HAL open science

Modeling the quenching of fluorescence from organic matter in Amazonian soils

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

To cite this version:

Amanda Maria Tadini, S. Mounier, Débora Marcondes Bastos Pereira Milori. Modeling the quenching of fluorescence from organic matter in Amazonian soils. Science of the Total Environment, 2020, 698, pp.134067 -. 10.1016/j.scitotenv.2019.134067 . hal-03488551

HAL Id: hal-03488551

https://hal.science/hal-03488551v1

Submitted on 21 Dec 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Version of Record: https://www.sciencedirect.com/science/article/pii/S0048969719340446
Manuscript_e17449659b51f58c62f8b40e5bcb3880

Modeling the quenching of fluorescence from organic matter in Amazonian soils

Amanda Maria Tadini[a,b,c*], Stephane Mounier[c], Débora Marcondes Bastos Pereira Milori[a]

[a] Embrapa Agricultural Instrumentation, São Carlos, SP, Brazil

[b] Institute of Chemistry of São Carlos, University of São Paulo, São Carlos, SP, Brazil

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

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

© 2019 published by Elsevier. This manuscript is made available under the CC BY NC user license
https://creativecommons.org/licenses/by-nc/4.0/

ABSTRACT

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

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

1. Introduction

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

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

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

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

2. Materials and Methods

2.1. Study area

The soil samples were obtained from a site located in the north of the municipality of Barcelos, near the Demeni River (0° 15’ 18’’N; 62° 46’ 36’’W) in the 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 $CuSO_4.5H_2O$ (0.01 mol L^{-1}) and $Al_2(SO_3).18H_2O$ (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

60% was chosen as the best one for each data set. For all the quenching experiments, the best model had two components, C1 and C2 as shown in Table 1.

Table 1: Components obtained by CP/PARAFAC for interactions of the humic acid and fulvic acid samples, extracted from the Amazonian Spodosols, with the Cu(II) and Al(III) ions.

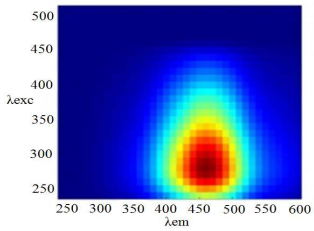
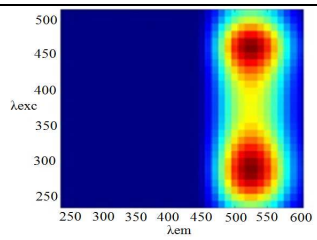
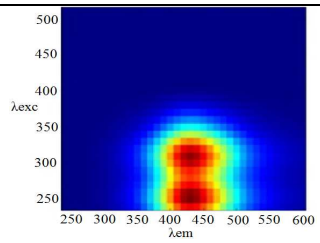
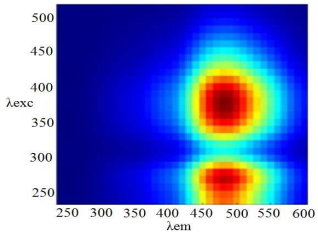
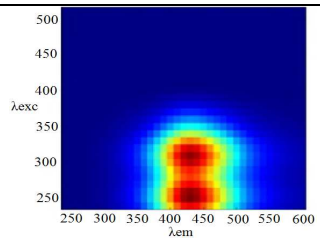
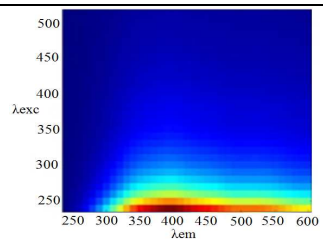
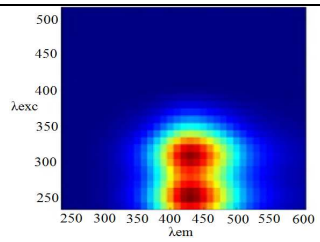
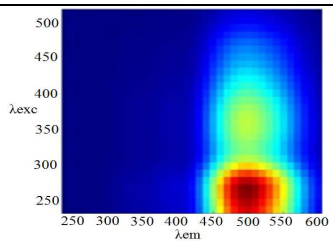
	Profiles	**Component 1**	**Component 2**
Denomination		**A**	**B**
Humic Acids	**P1** **P2** **P3** **P4**		
		Component 1	**Component 2**
Denomination		**C**	D
Fulvic Acids	**P1**		
Denomination		C	E
Fulvic Acids	**P2** **P4**		
Denomination		C	F
Fulvic Acids	**P3**		

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

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

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

$$L_1 + M \leftrightarrow ML_1 \qquad (1)$$

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

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

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

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

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

$$L_1 + M \leftrightarrow ML_1 \text{ and } L_2 + M \leftrightarrow ML_2 \quad (5)$$

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

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

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

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

where CL_1 and CL_2 are the total complexation sites present in the sample. Assuming that L_1, L_2, ML_1, and ML_2 have fluorescence quantum yields of Φ_{L1}, Φ_{L2}, Φ_{ML1}, and 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 $$BIAS_{1:1} = \Sigma[(IF_i - IF_{1:1}(CL_1, K_1, M_{0i})/IF_i]^2 \quad (9)$$

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

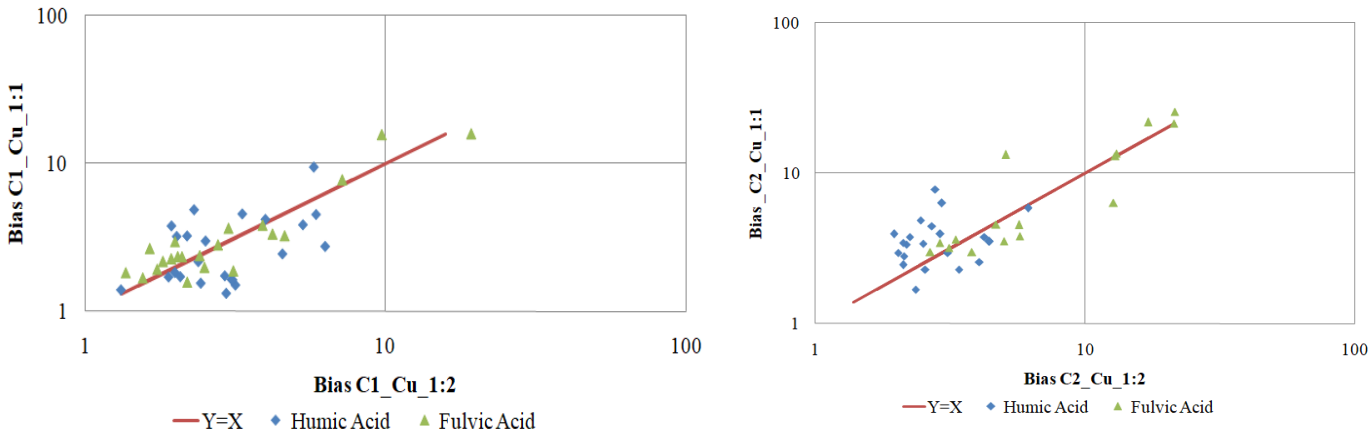
190

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 $BIAS_{1:1}$ (model 1:1) and $BIAS_{1:2}$ (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 $BIAS_{1:1}$ and $BIAS_{1:2}$ 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

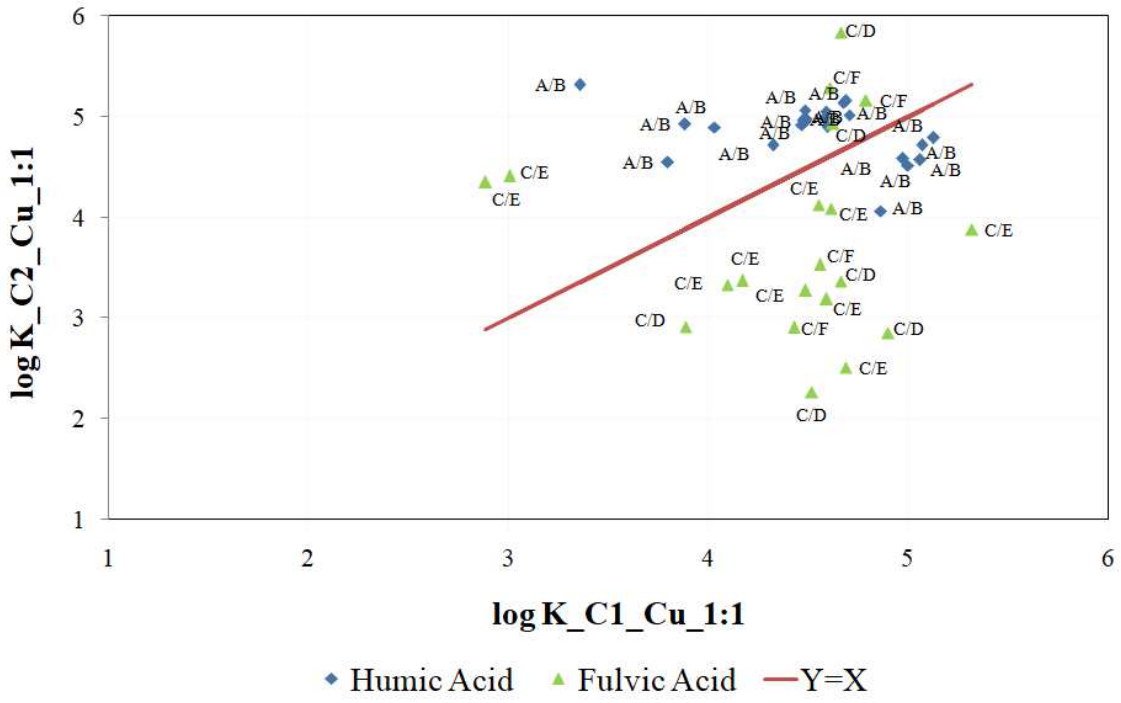
Figure 1: Comparison of the $BIAS_{1:1}$ and $BIAS_{1:2}$ models of the Component 1 (a) and Component 2 (b) for Cu(II). *Descriptions of terms - Bias C1_Cu_1:2: Bias for Component 1 by Cu(II) with 1:2 model; Bias C1_Cu_1:1: Bias for Component 1 by Cu(II) with 1:1 model; Bias C2_Cu_1:2: Bias for Component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



(a) (b)

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

Figure 2: Evaluation of the conditional stability constants for the interactions of Cu(II) with the humic acids (highlight blue) and fulvic acids (highlight green), using the 1:1 model, with each Component obtained by CP/PARAFAC. *Descriptions of terms - logK_C1_Cu_1:1: logarithm of conditional constant stability for Cu(II) by Component 1 for 1:1model; logK_C2_Cu_1:1: logarithm of conditional constant stability for Cu(II) by Component 2 for 1:1model; A/B: Component 1 (denominate A)/Component 2 (denominate B) for Humic acids from profiles P1, P2, P3 and P4; C/D: Component 1 (denominate C)/Component 2 (denominate D) for fulivc acids from profile P1; C/E: Component 1 (denominate C)/Component 2 (denominate E) for fulivc acids from profile P2 and P4; C/F: Component 1 (denominate C)/Component 2 (denominate F) for fulivc acids from profile P3; all denominates are present in Table 1.*



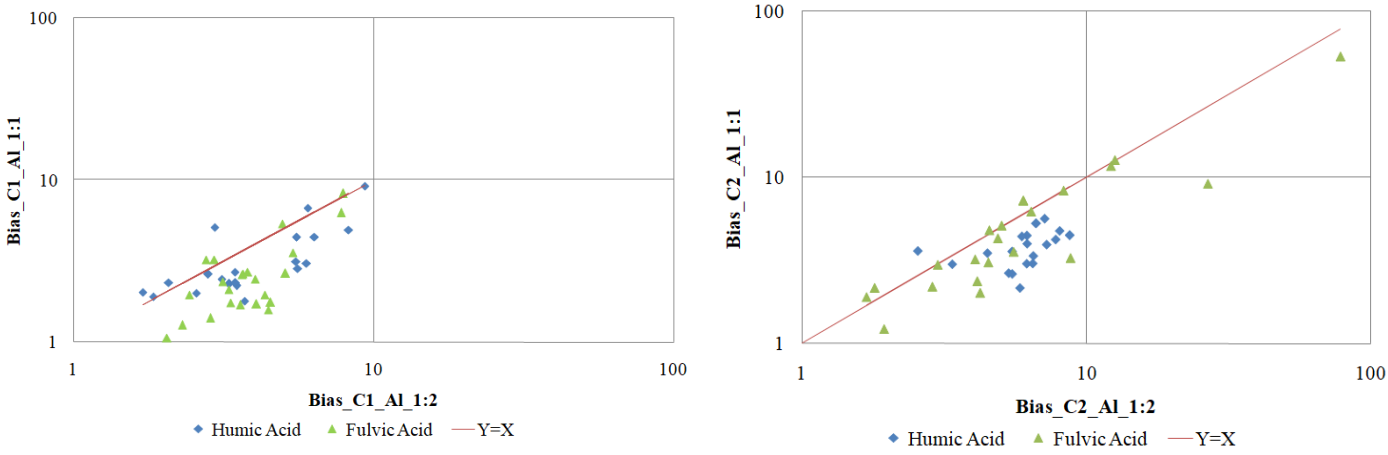
As shown in Figure 2, most of the fulvic acid samples showed higher conditional stability constant (K) values for CP/PARAFAC component 1 (Table 1). This component corresponded to a molecule with simpler structure that could be related to components described in the "*C/E*" and "*C/D*" chart (as shown in Table 1), with correlation to component C of the FA (the component with emission more towards the 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 $mol^{-1}L$. 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

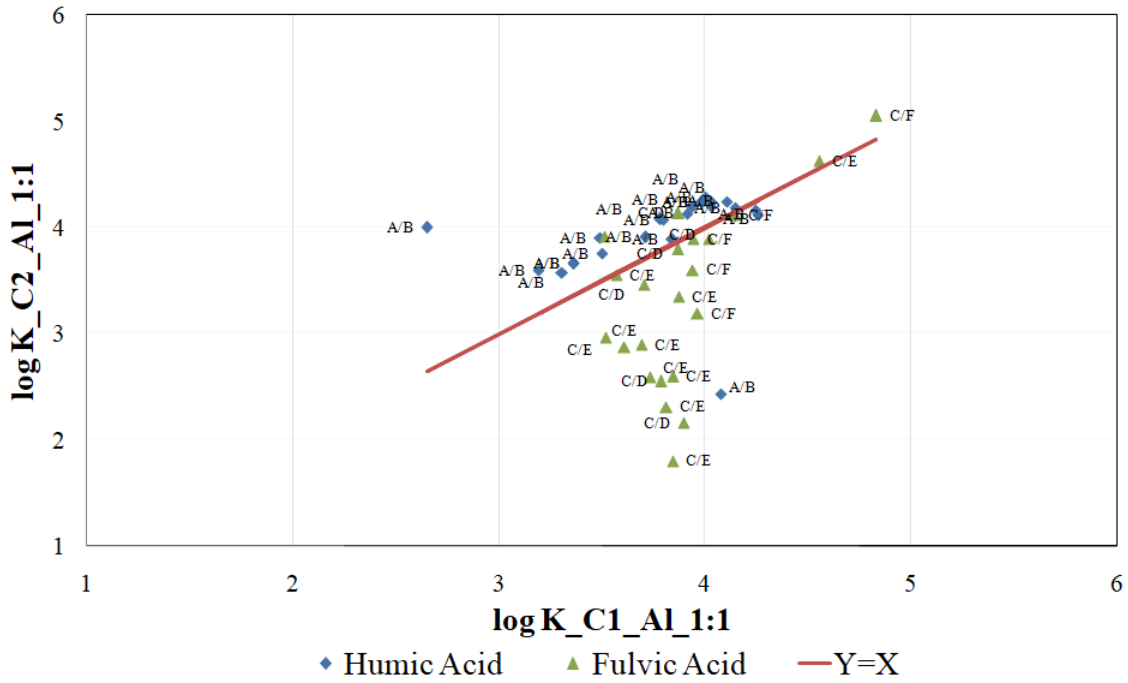
260 Figure 3: Comparison of the $BIAS_{1:1}$ and $BIAS_{1:2}$ values of the Component 1 (a) Component 2 (b) for
261 Al(III). *Descriptions of terms - Bias C1_Al_1:2: Bias for Component 1 by Al(III) with 1:2 model; Bias*
262 *C1_Al_1:1: Bias for Component 1 by Al(III) with 1:1 model; Bias C2_Al_1:2: Bias 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)

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

Figure 4: Evaluation of the conditional stability constant for the interactions of Al(III) with the humic acids (highlight blue) and fulvic acids (highlight green), using the 1:1 model, with each component obtained by CP/PARAFAC. *Descriptions of terms – logK_C1_Al_1:1: logarithm of conditional stability constant for Al(III) by Component 1 for 1:1model; logK_C2_Al_1:1: logarithm of conditional stability constant for Al(III) by Component 2 for 1:1model; A/B: Component 1 (denominate A)/Component 2 (denominate B) for Humic acids from profiles P1, P2, P3 and P4; C/D: Component 1 (denominate C)/Component 2 (denominate D) for fulivc acids from profile P1; C/E: Component 1 (denominate C)/Component 2 (denominate E) for fulivc acids from profile P2 and P4; C/F: Component 1 (denominate C)/Component 2 (denominate F) for fulivc acids from profile P3; all denominations are shown in Table 1.*



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

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

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

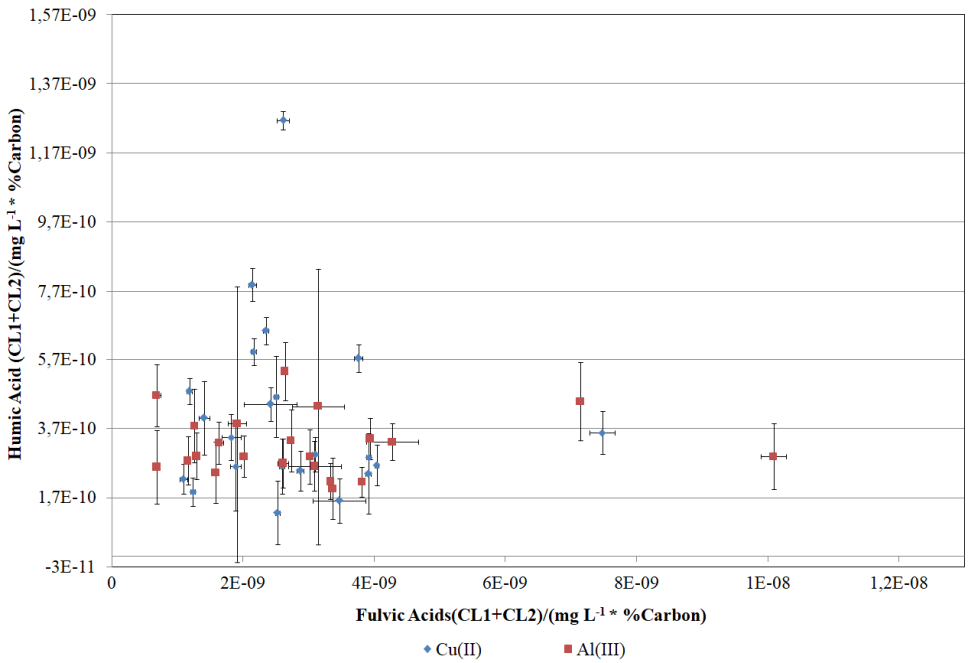


Figure 5, the average CL values were 1.18 10^{-9} to 2.40 10^{-8} for the humic and fulvic acids for Cu(II), respectively; and for Al(III), the average values were and 1.26 10^{-10} to 1.26 10^{-9} for the HA and FA, respectively. It can be seen from Figure 5 that the densities of complexation sites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

The results showed that the complexation capacity (considering the carbon content) was 10 times lower for the humic acid samples (scale in 10^{-10}) than for the fulvic acids (scale in 10^{-9}), as shown in Figure 5. The results shown that the humic acids required 10 times more carbon in their structures, in order to have the same complexing site density as the fulvic acids present in Spodosol Amazonian. Hence, the fulvic acid 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.

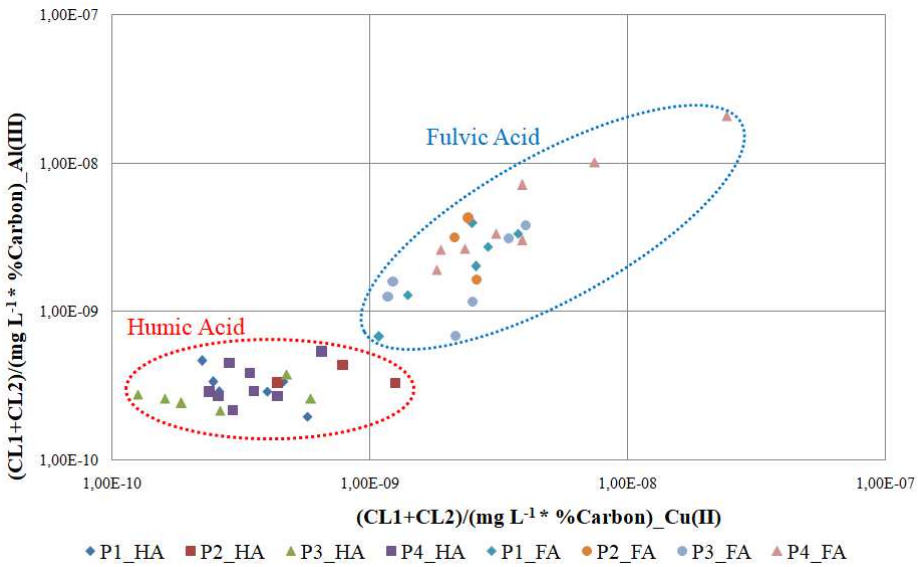
317 Esteves da Silva et al. (1998) used the Stern-Volmer model to determine logK
318 for the interactions of Cu(II) with humic and fulvic acids extracted from soil in Galicia
319 (Spain). The conditional stability constant (log K) was 4.60 and higher values for the
320 conditional stability constants 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 logK 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; Funtes 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

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

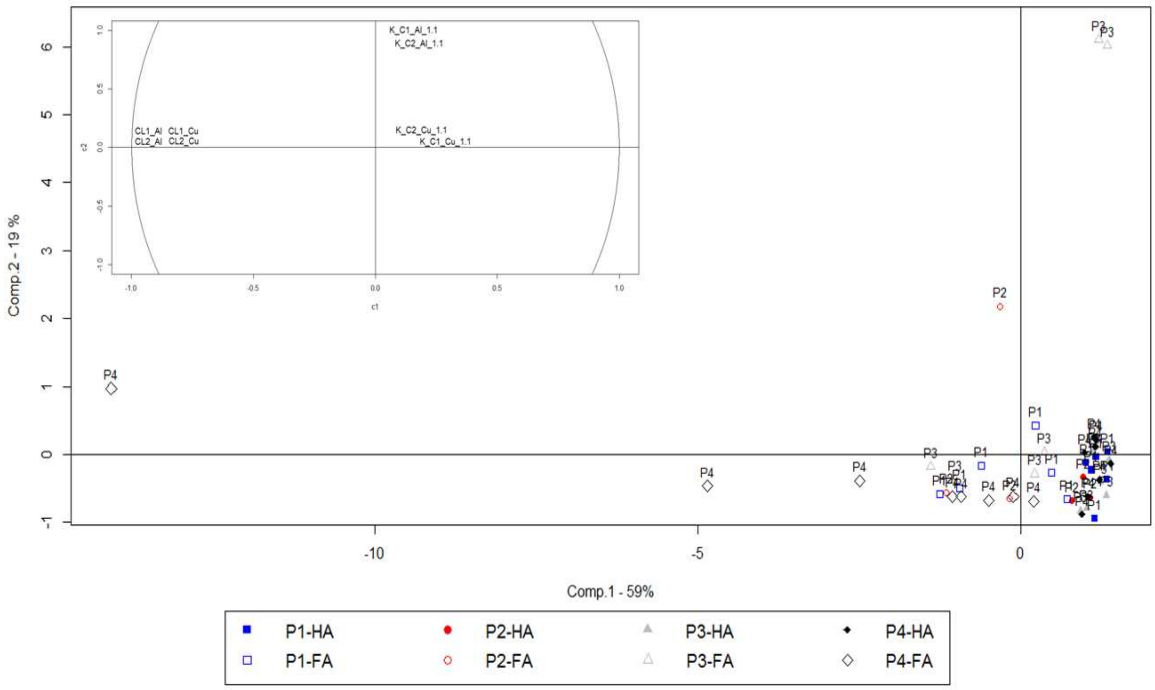
Figure 6 shows the correlation between the complexation capacities to the Cu(II) and Al(III) the humic fractions of the soil horizons and profiles evaluated in this study. It can be seen that the humic acid samples presented lower scattering for Al(III), indicative of a constant density of Al(III) sites,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

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



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

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



Humic acids with high molecular weights and highly condensed aromatic units tend to have higher affinity for binding with the Cu(II) ion (Plaza et al. 2005; Boguta et al. 2016). The literature reports few studies concerning quenching analysis of fulvic 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

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

Acknowledgments

The authors are grateful for the financial support provided by the São Paulo State Research Foundation (FAPESP, grant numbers 2011/03250-2, 2012/51469-6, 2013/07276-1, and 2013/13013-3), the Brazilian National Council for Technological and Scientific Development (CNPq, grant number 232225/2014-1-SWE), and the French ANR (Agence Nationale de la Recherche, grant number ANR-12-IS06-0002 "C-PROFOR"). We also thank Embrapa Agricultural Instrumentation for supporting the development of the research. The authors would like to thank Dr. Célia Regina Montes (ESALQ/USP, Piracicaba, São Paulo) for providing samples for analysis.

References

Bai, Y.C., Wu, F.C., Liu, C.Q., Li, W., Guo, J.Y., Fu, P.Q., Xing, B.S., Zheng, J., 2008. Ultraviolet absorbance titration for determining stability constants of humic

substances with Cu (II) and Hg (II). Anal. Chim. Acta 616, 115-121. https://doi.org/10.1016/j.aca.2008.04.003.

Boguta, P., D'orazio, V., Sokołowska, Z., Senesi, N., 2016. Effects of selected chemical and physicochemical properties of humic acids from peat soils on their interaction mechanisms with copper ions at various pHs. J. Geochem. Explor., 168, 119–126. https://doi.org/10.1016/j.gexplo.2016.06.004.

Bro, R., 1997. PARAFAC. Tutorial and applications. Chemometr. Intell. Lab. Syst., 38, 2, 149-171. https://doi.org/10.1016/S0169-7439(97)00032-4.

Cerri, C.C., Bernoux, M., Arrouays, D., Feigl, B.J., Piccolo, M.C., 2000. Carbon stocks in soils of the Brazilian Amazon. In: Kimble, R.L.J.M., Stewart, B.A. (eds.). Global Climate Change and Tropical Ecosystems. Advances in Soil Science. CRC Press, Boca Raton, Florida, E.U.A., p. 438.

Chen, W.B., Smith, D.S., Guéguen, C., 2013. Influence of water chemistry and dissolved organic matter (DOM) molecular size on copper and mercury binding determined by multiresponse fluorescence quenching. Chemosphere, 92, 351–359. https://doi.org/10.1016/j.chemosphere.2012.12.075.

Coble, P., Lead, J., Baker, A., Reynolds, D., Spencer, R.G.M., 2014. Aquatic Organic Matter Fluorescence. Environmental Chemistry. Ed. Cambridge, p. 418.

Coble, P.G., 1996. Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. Mar. Chem., 51, 325-346. https://doi.org/10.1016/0304-4203(95)00062-3.

Esteves da Silva, J.C.G., Machado, A.A.S.C., Ferreira, M.A., Rey, F., 1998. Method for the differentiation of leaf litter extracts and study of their interaction with

Cu(II) by molecular fluorescence. Can. J. Chem., 76, 1197–1209. https://doi.org/10.1139/v98-150.

Esteves da Silva, J.C.G., Machado, A.A.S.C., Oliveira, C.J.S., 1998. Fluorescence quenching of anthropogenic fulvic acids by Cu(II), Fe(III) and UO22+. Talanta 45, 1155–1165. https://doi.org/10.1016/S0039-9140(97)00224-5.

Esteves da Silva, J.C.G., Machado, A.A.S.C, 1996. Characterization of the binding sites for Al(III) and Be(II) in a sample of marine fulvic acids. Mar. Chem., 54, 293-302. https://doi.org/10.1016/0304-4203(96)00032-1.

Fuentes, M., Olaetxea, M., Baigorri, R., Zamarreño, A.M., Etienne, P., Laîné, P., Ourry, A., Yvin, J.C., Garcia-Mina, J.M., 2013. Main binding sites involved in Fe(III) and Cu(II) complexation in humic-based structures. J. Geochem. Explor., 129, 14–17. https://doi.org/10.1016/j.gexplo.2012.12.015.

Gerke, J., 1994. Aluminum complexation by humic substances and aluminum species in the soil solution. Geoderma, 63, 165-175. https://doi.org/10.1016/0016-7061(94)90004-3.

Hu, B., Wang, P., Wang, C., Quian, J., Hou, J., Cui, X., Zhang, N., 2017. The effect of anthropogenic impoundment on dissolved organic matter characteristics and copper binding affinity: Insights from fluorescence spectroscopy. Chemosphere, 188, 424-433. https://doi.org/10.1016/j.chemosphere.2017.09.023.

Huang, M., Li, H., Huang, B., Lu, N., Zhang, Q., Zhai, X., Zeng, G., 2018. Investigating binding characteristics of cadmium and copper to DOM derived from compost and rice straw using EEM-PARAFAC combined with two-dimensional FTIR correlation analyses. J. Hazard. Mater., 344, 539–548. https://doi.org/10.1016/j.jhazmat.2017.10.022

Brazilian Institute of Geography and Statistics (IBGE: Instituto Brasileiro de Geografia e Estatística), 2012. Manual técnico da vegetação brasileira, 2ª edição, Rio de Janeiro.

Kumke, M.U., Tiseanu, C., Braun, A.G., Frimmel, F.H., 1998. Fluorescence decay of natural organic matter (NOM) and influence of fractionation, oxidation, and metal ion complexation. J. Fluoresc., 8, 309-318.

Luciani, X., Redon, R., Mounier, S., 2013. How to correct inner filter effects altering 3D fluorescence spectra by using a mirrored cell. Chemometr. Intell. Lab. Syst., 126, 91–99. https://doi.org/10.1016/j.chemolab.2013.04.014.

Luster, J., Lloyd, T., Sposito, G., 1996. Multi-wavelength molecular fluorescence spectrometry for quantitative characterization of copper(II) and aluminum(III) complexation by dissolved organic matter. Environ. Sci. Technol., 30, 1565–1574. https://doi.org/10.1021/es950542u.

Manciulea, A., Baker, A., Lead, J.R., 2009. A fluorescence quenching study of the interaction of Suwannee River fulvic acid with iron oxide nanoparticles. Chemosphere, 76, 1023–1027. https://doi.org/10.1016/j.chemosphere.2009.04.067.

Matthews, B.J.H., Jones, A.C., Theodorou, N.K., Tudhope, A.W., 1996. Excitation-emission-matrix fluorescence spectroscopy applied to humic acid bands in coral reefs. Mar. Chem., 55, 317-332. https://doi.org/10.1016/S0304-4203(96)00039-4.

Milori, D.M.B.P., Martin-Neto, L., Bayer, C., Mielniczuk, J., Bagnato, V.S., 2002. Humification degree of soil humic acids determined by fluorescence spectroscopy. J. Soil Sci., 167, 739-749.

Mounier, S., Zhao, H., Garnier, C., Redon, R., 2011. Copper complexing properties of dissolved organic matter: PARAFAC treatment of fluorescence quenching. Biogeochemistry, 106, 107–116.

Nouhi, A., Hajjoul, H., Redon, R., Gagné, J.P., Mounier, S., 2018. Time-resolved laser fluorescence spectroscopy of organic ligands by europium: Fluorescence quenching and lifetime properties. Spectrochim. Acta A., 193, 219–225. https://doi.org/10.1016/j.saa.2017.12.028.

Paul, E.A., 2016. The nature and dynamics of soil organic matter: Plant inputs, microbial transformations, and organic matter stabilization. Soil Biol. Biochem., 98, 109–126. https://doi.org/10.1016/j.soilbio.2016.04.001.

Plaza, C., D'orazio, V., Senesi, N., 2005. Copper(II) complexation of humic acids from the first generation of EUROSOILS by total luminescence spectroscopy. Geoderma, 125, 177–18. https://doi.org/10.1016/j.geoderma.2004.07.012.

Plaza, C., Senesi, N., García-Gil, J.C., Polo, A., 2005. Copper (II) complexation by humic and fulvic acids from pig slurry and amended and non-amended soils. Chemosphere, 61, 711–716. https://doi.org/10.1016/j.chemosphere.2005.03.046.

Ryan, D.K., Weber, J.H., 1982. Fluorescence quenching titration for determination of complexing capacities and stability constants of fulvic acid. Anal. Chem., 54, 986-990. https://doi.org/10.1021/ac00243a033.

Santos, C.H., Nicolodelli, G., Romano, R.A., Tadini, A.M., Villas-Boas, P.R., Montes, C.R., Mounier, S., Milori, D.M.B.P., 2015. Structure of Humic Substances from Some Regions of the Amazon Assessed Coupling 3D Fluorescence Spectroscopy and CP/PARAFAC. J. Braz. Chem. Soc., 26, 1136-1142. http://dx.doi.org/10.5935/0103-5053.20150076.

Smith, D.S., Kramer, J.R., 2000. Multisite metal binding to fulvic acid determined using multiresponse fluorescence. Anal. Chim. Acta, 416, 211–220. https://doi.org/10.1016/S0003-2670(00)00900-4.

Stedmon, C.A., Markager, S., Bro, R., 2003. Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. Mar. Chem., 82, 239-254. https://doi.org/10.1016/S0304-4203(03)00072-0.

Stevenson, F.J., 1994. Humus chemistry: genesis, composition and reaction. 2nd Edition. New York: John Wiley & Sons.

Swift, R.S., 1996. Organic matter characterization (chap 35). pp. 1018-1020. In D.L. Sparks et al. (eds) Methods of soil analysis. Part 3. Chemical methods. Soil Sci. Soc. Am. Book Series: 5. Soil Sci. Soc. Am. Madison, WI.

Tadini, A.M., Nicolodelli, G., Marangoni, B.S., Mounier, S., Montes, C.R., Milori, D.M.B.P., 2019. Evaluation of the roles of metals and humic fractions in the podzolization of soils from the Amazon region using two analytical spectroscopy techniques. Microchem. J., 144, 454–460. https://doi.org/10.1016/j.microc.2018.10.009.

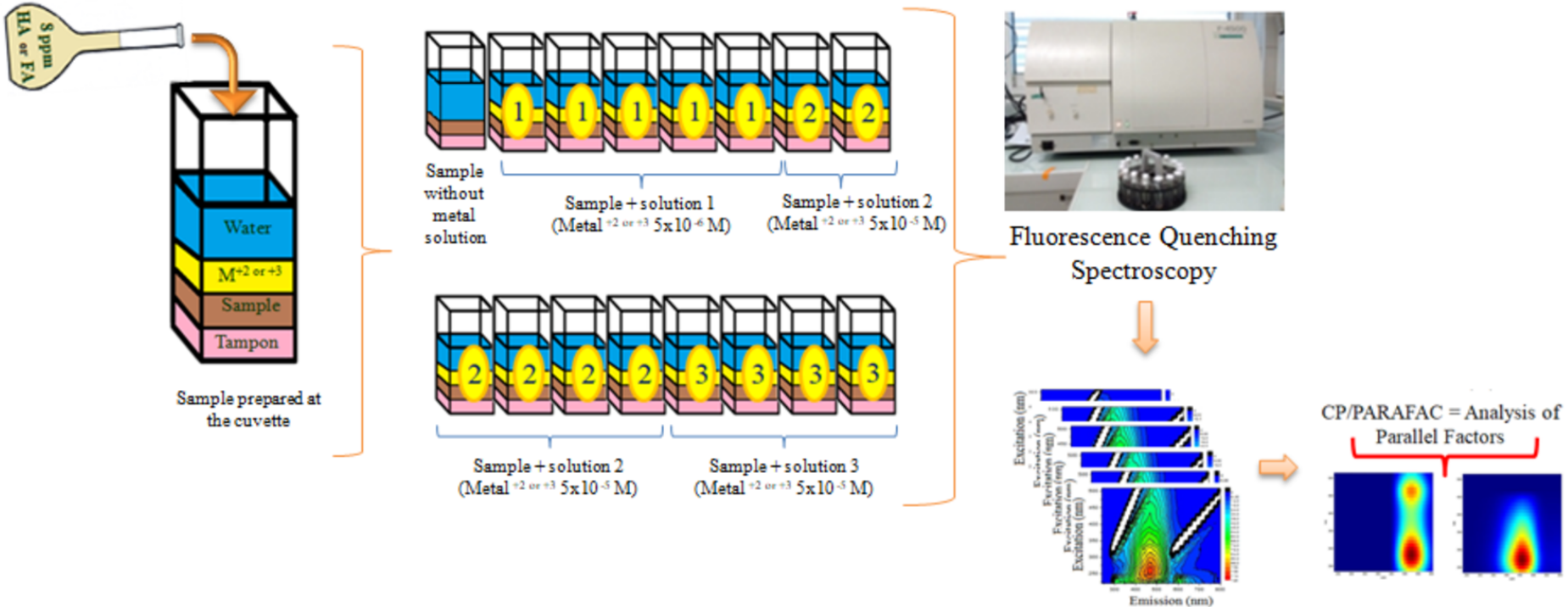
Tadini, A.M., Nicolodelli, G., Senesi, G.S., Ishida, D.A., Montes, C.R., Lucas, Y., Mounier, S., Guimaraes, F.E.G., Milori, D.M.B.P., 2018. Soil organic matter in podzol horizons of the Amazon region: Humification, recalcitrance, and dating. Sci. . Total Environ., 613, 160-167. https://doi.org/10.1016/j.scitotenv.2017.09.068.

Wu, F., Bai, Y., Mu, Y., Pan, B., Xing, B., Lin, Y., 2013. Fluorescence quenching of fulvic acids by fullerene in water. Environ. Pollut., 172, 100-107. https://doi.org/10.1016/j.envpol.2012.08.005.

Yamashita, Y., Jaffe, R., 2008. Characterizing the interactions between trace metals and dissolved organic matter using excitation–emission matrix and parallel

factor analysis. Environ. Sci. Technol., 42, 7374–7379. https://doi.org/10.1021/es801357h.

Zhu, B., Pennell, S.A., Ryan, D.K., 2014. Characterizing the interaction between uranyl ion and soil fulvic acid using parallel factor analysis and a two-site fluorescence quenching model. Microchem. J., 115, 51–57. https://doi.org/10.1016/j.microc.2014.02.004


5 ppm HA or FA
Water
M+2 or +3
Sample
Tampon
Sample prepared at the cuvette
1
2
Sample without metal solution
Sample + solution 1 (Metal +2 or +3 5x10-6 M)
Sample + solution 2 (Metal +2 or +3 5x10-5 M)
3
Sample + solution 2 (Metal +2 or +3 5x10-5 M)
Sample + solution 3 (Metal +2 or +3 5x10-5 M)
Fluorescence Quenching Spectroscopy
Excitation (nm)
Emission (nm)
CP/PARAFAC = Analysis of Parallel Factors