

## SUPPLEMENTARY MATERIAL

### Swelling and aggregation of Leonardite upon pH change and Pb(II) binding: an AFM study

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### Theory: The NICA-EPN model

The EPN model has been introduced and discussed elsewhere (Montenegro et al., 2014; Orsetti et al., 2010, 2013), thus here we will present a reduced account. In the EPN model, the HS particles are considered as divided in two fractions: an inner fraction  $g_f$ , which behaves as a gel in Donnan in equilibrium with the bulk solution, and an external fraction  $1 - g_f$ . The gel is treated, in a mechano-statistical sense, as an elastic polymeric network formed by  $N_2$  equivalent chains which are cross-linked and/or entangled; this network is a model of the structure of humic particles, generally considered as a supramolecular arrangement of relatively small molecules bound by a variety of interactions including hydrogen bond, van der Waals and electrostatic forces and metal complexation (Clapp et al., 2005; Simpson et al., 2002). Thus, HS are treated here as soft gel-like particles independently of its size, even in the case of fulvic acids (FA), which are composed of relatively small molecules (of a few kDa), because they are expected to be associated in natural environments. The gel can exchange ions and solvent with the surrounding solution, swelling and shrinking as a consequence of humic charge changes; the equilibrium volume for a given condition is found by minimization of the free energy of swelling:

$$\left. \frac{\partial \Delta A_{sw}}{\partial V} \right)_{T, N_2} = \frac{\partial}{\partial V} \left[ \Delta A_{mix} + \Delta A_{def} + \Delta A_{el} \right] \Bigg|_{T, N_2} = 0 \quad (S1)$$

where  $A$  is the Helmholtz free energy,  $T$  the temperature and  $V$  the gel volume. Three independent contributions are considered in Eqn (S1): the free energy of mixing between  $N_2$  chains and  $N_1$  solvent molecules (the solution is assumed to have the bulk properties of water),  $\Delta A_{mix}$ , the network deformation contribution,  $\Delta A_{def}$ , and the electrostatic interaction free energy  $\Delta A_{el}$ .

The electrostatic contribution to the free energy (repulsion between humic particle negative charges) can be obtained by assuming that the humic charged groups are initially in their equilibrium state (i.e. free or bound) but uncharged, and calculating the work to reversibly charge the network (Hill, 1952):

$$\Delta A_{el} \approx \Delta G_{el,rep} = \int_0^1 \psi(q\lambda) q d\lambda \quad (S2)$$

where  $G$  is the Gibbs free energy,  $q$  is the humic net charge and  $\psi$  the electrostatic potential. Eqn (S2) requires knowledge of the electric potential profile inside the gel, which in principle should be found through the Poisson-Boltzmann equation; in the EPN model it is assumed a constant potential inside the gel, equal to  $\psi_D$ , neglecting border effects (which also makes the model independent of the particle size), thus the Poisson-Boltzmann equation can be written for this case (Orsetti et al., 2010):

$$0 = \rho + \sum_i z_i c_i (1 - \varphi_2) \exp(-z_i F \psi_D / RT) \quad (S3)$$

where  $F$  is Faraday's constant, and  $\rho$  is the humic charge density (in molar units), given by

$$\rho = \frac{Z}{V} = g_f Q \delta \varphi_2 \quad (S4)$$

where  $Z$  is the net humic charge of the humic particle (mol),  $V$  the hydrated humic particle volume,  $Q$  is the net specific charge (mol kg<sup>-1</sup>) and  $\delta$  the density of the dry humic substance (following Dinar(Dinar et al., 2006)  $\delta = 1.5$  g cm<sup>-3</sup>).  $\varphi_2$  is the volume fraction of the humic substance in the gel:

$$\varphi_2 = \frac{V_2}{V} \quad (S5)$$

where  $V_2$  is the volume of dry humic substance. The factors  $(1 - \varphi_2)$  in Eqn (S3) and  $\varphi_2$  in Eqns (S4) and (S5) account for the charge density changes due to swelling (mixing of HS and electrolyte). Note that Eqn (S3) is the electroneutrality condition; it has no

analytical solution for  $\psi_D$  in the general case, thus it should be solved numerically. In the case of a 1:1 electrolyte, as it is well known, the Donnan potential can be found, being (Orsetti et al., 2010):

$$\psi_D = \frac{RT}{F} \operatorname{arcsinh} \left( \frac{\rho}{2I(1-\varphi_2)} \right) \quad (\text{S6})$$

where  $I$  is the ionic strength, equal to the electrolyte concentration in the 1:1 case; from the above equations it is found that (Orsetti et al., 2010):

$$\Delta A_{el} = ZRT \left[ \operatorname{arcsinh} \left( \frac{\rho}{2I(1-\varphi_2)} \right) - \sqrt{1 + 2 \left( \frac{2I(1-\varphi_2)}{\rho} \right)} + \frac{2I(1-\varphi_2)}{\rho} \right] \quad (\text{S7})$$

This equation is strictly valid for 1:1 electrolytes; for proton binding experiments, this is generally the situation found in the literature (Matynia et al., 2010; Milne et al., 2001; Orsetti et al., 2010), thus Eqns (S6) and (S7) are appropriate. In the presence of di- and trivalent metal salts, in principle this is not true, however in common cases where the metal concentrations are not high these equations can be applied (Montenegro et al., 2014).

The mixing and deformation contributions are found quite straightforwardly following Flory and Hill, resulting in the swelling equilibrium condition (Orsetti et al., 2010):

$$\frac{1}{v_1} \left[ \chi \varphi_2^2 + \varphi_2 + \ln(1-\varphi_2) \right] + \frac{1}{v_2} \left( \varphi_2^{1/3} - \frac{2\varphi_2}{f_p} \right) + 2I \left[ 1 - \sqrt{1 + \left( \frac{\rho}{2I(1-\varphi_2)} \right)^2} \right] = 0 \quad (\text{S8})$$

The humic binding sites outside the gel are in equilibrium with the bulk solution, whereas those inside the gel are in equilibrium at the internal ionic activity of species  $j$ ,  $a_j^{\text{int}}$ , which is affected by a Boltzmann factor as:

$$a_j^{\text{int}} = a_j f_B = a_j \exp \left( -z_j F \psi_D / RT \right) \quad (\text{S9})$$

The amount bound, in the NICA-EPN model, of species  $j$  per unit of humic mass in the presence of species  $l$  is given by

$$Q_j = g_f \sum_{k=1}^M \frac{n_{j,k}}{n_{1,k}} Q_{\max,k} \theta_{j,k,i} + (1 - g_f) \sum_{k=1}^M \frac{n_{j,k}}{n_{1,k}} Q_{\max,k} \theta_{j,k,e} + Q_{j,el} \quad (\text{S10})$$

where  $\theta_{j,k,e}$  is the fraction of external (outside the gel) type  $k$  sites occupied by species  $j$ , given by the NICA equation:

$$\theta_{j,k,e} = \frac{(K_{j,k} a_j)^{n_{j,k}}}{\sum_{l=1}^N (K_{l,k} a_l)^{n_{l,k}}} \times \frac{\left[ \sum_{l=1}^N (K_{l,k} a_l)^{n_{l,k}} \right]^{p_k}}{1 + \left[ \sum_{l=1}^N (K_{l,k} a_l)^{n_{l,k}} \right]^{p_k}} \quad (\text{S11})$$

whereas for the internal fractions  $\theta_{j,k,i}$  the internal activities  $a_j^{\text{int}}$  should be used in Eqn (S11). In Eqns (S10) and (S11),  $N$  is the number of species binding to the HS,  $M$  the number of site types,  $Q_{\max}$  the total number of sites per unity of mass,  $Q_{\max,k}$  the same for type  $k$  sites (assuming 1:1 binding),  $q_k$  the fraction of type  $k$  sites ( $Q_{\max,k} = q_k Q_{\max}$ ),  $n_{j,k}$  the nonideality parameter for species  $j$  on type  $k$  sites, ( $n_{1,k}$  for  $\text{H}^+$ ),  $p_k$  is the heterogeneity parameter of type  $k$  sites and  $K_{j,k}$  is the mean binding constant of species  $j$  on type  $k$  sites. Two site types are often considered: low affinity sites ( $k = 1$ ) and high affinity sites ( $k = 2$ ) (Kinniburgh et al., 1999; Milne et al., 2001; Molina, 2013) so  $M = 2$  (albeit some studies propose three types (Pernet-Coudrier et al., 2011)), and for binding of a metal cation in the presence of protons  $N = 2$ ; low and high affinity sites are often referred to as carboxylic-like and phenolic-like, respectively.  $Q_{j,el}$  is the excess amount of species  $j$  electrostatically bound in the gel phase, which usually is negligible (Montenegro et al., 2014; Orsetti et al., 2013).

## Experimental details

**Chemicals.** IHSS standard humic acid Leonardite was used as received.  $\text{NaClO}_4$  and  $\text{NaOH}$  Merck p.a.,  $\text{Pb}(\text{NO}_3)_2$  and  $\text{HClO}_4$  Fluka puriss. p.a. were used as received. Water was provided by a Millipore Milli-Q system.

**Acid-base titrations.** Titrations were carried out following a procedure similar to that described by Milne et al. (1995). The pH was measured with a Hanna pH 213 pH meter. Measurements were recorded when the drift was less than 0.02 pH units/minute, or when more than 20 minutes has elapsed.

Titrations were performed at three different ionic strengths: 0.1 M, 0.025 M and 0.007 M with NaClO<sub>4</sub> as support electrolyte. About 30 mg HA were dissolved in 1.0 mL of 0.1 M NaOH. The solution was deoxygenated with N<sub>2</sub>, acidified with 1 M HClO<sub>4</sub> and diluted to 25.0 mL with supporting electrolyte. The sample was then titrated with 0.1 M NaOH, recording the pH with either a Phoenix or a Radiometer glass electrode and a Ag/AgCl reference electrode (Radiometer REF201). The whole process was carried out under N<sub>2</sub> atmosphere and at a constant temperature of 25.0 ° C.

**Pb(II) potentiometric titrations.** Pb(II) potentiometric titrations of LHA were performed following known procedures (Benedetti et al., 1995; Kinniburgh et al., 1995). Free Pb(II) activity was measured by a Pb ion selective electrode (ISE, Radiometer ISE25Pb) with the same reference electrode. The ISE was calibrated and checked by running titrations of 2-picolinic acid with Pb(NO<sub>3</sub>)<sub>2</sub>, as in Orsetti et al. (2013).

In the LHA-Pb titrations, About 50 mg of HA were dissolved in a minimum amount of 0.1 M NaOH and then brought to 25,0 mL with supporting electrolyte (0.1 M NaClO<sub>4</sub>), adjusting the pH with HClO<sub>4</sub>; after each addition of Pb(NO<sub>3</sub>)<sub>2</sub>, small aliquots of HClO<sub>4</sub> or NaOH, were added to correct the pH as required. The added volume was recorded and the actual ionic strength computed at each point. N<sub>2</sub> was bubbled throughout.

Data processing and fitting to the NICA-EPN model of all titrations were conducted following basically the procedure detailed in Orsetti et al (2010), but no conditional

affinity spectra was obtained; instead, typical parameters for HA-H<sup>+</sup> and HA-Pb<sup>2+</sup> interactions were used as starting values in the fitting.

### **Image processing**

All the AFM images were processed and analyzed using Gwyddion software. The images were flattened applying the Gwyddion tools: 1) Align rows, 2) Remove scars, and 3) Flatten base.

Grains were marked by thresholding of 0.3 nm of height. The grains smaller than 6 pixel area or touching image edge were excluded. The heights of the highest pixel of each grain were taken for the statistical analysis with Eqs (1-4) of the main text.

### **Supplementary NICA-EPN results for proton and Pb(II) binding to Leonardite humic acid**

Figures S1 and S2 show the NICA-EPN model predictions for the relative humic radius ( $r/r_0$ ) and Donnan potential ( $\psi_D$ , in Volts) change with pH. Figure S3 shows the speciation of proton among the different sites. Figures S4 and S5 shows Pb(II) and H<sup>+</sup> speciation, respectively, in the Pb(II) titration experiments.

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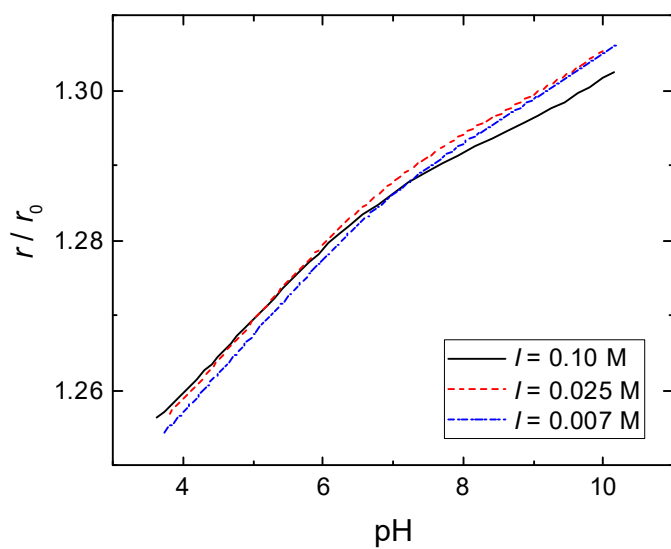


Figure S1. Results for the swelling of LHA particles as a function of pH, plotted as relative radius as a function of pH, as predicted by the NICA-EPN model.

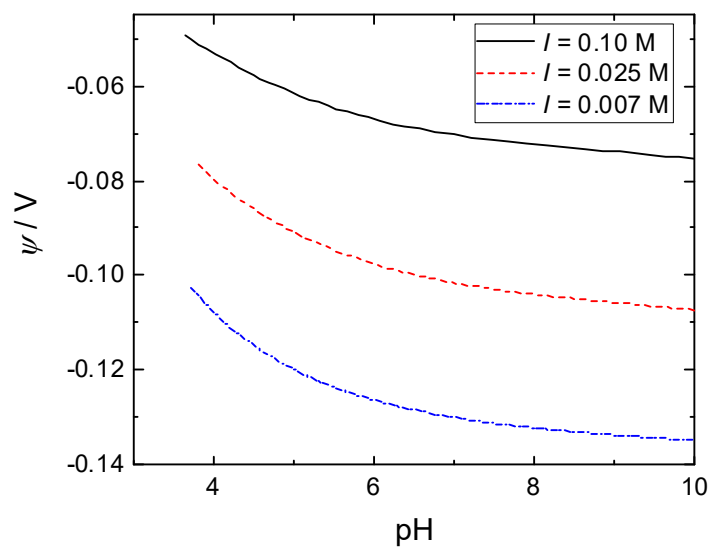


Figure S2. Donnan potential (Volts) as a function of pH for LHA as predicted by the NICA-EPN model.



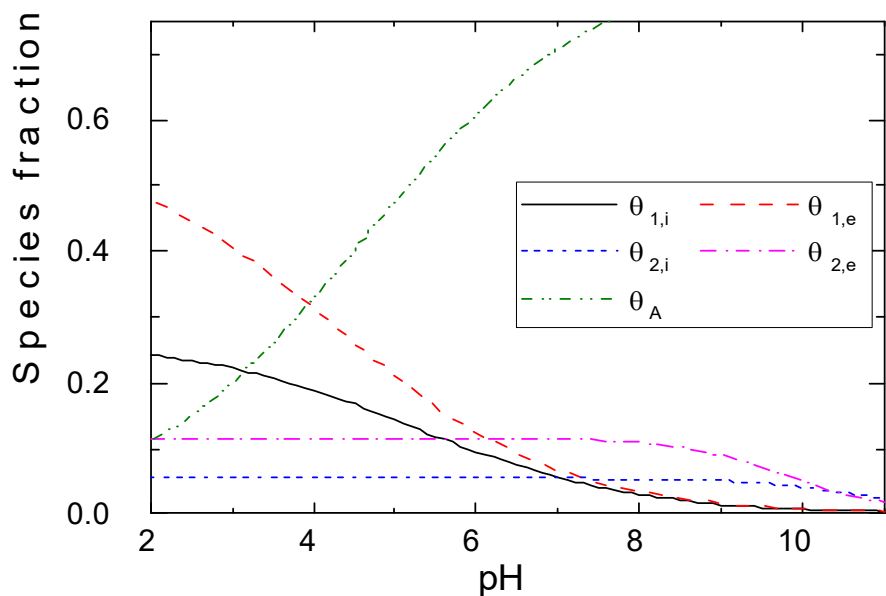


Figure S3. Speciation of proton binding groups in the NICA-EPN model at  $I = 0.1$  M.  $\theta_A$  is the total fraction of ionized (unprotonated) sites; subscript 1 indicates carboxylic type sites whereas 2 refers to phenolic type. Subscript “i” stands for internal (gel) sites and “e” stands for external (solution) sites.

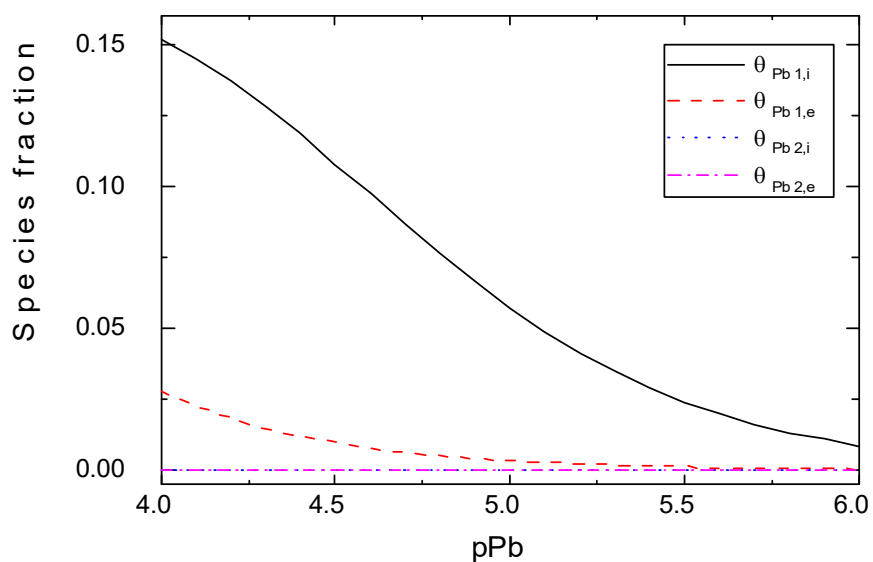


Figure S4. Speciation of Pb(II) binding groups as predicted by the NICA-EPN model at  $\text{pH} = 5.0$  and  $I = 0.1$  M; subscript 1 indicates carboxylic type sites whereas 2 refers to phenolic type. Subscript “i” stands for internal (gel) sites and “e” stands for external (solution) sites.

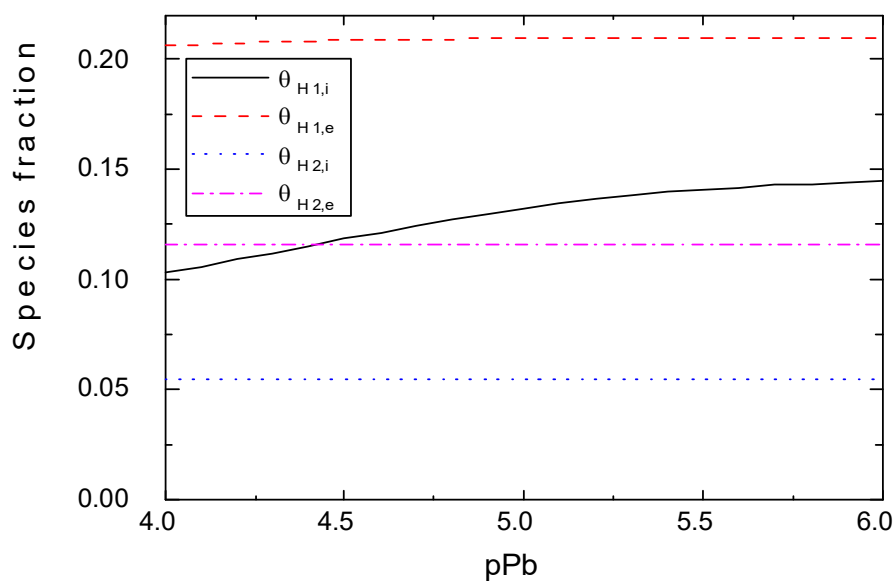


Figure S5. Speciation of proton among binding groups in the presence of Pb(II), as predicted by the NICA-EPN model, at pH = 5.0 and  $I = 0.1$  M; subscript 1 indicates carboxylic type sites whereas 2 refers to phenolic type. Subscript “i” stands for internal (gel) sites and “e” stands for external (solution) sites.

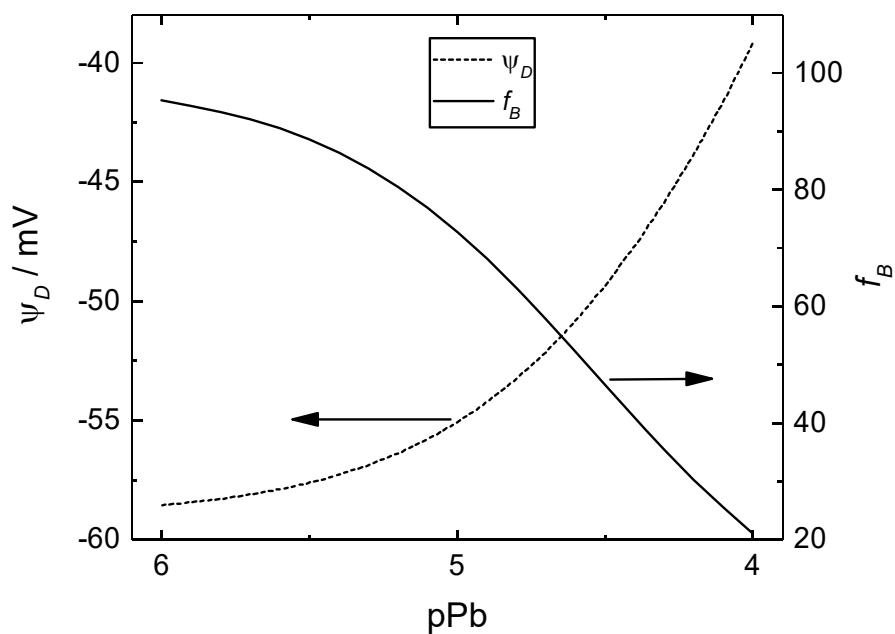


Figure S6. Donnan potential and Boltzmann factor for  $\text{Pb}^{2+}$  ions in the gel fraction, as a function of free Pb activity, as predicted by the NICA-EPN model, at pH = 5.0 and  $I = 0.1$  M.

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