

## Supplementary material

### **Isolation and purification treatments change the metal-binding properties of humic acids: effect of HF/HCl treatment**

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## Characteristics of FP and AE humic acids

Atomic ratios obtained using elemental analyses (Table S1) can be used to provide information concerning the structural characteristics of the humic acid. A smaller H/C ratio indicates a greater aromaticity, whereas a greater C/N ratio indicates a larger degree of decomposition of the organic matter, indicating that there was degradation of material and consequently increased formation of humic substances. Giovanela et al.<sup>[1]</sup> suggested that H/C ratios larger than 1 most probably indicate a vascular plant origin of the humic acids. The value of the H/C ratio obtained for the FP is closer to 1, which is similar to values reported in the literature for peat and soil humic acids.

The E4/E6 ratio is an important indicator of the degree of condensation of the humic macromolecule, and is generally associated with its aromaticity. According to the literature, ratios lower than 4 are indicative of a greater presence of condensed aromatic structures, while values greater than 4 indicate a lack of such structures<sup>[1,2]</sup>. Thus, it can be inferred that the AE has a higher degree of aromaticity when compared with the FP, the last being quite similar to the PPHA. These results suggest that the purification procedure alters to some extent the structural characteristics of the extracted humic materials.<sup>[3]</sup>

**Table S1. Elemental analysis (CHN), ash content, atomic ratios (H/C and C/N) and spectroscopic analysis (E<sub>4</sub>/E<sub>6</sub>) for several humic acids samples**

AEHA, alkaline extracted soil HA; FPHA, fully purified HA; PPHA, purified peat HA; KFHA,

Kinshozan F soil HA

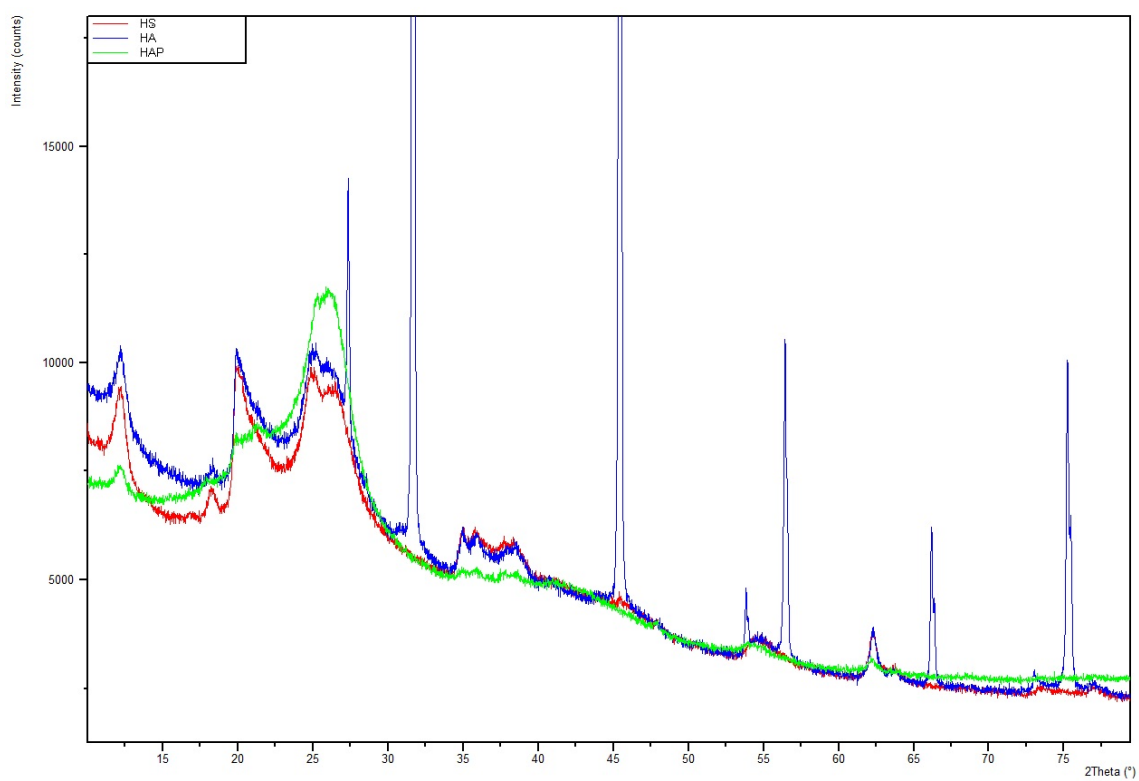
Sample	C	H	N	%Ash	H/C	C/N	E <sub>4</sub> /E <sub>6</sub>	Reference
AEHA	36.5	3.5	1.4	7.9	1.16	29.8	4.8	This work
FPHA	51.3	4.2	3.8	0.6	0.97	15.6	6.3	This work
PPHA	52.1	5.1	2.4	0.2	1.17	25.6	6.5	[4]
KFHA	57.1	4.5	3.9	–	0.94	16.9	–	[5]

**Table S2. NMR analysis for several humic acids samples**

AE, alkaline extracted soil HA from Mogi river; FP, fully purified HA from Mogi river; HAC1, Relict oak forest soil; HAHf, HA isolated from peat bog

Sample	Alkyl 0-50ppm	O-alkyl 50-110 ppm	Aromatic 110-160 ppm	Carboxyl 160-190 ppm	Reference
AEHA	32.5	46	19	2	This work
FPHA	25.5	40	15	4	This work
HAC1	25	39	24	11 <sup>A</sup>	[6]
HAHf	29	40	21	8	[7]

<sup>A</sup>160–220 ppm



**Fig. S1.** XRD analysis of the original soil material (HS) in red, the alkaline extracted (AE) in blue and the fully purified (FP) material in green. The large spikes in the AEHA material correspond to NaCl.

### Available metal contents in the AEHA sample (HNO<sub>3</sub> 0.43 M extraction)

**Table S3.** ICP-OES results for the extraction of major and minor cations present in the AE sample after extraction with 0.43 M HNO<sub>3</sub> for 2, 4 and 48 h

Concentration of major cations (mg kg <sup>-1</sup> )								
<i>t</i> (h)	Al	Ca	Cu	Fe	K	Mg	Mn	Na
2	16700	210	79	1370	90	27	10	69400
4	17401	230	73	1320	90	24	10	71700
48	19399	210	56	1440	80	26	10	71500

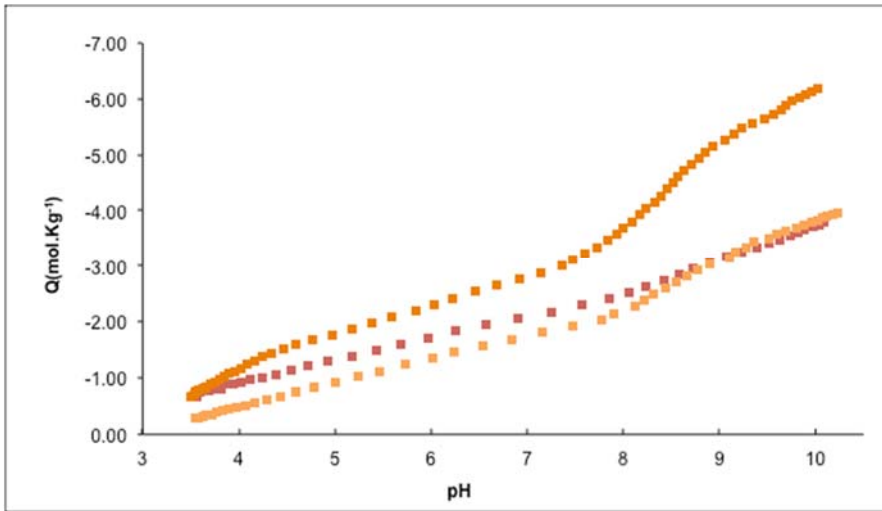
  

Concentration of minor cations (µg kg <sup>-1</sup> )					
<i>t</i> (h)	Zn	Cd	Pb	Ni	Co
2	3000	25	7609	1482	286
4	3000	26	7650	1500	283
48	3000	25	7131	1607	291

### AEHA proton titration

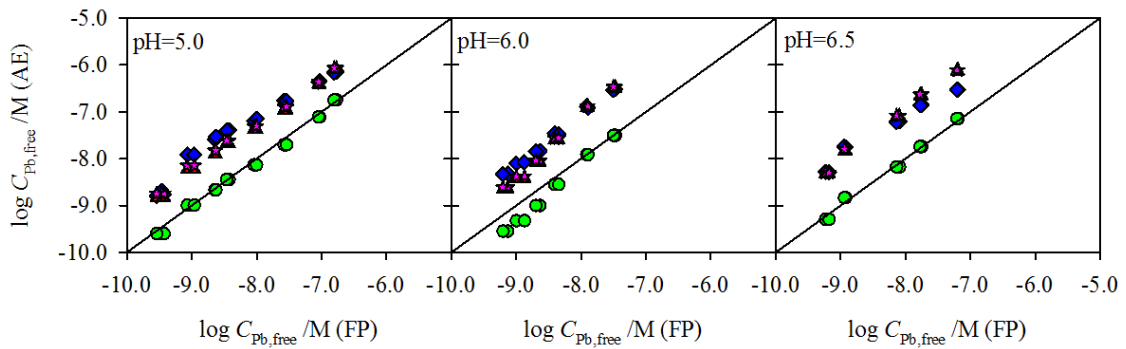
The AEHA proton titrations were performed according to the methodology proposed by Lopez et al. <sup>[8]</sup>, which consist of an acid calibration, between pH 2 and pH 3 at a certain ionic strength followed by the AE base titration at the same ionic strength. This methodology can be applied to lower concentrations of humic materials; however, the absence of an acid return titration does not allow the assessment of the hysteresis.

We observed that the repeatability of these titrations was very poor (Fig. S2), preventing us from applying the NICA Donnan modelling.

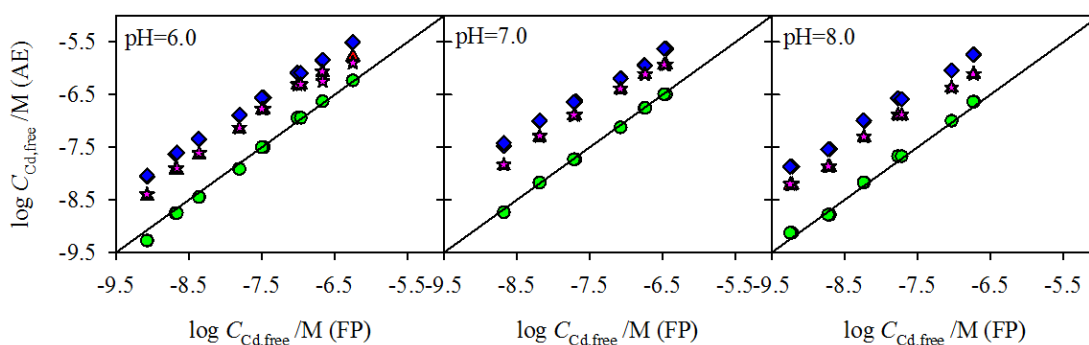


**Fig. S2.** Potentiometric titrations at  $I$  0.01 M of AEHA in triplicate (■ ■ ■) (adjusted with  $\text{NaNO}_3$ ).

### AEHA metal binding



**Fig. S3.** Logarithm of free  $\text{Pb}^{2+}$  in presence of AEHA vs. the logarithm of free  $\text{Pb}^{2+}$  in presence of FP for the same amount of organic material and at  $I$  0.01 M for pH 5.0, 6.0 and 6.5. Symbols represent:  $\blacklozenge$ , determined by AGNES,  $\bullet$ , fit results;  $\blacktriangle$ , adjusted Q1;  $\blackstar$ , adjusted Q1 with Al added.



**Fig. S4.** Logarithm of free  $\text{Cd}^{2+}$  in presence of AEHA vs. the logarithm of free  $\text{Cd}^{2+}$  in presence of FP for the same amount of organic material and at  $I$  0.01 M for pH 5.0, 6.0 and 6.5. Symbols represent:  $\blacklozenge$ , determined by AGNES;  $\bullet$ , fit results;  $\blacktriangle$ , adjusted Q1;  $\blackstar$ , adjusted Q1 with Al added.

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