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Amanda Maria Tadini, S. Mounier, Débora Marcondes Bastos Pereira Milori

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1 Modeling the quenching of fluorescence from organic matter in Amazonian
2 soils

3

4 Amanda Maria Tadini^{a,b,c*}, Stephane Mounier^c, Débora Marcondes Bastos Pereira
5 Milori^a

6

7 ^a Embrapa Agricultural Instrumentation, São Carlos, SP, Brazil

8 ^b Institute of Chemistry of São Carlos, University of São Paulo, São Carlos, SP, Brazil

9 ^c Laboratoire MIO - Equipe CEM, Université de Toulon - CS 60584, 83041 TOULON
10 CEDEX 9

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12 *Corresponding author e-mail: amandatadini@hotmail.com

13 **ABSTRACT**

14 Knowledge of the interactions of soil organic matter (SOM) with metal species
15 is important in order to obtain information concerning the fates of the metals in
16 environment, whose reactive functional groups present in SOM can provide high
17 complexation capacity. The aim of this study was to evaluate the interactions involving
18 humic acids (HA) and fulvic acids (FA), extracted from Amazonian soils, with Cu(II)
19 and Al(III) ions, using fluorescence quenching spectroscopy. The obtained results
20 showed that the data for the humic fractions of the Amazonian Spodosols could be fitted
21 with one to one complexation model, which provided the best representation of the
22 changes in fluorescence quenching after addition of Cu(II) or Al(III) ions. It was found
23 that the HA presented fewer complexation sites and lower stability constants, compared
24 to the FA samples. Furthermore, the FA showed selective interaction with the metals,
25 while the HA fraction was less selective and could be associated its homogeneous
26 structure. The results showed that the humic acids required 10 times more carbon in
27 their structures than fulvic acids for complexing a metal atom. This behavior can be
28 associated to the fact that the Fulvic Acid fraction has higher mobility and greater
29 interaction during the profile when compare humic acids. Hence, given the diversity of
30 metals to which the HA has affinity, this soil fraction appears to be mainly responsible
31 for soil fertility, while the FA fraction has higher mobility and greater interaction with
32 water.

33

34 **Keywords:** Humic Fractions; Complexation; Mobility; Amazonian Spodosol;

35 **1. Introduction**

36 Determination of the chemical properties of the soil organic matter, as well as its
37 interaction with metallic species, provides important information for understanding the
38 structures of the humic fractions. The humic substances (HS) are composed of aromatic
39 rings and reactive functional groups that are responsible for the high capacity for
40 complexation with metals, highlighting copper and aluminum species (Paul, 2016).

41 Quenching analysis is based on decrease of the fluorescence intensity of a
42 sample, usually due to molecular interactions (Manciulea et al. 2009). These
43 interactions include reactions in the excited state, molecular rearrangements, complex
44 formation in the ground state and collision energy transfer (Esteves da Silva et al. 1998;
45 Mounier et al. 2011). Quenching analysis of a sample, in the presence of metallic ions,
46 can involve two mechanisms: dynamic quenching and static quenching. Dynamic
47 quenching is a process involving collision, in which the fluorophore and the quenching
48 agent (for example, a metal) come into contact and energy transfer occurs between the
49 molecules in the excited state (collisional process). Static quenching involves an
50 equilibrium complexation process in which fewer (or no) fluorescent complexes of the
51 fluorophore in the ground state are formed (Kumke et al. 1998; Manciulea et al. 2009;
52 Wu et al. 2013).

53 In evaluation of quenching behavior, Luster et al. (1996) concluded that the
54 different types of bonding of copper metal with OM involved weak bonds, strong
55 bonds, and very strong complex bonds. Smith and Kramer (2000) proposed a multi-
56 response method to explain all the available fluorescence sites of Cu-OM binding, with
57 five different fluorescent components of OM being identified using a spectral resolution
58 technique. The application of statistical methods in fluorescence suppression studies is a

59 useful tool for evaluating intermolecular organic matter and trace metal interactions
60 (Yamashita and Jaffe 2008). In the literature several studies related to fluorescence
61 quenching involving copper and humic substances has been extensively investigated
62 (Esteves da Silva et al. 1998; Plaza et al. 2005; Hernández et al. 2006; Bai et al. 2008;
63 Chen et al. 2013; Fuentes et al. 2013; Zhu et al. 2014; Boguta et al. 2016; Hu et al.
64 2017; Huang et al. 2018). Fewer studies have investigated the interactions between
65 humic substances and aluminum (Gerke, 1994; Smith and Kramer, 2000). The
66 interactions of humic substances with aluminum are of great interest in research
67 concerning Spodosols, since natural organic matter plays a crucial role in the transport
68 of aluminum and iron along the depth profile (Santos et al. 2015; Tadini et al. 2019). In
69 this study will show a new data treatment model for fluorescence suppression in soil
70 samples based on the Ryan and Weber model in humic fractions extracted from
71 Amazonian soil. In addition, it will provide quantitative data on complexation capacity
72 and concentration of binding sites, as well as the determination of the conditional
73 stability constant for each component responsible for fluorescence. Therefore, the aim
74 of this study was to evaluate the interactions of humic and fulvic acid samples from an
75 Amazonian Spodosol with Cu(II) and Al(III) ions, using canonical polyadic/parallel
76 factor analysis (CP/PARAFAC) and fluorescence quenching spectroscopy.

77

78 **2. Materials and Methods**

79 *2.1. Study area*

80 The soil samples were obtained from a site located in the north of the
81 municipality of Barcelos, near the Demeni River (0° 15' 18''N; 62° 46' 36''W) in the
82 Middle Negro River Watershed region in the Amazon plains of Brazil. According to the

83 Brazilian Institute of Geography and Statistics (IBGE, 2012), the soils in this region are
84 classified as Spodosols. The soils collected were as follows: (a) a permanently
85 waterlogged podzol (P1: 0-350 cm); (b) a seasonally waterlogged podzol (P2: 0-290
86 cm; P3: 0-350 cm); and (c) a well-drained podzol (P4: 0-390 cm). Further details can be
87 found in a previous publication (Tadini et al. 2018).

88

89 *2.2. Extraction and purification of the humic fractions*

90 The extraction and purification of the humic and fulvic acids followed the
91 recommendations of the International Humic Substances Society (IHSS) and Swift
92 (1996), as described in detail by Tadini et al. (2019).

93

94 *2.3. Elemental analysis*

95 For elemental analysis of the carbon and nitrogen contents of the materials, 3 mg
96 portions of humic or fulvic acid were weighed out in tin capsules, using an analytical
97 balance. The samples were analyzed by combustion at 1000 °C, employing an elemental
98 analyzer (Model 2400, PerkinElmer). This procedure was performed in duplicate.

99

100 *2.4. Fluorescence spectroscopy*

101 *2.4.1. Sample preparation*

102 The studies involving the interactions with the Cu(II) and Al(III) ions were
103 performed using 8.0 mg L⁻¹ of HA or FA in buffered medium with pH of 4.0-5.0. The
104 titration experiments were prepared in sixteen fluorescence cuvette adding different
105 concentration of metal solutions. The metal solutions employed in this study were
106 CuSO₄·5H₂O (0.01 mol L⁻¹) and Al₂(SO₃)·18H₂O (0.01 mol L⁻¹). The concentrations of

107 the Cu(II) and Al(III) ions added to the solutions of the humic fractions varied from 0 to
108 105.9 mg L⁻¹ for each metal employed.

109

110 2.4.2. Excitation emission matrix (EEM)

111 Fluorescence measurements were performed with a luminescence spectrometer
112 (Model F4500, Hitachi). The spectra were acquired in the scanning ranges 240-700 nm
113 for emission and 220-510 nm for excitation. The excitation and emission slits were
114 fixed at 10 nm, the integration was set at 500 scans min⁻¹, and the detector voltage was
115 700 V. The data were obtained with an open filter and excitation wavelength increases
116 of 10 nm, giving 30 scans. The EEM obtained using this technique was treated using the
117 mathematical method known as canonical polyadic/parallel factor analysis
118 (CP/PARAFAC) (Bro, 1997; Mounier et al. 2011; Luciani et al. 2013; Nouhi et al.
119 2018).

120

121 3. Results and Discussion

122 Treatment of the EEM with CP/PARAFAC provided an EEM response model
123 with components representing the contributions to the sample fluorescence signal. The
124 results are presented in Table 1 for the four Amazonian Spodosol profiles. The
125 parameter used to define the best number of components is known as the core
126 consistency diagnostic (CORCONDIA). In this study the values ranging from 95.30 to
127 98.96% for the humic acids and from 93.93 to 99.88 % for the fulvic acids, in the
128 presence of Cu(II) ions. In the presence of Al(III) ions, the values were from 97.19 to
129 99.57% for the HA and from 95.60 to 99.65% for the FA. These data were obtained for
130 the CP/PARAFAC models with numbers of components ranging from 2 to 3. The
131 model presenting the greatest number of components with CORCONDIA values over

132 60% was chosen as the best one for each data set. For all the quenching experiments, the

133 best model had two components, C1 and C2 as shown in Table 1.

134

135 Table 1: Components obtained by CP/PARAFAC for interactions of the humic acid and fulvic acid

136 samples, extracted from the Amazonian Spodosols, with the Cu(II) and Al(III) ions.

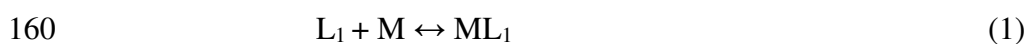
	Profiles	Component 1	Component 2
Denomination		A	B
Humic Acids	P1 P2 P3 P4		
		Component 1	Component 2
Denomination		C	D
Fulvic Acids	P1		
Denomination		C	E
Fulvic Acids	P2 P4		
Denomination		C	F
Fulvic Acids	P3		

137

138 Table 1 presents the main components obtained by CP/PARAFAC for the
139 interactions of the humic and fulvic acid samples from the Amazonian Spodosols with
140 the Cu(II) and Al(III) ions. In the literature (Luciani et al. 2009; Mounier et al. 2011;
141 Tadini 2015) its considered that the more the emission wavelength was blue-shifted, the
142 more the component was condensed and aromatic. In this study the Components B, C,
143 D, and F presented double excitation maxima, which could be attributed to different
144 energy levels of the chromophores or to different excitation moieties present in the
145 molecular structures leading to the same emission function.

146 For a better knowledge of components obtained by CP/PARAFAC, Table 1
147 shows the descriptions of the components, as such as, *denomination A* was assigned to
148 peak A, typical of humic-like substances; *denomination B* was low energy emission
149 peaks typical of terrestrial humic acid; *denomination C* referred to peaks A and C,
150 which are characteristic of humic substances from terrestrial environments, with peak A
151 being related to fulvic acid and peak C being related to humic acid; *denomination D and*
152 *F* referred to peak D, corresponding to fulvic acids of soils; and *denomination E*
153 referred to the T peak of the tryptophan protein (Coble, 1996; Matthews et al. 1996;
154 Stedmon et al. 2003; Coble et al. 2014). The humic acids showed only two components,
155 A and B (Table 1) for all profile study, while the fulvic acid samples exhibit three
156 components that varied between profiles, as shown Table 1.

157 Evaluation was made of two different metal complexation models, namely the
158 1:1 and 1:2 models (Ryan and Weber, 1982). The 1:1 model is represented by the
159 following equation:



161

162 where L_1 is a free complexing site, ML_1 is the complex, and M is the free metal (Cu or
163 Al). This chemical reaction is controlled by the conditional equilibrium constant and the
164 mass balance:

165

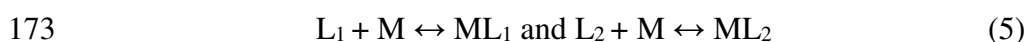
$$166 \quad K_1 = [ML_1] / [L_1] [M] \quad (2)$$

$$167 \quad CL_1 = [ML_1] + [L_1] \quad (3)$$

$$168 \quad M_0 = [ML_1] + [M] \quad (4)$$

169

170 The 1:2 model considers that for a single sample, there are two independent types of
171 complexing site present at the same time. This model is represented by the following
172 equations:



174 where L_1 and L_2 are complexing sites, ML_1 and ML_2 are the metal complexes with sites
175 L_1 and L_2 , respectively, and $M = \text{Cu or Al}$. This chemical reaction is controlled by the
176 conditional equilibrium constant and the mass balance:

177

$$178 \quad K_1 = [ML_1] / [L_1] [M] \text{ and } K_2 = [ML_2] / [L_2] [M] \quad (6)$$

$$179 \quad CL_1 = [ML_1] + [L_1] \text{ and } CL_2 = [ML_2] + [L_2] \quad (7)$$

$$180 \quad M_0 = [ML_1] + [ML_2] + [M] \quad (8)$$

181

182 where CL_1 and CL_2 are the total complexation sites present in the sample. Assuming
183 that L_1 , L_2 , ML_1 , and ML_2 have fluorescence quantum yields of Φ_{L1} , Φ_{L2} , Φ_{ML1} , and
184 Φ_{ML2} , respectively, it is possible to calculate the theoretical fluorescence responses

185 depending on the parameters $IF_{1:1} = f(L_1, K_1, M_0)$ and $IF_{1:2} = f(L_1, L_2, K_1, K_2, M_0)$, and
186 to fit them minimizing the following functions:

187

$$188 \quad \text{BIAS}_{1:1} = \sum[(IF_i - IF_{1:1}(CL_1, K_1, M_{0i})/IF_i]^2 \quad (9)$$

$$189 \quad \text{BIAS}_{1:2} = \sum[(IF_i - IF_{1:2}(CL_1, CL_2, K_1, K_2, M_{0i})/IF_i]^2 \quad (10)$$

190

191 where IF_i and M_{0i} are the component contribution and the total metal concentration
192 obtained with addition of the metal, respectively. The equations were adjusted using a
193 simplex algorithm with positive constraints on all the parameters, performed with a
194 modified “*fminsearch*” Matlab script (Matlab 2012Ra) obtaining two models. So, these
195 discrete models of complexation were chosen because they are fast and are useful for a
196 rapid overview of the complexant properties.

197 Firstly, identification of the best complexation model was achieved by
198 determining which model provided the best fits for the interactions of the humic
199 fractions with Cu(II) and Al(III), by comparing $\text{BIAS}_{1:1}$ (model 1:1) and $\text{BIAS}_{1:2}$ (model
200 1:2) in terms of the probability of components (C1 and C2) obtained by CP/PARAFAC.
201 Mounier and coauthors (2011) defined BIAS as the sum of the absolute value of the
202 difference between the experimental fluorescence intensity logarithm and the calculated
203 fluorescence logarithm intensity.

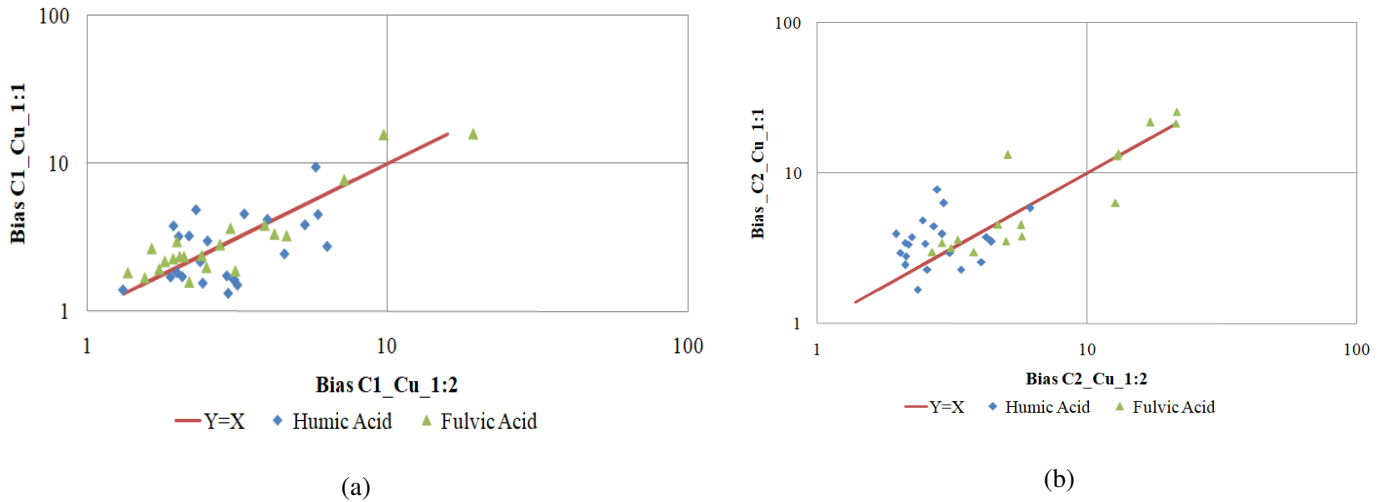
204 Figure 1 shows the $\text{BIAS}_{1:1}$ and $\text{BIAS}_{1:2}$ models for component 1 (Figure 1a)
205 and component 2 (Figure 1b) for the interactions of the HA and FA with Cu(II).

206

207

208

209 Figure 1: Comparison of the $BIAS_{1:1}$ and $BIAS_{1:2}$ models of the Component 1 (a) and Component 2 (b)
 210 for Cu(II). Descriptions of terms - Bias $C1_Cu_1:2$: Bias for Component 1 by Cu(II) with 1:2 model; Bias
 211 $C1_Cu_1:1$: Bias for Component 1 by Cu(II) with 1:1 model; Bias $C2_Cu_1:2$: Bias for Component 2 by
 212 Cu(II) with 1:2 model; Bias $C2_Cu_1:1$: Bias for Component 2 by Cu(II) with 1:1 model;



213

214 In comparison of the models, $BIAS_{1:1}$ (model 1:1) and $BIAS_{1:2}$ (model 1:2), the
 215 1:1 model showed lower BIAS values for most of the humic and fulvic acid fractions.
 216 The 1:1 model was therefore adopted in the study of the interaction of Cu(II) with the
 217 humic fractions. Other factor evaluate in this study were the conditional stability
 218 constants obtained for this model, which correlated for components obtained by
 219 CP/PARAFAC. The results for the component 1/component 2 (C1/C2) are shown in
 220 Figure 2.

221

222

223

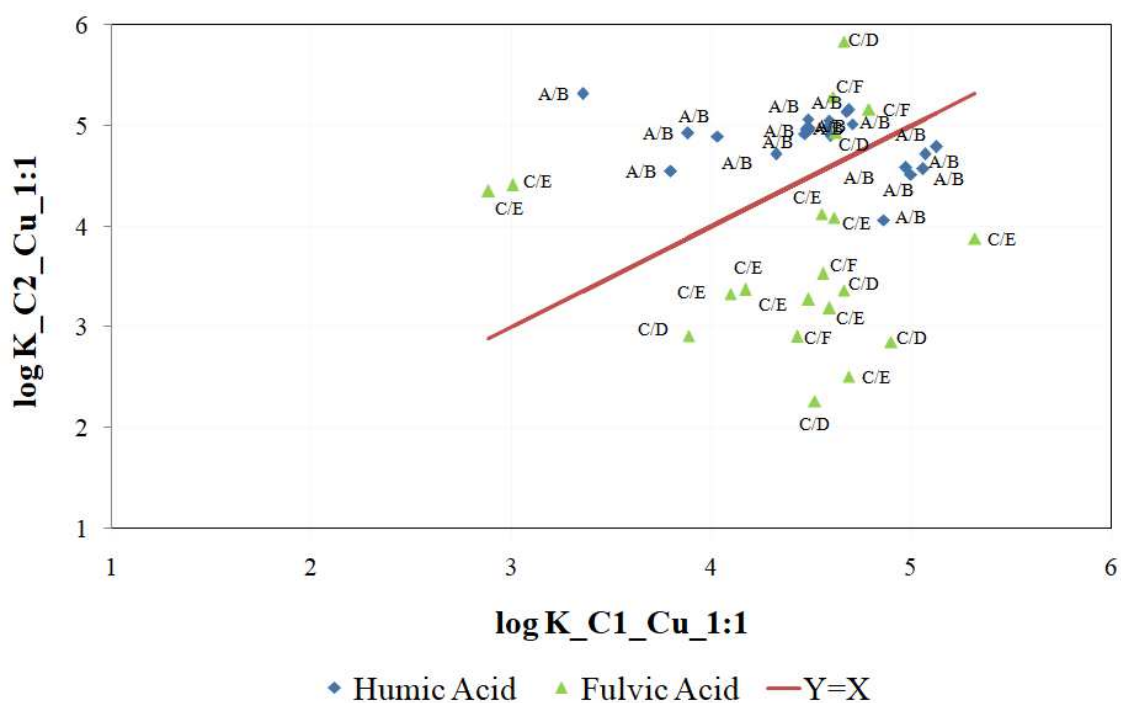
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225

226

227

228 Figure 2: Evaluation of the conditional stability constants for the interactions of Cu(II) with the humic
 229 acids (highlight blue) and fulvic acids (highlight green), using the 1:1 model, with each Component
 230 obtained by CP/PARAFAC. *Descriptions of terms - $\log K_{C1_Cu_1:1}$: logarithm of conditional constant
 231 stability for Cu(II) by Component 1 for 1:1model; $\log K_{C2_Cu_1:1}$: logarithm of conditional constant
 232 stability for Cu(II) by Component 2 for 1:1model; A/B: Component 1 (denominate A)/Component 2
 233 (denominate B) for Humic acids from profiles P1, P2, P3 and P4; C/D: Component 1 (denominate
 234 C)/Component 2 (denominate D) for fulvic acids from profile P1; C/E: Component 1 (denominate
 235 C)/Component 2 (denominate E) for fulvic acids from profile P2 and P4; C/F: Component 1 (denominate
 236 C)/Component 2 (denominate F) for fulvic acids from profile P3; all denominates are present in Table 1.*



238

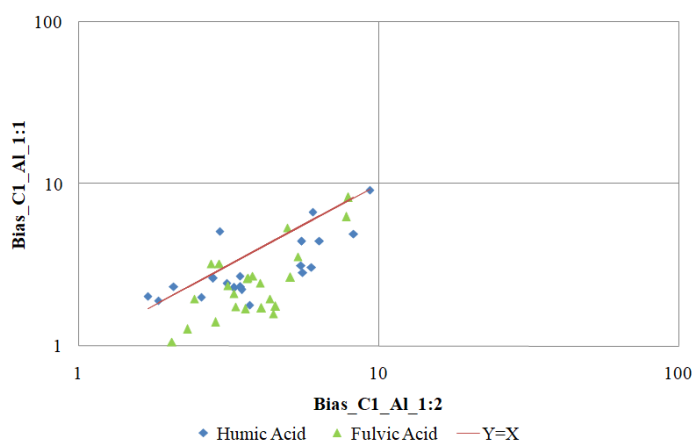
239 As shown in Figure 2, most of the fulvic acid samples showed higher
 240 conditional stability constant (K) values for CP/PARAFAC component 1 (Table 1).
 241 This component corresponded to a molecule with simpler structure that could be related
 242 to components described in the “C/E” and “C/D” chart (as shown in Table 1), with
 243 correlation to component C of the FA (the component with emission more towards the
 244 blue region, reflecting the presence of aliphatic groups). In the case of the humic acid

245 samples (Figure 2), different behavior was observed, with the highest values of K for
246 component 2, which represents more complex structures with emission in the red region
247 (corresponding to denomination “B” in Table 1). The humic substances samples
248 presented as a group gathered near the identity line, with a K value of around 50,000
249 mol⁻¹L. These results indicated that for copper, the HA were more complexing and more
250 homogeneous, compared to the FA, for which there was greater spread, with K values
251 ranging from 500 to 50,000 mol⁻¹.L.

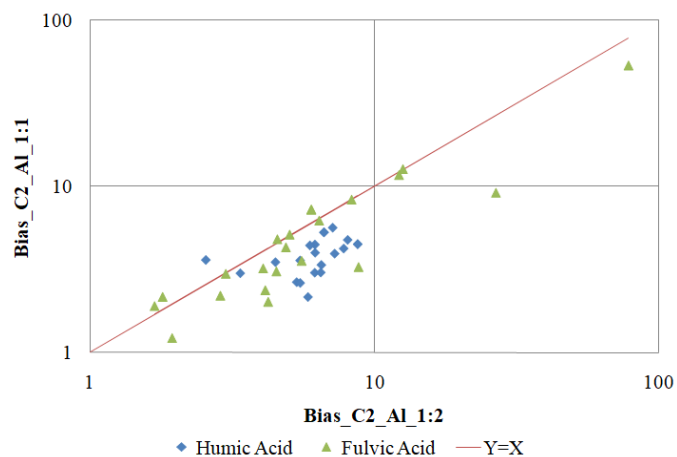
252 The same study was carried out using Al(III) ions. The BIAS values obtained by
253 the mathematical models are shown in Figure 3. It can be observed that the best
254 mathematical model representing the interaction of Al(III), specially humic acids was
255 the 1:1 model, because all the points were low BIAS for the Component 1 (Figure 3).
256 Interestingly, the difference between the two models was more marked for Al(III) than
257 for Cu(II), and comparison of the BIAS values showed that those obtained for Al(III)
258 were significantly lower than those for Cu(II), as shown in Figure 1a.

259

260 Figure 3: Comparison of the BIAS_{1:1} and BIAS_{1:2} values of the Component 1 (a) Component 2 (b) for
261 Al(III). *Descriptions of terms - Bias C1_Al_1:2: Bias for Component 1 by Al(III) with 1:2 model; Bias*
262 *C1_Al_1:1: Bias for Component 1 by Al(III) with 1:1 model; Bias C2_Al_1:2: Bias for Component 2 by*
263 *Al(III) with 1:2 model; Bias C2_Al_1:1: Bias for Component 2 by Al(III) with 1:1 model, all*
264 *denominations are shown in Table 1.*



(a)



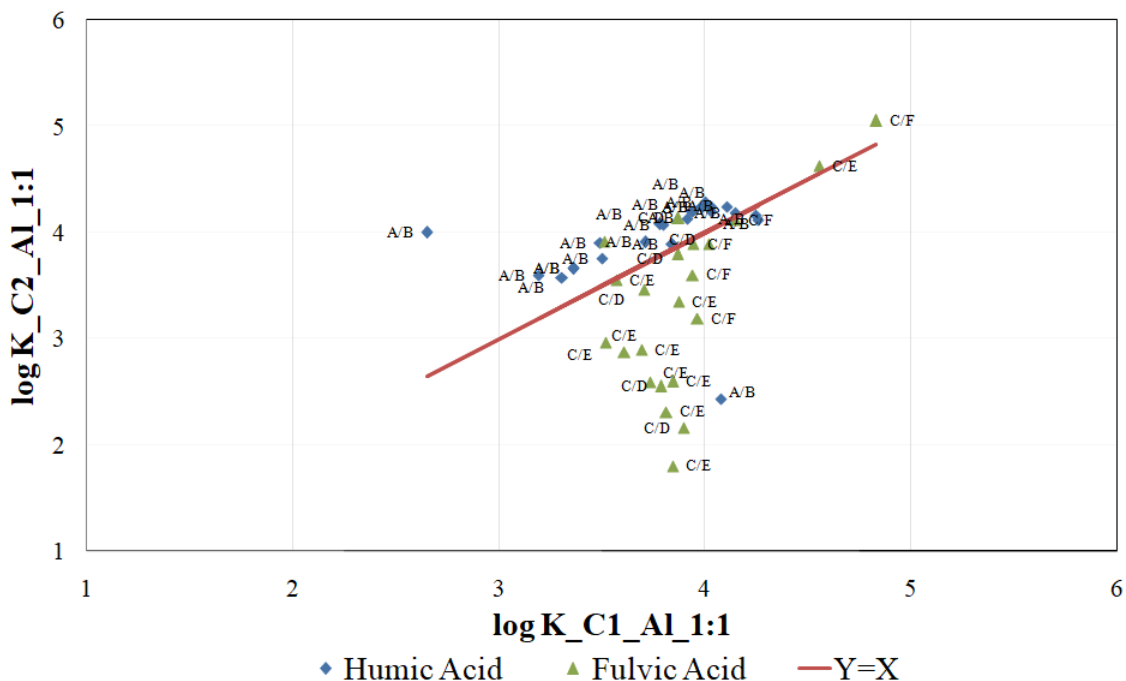
(b)

265

266 Figure 4 shows the behavior of the conditional stability constant (K) for the
 267 interactions of Al(III) with the humic fractions. The fulvic acids presented higher values
 268 of K for component 1, compared to the humic acids, with the latter showing little
 269 differentiation between components C1 and C2 (Table 1), as shown in Figure 4.

270

271 Figure 4: Evaluation of the conditional stability constant for the interactions of Al(III) with the humic
 272 acids (highlight blue) and fulvic acids (highlight green), using the 1:1 model, with each component
 273 obtained by CP/PARAFAC. *Descriptions of terms – $\log K_{C1_Al_1:1}$: logarithm of conditional stability
 274 constant for Al(III) by Component 1 for 1:1model; $\log K_{C2_Al_1:1}$: logarithm of conditional stability
 275 constant for Al(III) by Component 2 for 1:1model; A/B: Component 1 (denominate A)/Component 2
 276 (denominate B) for Humic acids from profiles P1, P2, P3 and P4; C/D: Component 1 (denominate
 277 C)/Component 2 (denominate D) for fulvic acids from profile P1; C/E: Component 1 (denominate
 278 C)/Component 2 (denominate E) for fulvic acids from profile P2 and P4; C/F: Component 1 (denominate
 279 C)/Component 2 (denominate F) for fulvic acids from profile P3; all denominations are shown in Table 1.*



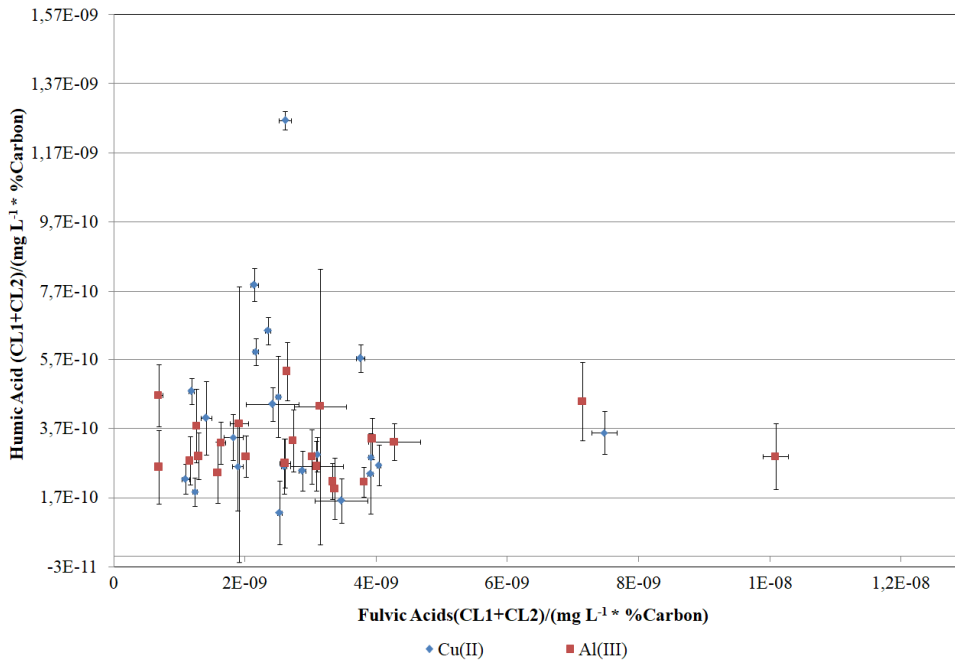
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281

282 As shown in Figure 4, the highest conditional stability constant values were
 283 shown by the fulvic acid samples for component 1 ($1000 \text{ mol}^{-1} \cdot \text{L}$), especially for the
 284 components denoted “C/E” and “C/F” (Table 1), whose characteristics corresponded to
 285 compounds with more aliphatic or less condensed structures, emitting fluorescence in
 286 the blue region. The humic acid samples did not present any substantial difference
 287 between components C1 and C2. The conditional stability constant values again showed
 288 less spread, compared to the values for the FA.

289 The numbers of sites able to complex the metals were determinate the
 290 complexation capacity ligand (CL) for the Components 1 and 2. This parameter was
 291 obtained by adding the CL1 and CL2 values of the components and dividing by the
 292 carbon content of the corresponding sample. The CL represents the density of
 293 complexing sites (in mol of complexing sites) for a metal (Cu or Al) per mol of carbon
 294 in the organic matter fractions. Figure 5 shows the CL values for the humic fractions,
 295 with respect to the metal ions Cu(II) and Al(III), with $\text{BIAS}_{1:1}$ as a proxy of fitting error.

296 Figure 5: Comparison between the CL (CL1 + CL2) values of the humic and fulvic acids, for Cu(II) ions
 297 (highlighted in blue) and Al(III) ions (highlighted in red), with the BIAS shown by the error bars of the
 298 samples.



299
 300
 301 Figure 5, the average CL values were $1.18 \cdot 10^{-9}$ to $2.40 \cdot 10^{-8}$ for the humic and
 302 fulvic acids for Cu(II), respectively; and for Al(III), the average values were and 1.26
 303 10^{-10} to $1.26 \cdot 10^{-9}$ for the HA and FA, respectively. It can be seen from Figure 5 that the
 304 densities of complexation sites for Cu(II) and Al(III) presented similar behaviors, since
 305 most of the horizons were grouped at around $4.00 \cdot 10^{-10}$ and $5.00 \cdot 10^{-9}$, respectively.

306 The results showed that the complexation capacity (considering the carbon
 307 content) was 10 times lower for the humic acid samples (scale in 10^{-10}) than for the
 308 fulvic acids (scale in 10^{-9}), as shown in Figure 5. The results shown that the humic acids
 309 required 10 times more carbon in their structures, in order to have the same complexing
 310 site density as the fulvic acids present in Spodosol Amazonian. Hence, the fulvic acid
 311 shown to have a greater quantity of complexation sites available to interact with the

312 Cu(II) and Al(III) ions, as shown in Figure 5. Results obtained in this work corroborate
313 with study realized by Mounier et al. (2011), that used the fluorescence quenching in
314 black water from Rio Negro-Brazil and they showed that PARAFAC enables
315 quantitative evaluation of complexing parameters for copper, and a good multi-response
316 methods.

317 Esteves da Silva et al. (1998) used the Stern-Volmer model to determine $\log K$
318 for the interactions of Cu(II) with humic and fulvic acids extracted from soil in Galicia
319 (Spain). The conditional stability constant ($\log K$) was 4.60 and higher values for the
320 conditional stability constants were obtained for the samples with higher concentrations
321 of FA (100 mg/L, pH = 6.0). It was concluded that the stabilities of the FA-Cu(II) and
322 HA-Cu(II) complexes were directly related to the numbers of binding sites present in
323 the structures of the humic substances. In the present work, the values of $\log K$ for the
324 HA were in the ranges 3.79-5.32 and 2.43-4.29 for Cu(II) and Al(III), respectively. The
325 corresponding values for the FA were in the ranges 2.26-5.31 and 1.80-4.14 for Cu(II)
326 and Al(III), respectively. These values were lower than those reported in previous
327 studies (Esteves da Silva et al. 1998; Plaza et al. 2005; Hernández et al. 2006; Funtet et
328 al. 2013), showing that the humic substances present in the Amazonian soil contained
329 greater quantities of bonding groups in their structures. The greatest affinities were
330 shown by the fulvic acid samples, which had the highest concentrations of sites
331 available for binding to Cu(II) and Al(III).

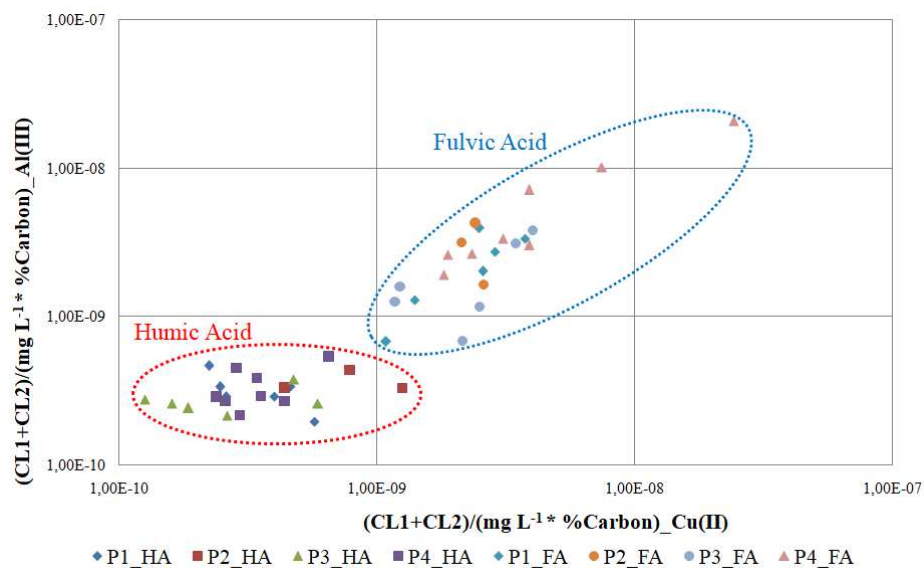
332 Esteves da Silva et al. (1996) studied the interaction of Al(III) with fulvic acids
333 from marine samples, employing synchronous fluorescence spectroscopy with evolving
334 factor analysis, obtaining a conditional stability constant of $\log_{10}K = 5.10$ at pH 4.
335 According to Esteves da Silva and Machado (1996), the concentration of binding sites

336 for marine fulvic acid samples was less than half of that for soil fulvic acid samples,
 337 suggesting existence of common pathways in the humification processes occurring in
 338 the soil and aquatic system.

339 Figure 6 shows the correlation between the complexation capacities to the Cu(II)
 340 and Al(III) the humic fractions of the soil horizons and profiles evaluated in this study.
 341 It can be seen that the humic acid samples presented lower scattering for Al(III),
 342 indicative of a constant density of Al(III) sites, while the density of Cu(II) sites varied.
 343 The HA appeared to have a constant specific density of complexing sites for Al(III),
 344 while the FA showed no such specificity, since the complexing capacity varied linearly
 345 for both Cu(II) and Al(III), maintaining the same ratio between the concentrations of
 346 complexing sites for the ions studied.

347

348 Figure 6: Correlation between the complexation capacities (CL1 + CL2) of the Amazonian Spodosol
 349 humic fractions for the Al(III) and Cu(II) ions. *Descriptions of terms* – ♦P1_HA: Profile 1 for Humic
 350 Acid; ■P2_HA: Profile 2 for Humic Acid; ▲P3_HA: Profile 3 for Humic Acid; ■P4_HA: Profile 4 for
 351 Humic Acid; ♦P1_FA: Profile 1 for Fulvic Acid; ●P2_FA: Profile 2 for Fulvic Acid; ●P3_FA: Profile 3
 352 for Fulvic Acid and ▲P4_FA: Profile 4 for Fulvic Acid;



353

354

355 A final treatment of the data was performed using principal component analysis
356 (PCA) (Figure 7). The results (Figure 7) showed clear separation of the humic fractions.
357 The points for the humic acids were more clustered, while those for the fulvic acids
358 were more dispersed. This separation was due to the influence of the complexing
359 capacity, with the grouping of the humic acids reflecting the lower complexing capacity
360 values. In addition, the best separation occurred to profile for the fulvic acid samples,
361 but some of the FA horizons presenting very different characteristics and the most
362 distant corresponding to the profiles P4 and P3. The differentiation between the FA and
363 HA was mainly due to the physical-chemical properties related to Al(III), corroborates
364 with the study of Boguta et al. (2016). The authors showed that the conditional stability
365 constant obtained for HA-Cu(II) complexes correlated with some of the physical-
366 chemical properties of the humic fraction, such as the predominance of -COOH and -
367 OH groups. The authors suggest that had been increased stability of the complexes, and
368 this group was associated with increase in the content of oxygen-containing structures,
369 indicating the formation of highly stable chelation complexes involving HA-Cu(II)
370 bonds, which presented greater stability when compared to simple and monodentate
371 complexes.

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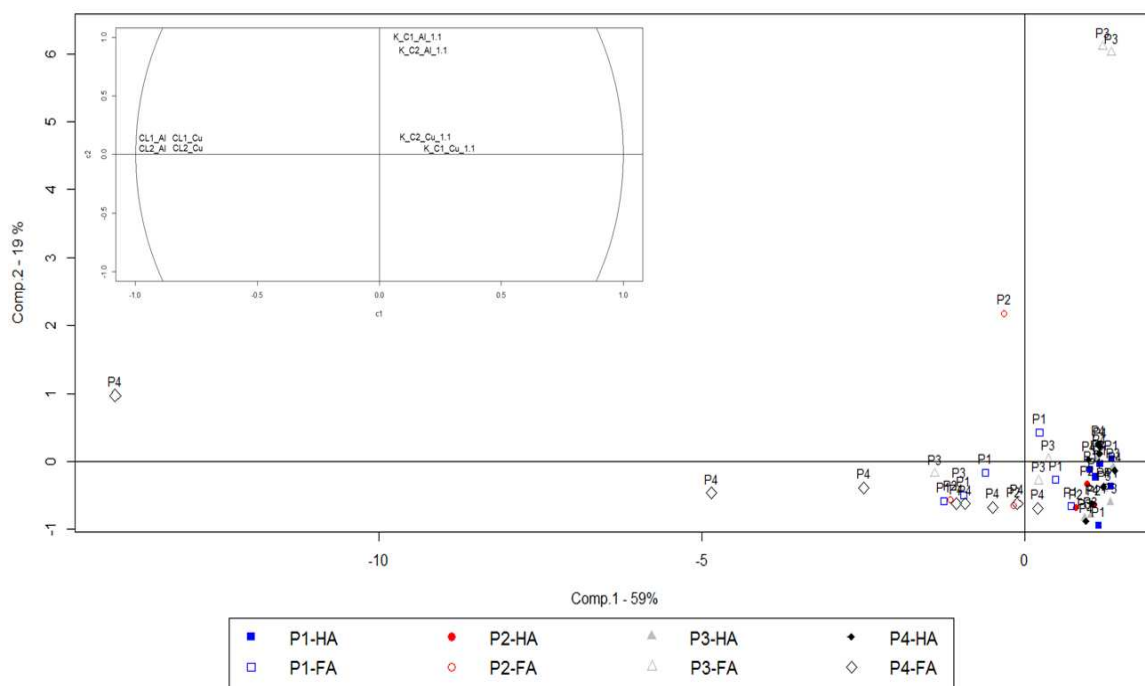
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379 Figure 7: Principal component analysis (PCA) of the quenching analysis results for the humic and fulvic
 380 acid samples, represented by profiles. *Descriptions of terms* – *CL1_Al: Complexation Capacity for*
 381 *Component 1 by Al(III) ions; CL2_Al: Complexation Capacity for Component 2 by Al(III) ions; CL1_Cu:*
 382 *Complexation Capacity for Component 1 by Cu(II) ions; CL2_Cu: Complexation Capacity for*
 383 *Component 1 by Cu(II) ions; K_C1_Al_1:1: Conditional stability constant for Al(III) by Component 1 for*
 384 *1:1model; K_C2_Al_1:1: Conditional stability constant for Al(III) by Component 2 for 1:1model;*
 385 *K_C1_Cu_1:1: Conditional stability constant for Cu(II) by Component 1 for 1:1model; K_C2_Cu_1:1:*
 386 *Conditional stability constant for Cu(II) by Component 2 for 1:1model; ■ P1_HA: Profile 1 for Humic*
 387 *Acid; ● P2_HA: Profile 2 for Humic Acid; ▲ P3_HA: Profile 3 for Humic Acid; ◆ P4_HA: Profile 4 for*
 388 *Humic Acid; □ P1_FA: Profile 1 for Fulvic Acid; ○ P2_FA: Profile 2 for Fulvic Acid; △ P3_FA: Profile 3*
 389 *for Fulvic Acid and ◇ P4_FA: Profile 4 for Fulvic Acid.*



390

391

392 Humic acids with high molecular weights and highly condensed aromatic units
 393 tend to have higher affinity for binding with the Cu(II) ion (Plaza et al. 2005; Boguta et
 394 al. 2016). The literature reports few studies concerning quenching analysis of fulvic
 395 acids, as shown in Table 2. There have been no previous studies related to the

396 fluorescence quenching of humic and fulvic acids extracted from Amazonian soils with
 397 Cu(II) and Al(III), indicating the novelty of the present work.

398

399 Table 2: Conditional stability constants (logK) and complexation capacities (CL) reported in the
 400 literature.

	Soil	Metal	Method	Conditional stability constant (logK)	Complexation capacity (CL / mmol g ⁻¹)	Reference
Humic Acid	Amazonian Spodosol	Cu(II)	Ryan-Weber	3.79 – 5.32	0.01 – 0.13	This study
	Amazonian Spodosol	Al(III)	Ryan-Weber	2.43 – 4.29	0.02 – 0.05	This study
	Calcic Luvisol	Cu(II)	Scatchard	6.02 – 7.04	0.22 – 0.86	Plaza et al. 2005
	EUROSOILS	Cu(II)	Ryan-Weber	5.14 – 5.36	1.80 – 3.70	Plaza et al. 2005
	Calcic Luvisol	Cu(II)	Ryan-Weber	4.71 – 5.25	1.01 – 2.84	Hernández et al. 2006
	Peat and Leonardite	Cu(II)	Ryan-Weber	4.58 – 5.10	0.21 – 1.33	Fuentes et al. 2013
	Peaty-muck	Cu(II)	Ryan-Weber	5.28 – 5.38 (pH 5) 5.46 – 5.77 (pH 7)	0.10 – 0.30 (pH 5) 0.27 – 0.42 (pH 7)	Boguta et al. 2016
Fulvic Acid	Amazonian Spodosol	Cu(II)	Ryan-Weber	2.26 – 5.31	0.11 – 2.44	This study
	Amazonian Spodosol	Al (III)	Ryan-Weber	1.80 – 4.14	0.07 – 2.08	This study
	Oak Forest	Cu(II)	Ryan-Weber	4.33 – 4.60	0.03 – 0.04	Esteves da Silva et al. 1998
	Calcic Luvisol	Cu(II)	Scatchard	5.96 – 6.41	0.20 – 0.52	Plaza et al. 2005

401

402 Plaza et al. (2005) determined the stability constants (logK) and complexation
 403 capacities (CC) for Cu(II) ions, using humic and fulvic acids extracted from soils that
 404 had received pig waste fertilization (Table 3). It was found that Cu(II) presented higher
 405 binding affinity for HA, compared to FA. Furthermore, the application of pig waste to
 406 the soil decreased the Cu(II) binding affinity for the humic fractions. The low values of
 407 CC and logK for the humic and fulvic acids from soil fertilized with pig waste, as well
 408 as for the fulvic acid from soil without fertilization, were suggested to be associated

409 with the presence of simple structural components with low degrees of humification and
410 low molecular weights. On the other hand, the higher values of CC and logK could be
411 associated with organic material with higher aromatic condensation, greater
412 humification, and higher molecular weight. The authors concluded that the presence of
413 more stable organic material with a higher degree of humification increased the affinity
414 for the Cu(II) ion.

415 The interaction of humic fractions of the Amazonian Spodosols with Cu(II) and
416 Al(III) ions showed that HA presented smaller amounts of complexation sites and a
417 lower stability constant when compared to FA according to Table 2. Fulvic acids
418 showed higher complexation capacity, which may be associated with their simpler
419 chemical structure, which allows a greater selectivity of interaction with these metals.
420 Whereas, humic acids presented a more complex structure and their metal selectivity
421 was lower as determined by the low complexation capacity.

422 Studies by Tadini et al. (2019) using the same soils presented in this work,
423 showed that the mobilization of suspended organometallic complexes and metallic
424 oxides were controlled by the presence of groundwater outcrops favored the movement
425 of the metals, especially in P1 and P4 profiles. The results showed that the FA fraction
426 were associated with high amounts of Al in the soils, while the HA fraction was more
427 selective towards Fe. The authors concluded that the transfer of Fe and Al in these
428 profiles is due to the type of chemical structure, the movement of the groundwater and
429 the process of formation of this soil. Therefore, it can be inferred that the FA have a
430 contribution from groundwater, whose chemical structure varies little with the soil
431 profile and a greater selectivity in the interaction with the metals, especially with the Al
432 metal. In contrast, HA were less selective because they may be associated with other

433 metals and have a greater variation in chemical structure with soil profile. Due to this
434 diversity of metals that HA has affinity for, it is suggested that they may be responsible
435 for soil fertility.

436 Therefore, this work allowed us to show a new data treatment model for
437 fluorescence suppression in soil samples, using the Ryan-Weber model and
438 CP/PARAFAC, obtaining quantitative data for EEM. A similar study was described by
439 Zhu and coauthors (2014) showed a new fluorescence suppression treatment based on
440 the Ryan-Weber model using the interaction of Cu(II) ions with tryptophan and
441 tyrosine. According to the authors modeling techniques are necessary to understand the
442 phenomenon of complexation that occurs in organic matter. Hence, the sensitivity and
443 simplicity of the fluorescence technique combined with statistical treatments capable of
444 analyzing interactions with metals at the molecular level (Esteves da Silva et al. 1998).
445 In this case, soils of great environmental relevance such as the present in the Amazon
446 region as discussed in this study.

447

448 **4. Conclusions**

449 Investigation of the humic fractions from Amazonian Spodosols showed that the
450 1:1 model was most suitable for modeling variations of fluorescence quenching with
451 Cu(II) or Al(III). It could be concluded from the results that the humic acids presented
452 only two components, for which the complexing properties did not vary with the soil
453 profile. The fulvic acid samples showed greater variation of these properties according
454 to the profile. For both Cu(II) and Al(III), the CP/PARAFAC components represented
455 typical humic material, with emission wavelengths ranging from 400 to 550 nm. The
456 humic acid samples generally presented complexes with blue shifts (C1), rather than red

457 shifts (C2) and more homogeneous than FA which obtained three different components.
458 For both metals studied, the humic acids presented smaller quantities of complexation
459 capacity (CL), compared to the fulvic acid samples. The HA showed a more
460 homogeneous of the constant stability and complexation capacity for Al(III), compared
461 to the FA, which were more heterogeneous characteristic for both metals, Cu(II) and
462 Al(III). The results shown that the humic acids required 10 times more carbon in their
463 structures, in order to have the same complexing site density as the fulvic acids, it can
464 be inferred that the FA fraction has higher mobility and greater interaction in this
465 Spodosol Amazonian.

466

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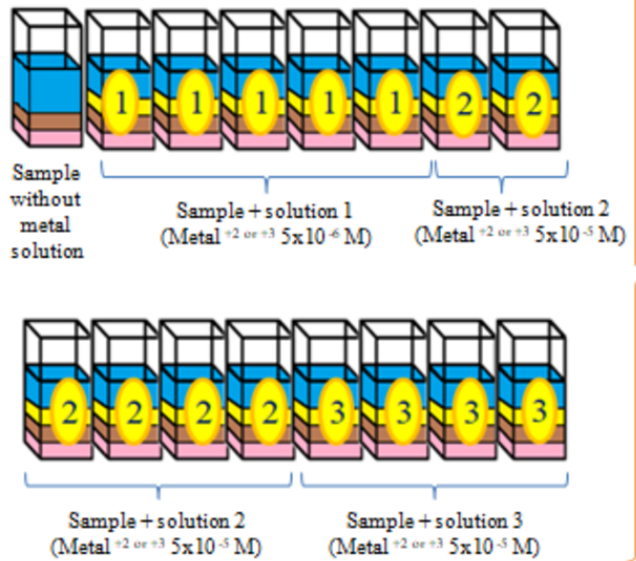
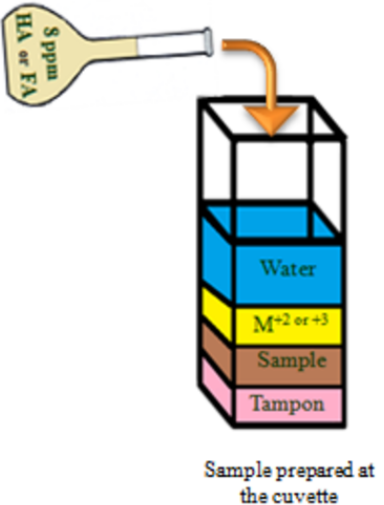
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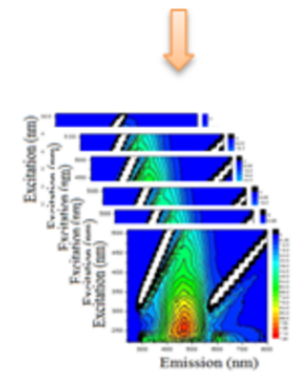
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Fluorescence Quenching Spectroscopy



CP/PARAFAC = Analysis of Parallel Factors

