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3 **Effect of mineral surface properties (alumina, kaolinite) on the sorptive**  
4 **fractionation mechanisms of soil fulvic acids: molecular-scale ESI-MS studies**

5

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24 **ABSTRACT**

25

26 We addressed the effects of mineral surface properties (kaolinite versus Al-oxide) on the sorption-driven  
27 fractionation of a soil fulvic acid (FA) at acidic pH, mainly by means of ESI(-)-FTMS analysis of initial and  
28 supernatant solutions of FA sorption batch experiments. The MS data provided clear molecular-scale evidence  
29 of distinct mechanisms and molecular parameters controlling the FA fractionation upon its sorption on clay  
30 and oxide surfaces, respectively.

31 Identification of sorbing and not-sorbing FA compounds in kaolinite-solution systems revealed a weak  
32 fractionation amongst members of  $-CO_2$  series of aliphatics or not-condensed aromatics (NCAs) at pH 3.8,  
33 and almost no sorption of poorly-oxygenated polycyclic aromatic compounds (PACs) and NCAs. This first  
34 molecular-scale description of a FA fractionation in a clay-solution system suggests that H-bonding with low  
35 affinity sites (aluminol/silanol) on the basal planes of the clay particles is the main mechanism of sorption.  
36 Due to the predominance of such weak and poorly-selective mechanism, the sorption of aliphatic and NCA  
37 molecules bearing oxygenated functionalities was prevented at pH 5, due to dissolved Al competing  
38 successfully for their coordination.

39 In contrast, a strong FA fractionation was observed onto alumina, with a preferential retention of PACs and  
40 highly-oxygenated aliphatics and NCAs. The major part of the poorly oxygenated aliphatics was left in  
41 solution. The sorption degree of NCAs and aliphatics was strongly correlated with molecular acidity. For  
42 PACs and poorly-oxygenated NCAs, the sorption was driven by reactions of surface ligand exchange (for the  
43 most oxygenated compounds) or by hydrophobic interactions (for the least oxygenated compounds).

44

45 **Key words:** humic substances; kaolinite; alumina; fractionation; mass spectrometry

46

## 47 1. INTRODUCTION

48

49 Humic substances (HSs) like fulvic acids (FAs) are complex heterogeneous mixtures of thousands of  
50 biochemically stable polymeric substances (Aiken et al., 1985) which are ubiquitous in all terrestrial surface  
51 systems and which account for a great part of organic carbon in soils. HSs show a variety in the composition,  
52 structure and reactivity of their constituents and functional groups (Swift, 1989; Plancque et al., 2001). It has  
53 long been known that HSs have the ability to interact with metals (Dobbs et al., 1989; Tipping, 2002; Milne et  
54 al., 2003; Janot et al., 2013), organic pollutants (Rebhun et al., 1996) and surfaces of (nano) particles of clays  
55 and Al- or Fe-oxyhydroxides (Ochs et al., 1994; Schlautman and Morgan, 1994; Feng et al., 2005; Janot et al.,  
56 2013), with the sorption of HSs resulting in its chemical fractionation between minerals and solution (Meier et  
57 al., 1999; Zhou et al., 2001; Reiller et al., 2006). Sorption-driven fractionation of HSs is expected to influence  
58 surface characteristics (reactivity, sorption capacity, charge) of minerals, dispersion behavior of suspended  
59 nanoparticles which are potential pollutants' carriers, and metal binding capacity of dissolved organic matter  
60 in many geochemical systems (Ochs et al., 1994; Schlautman and Morgan, 1994; Yang et al., 2013).  
61 Understanding of the fractionation of HS during the sorption at surfaces of mineral particles is thus of interest  
62 in many environmental issues including sorption / mobility of trace metal elements in soils and aquatic  
63 systems. It has some implications on the evaluation of the impact on health of the release of pollutants in the  
64 ecosystems and on the safety assessments of remediation processes of polluted soils or radioactive waste  
65 repositories in geological formations (Loffredo and Senesi, 2006; Bryan et al., 2012).

66 Building a comprehensive description of the coupled cycles of organic matter and pollutants in natural  
67 systems requires some basic knowledge of the fractionation trends exhibited by HSs during their sorption at  
68 mineral surfaces relevant to soils and waters, as well as a thorough understanding of the mechanisms and  
69 molecular parameters driving the fractionation -which is still lacking-. Speaking in a general way, several  
70 mechanisms of sorption of HSs onto minerals were proposed, including surface ligand exchange, specific  
71 adsorption, electrostatic interactions, hydrophobic effects, H-bonding and metal-ion bridging (Ochs et al.,  
72 1994; Schlautman and Morgan, 1994). In many studies, HSs were considered as bulk materials bearing polar  
73 functionalities and spectroscopic/modeling evidence was given that carboxyl functional groups of HS (Fu et  
74 al., 2005; Ghosh et al., 2009) are involved in the macroscopic sorption of HS at surfaces of iron or aluminum  
75 oxide minerals and that the contribution of phenol groups increases with pH (Filius et al., 2003; Claret et al.,

76 2008). In the last two decades, there has also been a growing interest in getting a detailed understanding of the  
77 behavior of HSs during their sorption at (nano) mineral - solution interfaces. Many spectroscopic evidences  
78 were provided that a strong sorptive fractionation takes place between the diverse classes of organic  
79 compounds of a humic substance (e.g. Meier et al., 1999; Claret et al., 2008; Ghosh et al., 2009). It was  
80 reported that the hydrophobic fractions of FAs and/or the organic molecules with high contents in aromatic  
81 moieties activated by oxygenated functionalities were preferentially sorbed –as compared to aliphatic  
82 fractions- on the surfaces of metallic oxide minerals (Meier et al., 1999; Zhou et al., 2001; Kaiser, 2003;  
83 Claret et al., 2008). Moreover, several studies combining size-exclusion-chromatography and spectroscopy  
84 analysis have revealed a size fractionation of fulvic or humic acids during their sorption at several mineral  
85 surfaces (e.g. Hur and Schlautman, 2004), which was explained by structural trends in the molecular weight  
86 fractions (degree of aromaticity, functionalities...) and the underlying sorption processes. Since recently, the  
87 development of an advanced technique, namely ultra-high resolution Fourier transform mass spectrometry  
88 (FTMS), has offered unique opportunities for identifying the molecules constitutive of a HA or FA (e.g.  
89 Kujawinski et al., 2002; D’Andrilli et al., 2010) and for approaching the issue of the HS-metallic oxide-  
90 solution interactions at the molecular scale (Reiller et al., 2006; Galindo and Del Nero, 2014; Galindo and Del  
91 Nero, 2015). Published FTMS data for the characterization of HSs (Plancque et al., 2001; Stenson et al., 2002;  
92 Sutton and Sposito, 2005) have supported the model proposed by Wershaw (1993) and by Engebretson and  
93 von Wandruszka (1994), and recently revived by Piccolo (2001) and Plancque et al. (2001), which describes  
94 an HS as a supramolecular association of small organic entities (“building blocks” of ca. 500 Da for FAs)  
95 assembled via weak bonds. This model has led to view an HS as a complex mixture of thousands of  
96 polycarboxylates of distinct molecular weight, degree of aromaticity or acidity, and chemical reactivity.  
97 Recently, the ESI-FTMS technique was applied by our research team to the identification of sorbing (and not  
98 sorbing) molecules of an aquatic fulvic acid (Galindo and Del Nero, 2014), and of a terrestrial humic acid  
99 (Galindo and Del Nero, 2015) during experiments of HS-alumina-solution interactions. A striking result was  
100 an inverse correlation existing between the degree of sorption of a molecule within a CH<sub>2</sub> series and its  
101 number of CH<sub>2</sub> groups, and a positive correlation between the degree of sorption and the number of CO<sub>2</sub>  
102 groups in a COO series, for homologous series containing up to nine members. In agreement with previous  
103 experimental work by Evanko and Dzombak (1998) on simple organic acids and modelling work by Janot et  
104 al. (2012) suggesting that enhanced sorption with increasing number of carboxyl groups was due to changes in

105 compound acidity, the correlations observed for FA by ESI-FTMS provided for the first time a molecular  
106 scale evidence that the sorptive fractionation of complex mixtures of thousands of organic compounds as  
107 fulvic acids was controlled by molecule acidity, which was remarkable. These ESI-FTMS findings also  
108 strongly supported that the FA subunits, held together by hydrophobic forces and hydrogen bonding, were  
109 disrupted during sorption because of strong FA–alumina surface interactions via surface ligand exchange  
110 mechanism, which was consistent with the concept of supramolecular assembly for HS (Piccolo, 2001;  
111 Piccolo et al., 2010). Hydrophobic interactions were moreover found to be a mechanism cooperating to the  
112 sorption onto alumina of the poorly-oxygenated polycyclic aromatic compounds (PACs) of a terrestrial HA,  
113 with the degree of molecule hydrophobicity influencing the degree of molecule sorption.

114 In contrast to the metallic oxide systems, very few information is available on the sorption-driven  
115 fractionation of HSs onto clay minerals and no molecular scale description of the process has been reported so  
116 far. Unlike for iron or aluminum (hydr)oxides, the aliphatic fractions of a humic acid were found to be more  
117 prone to sorption on the surface of kaolinite and montmorillonite than the aromatic fractions (Wang and Xing,  
118 2005; Ghosh et al., 2009). Such contrasting results between metallic oxides and clays have pointed the need to  
119 acquire detailed knowledge of the molecular characteristics and the sorption mechanisms driving HS sorptive  
120 fractionation in clay mineral – solution systems, which are relevant to many geochemical systems. In  
121 particular, such knowledge is critically needed for developing a comprehensive understanding of the sorptive  
122 fractionation of FAs in the various types of soils (rich in metal oxi-hydroxides or in clays).

123 In this study, we addressed the effects of mineral surface properties, i.e., clay versus aluminum oxide, on the  
124 molecular scale fractionation of a terrestrial FA during its sorption at mineral surface by mean of  
125 complementary techniques, traditional batch experiments, electro-kinetic measurements and electrospray  
126 ionization FTMS. The systems under investigation included an extracted FA (PPH) and kaolinite or alumina  
127 as a model sorbent taken as being representative of Al (hydr)oxides or clays in soils, respectively. First, batch  
128 sorption experiments and on-line electrophoretic mobility measurements were performed in PPH-containing  
129 mineral suspensions for studying the pH-dependency of sorption of PPH onto alumina and kaolinite and for  
130 gaining insights into surface charges of PPH-loaded particles. The use of a LTQ Orbitrap XL hybrid mass  
131 spectrometer allowed deriving the exact elemental composition of the molecules of PPH present in the  
132 solution before and after contact with the metal oxide or the clay mineral, for experiments performed under  
133 acidic and middle acidic solutions (pH 3.5 and 5.0, respectively). The molecular approach was used to retrieve

134 the distinct orders of relative affinity of the FA molecules for the surface of a clay and a metallic oxide,  
135 respectively, and to explore the relations existing between chemical characteristics of the PPH molecules  
136 (such as number of CO<sub>2</sub> functionalities, degree of aromaticity or acidity...) and their degree of relative  
137 sorption onto mineral. The molecular-scale results presented here represent a significant contribution to our  
138 understanding of the fractionation trends of a terrestrial FA during its sorption onto a clay mineral, and of the  
139 sorption mechanisms involved, and highlight the strong effects of mineral surface properties on the  
140 fractionation behavior of terrestrial fulvic acids.

141

## 142 **2. EXPERIMENTAL SECTION**

143

### 144 **2.1. Materials**

145

146 The fulvic acid denoted as “PPH” was extracted from a soil sample collected in a beech forest developed on  
147 sandstone, near La Petite-Pierre, Bas-Rhin, France. The extraction was carried out by using the International  
148 Humic Substances Society (IHSS, St. Paul, MN, USA) standard method for isolation of soil fulvic acids. This  
149 method, excerpted from Sparks et al. (1996), uses XAD-8 resin adsorption to isolate such acids. Due to the  
150 chemical properties of the resin, the fulvic acids used here only contain hydrophobic organic compounds.  
151 Stock solutions of PPH fulvic acid were prepared and stored frozen in the dark until used.

152 Alumina colloids were Alfa Aesar's  $\alpha$ -Al<sub>2</sub>O<sub>3</sub> crystallites (chemical purity: 99.95%, surface area: 7.6 m<sup>2</sup>/g)  
153 with a particle size of 280 nm as determined by dynamic light scattering ( $[\alpha\text{-Al}_2\text{O}_3] = 2.5 \text{ g.L}^{-1}$ , particle size  
154 determined in % of intensity,  $w_{50\%} = 90 \text{ nm}$ , polydispersity index = 0.12). The kaolinite sample used was the  
155 standard sample KGa-1b provided by The Clay Minerals Society. This sample was characterized by Pruett  
156 and Webb (1993), who reported a particle distribution of 57.8% < 2 $\mu\text{m}$  and 32.0% < 0.5 $\mu\text{m}$ , as well as a  
157 surface area of 11.7m<sup>2</sup>.g<sup>-1</sup>. This value for surface area of kaolinite has been used as such and no attempt was  
158 made to determine again this value.

159 All solutions were prepared using reagent grade chemicals and ultra-pure water (purity > 18 M $\Omega$ .cm).

160

### 161 **2.2. Experimental setup**

162

163 Experiments of FA sorption at acidic pH and 298K on alumina and kaolinite were carried out for analysis of  
164 the supernatants by ESI(-)-FTMS and study of the mineral surface characteristics on FA sorptive  
165 fractionation. The experiments were carried out at  $\text{pH } 3.8 \pm 0.1$  and  $4.9 \pm 0.1$  for both minerals.

166 Alumina and kaolinite suspensions were prepared in individual PEHD tubes with solid-to-solution ratios of 25  
167 and  $15 \text{ g.L}^{-1}$ , respectively. These values of solid-to-solution ratios were chosen so that the surface areas of  
168 alumina and kaolinite used in the experiments fall in the same range of values (ca. 170 and  $150 \text{ m}^2/\text{L}$ ,  
169 respectively). Efforts were made to adjust the concentration of PPH added in the individual tubes, so that the  
170 values of FA-to-mineral ratios ( $r$ ) of all experiments fall within a narrow range ( $r \sim 5 \text{ mg C/g}$ , or more  
171 precisely,  $r \sim 0.6$  and  $0.8 \text{ mgC/m}^2$  of mineral for sorption of FAs on kaolinite and alumina, respectively). No  
172 attempt was made to fix the ionic strength of the initial experimental solutions, as background salts were  
173 previously observed to alter considerably the ESI-MS response. After shaking of the tubes for 24 hours at  
174 298K, the final pH values of the suspensions were measured. The suspensions were then centrifuged at 8000  
175 rpm during 2.5 hours for solution-colloid separation and the supernatants were collected. Aliquots were taken  
176 for measurements of dissolved organic carbon, DOC, using a Shimadzu TOC  $-V_{\text{CPH}}$  analyzer, and of  
177 dissolved aluminum content using Inductively Coupled Plasma Mass Spectrometry (quantification limit:  
178  $5.3 \mu\text{g/L}$ ). All the remaining supernatant solutions were adjusted (using a 0.01M HCl solution) to a same pH  
179 value of  $3.8 \pm 0.1$  prior to ESI-MS analysis. Such an acidification procedure allows a direct comparison of the  
180 datasets obtained for the different experiments (as pH is known to affect ionization of compounds during ESI-  
181 MS analysis). An exception was however made for the supernatant of the PPH-kaolinite sorption experiment  
182 at pH 5.0, which was analyzed as collected because the ESI(-)MS spectra obtained for the acidified  
183 supernatant sub-sample were found to be of very poor quality due to the amount of salt introduced in the  
184 sample to obtain a pH of 3.8. It is to be noted that the ESI-MS data obtained from an analysis at pH 5.0 cannot  
185 be straightforwardly compared to those obtained from analysis at pH 3.8.

186 Complementary series of batch experiments were performed to study the macroscopic sorption of FA as a  
187 function of pH and to perform on-line electro-kinetic measurements of the FA-containing suspensions of  
188 kaolinite or alumina. Same procedure as that described above was used, except that an aliquot of sample was  
189 taken before centrifugation for particle electrophoretic mobility (EM) measurements, which were carried out  
190 using dynamic light scattering with a Malvern Zetasizer Nano ZS instrument. Values reported for EM  
191 correspond to mean values calculated on the basis of three replicates of a same measurement. Sorption



192 isotherms and EM values in the pH range 3.5-7 were determined for the alumina – PPH systems ( $r = 5-10$   
193 mgC/g). For the kaolinite – PPH system, both the effect of pH and ratio  $r$  on the percent of FA sorption and  
194 EM of particles were investigated ( $r$  value up to 50 mgC/g).

195

## 196 **2.3. ESI-MS instrumentation and data analysis**

197

### 198 **2.3.1. Instrumentation and acquisition parameters**

199

200 A Thermo Scientific LTQ Orbitrap hybrid mass spectrometer was used for the analysis in ESI negative  
201 ionization mode of native FA solutions and supernatants. Negative ion ESI appears to be the most appropriate  
202 ionization mode for the analysis of anionic compounds such as fulvic acids, which are already partly pre-  
203 ionized in solution. Ultra-pure water was used as the electrospray solvent as other solvents have been found to  
204 alter the FA properties (Piccolo et al., 2001). The samples were infused directly into the ESI source with a  
205 spray voltage of 3.5 kV at a flow rate of  $10 \mu\text{L}\cdot\text{min}^{-1}$ , and were analyzed in negative ion mode. Nitrogen was  
206 used as the drying and spraying gas. For each acquisition, 100 scans (2s/scan) were co-added using Xcalibur  
207 software. The Orbitrap MS was externally calibrated in negative ion mode on the 50-2000  $m/z$  range using a  
208 LTQ/FT-Hybrid ESI Calmix solution, for a mass accuracy better than 3 ppm. Instrument operating parameters  
209 (capillary voltage and temperature, tube lens voltage) were optimized one after the other for an optimal  
210 transmission of higher  $m/z$  ions. It was found that modifying the instrumental parameters did not affect  
211 important characteristics of spectra such as ion distribution, charge state or mass spacing patterns. The source  
212 capillary was finally held at -50 V and 275 °C, and a voltage of -240 V was applied to the tube lens.

213 All ESI(-) mass spectra were recorded in the ranges 120-400  $m/z$  and 400-798  $m/z$  in order to improve the  
214 mass accuracy in the region of low masses as well as the signal-to-noise ratio (S/N) for compounds having the  
215 highest values of  $m/z$  in samples.

216

### 217 **2.3.2. Elemental formula determination**

218

219 Elemental formulae were assigned to the measured  $m/z$  values using the Xcalibur software. The following  
220 criteria were chosen for assignment of molecular formulas to the peaks detected on the mass spectra:

- 221 – Signal-to-noise ratio of peak > 4,  
 222 – Mass accuracy better than 3 ppm,  
 223 – Application of the following ranges:  $^{14}\text{N} \leq 1$ ;  $^{16}\text{O} \leq 50$ ;  $^{12}\text{C} \leq 200$ ;  $^1\text{H} \leq 600$  (N, O, C and H being  
 224 respectively the number of Nitrogen, Oxygen, Carbon and Hydrogen atoms).  
 225 –  $0,2\text{C} \leq \text{H} \leq 2\text{C} + 3$   
 226 Double Bond Equivalent  $\frac{2+2\text{C}-\text{H}+\text{N}}{2} \geq 0$

227

228 Elemental analyses showed that the PPH sample contains low amounts of Nitrogen (< 3% w/w). Sulfur and  
 229 Phosphorus contents were assumed negligible (<1% w/w). Odd m/z values correspond to even mass, and  
 230 relate to chemical formulae with even number of nitrogen atoms. As the nitrogen content in the sample does  
 231 not exceed a few percent, we assumed that compounds peaking at odd m/z primarily contain C, H and O  
 232 atoms. All possible formula attributable to a given odd m/z value were thus calculated by considering only C,  
 233 H and O atoms. Due to low nitrogen content in HS, species peaking at even m/z were considered to contain at  
 234 most one N atom, in addition to C, H and O atoms. Ions corresponding to  $^{13}\text{C}$  isotopes and elemental formulae  
 235 that did not match the above criteria were not taken into account.

236

### 237 2.3.3. *Van krevelen diagrams*

238

239 The Van Krevelen (VK) diagram plots the H/C ratio vs. the O/C ratio of a molecule and is an effective  
 240 graphical method for displaying complex data from NOM analysis by ultrahigh-resolution mass spectrometry  
 241 (Kim et al., 2003). On the VK diagrams, the identified compounds are classified into three categories on basis  
 242 of their value of Aromaticity Index (A.I.) proposed by Koch and Dittmar (2006). The authors proposed a  
 243 modified formula for calculating A.I., considering that half of the oxygen is  $\sigma$ -bound:

$$\text{Aromaticity Index (A.I.)} = \frac{1 + \text{C} - \frac{\text{O}}{2} - \frac{\text{H}}{2}}{\text{C} - \frac{\text{O}}{2} - \text{N}}$$

244 They defined two threshold values of AI to distinguish between three categories of compounds, i.e., between  
 245 compounds having an unambiguous polycyclic aromatic structure (PACs, having  $0.67 < \text{A.I.}$ ), a not-  
 246 condensed aromatic structure (NCAs, having  $0.5 < \text{A.I.} < 0.67$ ), and a more or less pronounced aliphatic

247 character (A.I. < 0.5).

248

#### 249 **2.3.4. Definition of I**

250

251 In order to explore the mechanisms of the sorptive fractionation of FAs, we used the parameter I defined by  
252 Galindo and Del Nero (2014). The value of I for a given ion is equal to the ratio of the normalized peak  
253 intensity of this ion in the supernatant (after sorption) on the normalized peak intensity of the ion in the initial  
254 solution (before sorption), with the normalization being made to the sum of the intensities of all the ions  
255 (Total ion current, TIC) of the considered solution. Trends in the values of I for compounds in a FA supply  
256 information on trends existing in the orders of relative affinity of compounds for the mineral surface: the  
257 lower the value of I of a FA ion, the higher its relative affinity (by comparison with that of the other detected  
258 FA ions) for the surface of the mineral. Ions with  $I = 0$  are totally sorbed at the surface of mineral, and ions  
259 with  $I \neq 0$  are distributed between the surface and the solution.

260 It is well known that the intensity of an ion on a mass spectrum not only depends on its concentration but also  
261 on its ionization efficiency and on the matrix, i.e. on the number, the quantity and the structure of the other  
262 ions present in the sample. A simple dilution of initial FA solution can thus possibly have an effect on the  
263 relative peak intensities of the ions in the solution. As bulk concentration of PPH is lower in the supernatants  
264 collected at the end of the sorption experiments than in the native PPH solution (due to PPH sorption at the  
265 surface of the minerals), it is necessary to determine the effect of such a sorption-driven decrease in solution  
266 concentration on the I values of the FA ions and to differentiate it from the effect on I of the sorptive  
267 fractionation process itself. To this end, we compared the ESI(-)MS spectra recorded for initial PPH solution  
268 at pH 3.8 and at pH 4.9 used in the experiments to those obtained for PPH native solutions diluted by a factor  
269 2, and we calculated the “I values” of the FA ions resulting from a pure dilution of samples. Dilution by a  
270 factor of 2 was chosen because approximately 50% of total organic carbon was found to be removed from  
271 solution during the experiments of PPH sorption onto alumina and kaolinite. It was found (cf. Fig. A1 in the  
272 Appendix) that all PPH ions display I values equal to  $0.85 \pm 0.15$  due to dilution effect only (except for the  
273 400-798 m/z range at pH 3.8 where the values were equal to  $1.05 \pm 0.15$ ). Applying these results of the  
274 dilution tests to the interpretation of the I values of ions derived from the sorption experiments, it can be  
275 considered that a value of I lower than 0.7 (or 0.9 for the range 400-798 m/z range at pH 3.8) indicate a rather

276 good sorption of an ion on the mineral surface, while ions showing values comprised between 0.7 and 1 (or  
277 0.9 and 1.2 for the range 400-798 m/z range at pH 3.8) are poorly sorbed or not sorbed at all, and ions  
278 showing values higher than 1 (or 1.2) are not sorbed at all during the experiment.

279

### 280 3. RESULTS

281

#### 282 3.1. Macroscopic description of the FA-mineral systems

283

284 Fig. 1 shows the percentage of organic carbon of PPH sorbed onto alumina or kaolinite as a function of pH,  
285 for different FA/mineral ratios ( $r$ ). The results are as follows.

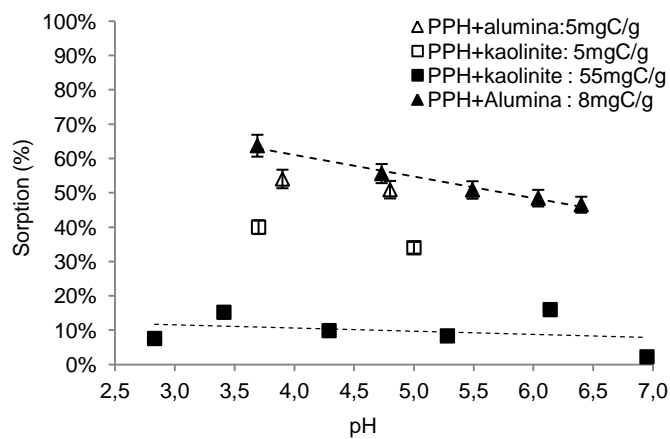
286 Regarding alumina, first, the sorption isotherm obtained for PPH indicate a slight pH-dependency of the  
287 sorption, i.e., a slight decrease of the percentage of sorption with increasing pH. Such a decrease with  
288 increasing pH value is consistent with an increase of the rate of deprotonation of both the reactive groups of  
289 the FA molecules and the aluminol sites on the surface of alumina. It has been widely reported that the  
290 carboxyl and phenol functional groups of FAs exhibit wide distributions of pK values, which are mostly lower  
291 than 4 for carboxyl groups (e.g. Ritchie and Perdue, 2003) and higher than 8 for phenol moieties (e.g. Gu et  
292 al., 1995). Moreover, the IEP of the alumina particles used here was previously found to be equal to 8.5 (Del  
293 Nero et al., 2010). An increase of the deprotonation with pH thus has the effect of diminishing electrostatic  
294 attractions between aluminol sites on the mineral surface and FA molecules, which is unfavorable to a close  
295 approach of the organic molecules to the vicinity of the surface. Thus, such features may lead to a slight  
296 decrease of the percentage of macroscopic sorption with pH observable in Fig. 1. The results of EM  
297 measurements performed on the systems investigated are presented on Fig. 2. The data show a sign reversal of  
298 the surface charge of the suspended alumina particles in the presence of PPH, evidencing that the FA-alumina  
299 interactions are not of electrostatic nature only but involve the formation of inner-sphere fulvate complexes on  
300 the surface of alumina. Chemical sorption is indeed the only sorption process capable of inducing such a sign  
301 reversal (Fairhurst and Warwick, 1998).

302 Fig. 1 shows that PPH has a lower overall affinity for the kaolinite surface than for the alumina surface (cf.  
303 data at  $r= 5\text{mgC/g}$ ). There also appeared that the percentage of PPH sorption decreases with an increase of the  
304 PPH / kaolinite ratio (cf. data at  $r= 5$  and  $55 \text{ mgC/g}$ ). Such a feature indicates that multiple sites on the mineral

305 surface were involved in the sorption of the PPH molecules and/or several types of surface complexes of very  
 306 distinct values of formation constant were formed. The former hypothesis is consistent with results of many  
 307 previously published studies reporting the existence of weak surface sites at basal planes of kaolinite particles  
 308 and of strong sites (high-affinity sites) at edges and/or defects of kaolinite(Sverjensky and Sahai, 1996). The  
 309 high-affinity sites existing in limited amounts onto the kaolinite particles might be saturated in our  
 310 experiments at low surface loadings of FA. High-affinity sites have long been reported to be protonated under  
 311 acidic and neutral pH conditions whereas the early deprotonation of basal aluminol sites (Wieland and  
 312 Stumm, 1992) is (partly) responsible for an overall negative surface charge of kaolinite (e.g. data on Fig. 2  
 313 obtained in the absence of FA). EM measurements reported in Fig. 2 shows that the sorption of the PPH  
 314 molecules imparts negative charges to the kaolinite surface, which is consistent with involvement of the high-  
 315 affinity sites in the sorption.

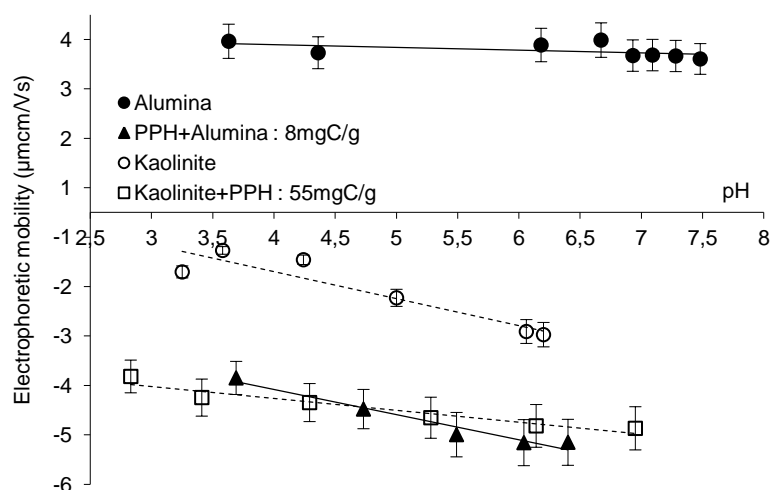
316 In summary, surface properties of mineral have an effect on the macroscopic behavior of sorption of FA at  
 317 acidic/near neutral pH, which highlights a need for further understanding their effect on the fractionation of  
 318 FA.

319



320

321 **Fig. 1:** Sorption isotherms of organic carbon obtained for different PPH-to-mineral ratios in alumina or  
 322 kaolinite suspensions.



323

324 **Fig. 2:** Electrophoretic mobility (EM) of alumina or kaolinite particles as a function of final pH of mineral-  
 325 solution suspensions, in the absence of PPH or at different PPH-to-mineral ratios.

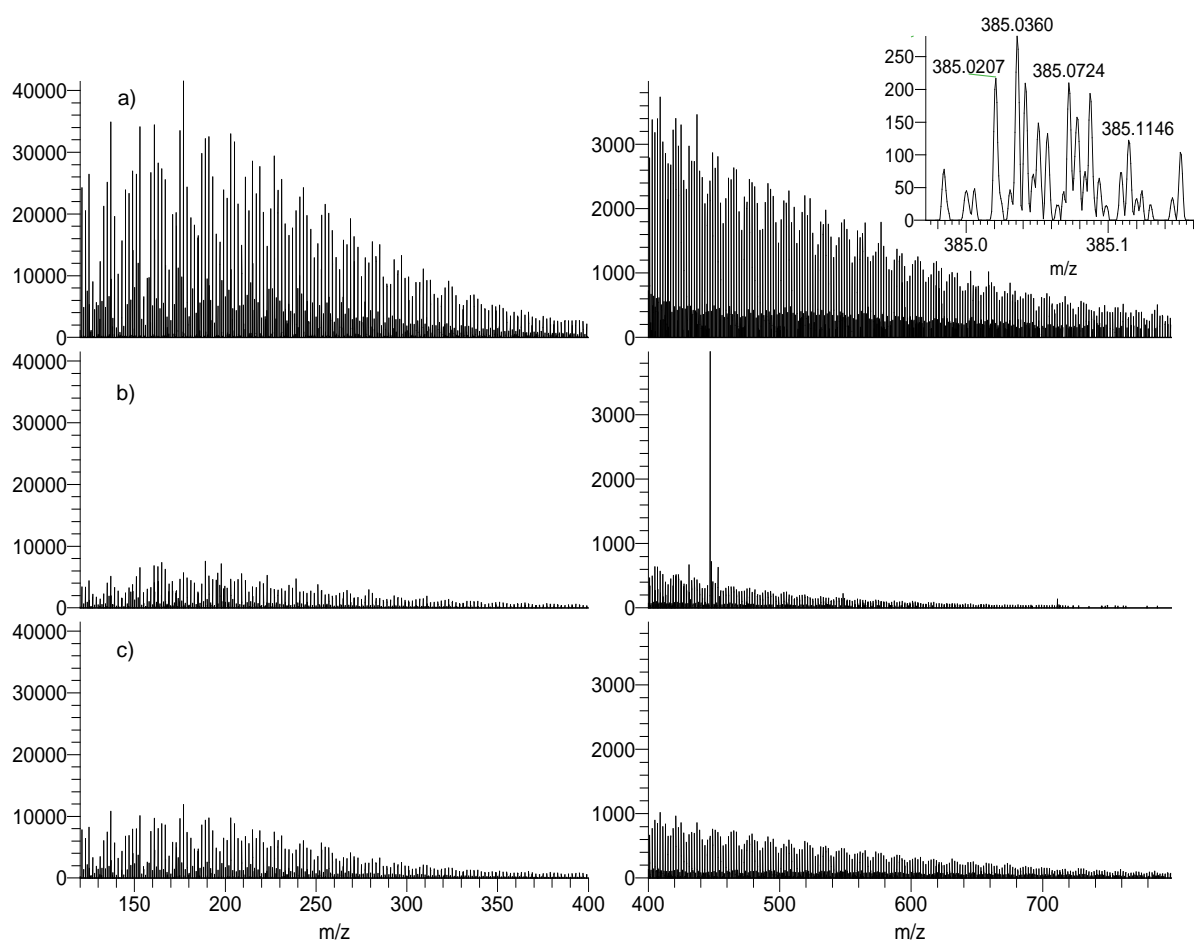
326

### 327 3.2. ESI(-)-FTMS spectra of initial solution of PPH and chemical formula assignments

328

329 The full mass spectrum recorded in the mass range 120-2000m/z for the native solution of PPH, using the  
 330 negative ionization mode, showed a mono-modal distribution of peaks lying between 120-800m/z (cf. Fig. A2  
 331 in the Appendix). The presence on our mass spectra of peaks at m/z<800 only is globally consistent with  
 332 previously published ESI-MS spectra of fulvic acids, which exhibit compounds of molecular weight no higher  
 333 than a few kDa (Piccolo, 2001; Sutton and Sposito, 2005; Reemtsma, 2009). Several authors have underlined  
 334 that their mass spectrometry data support the view of HS as being a supramolecular assembly, which can be  
 335 dissociated during ionization in the ESI probe, in particular when high voltages are applied to the transfer  
 336 optics (Piccolo et al., 2010). Fig. 3 shows the ESI(-) mass spectra recorded for the native solution of PPH in  
 337 the ranges 120-400 m/z and 400-798 m/z. Circa 1725 and 1190 peaks were detected with S/N > 4 in the  
 338 aforementioned mass ranges, respectively. The spectra display peaks of high intensity at odd-integral values  
 339 of m/z and peaks of lower intensity at even-integral values of m/z, with the latter peaks having been reported  
 340 in previously published studies to correspond to either the <sup>13</sup>C-containing isotopes of the odd m/z ions or to  
 341 components containing one nitrogen atom (Brown and Rice, 2000; Leenheer et al., 2001; Stenson et al., 2002;  
 342 Kim et al., 2003). No spacing equal to (1.0034/n)m/z (with n = 2, 3...) was detected between the mono-  
 343 isotopic peak and the corresponding peak for the one <sup>13</sup>C-containing isotope, showing that no multiply  
 344 charged species were generated in the ESI probe during analysis of PPH. The PPH mass spectra display a

345 common 14.0156 amu repeating unit due to the existence of homologous series of species differing by  $-CH_2$   
346 groups (Stenson et al., 2003; Reemtsma et al., 2008; Hertkorn et al., 2008), as reported previously for spectra  
347 of DOM analyzed by using FT-ICR MS (Stenson et al., 2002; Stenson et al., 2003; Reemtsma et al., 2008;  
348 Hertkorn et al., 2008). Homologous  $-CH_2$  series identified for PPH by Kendrick mass analysis were found to  
349 contain up to 14 molecules, which suggests that the  $-CH_2$  group is an important constitutive element in the  
350 structure of FAs (e.g. Fig. A3 in the Appendix). Numerous homologous series were observed and accounted  
351 for the large number of different basic structures in PPH, similar to what was reported for DOM samples  
352 (Kujawinski, Hatcher, et al., 2002).  
353

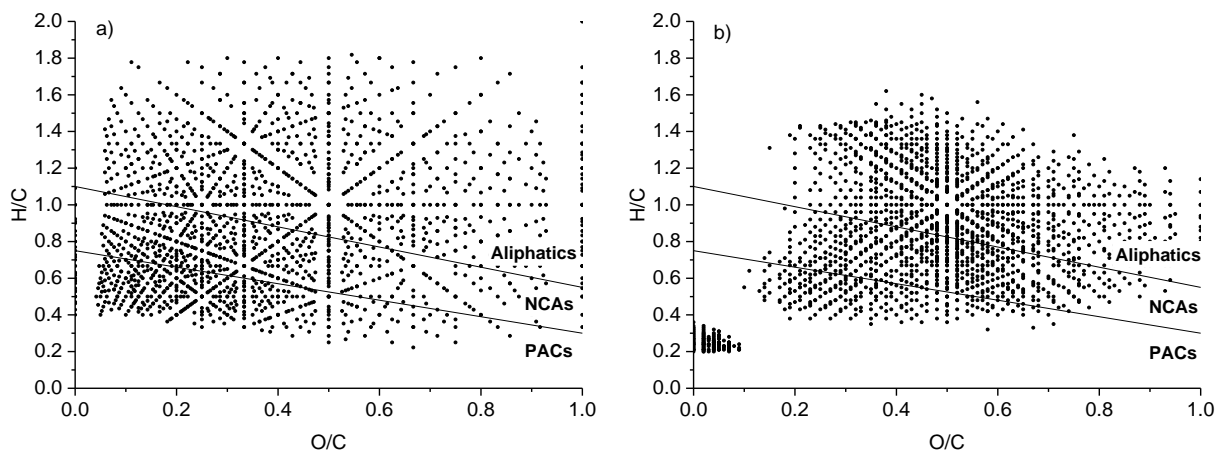


354  
355 **Fig. 3:** Negative ion ESI mass spectra in the ranges 120-400 m/z and 400-798 m/z of PPH native solution (a),  
356 and of the supernatants of PPH sorption experiments (pH =  $3.8 \pm 0.1$ ) onto alumina (b), and kaolinite (c).

357  
358 Chemical formulae were assigned to 74% and 98% of the PPH compounds detected with S/N > 4 in the mass  
359 ranges 120-400 m/z and 400-798 m/z, respectively. Fig. 4 shows that the chemical compositions of PPH  
360 compounds are distributed in a wide region of the VK diagrams and belong to the three following categories,

361 i.e., polycyclic aromatic compounds (PACs), not-condensed aromatics (NCAs), and compounds of aliphatic  
362 character. Such VK diagrams with a wide distribution of compounds is somehow contrasting with most of  
363 those previously published for soil humic substances (Rice and MacCarthy, 1991; D'Andrilli et al., 2015)  
364 showing data-points gathered in more restricted areas of O/C. However, there are observed in Fig. 4b and Fig.  
365 4a two sets of points that show slightly stronger densities and are mainly made up of strongly oxygenated  
366 ( $0.5 < O/C < 0.8$ ) NCAs or aliphatics with  $H/C < 1.1$  and of poorly oxygenated PACs with  $0.1 < O/C < 0.35$  (as  
367 well as a few NCAs with  $H/C < 0.8$ ), respectively.

368



369

370 **Fig. 4:** VK diagram showing elemental formulae assigned to ESI(-) FTMS peaks detected at pH = 3.8 on the  
371 ranges 120-400 m/z (a) and 400-798m/z (b) for the PPH native solution.

372

### 373 3.3. Molecular-scale study of the sorptive fractionation of PPH

374

#### 375 3.3.1. Influence of mineral surface characteristics

376

377 At first examination, ESI(-) spectra provide strong evidence that PPH fractionated during the sorption process.  
378 Fig. 3b and Fig. 3c report spectra of supernatants collected in PPH sorption experiments which were  
379 conducted at  $pH\ 3.8 \pm 0.1$  (and PPH-to-solid ratio of 5mgC/g) and which led to 63% and 40% sorption of  
380 organic carbon onto  $\alpha$ -alumina and kaolinite, respectively (Fig. 1). The total ion currents recorded for the  
381 mass spectra of the supernatants were lower than those recorded for the initial solution. Moreover, peak  
382 intensities were lower in the mass spectra of the supernatants than in those of PPH. Circa 19% and 11% of  
383 compounds detected with  $S/N > 4$  on the PPH mass spectra were not detected on the supernatants' spectra,



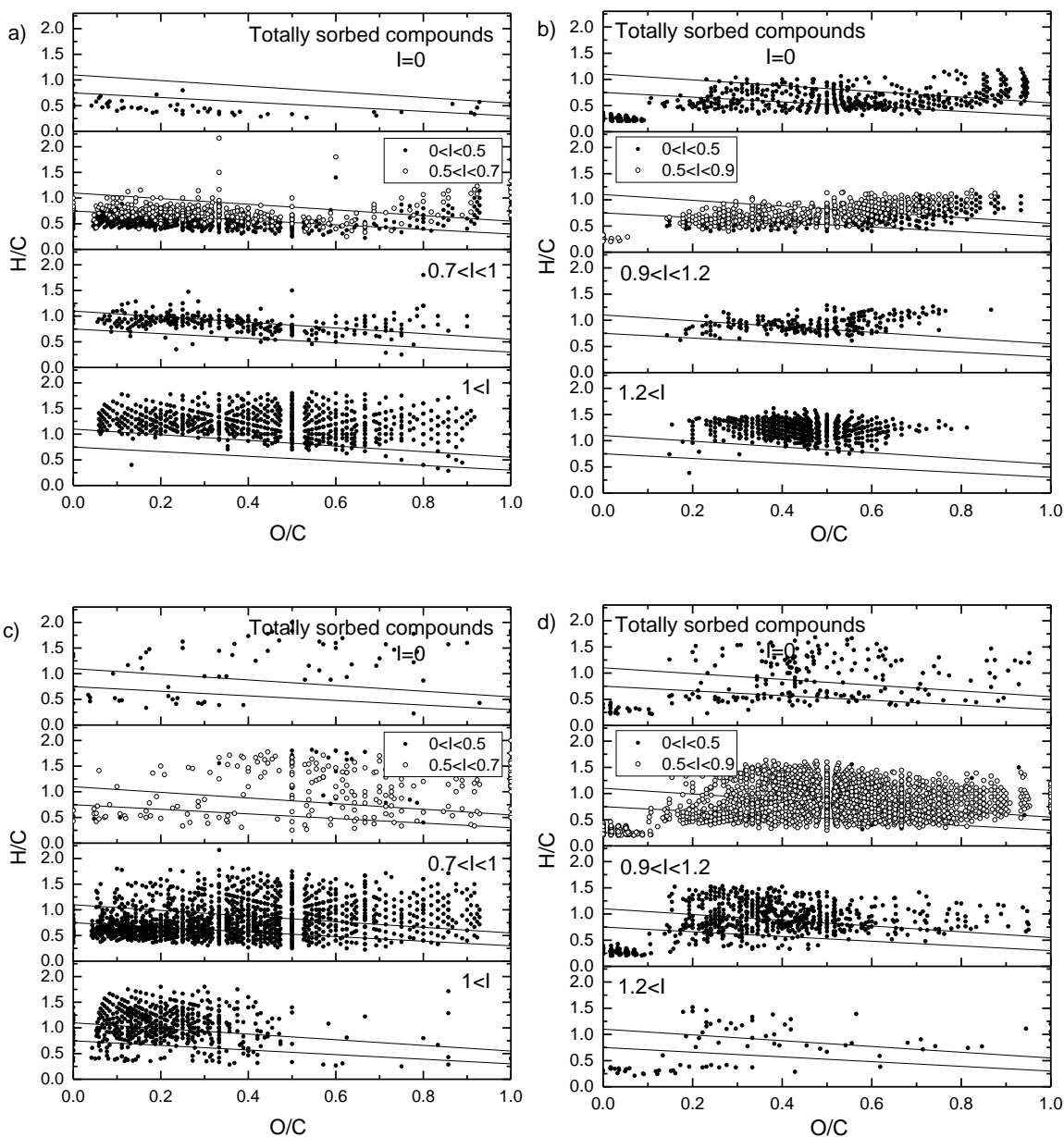
384 indicating that the compounds were totally sorbed on the surfaces of alumina and kaolinite during the  
385 experiments, respectively. On the contrary, some ions were detected on the mass spectra of the supernatants  
386 only. This phenomenon has already been reported by Reiller et al. (2006) and Galindo and Del Nero (2015) in  
387 their ESI MS studies of sorption of HA onto hematite and HSs onto alumina, respectively. Actually, the  
388 intensity of an ion on a mass spectrum not only depends on its concentration but also on its ionization  
389 efficiency and on the matrix, i.e. on the number, the quantity and the structure of the other ions present in the  
390 sample. A possible explanation for the detection of some ions in the supernatants' spectra only is that ions  
391 undetectable in the native solutions of PPH because of matrix complexity might be ionized more efficiently in  
392 the conditions of the supernatants' matrixes due to combined effects of their low sorption onto minerals and of  
393 a release of the ionization charge upon preferential sorption of certain PPH compounds. In summary,  
394 important features of the spectra were that the extent of the lowering of peak intensities from initial solution to  
395 supernatants was varying considerably with the considered ion -going from a peak disappearance to a  
396 lowering higher than that of the TIC-, and with the type of mineral. Hence, the degree of sorption of a PPH  
397 compound was dependent on both the type of compound and the characteristics of mineral surface.

398

399 VK diagrams in Fig. 5 provide information on the order of relative affinity of PPH molecules (sorted as a  
400 function of their values of I) for the surfaces of alumina and kaolinite. A main result is that PPH is strongly  
401 fractionated between mineral surfaces and solution. Some compounds of low values of I ( $0 < I < 0.7$  in Figs. 5a,c  
402 and  $0 < I < 0.9$  in Fig. 5b,d) are well sorbed, while those of highest values of I ( $I > 1$  in Figs. 5a,c and  $I > 1.2$  in  
403 Fig. 5b,d) are unambiguously not sorbed at all. Compounds of intermediate values of I are only poorly sorbed  
404 or even not sorbed at all (cf. section 2.3.4. for detailed explanation). The fact that distinct ions show very  
405 different degrees of sorption on alumina is a first indication that the supramolecular FA structure is not  
406 preserved during sorption on this mineral surface, i.e. that the sorption involves relatively strong forces that  
407 are likely to modified to some extent the assembly of building blocks holding together thanks to weak  
408 cohesion forces. Another main result is that the sorption scheme of PPH onto alumina and kaolinite appear to  
409 be quite different.

410 Clear overall trends are observed for alumina (Figs. 5a,b). Firstly, the affinity of a PPH compound for the  
411 surface decreases with an increase of its H/C ratio. Secondly, PACs are totally sorbed ( $I=0$ ) or have a high  
412 affinity for the surface ( $I < 0.5$ ) whereas NCAs and aliphatic compounds exhibit a wide range of I, with the

413 latter compounds showing the largest distribution. For NCAs, the cloud of data-points is moved towards lower  
414 values of O/C with increasing values of I, which suggests that increasing amounts of oxygenated  
415 functionalities for these molecules increases their affinity for the surface. For the aliphatic compounds, the  
416 cloud of data-points is displaced towards both lower values of O/C and higher values of H/C, which suggests  
417 that the amount of oxygenated functionalities and/or the length (or number) of aliphatic chains are parameters  
418 influencing the affinity for surface. Finally, all the strongly oxygenated molecules ( $O/C > 0.6$ ) amongst NCAs,  
419 and the H-depleted molecules ( $H/C < 1.2$ ) amongst aliphatics, show a high affinity for the surface of alumina.  
420 Differences existing between the sorptive fractionation patterns of PPH in the alumina suspension and  
421 kaolinite suspension (Fig. 5c,d) are rather striking. Firstly, the totally sorbed compounds ( $I = 0$ ) are fewer for  
422 kaolinite than for alumina and they are moreover dispersed over a wide region of VK diagram. Secondly, no  
423 effect of the H/C ratio on the sorption is observed. Unlike for alumina, compounds showing an affinity for the  
424 surface of kaolinite extend to aliphatic compounds of intermediate O/C and high H/C -and include as well the  
425 same compounds as those displaying a high affinity for alumina, such as NCAs with  $O/C > 0.4$  and aliphatics  
426 with  $O/C > 0.6$  (together with some PACs of  $0.2 < O/C < 0.6$ )-. It is also to be noted that most of the  
427 compounds sorbed onto kaolinite show I values in a narrow range ( $0.5 < I < 0.9$ ). All these features clearly  
428 indicate that the surface of kaolinite is weakly selective, and that unlike for alumina, the supramolecular  
429 structure of the FA is likely to be globally preserved during sorption onto kaolinite. Thirdly, another striking  
430 difference between kaolinite and alumina is that a great part of PACs, including those having weak O/C ratios,  
431 are only slightly sorbed or not sorbed at all onto kaolinite (Fig. 5c,  $0.7 < I < 1$ ). All the differences described  
432 above suggest that different molecular characteristics and sorption mechanisms are influencing the sorptive  
433 fractionation of PPH onto kaolinite and alumina.  
434



435

436

437 **Fig. 5:** VK diagrams for PPH compounds sorted as a function of their relative affinity for the surface of  
 438 alumina at pH 3.9 (a,b) and for the surface of kaolinite at pH 3.7 (c,d) for the ranges 120-400m/z and 400-  
 439 798m/z, respectively.

440

441 **3.3.2. Relative affinity order for mineral surfaces of PPH compounds in  $-\text{CO}_2$  and  $-\text{CH}_2$  series**

442

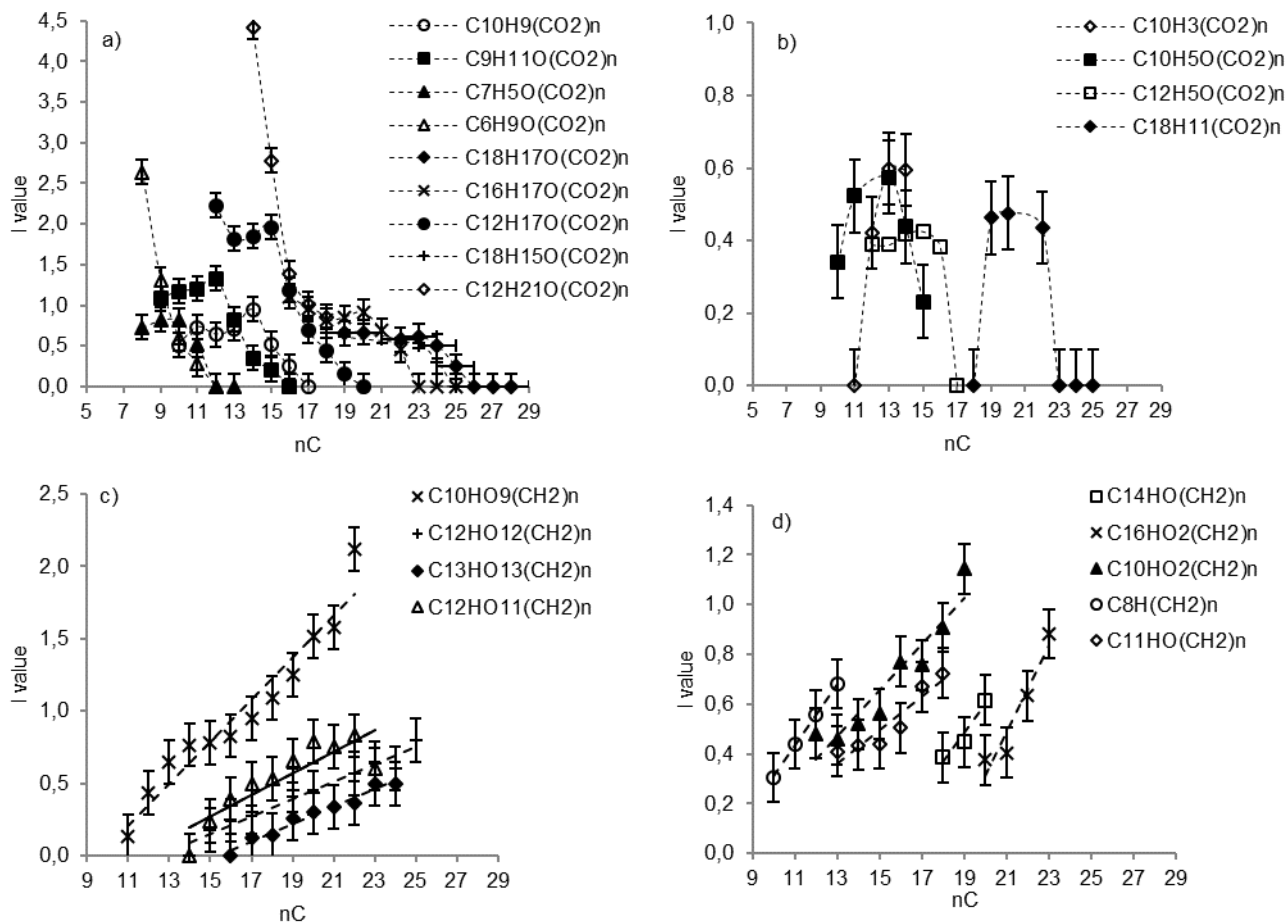
443 Relations existing between the sorption degree of a molecule in a  $-\text{CO}_2$  series and its number of  $\text{CO}_2$  groups,  
 444 as representative for the number of carboxyl functionalities, or between the sorption degree of a molecule in  $-\text{CH}_2$   
 445 series and its number of  $-\text{CH}_2$  groups, as representative for length/number of aliphatic chains, were  
 446 investigated to gain further information on the mechanisms involved in the sorption of PPH onto alumina and

447 kaolinite.  $-\text{CO}_2$  series or  $-\text{CH}_2$  series are made up by compounds whose chemical formula differ only by their  
448 number of  $\text{CO}_2$  or  $\text{CH}_2$ , respectively.

449

450 Figs. 6a,b report, for alumina-solution systems at pH 3.9, the evolution of I as a function of number of carbon  
451 atoms ( $n_c$ ) of PPH compounds in  $-\text{CO}_2$  series of aliphatic compounds or oxygenated NCAs compounds  
452 ( $\text{O}/\text{C}>0.2$ ), and in  $-\text{CO}_2$  series of PACs or poorly-oxygenated NCAs, respectively. Figs. 6c,d refer to the  
453 results obtained for the  $-\text{CH}_2$  series. A striking result is the difference in the  $n_c$ -I relations existing between the  
454 two sets of series. For the aliphatic compounds or the NCAs with  $\text{O}/\text{C}>0.2$  (Fig.6a), the  $-\text{CO}_2$  series show an  
455 inverse correlation between the value of I and the number of carbon atoms,  $n_c$ . This indicates that the degree  
456 of sorption of a molecule in the series is directly related to its number of carboxyl groups. For the  $-\text{CH}_2$  series,  
457 a sharp increase of I with  $n_c$  was observable (Fig. 6c) indicating that the affinity for surface of a molecule  
458 within a series decreases with an increasing of the length/number of aliphatic chains. Such remarkable and  
459 monotonous  $n_c$ -I relations, which were observed for all the  $-\text{CO}_2/-\text{CH}_2$  series investigated, suggest that a  
460 single mechanism controls the degree of sorption onto alumina of aliphatic compounds and oxygenated  
461 NCAs. The fact that distinct molecules of a same  $-\text{CO}_2/-\text{CH}_2$  series display very different degrees of sorption  
462 -some being totally sorbed ( $I = 0$ ) and others being not sorbed at all ( $I > 1.2$ )- clearly indicate that the  
463 supramolecular structure of the FA existing in solution is greatly modified during sorption. Probably, the  
464 sorption implies strong forces which compete efficiently against the weak cohesion forces of the  
465 supramolecular assemblies, leading to different sorption behaviors for the distinct FA building blocks at the  
466 surface of alumina. Regarding the PACs and the poorly-oxygenated NCAs, more complex features were  
467 observed. The degree of sorption of a molecule within a  $-\text{CH}_2$  series was found to be only weakly correlated  
468 with its number of  $\text{CH}_2$  groups (only a small increase of I with  $n_c$  is observable in Fig. 6d). In contrast,  $-\text{CO}_2$   
469 series showed a noticeable and not monotonous evolution of I with  $n_c$ . Indeed, bell-shaped curves were  
470 observed: the last members of the  $-\text{CO}_2$  series showed a decrease of I with an increase of  $n_c$  while the first  
471 members of the series showed the opposite (Fig. 6b). Hence, PACs and NCA compounds with  $\text{O}/\text{C}<0.2$  show  
472 different sorption trends according to their molecular  $\text{O}/\text{C}$  ratio, which suggests that within a  $-\text{CO}_2$  series of  
473 compounds two different mechanisms of sorption (at least) operate, one favoring the retention of molecules  
474 containing some oxygen and another favoring the retention of molecules depleted in oxygen.

475



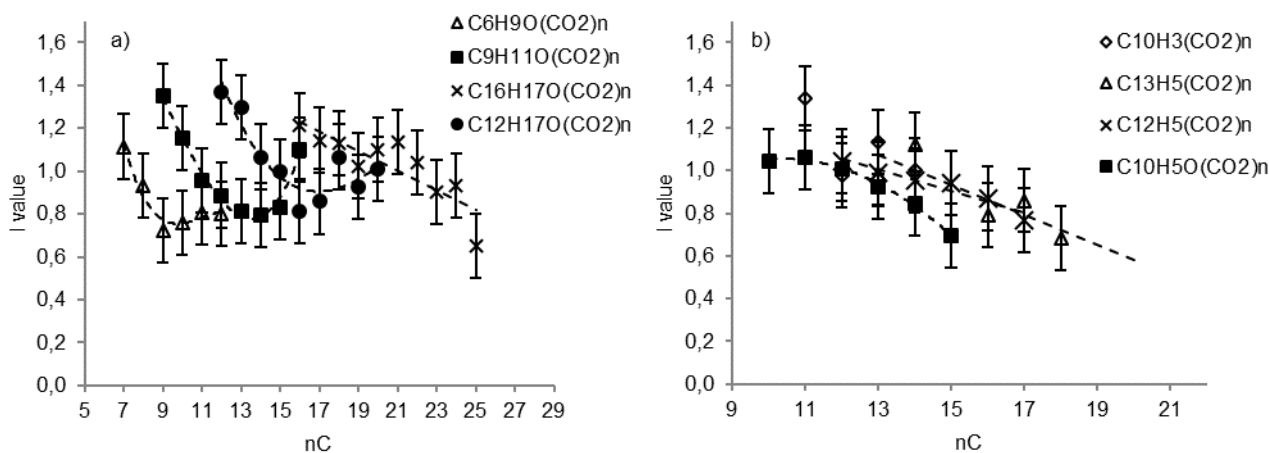
476

477 **Fig. 6:** Evolution of value of I of PPH compounds as a function of number of carbon atoms ( $n_C$ ) for aliphatics  
 478 and NCAs with  $O/C > 0.2$  in  $-\text{CO}_2$  (a) and  $-\text{CH}_2$  (c) series, and for NCAs depleted in oxygen and PACs in  $-\text{CO}_2$  (b) and  $-\text{CH}_2$  (d) series, in alumina-solution systems at pH 3.9.

480

481 Figs. 7a,b show, for kaolinite-solution systems at pH 3.7, plots of I versus  $n_C$  for  $-\text{CO}_2$  series of aliphatic  
 482 compounds or oxygenated NCAs compounds ( $O/C > 0.2$ ) and for  $-\text{CO}_2$  series of PACs or poorly-oxygenated  
 483 NCAs, respectively. The same series as those described above for alumina were considered for kaolinite. The  
 484 most striking result is that the values of I for  $-\text{CO}_2$  series of aliphatics or highly oxygenated NCAs (Fig. 7a)  
 485 are in a very limited range, showing that all compounds in a series have almost a similar relative affinity for  
 486 the surface of kaolinite. Such a trend in kaolinite-solution systems is also visible for the  $-\text{CO}_2$  series of PACs  
 487 and oxygen-depleted NCA molecules (Fig. 7b). These results contrast markedly with those reported above for  
 488 the surface of alumina, for which values of I varied largely amongst compounds of a  $-\text{CO}_2$  series (cf. Figs.  
 489 6a,b). It is also to be noted that, in the PPH-kaolinite system, none of the  $-\text{CO}_2$  series investigated showed  
 490 well-sorbed molecules with values of I lower than 0.5. All these results confirm that the clay surface is less  
 491 selective than the Al-oxide surface and that molecular characteristics such as number of  $-\text{CO}_2$  groups and/or

492 number/length of aliphatic chains are not determining parameters regulating the sorptive fractionation of PPH  
 493 onto kaolinite.  
 494



495  
 496 **Fig. 7:** Evolution of the values of I within  $-\text{CO}_2$  series of aliphatics or NCAs with  $\text{O}/\text{C} > 0.2$  (a), and  $-\text{CO}_2$   
 497 series of NCAs depleted in oxygen and PACs (b), after sorption of PPH onto kaolinite at pH 3.7.

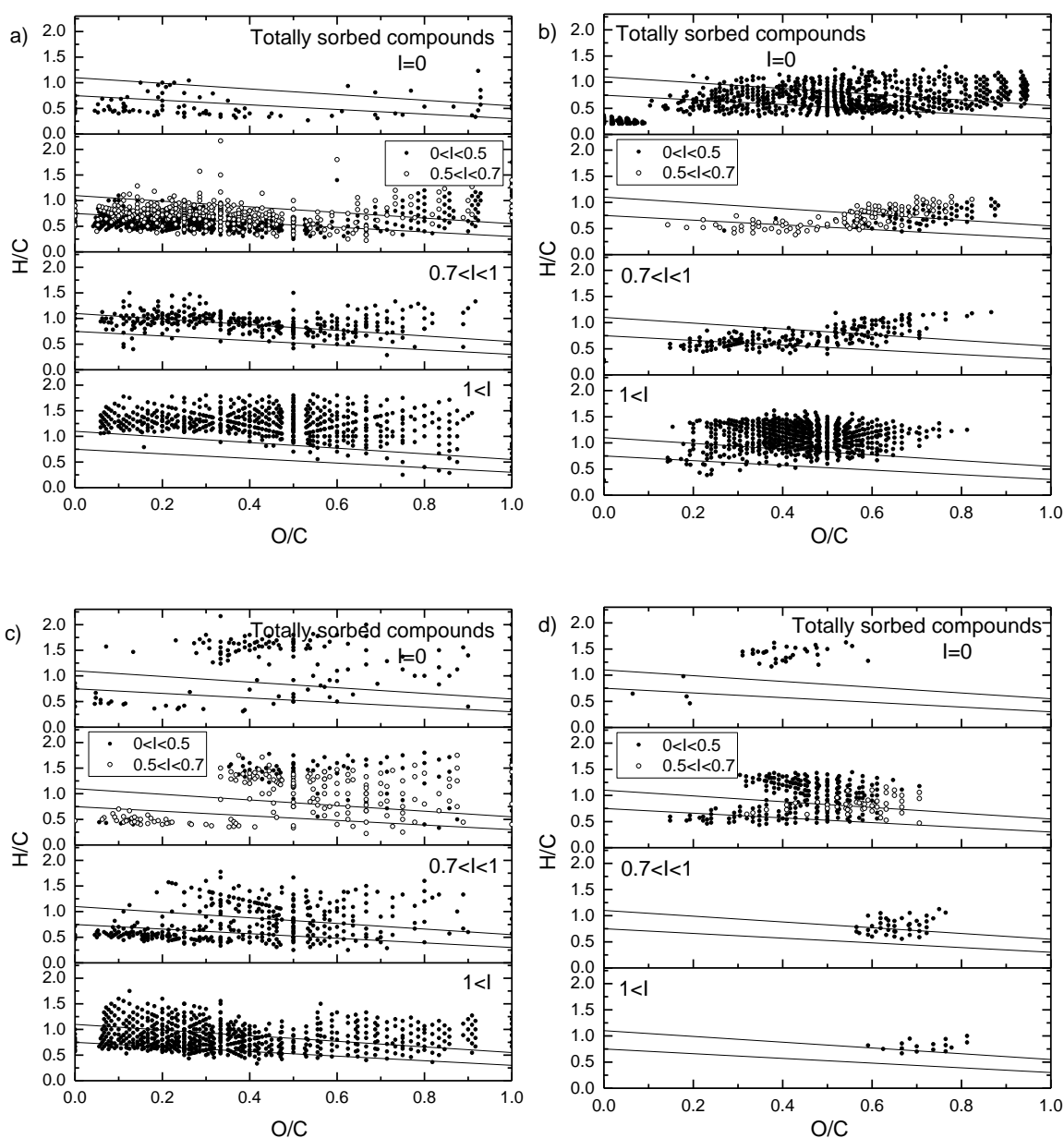
### 3.3.3. Effect of pH on the sorptive fractionation of PPH

500  
 501 Regarding the sorption onto alumina, it appears that the relative affinities of PPH molecules for the surface do  
 502 not change significantly in the acidic pH range 3-5, as shown by comparing VK diagrams obtained for the  
 503 system at pH = 4.8 (Figs. 8a,b) and at pH = 3.9 (cf. Figs. 5a,b). Correlations observed at pH 4.8 between the  
 504 degree of molecule sorption and molecular parameters within  $-\text{CO}_2$  or  $-\text{CH}_2$  series (data not shown) were  
 505 similar to those described for the pH 3.9 (cf. paragraph 3.3.2).

506  
 507 Regarding the sorption onto kaolinite, it is first to be noted that fewer compounds are represented on the VK  
 508 diagrams reporting the relative affinities of PPH molecules for surface at pH 5.0 (Figs. 8c,d) than at pH 3.7  
 509 (Figs. 5c,d). This is due to the fact that supernatants for the kaolinite-PPH-solution systems had to be analyzed  
 510 at pH 5.0 (cf. section 2.2), which leads to an alteration of the MS response (for illustrative purposes, compare  
 511 VK diagrams of native PPH solution analyzed at pH 3.8 in Fig. 4 and at pH 5.0 in Fig. A4 in the Appendix).  
 512 Despite fewer compounds are detected for the native solution of PPH at pH 5.0 than at pH 3.8, some key  
 513 trends in the pH-dependency of the sorption of PPH onto kaolinite can still be determined. Some highly  
 514 oxygenated aliphatics and NCAs are poorly sorbed at pH 5.0 (while they display a good affinity for the

515 surface at pH 3.7). This suggests the existence of a process that competes against sorption at pH 5.0 and tends  
 516 to keep these highly oxygenated molecules in solution. Interestingly, the final concentration of dissolved  
 517 aluminum in kaolinite-PPH suspensions at pH 5.0 was found to be ca. four times higher than in kaolinite  
 518 suspensions without PPH (cf. Fig. A5 in the Appendix). This supports the formation in kaolinite-PPH systems  
 519 of solution complexes implying the most oxygenated aliphatic and NCA compounds and the dissolved  
 520 aluminum originating from the dissolution of kaolinite.

521



522

523

524 **Fig. 8:** VK diagrams for PPH compounds sorted as a function of their relative affinity for the surface of  
 525 alumina at pH 4.8 (a,b) and for the surface of kaolinite at pH 5.0 (c,d) for the ranges 120-400m/z and 400-  
 526 798m/z, respectively.

527

#### 528 4. DISCUSSION

529

530 A main finding is that the trends in the sorptive fractionation of PPH in kaolinite-solution systems are very  
531 different than those observed for alumina, in the present work or in previously published studies of molecular-  
532 scale alumina-HS-interactions (Galindo and Del Nero, 2014; Galindo and Del Nero, 2015). Such a finding  
533 provides strong evidence that the mineral surface properties are a key parameter governing the sorptive  
534 fractionation of HS.

535 All the results obtained at the molecular scale for the kaolinite-solution system suggest that the sorption of  
536 PPH onto kaolinite is mostly the result of a weakly selective mechanism. For example, the dispersion of the  
537 totally sorbed PPH compounds over a wide region of VK diagram and the closeness of the I values of the  
538 molecules in  $-CO_2$  series well exemplify that the number of  $CO_2$  groups in a molecule and, more generally,  
539 molecular acidity, have a slight influence on the degree of sorption of NCAs and aliphatics onto kaolinite.  
540 These features rule out a substantial contribution to PPH sorption of a surface ligand exchange mechanism  
541 between the hydroxyl groups existing on the kaolinite surface and the carboxyl groups of PPH. Though, it has  
542 been widely reported that high affinity aluminol sites on singly coordinated oxygens at edges and steps of  
543 kaolinite surface have chemical properties close to the surface sites of alumina (e.g. Kummert and Stumm,  
544 1980). These sites probably have a strong affinity for the highly oxygenated compounds of PPH but are not  
545 numerous enough to have a measurable effect on the overall sorptive fractionation pattern of PPH.

546 Based upon the structure of fulvic acid molecules, which have numerous functional groups with hydrogens  
547 bound to heteroatoms (N, O), hydrogen bonding seems to be a good candidate because such a mechanism is  
548 not as selective as surface ligand exchange. Hydrogen bonding well accounts for the lack of preferential  
549 sorption observed amongst all aliphatics and NCA molecules of  $O/C > 0.2$  (Fig. 5d). Sorption of most of PPH  
550 compounds, including the hydrogenated compounds, may thus imply aluminol or silanol groups existing on  
551 the doubly coordinated oxygens located on the basal planes of kaolinite, which are poorly selective sites  
552 expected to be involved in weak sorption mechanisms such as hydrogen bonding. Probably, such weak  
553 sorption mechanisms do not compete efficiently against the cohesion forces of the supramolecular FA  
554 assemblies. The supramolecular structure would thus be globally preserved during sorption, accounting for the  
555 similar affinities of the different FA compounds for the surface of kaolinite. Implication of these low affinity



556 sites not only accounts for a weak fractionation amongst members of  $-\text{CO}_2$  series of aliphatics or NCAs  
557 during their sorption onto kaolinite, but also for the very low degree of sorption of PACs. No positive  $n_c$ -I  
558 correlation were observed in kaolinite-solution systems for  $-\text{CO}_2$  series of PACs with low O/C ratios,  
559 suggesting a poor contribution of hydrophobic interactions between PACs and kaolinite surface. Possibly, the  
560 rather limited degree of sorption onto kaolinite of the PACs bearing oxygenated functionalities (due to low  
561 amounts of high affinity surface aluminol sites) resulted in few amounts of hydrophobic domains available, in  
562 the first layer of PPH molecules sorbed at the clay surface, for further interactions with hydrophobic moieties  
563 of NCAs and PACs of low O/C present in solution. Moreover, the first layer of sorbed compounds is only  
564 weakly bonded on the basal planes of the kaolinite surface, preventing a strong attach of poorly-oxygenated  
565 PACs on the surface.

566 Implication of the low affinity sites on the basal planes of kaolinite also accounts for the pH-dependency of  
567 the sorption of the highly oxygenated aliphatic and NCA compounds of PPH. A decrease of the degree of  
568 sorption of these molecules at pH 5.0 results from their complexation with aluminum in solution competing  
569 against their sorption at the surface of the mineral. Indeed, an increase in pH value induces the deprotonation  
570 of both the aluminol/silanol groups on the basal planes of kaolinite, as well as of the carboxyl groups of PPH.  
571 Such a deprotonation has the effect of preventing hydrogen bonding of PPH molecules on the kaolinite  
572 surface sites, and of decreasing the degree of sorption of the highly oxygenated aliphatic and NCA  
573 compounds. Moreover, the deprotonation of the carboxyl groups of these molecules also favors the formation  
574 of FA-aluminum complexes in solution. The pH-dependency of the sorption of highly oxygenated aliphatic  
575 and NCA compounds in PPH is thus the result of both these processes.

576 The molecular-scale findings on the sorptive fractionation trends of PPH during sorption at the clay surface  
577 are quite contrasting with those observed for the alumina-solution-PPH systems, for which molecules showing  
578 the highest affinity for the mineral surface are the PACs and the highly oxygenated compounds amongst  
579 aliphatics or NCAs. For the latter compounds, strong evidence is provided that a single mechanism controls  
580 their sorption at the alumina surface, as shown, for example, by the remarkable positive correlations existing  
581 for PPH compounds in  $\text{CO}_2$  series between the degree of sorption of a molecule and its molecular O/C ratio.  
582 Highly-oxygenated NCA and aliphatic compounds carry multiple carboxyl and phenol groups which are well  
583 known to be involved in ligand exchange reactions (specific adsorption) with surface aluminol groups, e.g.  
584 (Schlautman and Morgan, 1994). The positive correlation observed between affinity for the surface of alumina

585 and number of carboxyl groups of a PPH compound in a  $-\text{CO}_2$  series even includes molecules containing up to  
586 5 groups or more (e.g. Fig. 6). As discussed in previously published papers by our team (Galindo and Del  
587 Nero, 2014), it is unlikely that more than three of carboxyl groups of a molecule are involved simultaneously  
588 in multiple bonding with aluminol groups at the surface of alumina. The concomitant increase of affinity for  
589 alumina surface and  $n\text{CO}_2$  of compounds in  $\text{CO}_2$ -series of the types highly-oxygenated NCAs or aliphatics is  
590 thus due likely to a change in molecule acidity. Such an interpretation was already proposed for simple  
591 organic acids by Evanko and Dzombak (1998), who suggested that enhanced sorption with increasing number  
592 of carboxyl groups of acids was due to changes in the acidity of compound rather than to multiple bonding,  
593 and was supported by the results of Janot et al. (2012). The interpretation is also supported by our results  
594 showing that a lengthening of aliphatic chains of a molecule within a  $-\text{CH}_2$  series, which is expected to  
595 decrease the molecule acidity due to donor inductive effect, decreases the affinity for the surface of alumina.  
596 Regarding the NCAs with  $\text{O/C} < 0.2$  and the PACs, the bell shape of the curves reporting affinity of  
597 molecules in  $\text{CO}_2$ -series for alumina surface vs. number of carboxyl groups indicate that different mechanisms  
598 are responsible for the sorption of molecules of lowest  $\text{O/C}$  ratios and highest  $\text{O/C}$  ratios, respectively. For the  
599 latter molecules, acidity is the key parameter controlling the degree of sorption (same types of  $n_{\text{C-I}}$  inverse  
600 correlation as those observed for the aliphatics), and surface ligand exchange is the mechanism driving the  
601 sorption. For the former compounds, the positive  $n_{\text{C-I}}$  correlation observed rules out acidity as a parameter  
602 governing sorption. The existence of this correlation for compounds having many hydrophobic moieties (e.g.  
603 aromatic structures) and few oxygenated functional groups provides strong evidence that their degree of  
604 hydrophobicity determines their affinity for the surface of the oxide. A possible explanation is as follows. As  
605 evidenced by the impact of molecule acidity on the degree of sorption of a FA molecule onto alumina, - either  
606 for aliphatics, NCAs and PACs-, the supramolecular structure of FA appears to be not preserved during  
607 sorption, allowing : (i) the sorption of the most highly acidic FA compounds to be sorbed via surface ligand  
608 exchange involving their hydrophilic moieties and (ii) the release into solution of PACs with very low  $\text{O/C}$   
609 and their subsequent sorption via interaction between their hydrophobic moieties and those of the strongly  
610 sorbed PACs (which form a first sorption layer onto alumina). Another possible interpretation is that the  
611 electric fields induced by the surface charge of alumina influence the dielectric constant of water (Booth,  
612 1951). Water at the surface of alumina would thus have a lower apparent dielectric constant, which favors the  
613 adsorption of hydrophobic molecules. Molecular-scale evidence that the degree of hydrophobicity governs the

614 degree of sorption of poorly-oxygenated PACs and NCAs onto a metallic oxide has been published recently  
615 for a peat humic acid (Galindo and Del Nero, 2015) but has never been so far demonstrated for fulvic acids.  
616 The molecular-scale results presented here for the sorption of PPH are useful for rationalizing the contrasting  
617 results of spectroscopic studies published on the sorption of bulk FAs at the surfaces of metallic (hydr)oxides  
618 and clays, respectively. Ghosh et al. (2009) studied by spectroscopy techniques the fractionation behavior of  
619 HS on minerals with different surface properties. They reported that aliphatic fractions of HS are more prone  
620 to sorption at the surface of clay minerals than the highly aromatic fractions, while carboxylic functional  
621 groups play a significant role in the sorption of HA on goethite. Spectroscopic studies have long reported  
622 preferential sorption of aromatic structures bearing oxygenated functionalities of HS at the surface of metallic  
623 (hydr)oxides (Meier et al., 1999; Zhou et al., 2001; Kaiser, 2003; Claret et al., 2008). For example, Kaiser  
624 showed for HS-solution-goethite systems a preferential removal from solution of aromatic and carboxyl C,  
625 whereas alkyl-C accumulated in the solution. Claret et al. (2008) reported a preferential sorption at the surface  
626 of alumina of the hydrophobic fractions of FA and/or of the FA compounds showing high contents in aromatic  
627 moieties and functional groups, as compared to aliphatic fractions. In the present study, on the one hand, we  
628 provide molecular-scale evidence that most of the acidic, aliphatic compounds of a FA display a greater  
629 affinity for the kaolinite surface than PACs because the most effective mechanism of sorption of FA  
630 molecules at such a clay surface is a weak sorption via H-bonds at the aluminol / silanol sites on the doubly  
631 coordinated oxygens located on the basal planes of the clay. On the other hand, the present molecular-scale  
632 study also confirms unequivocally that clear relationships exist between molecular parameters such as acidity  
633 or hydrophobicity of a molecule and its degree of sorption in a metallic oxide–solution system. The sorptive  
634 fractionation of FAs in such systems, in contrast to clay systems, is due to a competition between protons and  
635 metallic centers at the surface of the mineral for coordination of FA molecules, and to the subsequent sorption  
636 of poorly-oxygenated PACs by hydrophobic forces involving hydrophobic moieties in the first layer of sorbed  
637 FA molecules on the oxide surface.

638

## 639 **5. CONCLUSION**

640

641 The results of the present study on the fractionation behavior of a soil FA onto alumina and kaolinite provide  
642 valuable molecular-scale insights on the relationships existing between the chemical characteristics of the

643 molecules and their distribution between aqueous and mineral phases, as well as on the distinct mechanisms  
644 involved in the fractionation of a FA during its sorption onto minerals of different surface properties. A main  
645 finding is that the molecular-scale fractionation of FA in kaolinite suspensions is highly contrasting with that  
646 observed here for alumina or with those reported in previously published studies for aluminum oxide (Galindo  
647 and Del Nero, 2014; Galindo and Del Nero, 2015). Such a finding highlights that the key parameter  
648 controlling the sorptive fractionation trends of FAs is the nature, i.e. oxides vs. clays, and the surface  
649 properties of sorbing minerals.

650

651 The data presented here confirm previous work by our team (Galindo and Del Nero, 2014) indicating that  
652 acidity of FA molecules is a main parameter determining the relative degree of sorption onto Al-oxides of FA  
653 molecules, with the highly acidic molecules bearing oxygenated functionalities being sorbed preferentially via  
654 surface ligand exchange involving the high-affinity aluminol surface sites. Our study also reveals that degree  
655 of hydrophobicity of PACs and NCAs of low O/C ratio is another key molecular parameter influencing the  
656 degree of sorption of these FA compounds, which are able to interact with hydrophobic domains of organic  
657 molecules (probably mainly PACs) strongly retained at the Al oxide surface within a first layer of  
658 specifically-sorbed organic molecules. The present study establishes that, whatever the FA composition and in  
659 a range of acidic pH values, surface ligand exchange and hydrophobic interactions are the main (cooperative)  
660 mechanisms leading to the preferential retention on metal oxides of most of PACs, of NCAs with high O/C  
661 ratios ( $>0.5$ ) and of highly-oxygenated aliphatic compounds ( $O/C > 0.7$ ), whereas hydrogenated and less  
662 oxygenated NCAs and aliphatics are left in the solution.

663

664 The first molecular-scale description presented here of the fractionation of a FA in a clay mineral-solution  
665 system reveals that, unlike for metallic oxides, molecular acidity has no substantial effect on the degree of  
666 sorption of a FA molecule onto a clay, and that surface ligand exchange is not the mechanism driving the  
667 sorptive fractionation of FA in such systems. Instead, the main mechanism of sorption is hydrogen bonding of  
668 FA molecules at the low affinity sites (aluminol or silanol) present on the basal planes of the clay, as  
669 evidenced here by the weak sorptive fractionation existing amongst members of  $-CO_2$  homologous series of  
670 aliphatics or NCAs during their sorption onto kaolinite at low pH. In contrast, at slightly acidic pH (5), a  
671 fractionation occurs to a certain extent between molecules within a same class of FA compounds (for

672 aliphatics as well as NCAs), due to a decreasing with pH of the degree of sorption of highly acidic molecules  
673 bearing oxygenated functionalities. Such a fractionation is explained by the fact that dissolved Al has the  
674 ability to compete successfully for the coordination of such reactive organic molecules against a weak  
675 sorption process such as H-bonding. Finally, the predominance of a weak and poorly-selective mechanism of  
676 sorption of FA molecules like H-bonding indirectly accounts for a very low degree of sorption of PACs or of  
677 low O/C NCAs onto kaolinite. Absence of preferential and specific sorption at surface of kaolinite of aromatic  
678 structures activated by oxygenated functionalities prevents the formation of a first layer of strongly-sorbed  
679 molecules exhibiting hydrophobic domains which would be available for further sorption of FA compounds  
680 via hydrophobic interactions.

681

682 The results of the present molecular scale studies have several implications on our knowledge of storage of  
683 natural organic matter in soils as well as on the cycle of metals. It is to be expected that clayey soils trap lesser  
684 amounts of condensed aromatics than metal oxide-rich soils and may retain non-selectively most of FA  
685 compounds of the types of aliphatics or not-condensed aromatics at acidic pH, via weak sorption mechanisms.  
686 In turn, solutions percolating through clayey soils, if being slightly acidic, would contain a multiplicity of the  
687 FA organic acids bearing multiple oxygenated functionalities, owing to the high ability of these FA molecules  
688 for binding dissolved metals and their only weak sorption at clay surfaces. Such a feature would promote the  
689 transport of metal pollutants with soil solutions. Consequently, (long term) metal sorption capacities of clayey  
690 soils are expected to be rather low compared to that of soils rich in Al oxi-hydroxides.

691

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695

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