok and Fein, 2005; Moon and Peacock, 2011; 65 Liu et al., 2013).

Soil solid components, which include bacteria and clay minerals, often exist as composites in natural environments. The component additivity (CA) approach has been widely used in the application of surface complexation models for describing heavy metal adsorption on mixtures and on the composites of soil solid components. The component additivity-diffuse layer model (CA-DLM) has been successfully applied to model the adsorption of Co^{2+} (Landry et al., 2009), Cd^{2+} (Reich et al., 2010),





72	and Pb ²⁺ (Reich et al., 2010), on mixtures of hydrous ferric oxide (HFO) and quartz
73	with kaolinite. Dong and Wan (2014) indicated that the CA-SCM can provide
74	excellent predictions for uranium (VI) adsorption on quartz-sand dominated sediments.
75	Fowle and Fein (1999) concluded that the CA approach can be used to model the
76	adsorption of Ca^{2+} , Cu^{2+} , Cd^{2+} , and Pb^{2+} on mixtures of <i>Bacillus licheniformis</i> and <i>B</i> .
77	subtilis. Additionally, CA could successfully describe Cd, Co, Sr, and Zn adsorption
78	onto the mixtures of 10 species of Gram-positive bacteria (Yee and Fein, 2001). For
79	the mixtures of minerals and organic systems, the extended constant capacitance
80	model was used to depict Cd^{2+} adsorption onto goethite, kaolinite, and muloorina illite
81	in the presence of citric acid (Lackovic et al., 2004a,b). The linear additivity model
82	has succeeded in predicting Cu^{2+} adsorption, but has failed to simulate Ca^{2+}
83	adsorption on goethite in the presence of fulvic acid (Weng et al., 2008). The
84	adsorption of Cd ²⁺ onto Comamonas sppferrihydrite composite could be accurately
85	predicted by the addition of the reaction between end-member ferrihydrite and
86	bacteria (Song et al., 2009). However, the amount of Cd^{2+} adsorption onto the
87	composite was up to 10% lower than that predicted by the additive approach. In the
88	study of Alessi and Fein (2010) the CA approach was applied to describe the extent of
89	Cd adsorption on the surface of two- and three-component mixtures of B. subtilis cells,
90	HFO, and kaolinite at various mass ratios in the presence of acetate. The models
91	matched well with experimental data in the absence of acetate, whereas they
92	underestimated Cd adsorption in the presence of acetate. Moon and Peacock (2013)





- 93 found that Cu adsorption behavior on ferrihydrite–*B. subtilis* composites with 94 ferrihydrite as the major component can be modeled by a component additivity 95 approach. By contrast, composites with a majority of bacteria component cannot be 96 simulated by a component additivity approach, and significant deviations were 97 observed.
- 98 Previous studies concerning surface complexation models have mainly focused 99 on heavy metal adsorption by individual minerals or bacteria or mineral-organics and 100 metal oxide-bacteria composites. To the authors' knowledge, no SCM investigation 101 using 2:1 clay mineral-bacteria composites, has been performed. Montmorillonite, a 102 representative of the 2:1 phyllosilicates, exhibits large specific surface area and high 103 cation exchange capacity, which make it an effective adsorbent for heavy metals. The 104 clay mineral is widely distributed in a variety of soils in temperate zone. The model 105 developed in clay mineral-bacteria composites will provide insights into the 106 behaviors of heavy metals in contaminated soils of these regions. Thus, in this study, 107 we first develop surface complexation models for Cd adsorption on end-member 2:1 108 clay mineral montmorillonite, Gram-positive bacteria Bacillus subtilis, and finally 109 their mineral-organic composite by using the component additivity (CA) approach. 110 Moreover, linear combination fitting of the extended X-ray absorption fine structure 111 (EXAFS) spectra were applied to verify the distribution ratio of Cd between different 112 components derived from CA-SCM.
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114 2. Materials and Methods





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116	2.1. Adsorbent
117	Montmorillonite, (Sanding Group Co. Ltd., Shaoxing, Zhejiang, China), was
118	prepared following previously outlined procedures (Hong et al., 2012; 2013). The
119	clays were oxidized using H_2O_2 (30%) to remove any residual organic matters. The <
120	$2\ \mu m$ colloidal fractions were isolated through sedimentation. The specific surface
121	area, determined by the BET method (N $_2$ adsorption), was found to be 60.2 $m^2\ g^{\text{-1}}.$
122	Previous study showed that a particle was estimated to be formed of nearly 20 stacked
123	layers in the dehydrated state used in the gas adsorption experiment (BET' method),
124	whereas it was estimated to be composed of only 1 or 2 layers in aqueous suspension
125	(Tournassat et al., 2003). Thus, the values derived from BET' method usually
126	underestimate the actual total surface area in aqueous. Herein, we set the SSA of
127	montmorillonite to be 800 m ^{2} g ⁻¹ , and the detailed processing procedures are shown in
128	Supporting Information.

129 The test bacteria used in this study came from the aerobic Gram-positive species B. subtilis, which were obtained from the State Key Laboratory of Agricultural 130 131 Microbiology. The microorganisms were inoculated into 10 mL LB medium and 132 cultured for 7 h at 28 °C to reach the exponential phase with an optical density of 1.4 133 at 600 nm (OD600 nm). Subsequently, 2 mL of the resulting bacterial suspension was transferred to 200 mL LB medium for another 18 h inoculation (OD_{600nm}=2.7) to 134 135 reach the mid-exponential phase. Cells were pelleted by centrifugation at 136 approximately 7200 g for 5 min and rinsed three times in ultrapure water. The ratio of





- 137 the wet weight to dry (60 °C in drying oven) weight of the biomass is 9:1. The
- 138 biomass mentioned in this paper all refer to dry weight.

139 Montmorillonite and the bacterial cells were suspended in 0.01 M NaNO₃ to 140 produce the suspensions that experiments. The were used in the 141 montmorillonite-bacteria composite was prepared following a montmorillonite: B. 142 subtilis mass ratio of 7:1 to produce suspensions (4 h, 28 °C). The resultant 143 montmorillonite-bacteria composite suspensions were used for subsequent experiments. Preliminary isothermal adsorption (pH 8) of bacteria to montmorillonite 144 145 results showed that the maximum adsorption predicted by Langmuir equation was 146 approximately 0.14 g (dry cell)/ g (clay) which was equal to a mass ratio (clay to 147 bacteria) of 7:1 (Supporting Information, Figure S1). Clearly adsorption of bacteria on 148 montmorillonite decreased with the increase of pH, thus bacteria can be completely 149 adsorbed by montmorillonite to a large extent in all experimental conditions (pH 150 3-8.5) at this ratio. The bacterial cells were remain intact under the experimental 151 condition (see AFM images of the Supporting Information, Figure S2).

152 2.2. Reagents

153 Analytical reagent grade chemicals were used throughout the study. $Cd(NO_3)_2$ 154 was used to prepare 178.0 μ mol L⁻¹ Cd(II) stock solution. Water was Milli-Q reagent 155 grade.

156 2.3. Zeta potential analysis

Zeta potential analysis was conducted to study the surface charge properties of
 montmorillonite, *B. subtilis*, and montmorillonite–*B. subtilis* composite. Suspensions





- 159 were prepared the same as section 2.1, over the pH range of 3 to 8. Samples were
- 160 injected into a zeta potential analyzer (Zetaplus90, Brookhaven), and measured in
- 161 triplicate.
- 162 2.4. Potentiometric titrations
- 163 Titration experiments were performed (in duplicate) according to the procedures 164 described by Fein et al. (2005). The suspensions (1 g L⁻¹, 40 mL) of montmorillonite 165 and *B. subtilis* and the composite in 0.01 M NaNO₃ were placed in a sealed titration 166 vessel maintained under a positive pressure of N₂. Titrations were conducted using an 167 automatic potentiometric titrator (Metrohm titrator 836, Switzerland). Each 168 suspension was first titrated to pH 3 by using 0.1136M HNO₃ solutions and then to 169 pH 8.5 by using 0.0976 M NaOH. At each titration step, a stability of 0.01 mV S⁻¹ was
- 170 attained before adding the next drop of the titrant.
- 171 Gran plot was adopted to determine the specific volume of titrant added at the
- 172 equivalence point (Ve) and to derive the total H⁺ concentration (TOTH) (Liu et al.,
- 173 2013; Du et al., 1997; Liu et al., 1999; Chu et al., 2002).
- 174 The values for the Gran function (G) were calculated as follows:
- 175 On the acidic side: $G_a = (V_0 + V_{at} + V_b) \times 10^{-pH} \times 100$
- 176 On the alkaline side: $G_b = (V_0 + V_{at} + V_b) \times 10^{-(13.8-pH)} \times 100$

Gran plots for titration data on montmorillonite and *B. subtilis* are illustrated in Figure S3, where V_0 stands for the initial volume of the titrimetric suspension; and V_a and V_b represent the total volume of acid and alkaline solution added at each titration point, respectively. In the Gran plots, the OH⁻ added to a suspension firstly neutralizes





181 the excess H⁺ before V_{eb1} , then reacts with the functional group on the surface of the 182 sorbent between V_{eb1} and V_{eb2} , finally changes the pH after V_{eb2} . No apparent surface 183 reactions consuming OH⁻ before V_{eb1} were noted. The total concentration of reacting 184 H⁺ at each titration point (TOTH) was calculated using the following equation, where 185 C_b stands for NaOH concentration:

186
$$TOTH = \frac{-(V_b - V_{eb1}) \times C_b}{V_0 + V_{at} + V_b} \quad (mol/L),$$

187 **2.5. Adsorption experiment**

188 The adsorption edge experiments were conducted in 0.01 M NaNO₃ solutions to investigate the adsorption of Cd onto montmorillonite, B. subtilis and the composite. 189 190 15 mL 178.0 μ mol L⁻¹ Cd(II) solution and 15 mL 2 g L⁻¹ adsorbent suspensions were 191 added to 50 mL centrifuge tubes. A suitable volume of concentrated HNO₃ or NaOH 192 solutions was added to adjust the pH of the suspensions from 3 to 8. The finial concentration of Cd and adsorbents were 89.0 μ mol L⁻¹ and 1 g L⁻¹, respectively. 193 Fixed pH isotherm experiments were performed at pH 6 and 8 in 0.01 M NaNO₃ 194 electrolyte. The initial concentrations of Cd ranged from 8.9-89.0 µmol L⁻¹ with a 195 sorbent concentration of 1 g L⁻¹. The mixtures were allowed to equilibrate for two 196 197 hours at 28 °C. After equilibration, the final suspensions were centrifuged at 12557 g 198 for 5 min, and the clear supernate was analyzed using atomic adsorption spectroscopy 199 (AAS; Varin AAS240FS) to determine the residue Cd.

200 Solution speciation of 89.0 μ mol L⁻¹ Cd(II) in 0.01M NaNO₃ was calculated as a 201 function of pH using the geochemical software Visual MINTEQ 3.1. Cd²⁺ was





- 202 determined to be the main species at the experimental conditions. Cd(OH)₂, CdOH⁺,
- and Cd_2OH^{3+} were also found at pH > 7.8, but in relatively small percentages.

204 2.6. X-ray absorption spectroscopy

We selected pH 6 sample of the adsorption edge experiments in section 2.5 as 205 206 representative to perform the XAS experiments. The solid phases after centrifugation 207 were transformed immediately to collect XAS spectra in 12 h. The sample holders were packed together with moist tissues (stored at ~ -4° C) to minimize sample drying 208 209 during shipment to the Shanghai Synchrotron Radiation Facility (SSRF), China. The X-ray absorption data at the Cd K-edge (~26700 keV) of the samples were recorded at 210 211 room temperature in the fluorescent mode with silicon drift fluorescence detector. The 212 station was operated with a Si(III) double crystal monochromator. During the 213 measurement, the synchrotron was operated at energy of 3.5 GeV and a current 214 between 150-210 mA. EXAFS spectra were collected from 26511 to 27551 eV. 215 Background subtraction and normalization of were conducted in ATHENA, a program 216 in the IFEFFIT package (Ravel and Newville, 2005). The background subtraction was 217 performed using PySpline. The pre-edge was fit to a linear function and the post-edge 218 background to two 2nd-order polynomial segments. To obtain information on the 219 partitioning of Cd between the different fractions of the composites, the spectra were 220 fitted using a linear combination fitting procedure (LCF). Cd-adsorbed 221 montmorillonite, Cd-bacteria were used as end-member for the 2-component fitting of the binary composites, with linear combination performed over the k^3 -range of 2.3-10 222 $Å^{-1}$ (force weight to sum to 1). The goodness of the fitting was evaluated by the 223 10





best-fit criterion values (*R*) (Du et al., 2016).

225 2.7. Surface complexation modeling

226	The program FITEQL 4.0 was utilized to determine functional group site
227	concentrations, proton, and metal binding stability constants (Herbelin and Westall,
228	1999). A constant capacitance model was implemented (Tertre et al., 2006). The
229	activity coefficient correction was made within FITEQL using the Davies equation.
230	An indicator of the goodness of the fitting is the overall variance, V, in Y. ⁴⁴
231	$V_{y} = \frac{WSOS}{DF}$, where WSOS is the weighted sum of squares of the residuals and DF is
232	the degrees of freedom. Lower $V_{\rm Y}$ values signify better fits. The systematic process of
233	developing the SCM was started by fitting the model to the experimental data for
234	montmorillonite and <i>B. subtilis</i> .
235	The potentiometric titration data was fit to generate the acid-base constants and

surface site densities (Figure S4). Acid-base reactions on the surface ofmontmorillonite are represented by the following equations:

$$238 \qquad \text{SOH} \Leftrightarrow \text{SO}^- + \text{H}^+ \tag{1}$$

$$239 \qquad \text{SOH} + \text{H}^+ \, \Leftrightarrow \, \text{SOH}_2^+ \tag{2}$$

$$240 \qquad \text{WOH} \, \Leftrightarrow \, \text{WO}^- + \text{H}^+ \tag{3}$$

 $241 X^{-} + H^{+} \Leftrightarrow XH (4)$

242
$$X^{-} + Na^{+} \Leftrightarrow XNa$$
 (5)

where SOH and WOH are aluminol and silanol sites on the surface of montmorillonite, respectively. X⁻ is the negative exchange site that can react with both H⁺ and Na⁺. The total concentration of exchange site and the binding constants of XH,





246	XNa were used as fixed input parameters when the titration data was fitted. These
247	three values were 8.7×10^{-4} mol/g, -2.2 and 1.4, respectively (Table S1), which come
248	from Tertre et al. ⁴⁶ and Soltermann et al. ¹⁰ The acid-base constant and the site
249	density of SOH and WOH were set as iterated parameters, in addition, these
250	parameters were iterated simultaneously.

251 The acid-base reactions on the surface of *B. subtilis* were represented by

252
$$\operatorname{R-L}_{i}\operatorname{H}^{0} \Leftrightarrow \operatorname{R-L}_{i}^{-} + \operatorname{H}^{+}$$
 (6)

where R represents the bacterium to which each functional group is attached. Carboxyl sites, phosphate sites, and hydroxyl sites were considered to be the main functional groups on the surface of *B. subtilis* (Yee and Fein, 2001). L_iH^0 represents these three groups.

Acid-base equilibrium constants and site densities (Tables S1 and S2) were used as fixed input parameters in the modeling of the Cd pH adsorption edge and fixed pH isotherm data. The constants of Cd complexes with the functional groups on each sorbent were iterated simultaneously. The following equilibria were used to describe Cd(II) adsorption onto montmorillonite:

$$262 \qquad 2X^{-} + Cd^{2+} \Leftrightarrow X_2Cd \tag{7}$$

263
$$\operatorname{SOH} + \operatorname{Cd}^{2+} \Leftrightarrow \operatorname{SOCd}^+ + \operatorname{H}^+$$
 (8)

Due to their higher pK_a , hydroxyl sites are not deprotonated over the range of pH 3 to 8. To simplify the model, hydroxyl sites were not invoked. Cd(II) adsorption on *B*. *subtilis* was described with the following reaction equilibria:





267		
268	$R-COO^{-} + Cd^{2+} \Leftrightarrow R-COOCd^{+}$	(9)
269	$R-PO^{-} + Cd^{2+} \Leftrightarrow R-POCd^{+}$	(10)
270	Finally, we combined the model of Cd(II) adsorption on montmorillo	nite and <i>B</i> .
271	subtilis to simulate the adsorption behavior of Cd(II) on the composite. E	nd-number
272	montmorillonite and B. subtilis models were combined in FITEQL 4.0 us	ing the CA
273	approach.	
274		
275	3. Results	
276		
277	3.1. Zeta potential	
278	The zeta potential of montmorillonite, B. subtilis, and montmorillonite	rillonite– <i>B</i> .
279	subtilis composite are given in Figure 1. Over the whole pH range stu	idied, both
280	montmorillonite and the composite had negative surface charge with very	low pH _{pzc}
281	around pH 3.1. The bacteria displayed a negative surface charge at $pH > 3.5$	5 and had a
282	higher pH_{pzc} than the value reported by Fein et al. (2005) (pH_{pzc} < 2). The	e negative
283	electrokinetic charge of all sorbents increased with pH and remained con	stant when
284	pH was higher than 5. The zeta potential of montmorillonite and bacteria	were -2.29
285	mV and 12.27 mV at pH 3, -26.70 mV and -19.46 mV at pH 8. The sur	face of the
286	montmorillonite-bacteria composite was more negatively charged that	an that of
287	montmorillonite and <i>B. subtilis</i> at $pH > 5$, the zeta potential of the com-	posite was
288	-20.30 mV at pH 3.	
289	3.2. Cadmium adsorption	





290	Figure 2 shows the pH dependence of Cd adsorption on montmorillonite, B.
291	subtilis, and montmorillonite-B. subtilis composite. Adsorption onto montmorillonite
292	increased slowly over the pH ranges, and about 55% of the total Cd(II) was adsorbed
293	at pH 8. Adsorption onto B. subtilis increased sharply between pH 3 and 7 and then
294	reached a plateau at pH 7-8. Nearly 90% of total Cd(II) was adsorbed at pH 8. In
295	comparison to bacteria, the uptake of Cd on montmorillonite-B. subtilis composite
296	increased more slightly over the range of pH 3 to 8. The extent of adsorption onto the
297	composite increased swiftly from pH 3 to 5, and varied slightly at pH 5-8. About 60%
298	of the total Cd(II) ions were adsorbed onto the composite at pH 8.
299	3.3. Modeling of Cd adsorption onto montmorillonite and B. subtilis
300	The titration data for montmorillonite and <i>B. subtilis</i> are presented in Figure S4.
301	Bacteria exhibited significant buffering capacity over the range of pH 3 to 8.5, which
302	was assigned to the deprotonation of functional groups on cell walls. ^{19,22,28}
303	Montmorillonite presented strong buffering capacity only at $pH < 4$ and $pH > 7.5$.
304	This result was in line with the report of Ikhsan et al. (2005), which also showed
305	similar buffering behaviors of a Ca-montmorillonite over these pH ranges. The
306	titration data of montmorillonite and B. subtilis were successfully modeled with
307	reactions (1)-(5) and (6). The protonation constants and site densities of sorbents
308	calculated from titration data are indicated in Tables S1 and S2.
309	Adsorption of Cd onto the surface of montmorillonite and B. subtilis were

successfully modeled with reactions (7)–(10). The best-fit for cadmium adsorption on
montmorillonite is depicted in Figure S5 (a). The adsorption of Cd(II) onto





312	montmorillonite was modeled using two adsorbed species, X_2Cd and $SOCd^+$. The
313	calculated complexation constants of these two species were 8.30 and -1.68,
314	respectively (Table S1). The outer-sphere complexation of $Cd(II)$ onto two X ⁻ sites
315	(X ₂ Cd) dominated at $pH<4.5$ while monodentate inner-sphere complexation onto
316	SOH sites of the crystal edges (SOCd ⁺) predominated at $pH > 4.5$. The V _Y value of
317	the model which describes the Cd sdsorption on montmorillonite was 5.6. The best-fit
318	model for the Cd adsorption onto B. subtilis is presented in Figure S5 (b). The
319	optimized $\log K_{R-COOCd+}$ and $\log K_{RPOCd+}$ values were 2.54 and 2.87, respectively
320	(Table S2). Surface complexation model successfully simulated the adsorption edge of
321	B. subtilis over the pH ranges of 3 to 8. The model predicted that one inner-sphere
322	monodentate complexation (R-COOCd ⁺) dominated at low pH while another
323	inner-sphere monodentate complexation (R-POCd ⁺) dominated at high pH. The $V_{\rm Y}$
324	value of the model which described the Cd adsorption on bacteria was 3.8.
325	3.4. Modeling of Cd adsorption onto montmorillonite-B. subtilis composite
326	The parameters for the model of Cd adsorption onto montmorillonite-B. subtilis

327 composite are shown in Table 1. As per the CA approach, the constants for 328 protonation and the acid-base reactions on the surface of the composite were the same 329 as those for the end-member montmorillonite and B. subtilis. The operational site 330 densities of functional groups used in the model of Cd sorption on the composite were 331 calculated based on the end-member site densities weighted to the 332 montmorillonite:bacteria mass ratio (Moon and Peacock, 2013). The specific 333 capacitance and surface area were calculated in the same way. Four Cd complexation 15





334	species were considered in the model of Cd sorption on the composite, i.e.,
335	outer-sphere (X ₂ Cd) and inner-sphere complex (SOCd ⁺) on montmorillonite; two
336	inner-sphere complexes on bacteria. The complexation constants of the four species
337	were the same as those optimized by the models of Cd adsorption on montmorillonite
338	and <i>B. subtilis</i> .

339 The adsorption behavior of Cd on montmorillonite-B. subtilis composite was well described by CA-SCM, except some deviations were observed at pH < 5 (Fig. 3, 340 V_Y=15.4). The outer-sphere complex X₂Cd dominated below pH 4. At pH 4.5, the 341 342 inner-sphere complex SOCd⁺ was the major species with deprotonation of the SOH 343 surface sites. At pH 3–5.5, the inner-sphere complex R-COOCd⁺ on the surface of 344 bacteria contributed 10-20% of the total Cd adsorption. At pH above 5.5, the 345 inner-sphere complex on the bacterial surface (R-POCd⁺) played an important role 346 and amounted to 10-20% of the total Cd adsorption. The CA-SCM provided an 347 adequate fit to the adsorption isotherms of Cd on the composite at pH 6 and pH 8, 348 with V_Y values of 4.3 and 2.4, respectively (Fig. S6). Figure 4 depicts the distribution 349 of the total adsorbed Cd between the composite fractions. Montmorillonite played a 350 dominating role in Cd sorption on the composite over the whole pH range studied. 351 The percentage for the adsorption of Cd on montmorillonite and B. subtilis in the 352 composite at pH 6 was 71.4% and 28.6%, respectively. The V_Y value ranging from 0.1 353 to 20 can generally be considered a good fit. Overall, within the constructs of FITEQL 354 SCM, CA-SCM is adequate to describe the Cd adsorption behavior on the surface of





355 the composite.

356 **3.5.** Distribution of Cd on montmorillonite–*B. subtilis* composite by XAS.

357 Since the model successfully described Cd adsorption on bacteria-clay composite at pH > 5, herein we selected pH 6 samples to investigate whether model predictions 358 359 match well with the EXAFS prediction. Linear combination fitting (LCF) procedure 360 has been successfully applied to obtain the partitioning of metal adsorption on 361 bacteria-mineral composites (Du et al., 2016; Templeton et al., 2003; Moon and Peacock, 2012). This approach is based on the intermediate adsorption behavior of 362 metals between the end-member component. The Cd K-edge (EXAFS) spectra of 363 364 montmorillonite, Bacillus subtilis and their composite are shown in Figure 5. A few 365 differences between the spectrum of mineral and bacteria are pronounced at k-values 366 of 6-10 Å⁻¹. We were able to produce excellent fits to the Cd-composite spectra with a linear combination of Cd-adsorbed montmorillonite and Cd-adsorbed B. subtilis 367 368 clusters (*R*-factor < 0.1). Linear combination results for the distribution of total 369 adsorbed Cd by the composite yielded a ratio of 69.1%:30.9% for montmorillonite 370 and bacterial cells, respectively. This ratio closely approaches the value obtained from 371 model speculation, which is 71.4% : 28.6%.

372

373 4. Discussions

A number of investigators have employed CA-SCM to describe metal adsorption onto
mineral-bacteria composites (Song et al., 2009; Alessi and Fein, 2010; Moon and
Peacock, 2013). However, to the authors' knowledge, this is the first information on





377	the modelling of Cd adsorption by 2:1 clay mineral-bacteria composites. The species
378	and distribution of Cd on the surface of montmorillonite-B. subtilis composite were
379	modeled using CA-SCM in this study. Cadmium was found to be adequately
380	described by adsorption to the nominally assigned RCOOH and POH sites, and the X
381	and SOH groups on the surface of montmorillonite-B. subtilis composite. Our results
382	imply that all these groups have to be considered for a successful simulation of Cd
383	adsorption on 2:1 clay mineral-bacteria mixtures, compared to ferrihydrite-bacteria
384	composites where, of the bacterial component, only carboxyl (RCOOH) groups were
385	found to be responsible for heavy metal adsorption over the entire pH range studied. ³⁴
386	The model of Cd adsorption on montmorillonite-B. subtilis composite in our study
387	suggested that X_2Cd was the major species on the composite at $pH < 4$. Interestingly,
388	montmorillonite played a dominant role in Cd sorption on the composite (the mass
389	ratio of montmorillonite to bacteria was 87.5%:12.5%) over the entire pH range
390	studied. However, the contribution to Cu adsorption from the components of a
391	ferrihydrite–bacteria mixture with a mass ratio of 82% :18% was dependent on pH 34 .
392	Bacteria and ferrihydrite governed Cu adsorption at low and high pH values,
393	respectively. The montmorillonite has a permanently charged XH site, which provides
394	a certain level of adsorption capacity independent of pH, whereas the
395	ferrihydrite-bacteria composites to which the authors are contrasting their work
396	possess only amphoteric sites (Moon and Peacock, 2013). Our data revealed that the
397	percentage of total adsorbed Cd on montmorillonite in the composite was in the range





of 87.3–69.1% from pH 3 to 8 (Figure S7). These results suggest that the bacteria fraction in the composite plays an increasingly important role in the retention of cadmium ions from pH 3 to 8, although slight decreases in Cd attachment were observed for the bacteria from pH 4 to 5. Some of the Cd ions may have been transferred from the montmorillonite surface to the bacteria in the composite with the rise of pH.

The modelled distribution of Cd on individual components in our 404 montmorillonite-B. subtilis composite was supported by the EXAFS investigations at 405 406 pH 6. The ratio of Cd adsorption onto montmorillonite to that of the bacterial cells 407 present in the composite at pH 6 was 69.1% : 30.9%, as obtained from EXAFS 408 spectra analysis. The ratio also closely coincides with the value obtained from model 409 speculation. Templeton et al. (2003) reported that 71% and 29% of Pb ions were 410 adsorbed by goethite and bacteria in a goethite-bacteria composite with a mass ratio of 70% : 30% at pH 7. Moon and Peacock ⁴⁹ found that the distribution of Cu 411 412 adsorption between ferrihydrite and bacteria fractions in a ferrihydrite-bacteria 413 composite (with a mass ratio 65% : 35%) was 80% : 20% at pH 6.4. Both the model 414 simulation and EXAFS observation from our study suggested that the fate of Cd is 415 largely affected by the presence of montmorillonite in soils and sediments, 416 particularly in locations where the mineral exists as the major clay mineral component. 417 Our work corroborates the findings of Moon and Peacock (2012) who predict that, in 418 mineral-organic composites dominated by the mineral component, the mineral will be





- responsible for the majority of the heavy metal adsorption and the composites will
 behave approximately additively. Moreover, this study has extended their work which
 was based on iron (hydr)oxide-organic composites, to clay mineral-organic
 composites. The established models from these investigations may provide a universal
 rule to predict the behavior of trace metals in more complicated ecosystems bearing
 mineral–organic complexes.
- 425

426 Environmental Implication

427 Although surface complexation modeling (SCM) has been widely applied to 428 describe trace metal adsorption behaviors on various soil solid components, how to 429 apply it in complex systems is still challenging. The present results indicate that Cd 430 adsorption behaviors on montmorillonite-bacteria composite is successfully described 431 by CA-SCM model, except for some deviations at pH < 5. Current SCM models 432 might be useful for the prediction of the distribution of Cd between solid and solution 433 phases in a variety of settings. Soils and various sediments are composed of clay 434 minerals, organic matter and microorganisms. It is critical to model Cd adsorption and 435 availability at the interface of multi-components interactions which is fundamental for 436 the risk evaluation of Cd-contaminated environments. Secondly, the fact that the 437 distribution of Cd between the end-member component is dependent on pH, has 438 important implication for Cd mobility. The adsorbed Cd complexes, either 439 outer-sphere or inner-sphere, may exhibit different exchange or desorption behaviors





440	during the changes of environmental factors, e.g., cations, redox potential. The
441	relative mobility of clay- and organic -adsorbed Cd might differ. Under this premise,
442	mineral-organic composite is a suitable analogue for a good predictor of the mobility
443	and thus eventual fate of Cd in natural environments. Lastly, our study has
444	implications for the remediation of Cd contaminant soils and sediments where 2:1
445	phyllosilicates dominate the mineral composition, in particular, when various organic
446	agents are applied, e.g., straw, manure or engineering microorganisms. These
447	substances are easily bound to soil mineral surface. In these cases, it is vital to
448	consider the fate, mobility of heavy metals with respect to mineral-organic composites,
449	in addition to pure mineral phases.

450

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600	Figure Captions
601	
602	Figure 1. Zeta potential of montmorillonite, B. subtilis, and montmorillonite-B.
603	subtilis composite at different pH values.
604	Figure 2. Adsorption of Cd(II) to montmorillonite, <i>B. subtilis</i> , and montmorillonite– <i>B</i> .
605	subtilis composite as a function of pH in the presence of 0.01 M NaNO ₃ (symbols
606	are data points and lines are model fits). The sorbent and initial Cd concentration
607	is 1 g L^{-1} and 10 ppm, respectively.
608	Figure 3. Speciation diagrams for Cd (II) adsorption onto montmorillonite-B. subtilis
609	composite at 28 °C in the presence of 0.01 M NaNO ₃ , calculated from the surface
610	complexation models (filled squares represent the experimental adsorption data).
611	Cd(II) :10 ppm, composite: 0.875 g L^{-1} Mont +0.125 g $L^{-1}B$. subtilis.
612	Figure 4. Distribution of the total adsorbed Cd between montmorillonite and B.
613	subtilis fractions in the composite predicted by CA-SCM (symbols are data points,
614	lines are model fits) at 28 °C in the presence of 0.01 M NaNO ₃ . Cd(II) :10 ppm,
615	composite : 0.875 g L^{-1} Mont +0.125 g L^{-1} <i>B. subtilis.</i>
616	Figure 5. Normalized Cd K-edge k3-weighted EXAFS spectra of B. subtilis (a),
617	montmorillonite (b) and montmorillonite-B. subtilis composite (c) at pH 6.Dotted
618	(blue) line display the best 2-component linear combination fit.
619	







- Figure 1. Zeta potential of montmorillonite, *B. subtilis*, and montmorillonite–*B*. *subtilis* composite at different pH values.





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Figure 2. Adsorption of Cd(II) to montmorillonite, *B. subtilis*, and montmorillonite–*B. subtilis* composite as a function of pH in the presence of 0.01 M NaNO₃ (symbols are data points and lines are model fits). The sorbent and initial Cd concentration is 1 g L^{-1} and 10 ppm, respectively.

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Figure 3. Speciation diagrams for Cd (II) adsorption onto montmorillonite–*B. subtilis* composite at 28 °C in the presence of 0.01 M NaNO₃, calculated from the surface complexation models (filled squares represent the experimental adsorption data). Cd(II) :10 ppm, composite: 0.875 g L⁻¹ Mont +0.125 g L⁻¹ *B. subtilis*.







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Figure 4. Distribution of the total adsorbed Cd between montmorillonite and *B*. *subtilis* fractions in the composite predicted by CA-SCM (symbols are data points,
lines are model fits) at 28 °C in the presence of 0.01 M NaNO₃. Cd(II) :10 ppm,
composite : 0.875 g L⁻¹ Mont +0.125 g L⁻¹ *B. subtilis*.







646

- 647 Fig. 5. Normalized Cd K-edge k^3 -weighted EXAFS spectra of *B. subtilis* (a),
- 648 montmorillonite (b) and montmorillonite–*B. subtilis* composite (c) at pH 6. Dotted
- 649 (blue) line display the best 2-component linear combination fit.





651	Table 1 Surface complexation model parameters for Cd(II) sorption on		
652	montmorillonite-B. subtilis composite.		
	Surface area (m ² /g)		422.6 ^b
	Total concentration of sites (mmol/g)		
	SOH		0.13 ^b
	WOH		0.50^{b}
	X ⁻		0.37 ^b
	R-COOH		0.09 ^b
	R-POH		0.07 ^b
	Specific capacitance (F/m ²)		5.54 ^b
	Surface reactions		
	$LogK_{XH}$	$X^{-} + H^{+} \iff XH$	-2.2 ^a
	LogK _{XNa}	$X^{-} + Na^{+} \iff XNa$	1.4^{a}
	LogK _{SO}	SOH \Leftrightarrow SO ⁻ + H ⁺	-4.83±0.06 ^a
	$LogK_{SOH2}^+$	$SOH + H^+ \iff SOH_2^+$	1.86 ± 0.06^{a}
	LogK _{WO}	WOH \Leftrightarrow WO ⁻ + H ⁺	-8.36±0.08 ^a
	LogK _{R-COO}	$R\text{-}COOH \iff R\text{-}COO^{-} + H^{+}$	-3.32 ± 0.08^{a}
	LogK _{R-PO}	$R\text{-POH} \iff R\text{-PO}^- + H^+$	-5.38±0.02 ^a
	LogK _{X2Cd}	$2X^{-} + Cd^{2+} \iff X_2Cd$	8.30±0.04 ^a
	$LogK_{SOCd}^+$	$SOH + Cd^{2+} \iff SOCd^+ + H^+$	-1.68±0.005 ^a
	$LogK_{R-COOCd}^+$	$R-COO^{-} + Cd^{2+} \Leftrightarrow R-COOCd^{+}$	$2.54{\pm}0.005^{a}$
	$LogK_{R-POCd}^+$	$R-PO^{-}+Cd^{2+} \Leftrightarrow R-POCd^{+}$	2.87 ± 0.02^{a}

653 ^a Fixed at those determined for montmorillonite-bacteria composite (obtained from

Tables S1 and S2)

^b Calculated using the surface site densities or surface areas or the specific capacitance

of the end-member montmorillonite and *B. subtilis* weighted to the mineral:bacteria

mass ratio of 7:1.