Combination of 13 C/ 113 Cd NMR, potentiometry, and voltammetry in characterizing the interactions between Cd and two models of the main components of soil organic matter

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ORIGINAL PAPER

Combination of $^{13}C/^{113}Cd$ NMR, potentiometry, and voltammetry in characterizing the interactions between Cd and two models of the main components of soil organic matter

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Abstract This work allowed the characterization of the Cd-binding sites of two compounds taken as models for exudates, the main components of soil organic matter (SOM). The studied compounds were exopolysaccharides (EPS), specifically exudates of roots (polygalacturonic acid) and of soil bacteria (Phytagel). Potentiometric acid–base titrations were performed and fitting of the obtained results indicated the presence of two main classes of acidic sites, defined by their pK_a values, for both EPS but of a different nature when comparing the two compounds. The two studied exopolysaccharides presented different acidic/basic site ratios: 0.15 for Phytagel and 0.76 for polygalacturonic acid. Spectroscopic techniques ($^{13}C/^{113}Cd$ NMR, FTIR) distinguished different Cd surroundings for each of the studied EPS, which is in agreement with the titration results. Furthermore, these analyses indicated the presence of –COOH and –OH groups in various proportions for each exopolysaccharide, which should be linked to their reactivity towards cadmium. Cadmium titrations (voltammetric measurements) also differentiated different binding sites for each compound and allowed the determination of the strength of the Cd-binding site of the EPS. Fitting of the results of such voltammetric measurements was performed using PROSECE (*Programme d'Optimisation et de Speciation Chimique dans l'Environnement*), a software coupling chemical speciation calculation and binding parameter optimization. The fitting, taking into account the Cd^{2+}/H^+ competition towards exopolysaccharides, confirmed the acid-base titrations and spectroscopic analyses by revealing two classes of binding sites: (i) one defined as a strong complexant regarding its Cd^{2+}–EPS association (log$K$ = 9–10.4) and with basic functionality regarding H^+–EPS association (pK_a = 11.3–11.7), and (ii) one defined as a weak complexant (log$K$ = 7.1–8.2) and with acidic functionality (pK_a = 3.7–4.0). Therefore the combination of spectroscopic analyses, voltammetry, and fitting allowed the precise characterization of the binding sites of the studied exopolysaccharides, mimicking the main SOM components. Furthermore, the binding parameters obtained by fitting can be used in biogeochemical models to better define the role of key SOM compounds like exudates of roots and of soil bacteria on trace metal transport or assimilation.

Keywords Cadmium complexation · Soil organic matter · Complexation modeling · $^{13}C/^{113}Cd$ NMR · Voltammetry

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Introduction

Natural organic matter (NOM) is ubiquitous in the environment and consists of a complex mixture [1], depending on the origin and age of the material [2, 3]. NOM is also known to play important roles in the fate of many contaminants due to its complexing properties. A better comprehension of NOM structural and functional properties can greatly improve our understanding of the underlying mechanisms responsible for heavy metal complexation [4]. This, in turn, may enhance our predictive capabilities regarding the behavior of NOM and environmental inorganic pollutants in natural ecosystems.

Reactivity of soil organic matter (SOM) towards cadmium, a quite toxic element, is of particular interest, especially in the case of polluted soils and their (phyto-)remediation [5–7], but also to better quantify the bioavailability of Cd towards the plants. Many studies have investigated implications of SOM in Cd transport and bioavailability, e.g., in a rhizosphere soil [8], but with a less important combination of analytical tools than in the study presented here.

Many microorganisms, especially in soils, produce extracellular polysaccharides (EPS) with a high molecular weight, consisting of polysaccharides, proteins, and nucleic acids [9]. These biopolymers are known to complex heavy metals [10–12] and represent the main components of SOM in natural systems [13, 14].

No single analytical tool can provide structural or functional information about NOM because of its heterogeneous, complex nature. Thus, a combined application of various analytical techniques is more suitable. Among characterization methods, spectroscopic techniques appear the most useful, since they are non-destructive, usually require no or little sample preparation, and they provide valuable information on molecular structure and chemical or functional NOM properties [15]. On the other hand, voltammetry is a suitable technique to study the characterization of NOM metal-binding sites, as it is a sensitive method that does not need any physical or chemical sample preparation.

The EPS structure can be characterized by using spectroscopic techniques, e.g., FTIR and ^{13}C solid-state NMR spectroscopy. NMR analysis is a direct probe of the studied nuclei surroundings [16] and the chemical shifts of the targeted nuclei have a high sensitivity to variations in the local chemical surroundings. Regarding Cd complexation, ^{113}Cd NMR spectroscopy has already been used as a probe of Cd^{2+}complexation by a known functional group [17, 18], by natural organic matter [19, 20], and by biomaterial [21].

Differential pulse anodic stripping voltammetry (DPASV) is another technique that is able to recognize metal–ligand complexation by directly measuring the non-organic metal fraction [22, 23]. The metal fraction analyzed by DPASV referred to as labile, usually corresponds to the sum of free metal and labile inorganic complexes. Therefore by knowing the total metal concentration, the organically bound metal fraction can be calculated.

With voltammetry results fitted by using Scatchard [24] and Ružić [25] linearization methods one can estimate if the number of SOM apparent binding sites is one or more. However, as these methods often generate important errors in terms of the ligand binding properties [22], a non-linear fitting has to be applied afterwards. Nowadays, more complex and flexible fitting programs have been developed that include models with various chemical interactions [26–29]. One such program is PROSECE (*Programme d'Optimisation et de Speciation Chimique dans l'Environnement*), software that allows the determination of complexation properties using a discrete model distribution of binding sites [22, 30, 31]. PROSECE be considered as an alternative to the MODEL VI or NICA-DONNAN approaches involved in WHAM and FITEQL software, respectively [27, 29].

In this study, two exopolysaccharides (EPS) used as models for exudates of roots and of soil bacteria were studied for their reactivity towards Cd and their implications in terms of Cd speciation and bioavailability in soils. The selected EPS were of bacterial (Phytagel) and plant (polygalacturonic acid, hereafter named PGA) origin. Both have well-known structures [32, 33], but their reactivity towards trace metals (i.e., in order to study their role in metal bioavailability) has not been investigated much. By combining spectroscopic techniques, potentiometry, and voltammetry (which has scarcely been presented in the literature) and by fitting of the measured data to the corresponding model, conditional binding parameters (e.g., log$K$, pK_a, and binding site concentrations) are proposed in this work. These parameters can further be used in any geochemical model currently lacking these kinds of values.

Experimental

Chemicals

All reagents were of Normapur quality. Deionized water was obtained with a Milli-Q water purification system. Polygalacturonic acid and Phytagel were of Analytical Grade and were both provided by Sigma.

Procedures for EPS characterization and EPS–Cd complexation study

Potentiometry experiments were conducted on 50 mL of 250 mg L^{-1} PGA and Phytagel solutions (I = 0.01 M, $NaNO_3$), which corresponds to 12.5 $mmol_{DOC}$ L^{-1} and 7.85 $mmol_{DOC}$ L^{-1} of carbon content, respectively.

Spectroscopic analyses of cadmium complexation was performed by addition of cadmium (as 1 mg Cd/mL in 2% HNO_3 solution, Inorganic Ventures Labs) to PGA and Phytagel solutions, with a fixed carboxylic group to metal quantity ratio of 10/1 or 10:1 (0.48 $mmol_{COOH}$ L^{-1} and 0.044 $mmol_{Cd}$ L^{-1} in 50 mL), in a solution of pH 6. After 5 days of contact time, the solution was freeze-dried and analyzed by FTIR, ^{13}C and ^{113}Cd solid-state NMR spectroscopy.

Voltammetric cadmium titrations were carried out at different pH values (3, 8, and 10) in order to determine proton and cadmium competition for the binding sites. For each EPS (3.33 $mmol_{DOC}$ L^{-1}, I = 0.01 M, $NaNO_3$), and at the various studied pH values, standard additions in a logarithmic addition mode [22, 31] were performed; total concentration ranged from 10^{-9} to 10^{-7} mol L^{-1}. For each metal addition an equilibration time of 1 h was set up, which appears sufficient to reach complexation equilibrium. Each measurement was duplicated, and analytical uncertainty is around 3%.

Analytical tools

Total organic carbon in solution was measured with a Shimadzu TOC 5000 with an accuracy of 0.1 mg C L^{-1}.

Fourier transform infrared (FTIR) analyses were carried out with KBr pellets (100 mg KBr + 1 mg sample) on an Equinox55 (Bruker) spectrometer. Spectra were recorded in transmission mode with 16 scans for each spectrum, between 4,000 and 400 cm^{-1}, with a 4-cm^{-1} resolution.

All solid-state cross polarization nuclear magnetic resonance spectroscopy with magic angle spinning (CPMAS NMR) spectra were obtained on a Bruker Avance 400-MHz NMR spectrometer operating at a ^{13}C and ^{113}Cd resonance frequency of 101.6 MHz and 88.8 MHz, respectively. ^{13}C and ^{113}Cd CPMAS experiments were performed with a commercial Bruker double-bearing probe with zirconium dioxide rotors of 4-mm outer diameter. The CP technique [34] was applied during MAS of the rotor at 10 kHz. A ramped ^{1}H pulse starting at 100% power and decreasing to 50% was used during contact time to circumvent Hartmann–Hahn mismatches [35, 36]. For ^{13}C and ^{113}Cd CPMAS, contact times were 2 ms and 5 ms, respectively, and the number of scans were 2,048 and 50,000 (delay of 2 s and 5 s, respectively) [37]. To improve the resolution, dipolar decoupling on the proton channel was applied with TPPM-15 sequence during acquisition. The ^{13}C and ^{113}Cd chemical shifts were referenced to tetramethylsilane and 0.1 M Cd $(ClO_4)_2$ in aqueous solution, respectively [37].

EPS acidic functions distribution was analyzed by potentiometric titrations [26, 38–41]. Acid–base titrations were carried out following the procedure detailed elsewhere [30]. Briefly, experiments were conducted in thermostated cells at 25±0.2° C, by additions of HNO_3 (0.20 M, from HNO_3 69% J.T. Baker) until pH 2 was reached, then additions of KOH (0.10 M, from KOH 0.5033 M Sigma-Aldrich) up to a value of 11.9 were performed. $NaNO_3$ concentration in KOH and HNO_3 standard solutions was 0.10 M. The micro-titration stand (Metrohm) was equipped with two Titrino 716 titrators controlled by Tinet2.4 software. The combined pH–micro-electrode used (Ross 8103SC, Orion) was calibrated by pH buffer solutions (HANNA 4.01, 7.01, and 10.01) before each titration and controlled after.

Voltammetric measurements were performed on a Metrohm-EcoChemie stand controlled by the GPES 4.9 software [23]. This apparatus is composed of a potentiostat/galvanostat (PGSTAT12) connected to the measurement stand (VA663), comprising a static mercury drop electrode (SMDE), a reference (Ag/AgCl/KCl 3 mol L^{-1}) and an auxiliary glassy carbon electrode. After each DPASV measurement, carried out in a thermostated Teflon® cell (25±0.2° C), pH was monitored by a combined micro-electrode (Mettler, Inlab422) linked to a pHM713 (Metrohm). The voltammetric measurements involved N_2 purging through the cell for 120 s, a deposition time of 600 s at a potential of −0.9 V, then a scan in differential pulse mode towards oxidative potentials, from −0.9 to 0.1 V.

Results and discussion

NOM characterization

^{13}C NMR and FTIR spectra (Fig. 1, thin lines) confirm the known structure of EPS (see Table 1).

A Phytagel unit is composed of four tetrasaccharides (i.e., a 24-carbon moiety), and PGA is composed of a monosaccharide (i.e., a six-carbon moiety). Thus, each carbon should contribute to the signal with a proportion of 4.2% for Phytagel and 16% for PGA. The ^{13}C NMR carbon distributions are presented in Table 1. The proportions derived from the fitting are close to the theoretical values, thus showing that the applied CPMAS pulse sequence using a ^{1}H ramp pulse during cross polarization [42] and an optimized contact time (2 ms) allows a (semi-)quantitative interpretation of the ^{13}C NMR spectra.

PGA acide–base titration is presented in Fig. 2; the titration of Milli-Q water that does not present any acidic sites. Acid–base titrations results were fitted (Fig. 2) by PROSECE to determine the distribution of the discrete acidic sites [30]. In the literature, distribution of natural organic matter acidic sites is reported to range from two [40] to six sites [38]. For each studied EPS, the best PROSECE fitting of the acid–base titration (see Fig. 2 and Table 2) was obtained with four classes of acidic sites, with two preponderant in percentage, expressing the presence of various kinds of surroundings for these sites.

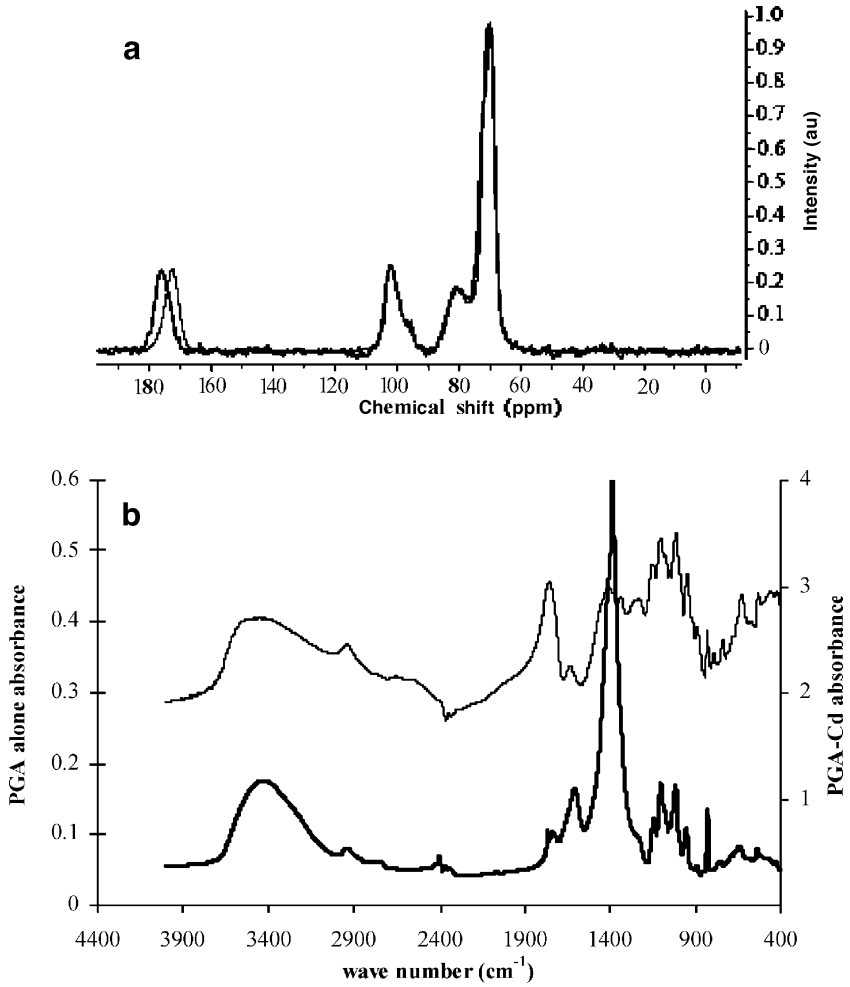


Fig. 1 ^{13}C NMR spectra (**a**) and FTIR spectra (**b**) of PGA alone (*thin line*) and PGA-complexed Cd (*thick line*). Analyses were performed with a fixed EPS carboxylic group to metal quantity ratio of 10:1 (0.48 $mmol_{COOH}\ L^{-1}$ and 0.044 $mmol_{Cd}\ L^{-1}$ in 50 mL) solution at pH 6, freeze-dried after 5 days of contact time and analyzed

All models applied to NOM aim at defining groups of acidic sites, distinguishing those with acidic pK_a in the range usually attributed to carboxylic groups (pK_a<7, thereafter named acidic sites A) and those with basic pK_a in the range usually attributed to phenolic groups (pK_a>7, thereafter named acidic sites B) [26, 30, 38, 39]. For the studied EPS (Table 2), acidic sites A and also acidic sites with higher pK_a, in the range of acidic sites B, were present. The observation is consistent with others NOM acidic sites identification [26, 30, 38, 40, 41]. Furthermore, this result has already been observed for acidic binding sites of simpler structures (e.g., EDTA and glucuronic acid), whose acidic sites showed pK_a values in the range of acidic sites B [43].

Studies on PGA and gellan (Phytagel-like polymer family) structures revealed a helix-based structure of the chains [32, 33], leading to an overall conformation that forms a three-dimensional network. Therefore, the observed acidic sites distribution could be linked to the helix structure, leading to different but repetitive surroundings of the carboxylic groups.

Results also emphasize the importance of the carboxylate and hydroxyl groups and of the hydrogen bonds in the interchain interactions, as the pK_a of such sites (e.g., C4 on PGA) is around 11–12 [44].

Although the total acidic sites density is almost the same for the two studied exopolysaccharides (14.6 and 13.2 meq g_{DOC}^{-1}; Table 2), the distribution is completely different: Phytagel acidic sites are mainly acidic sites B, with a ratio A/B of 0.15, whereas for PGA, the proportion of acidic sites B is in smaller excess (ratio A/B of 0.76). These results can be explained by the different environments of the carboxylic groups inside the three-dimensional polymer structure [32, 33, 45] and the OH distribution.

Therefore characterization highlighted the presence of various classes of acidic binding sites for each studied compound.

Exopolysaccharide–Cd complexation

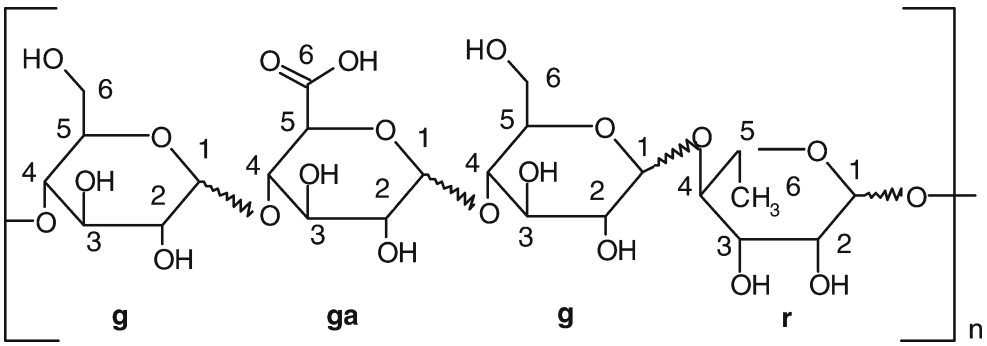
It is well known that only a minor fraction of the measured acidic sites display a high affinity towards metals [46]. Both our spectroscopic and voltammetric complexation investigations aimed at determining the nature of these high energy sites, i.e., whether sites A or B are involved in

Table 1 Assignment of ^{13}C CPMAS spectra of PGA and Phytagel, along with their schematic representations

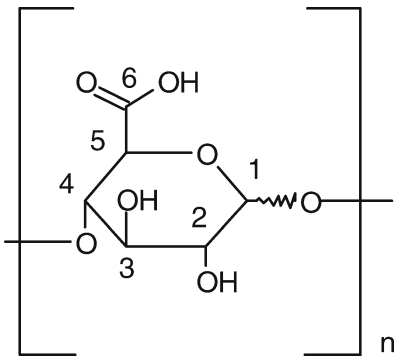
Chemical shift (ppm)	Proportion	Assignment[a]
PGA		
68–75	53.1	C2p, C3p, C5p
81	16.0	C4p
96–102	17.0	C1p
172	13.9	C6p
Phytagel		
18	4.1	C6r
62	6.5	C6g
68–77	54.6	C2 3 5g / C2 3 5ga / C2 3 5r
83	15.0	C4g / C4ga / C4r
104	16.4	C1g / C1ga / C1r
176	3.4	C6ga

[a] *p* PGA, *g* glucose, *ga* glucuronic Acid, *r* rhamnose

Phytagel schematic representation



PGA schematic representation



cadmium binding. The obtained results should allow the precise characterization of the Cd-binding sites of the studied SOM exopolysaccharides.

Spectroscopic study of cadmium complexation

Cadmium complexation by the studied EPS was assessed by ^{13}C and ^{113}Cd NMR and FTIR measurements.

^{13}C NMR spectrum of PGA in the presence of Cd (Fig. 1a, thick line) revealed a 4-ppm downfield shift of the 172-ppm peak with respect to the spectrum without Cd, indicating the change in the surroundings of the carboxyl group(s). This change can also be seen by FTIR analysis (Fig. 1b, thick line). The main difference is the presence of COOH in PGA without Cd (1,739-cm^{-1} band) which almost disappears when in the presence of cadmium, being replaced by COO^- (1,593-cm^{-1} band). The sharp band at 1,381 cm^{-1} corresponds to the nitrate of the added cadmium salt.

In contrast, no differences were observed between ^{13}C NMR spectra of Phytagel and Phytagel–Cd (data not

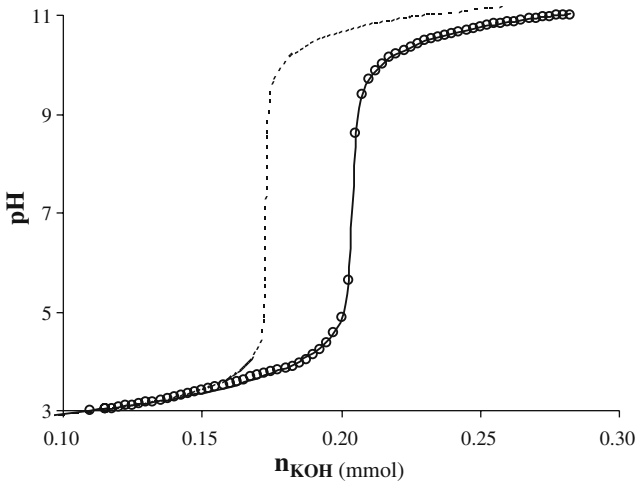


Fig. 2 PGA potentiometric measurements (*open circles*) as compared to Milli-Q titration (no acidic sites; *dotted line*). Experiments were conducted in a thermostated cell (25±0.2 °C), on 50 mL of 250 mg L^{-1} PGA solution (I=0.01 M, $NaNO_3$), which corresponds to 12.5 $mmol_{DOC}$ L^{-1} of carbon content. *Solid line* results of fitting

shown). This observation is consistent with the titrations results. Indeed, to avoid $Cd(OH)_2$ precipitation, the experiment was performed at pH 6, i.e., a pH value too low to allow quantitative deprotonation of acidic sites B, which account for the vast majority of the measured sites. The proportion of sites reacting with Cd was small (around 13%, i.e., sum of % of site 1 and site 2, Table 2). Therefore, Cd complexation though occurring, did not lead to any significant NMR downfield shift. The same conclusions can be drawn from the FTIR data.

^{113}Cd NMR measurements showed (Fig. 3) downfield shift and broadening of signals of PGA–Cd and Phytagel–Cd compared with unreacted solid-state mixtures of the Cd salt with the studied exopolysaccharides, confirming strong interactions between Cd metal and organic materials.

Additionally, Fig. 3 shows a different shift of signal for Cd in PGA and Phytagel, −38 and −88 ppm respectively, which can be due to a different cadmium complexation on the two studied EPS.

Therefore, spectroscopic analyses added to characterization and also proved that cadmium complexation is different when considering the two studied EPS.

Table 2 Acidic sites distribution and total acidic sites density for Phytagel and PGA

	Phytagel			PGA		
Total acidic sites density (meq g_{DOC}^{-1})	14.6			13.2		
	pK_a	%	Type	pK_a	%	Type
Site 1	4.0	10.9	A	3.6	43.1	A
Site 2	6.6	2.0	A	8.2	2.2	B
Site 3	9.3	3.6	B	10.3	7.7	B
Site 4	11.2	83.4	B	11.7	47.0	B

A acidic sites with acidic pK_a usually attributed to carboxylic functionality, *B* acidic sites with basic pK_a usually attributed to phenolic functionality

Voltammetric study of cadmium complexation

Voltammetric cadmium titrations were carried out at pH values 3, 8, and 10 to determine proton and cadmium competition for the binding sites. These analyses allow a more precise definition of the binding sites involved in cadmium complexation.

Experiments conducted at pH 3 showed no cadmium complexation by the studied exopolysaccharides (results not shown). Therefore, the concerned EPS complexing sites seemed not to be deprotonated at this pH, which correspond to pK_a values above 3. This observation corroborates the potentiometric titrations results.

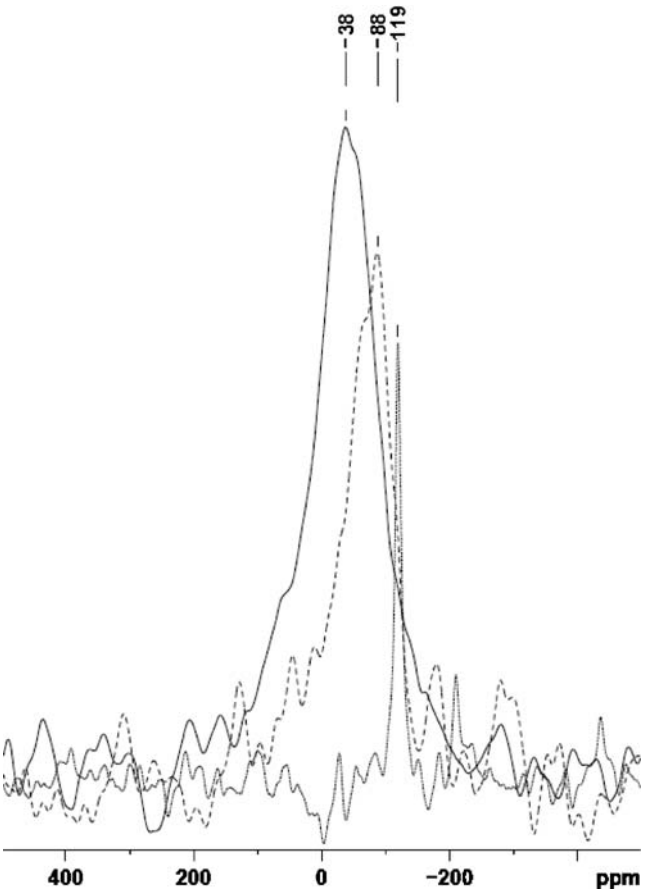


Fig. 3 ^{113}Cd NMR analysis for PGA-complexed Cd (*thick line*), Phytagel-complexed Cd (*dashed line*), Phytagel, and cadmium simple contact (*dotted line*). Experimental conditions for complexation analysis are 0.48 $mmol_{EPS}$ L^{-1} and 0.044 $mmol_{Cd}$ L^{-1} dissolved in 50 mL; for the simple contact experiment, the same proportion of EPS and Cd (both as solids) were mixed but not dissolved

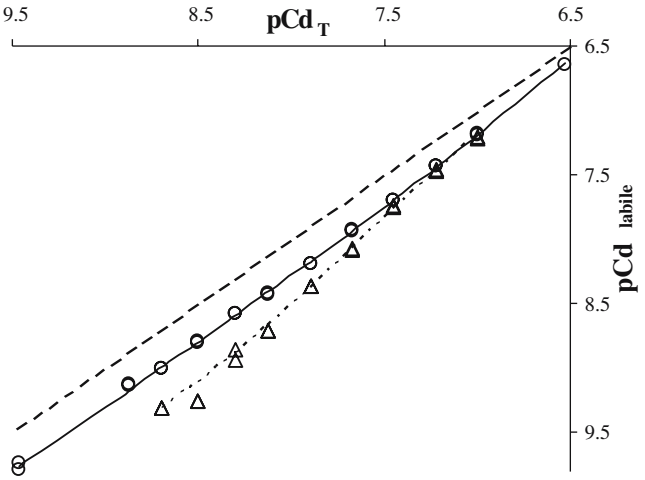


Fig. 4 Labile (pCd_{labile}, expressed as $-\log[Cd_{labile}]$) vs. total Cd (pCd_T, expressed as $-\log[Cd_T]$) concentration in a solution of 3.33 $mmol_{DOC}\ L^{-1}$ of PGA. The metal fraction referred to as labile corresponds to the sum of free metal and labile inorganic complexes. Various experiments are presented: pH 8 (*open circles*), pH10 (*open triangles*), and non-complexed Cd (*dashed line*) as well as the results of fitting for pH 8 (*solid line*) and pH 10 (*dotted line*)

Acidic sites A and B involved in the complexation should present different affinities towards cadmium. The presence of acidic sites B should be confirmed by a significant increase of the strength of EPS complexation between experiments carried out at pH 8 and pH 10, according to their pK_a values.

Cadmium titration results obtained with PGA at pH 8 and 10 are presented in Fig. 4. As data points are clearly below the non-complexation line (dashed line) it underlines that interactions between exopolysaccharides and cadmium dominate Cd speciation. So, natural exudates of bacteria and of roots could largely control cadmium distribution in soils. Moreover, interactions increased between pH 8 and pH 10, suggesting Cd^{2+}/H^+ competition towards EPS binding sites and the influence of acidic sites B.

Fitting of metal titrations results using Scatchard [24] and Ružić [25] linearizations showed a curvature, i.e., that it is not a one-site complexation that is occurring (data not shown). Therefore, there is more than one class of binding site, which agrees with the different cadmium surroundings highlighted by our ^{113}Cd NMR measurements. This also agrees with a previous study on cadmium complexation by bacterial exopolysaccharides involved in the adhesion to surfaces and protection from environmental stress ([14 and references therein).

The experiments carried out were fitted by PROSECE using a discrete distribution of binding sites to characterize exopolysaccharides/Cd reactivity. Inorganic chemical composition of the solution was taken in account to calculate the inorganic speciation of Cd, using thermodynamic stability constants from MINEQL and MINTEQ databases [47, 48]. Best fitting was obtained defining two classes of sites (L1 and L2), both acidic and complexing, each defined by a binding site density (μeq g_{DOC}^{-1}), an acidic constant (pK_a), and a stability constant (log$K$) towards Cd, i.e., six unknown parameters for each EPS. The values of these six parameters were optimized using PROSECE, by fitting simultaneously the experimental data (i.e., H^+ and labile Cd concentrations vs. total Cd ones) obtained at pH 8 and 10, taking account of the EPS acidic parameters modeled previously (see Table 2). The obtained results are summarized in Table 3, and results of fitting by the proposed model of the experiments carried out on PGA are shown in Fig. 4.

For each studied EPS, a "strong" (L1) and a "weak" (L2) site can be defined in terms of the obtained complexation constant values. According to the pK_a values obtained, these sites were described as follows: the "strong" binding sites are a part of the acidic sites B, the "weak" binding sites are a part of the acidic sites A.

Therefore, the different binding sites involved in Cd complexation, highlighted by characterization and spectroscopic measurements, were identified by voltammetry and fitting. However, differences appeared between PGA and Phytagel in terms of the sites densities, the stability constants, and the acid constants.

There was an important effect of pH variation on PGA complexation (Fig. 4), particularly at low Cd concentrations. This has been simulated (Table 3) by a small proportion (0.41 μeq g_{DOC}^{-1}) of acidic sites B (strong Cd-binding site L1 with log$K$ 10.4) and an important proportion (1.87 μeq g_{DOC}^{-1}) of acidic sites A (weak Cd-binding site L2 with log$K$ 7.1).

In contrast, with Phytagel there were almost no significant pH effects on the complexation on the scanned Cd concentrations (data not shown), and fitting (Table 3) showed a major proportion (1.32 μeq g_{DOC}^{-1}) of acidic sites B (strong Cd-binding site L1 with log$K$ 9.0). The difference of stability constant between PGA and Phytagel acidic site B has to be noted, and is probably due to the different surroundings highlighted by the spectroscopic techniques.

Table 3 PROSECE fitting results (site densities, stability constants, and pK_a) for PGA and Phytagel at the various studied pH values

	PGA		Phytagel	
	L1	L2	L1	L2
Site densities (μeq g_{DOC}^{-1})	0.41	1.87	1.32	0.33
Stability constants (log$K$)	10.4	7.1	9.0	8.2
pK_a	11.7	3.7	11.3	4.0

Regarding to their quite basic pK_a values (Table 3), these strong sites (L1) should not be efficient toward cadmium speciation in neutral to acidic conditions occurring in soil environments, due to a high H^+/Cd^{2+} competition effect. Yet these exopolysaccharides present a second class of sites, much more concentrated but weaker complexants (L2) with acidic pK_a values (around 4). Therefore, it can be concluded that natural exudates must conserve an important role in cadmium speciation in soils.

As already mentioned, only a few studies reported in the literature have dealt with Cd complexation by exudates and they do did not involve the same analytical tools or fitting models employed here [14, 49]. Nevertheless, these studies also pointed out the presence of two binding sites [14, 49]. Lamelas et al. [14] obtained complexation parameters for the two binding sites that were different from ours (log$K$ 2.4 and 2.95) when dealing with H^+ and Cd^{2+} binding properties of bacterial exopolysaccharides. However, the two sets of results are barely comparable as the fitting model involved continuous distribution of organic matter binding sites. The complexation parameters found by Karlsson et al. [49] for Cd complexation on functional groups of an organic soil are in the same range as our results: one strong complexant site in terms of its Cd^{2+}–SOM association (log$K$ = 11.2–11.6) and with basic functionality regarding H^+–SOM association (pK_a = 9.96), which the authors attributed to thiol functionality; and one weak complexant site (log$K$ 3.2) and with acidic functionality (pK_a= 3.5) [49]. However, as the analytical tools are different (ion-selective electrode and EXAFS) the comparison is once again not easy.

The characterization of our model EPS has therefore led to a better understanding of the binding capacities of SOM main components towards cadmium and thereby to a better knowledge of the role of these key SOM compounds.

Conclusion

Owing to the combination of various analytical techniques and fitting, the characterization of the Cd-binding sites of the main components of soil organic matter (exudates of roots and of soil bacteria) was precisely investigated in this work.

Potentiometric results revealed the presence of acidic sites with acidic functionality in terms of H^+–EPS association (sites A; pK_a = 3.7–4.0) and acidic sites with basic functionality (sites B; pK_a= 11.3–11.7), for both studied EPS. The ratio A/B is different for the two exopolysaccharides, probably reflecting the presence of various kinds of binding sites for the studied EPS.

Spectroscopic techniques (^{13}C and ^{113}Cd NMR, FTIR) corroborated this result and especially proved that cadmium surroundings are different when considering complexation by the two studied exopolysaccharides.

Furthermore, voltammetric experiments combined with fitting allowed the characterization of each EPS binding site, including fractions of the defined acidic sites A and B. These binding sites present various intrinsic properties (various pK_a ranges), and complexation ability towards cadmium is different (log$K$ = 7.1–8.2 for sites A; log$K$ = 9–10.4 for sites B). The binding sites could thereby be defined and differences between the two exopolysaccharides could be fitted by the proposed model.

This combination of analytical tools and fitting should be used whenever characterization of SOM complexation properties is concerned. It allows one to better take into account the intrinsic reactivity of the main compounds of soil organic matter, mimicking SOM usually found in a plant–soil system, through their complexation ability. Furthermore, this study allowed an easier comprehension of cadmium speciation at the plant/soil interface. The parameters determined in this study can therefore be considered as trustworthy data for biogeochemical models (e.g., for metal-transfer modeling), to improve our knowledge and understanding of metal transfer from a soil to a plant.

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