Characterization of Organic Matter in Spodosol Amazonian by Fluorescence Spectroscopy

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***Abstract*—**Soil organic matter (SOM) plays an important role in maintaining soil productivity and accounting for the promotion of biological diversity. The main components of the SOM are the humic substances which can be fractionated according to its solubility in humic acid (HA), fulvic acids (FA) and humin (HU). The determination of the chemical properties of organic matter as well as its interaction with metallic species is an important tool for understanding the structure of the humic fractions. Fluorescence spectroscopy has been studied as a source of information about what is happening at the molecular level in these compounds. Specially, soils of Amazon region are an important ecosystem of the planet. The aim of this study is to understand the molecular and structural composition of HA samples from Spodosol of Amazonia using the fluorescence Emission-Excitation Matrix (EEM) and Time Resolved Fluorescence Spectroscopy (TRFS). The results showed that the samples of HA showed two fluorescent components; one has a more complex structure and the other one has a simpler structure, which was also seen in TRFS through the evaluation of each sample lifetime. Thus, studies of this nature become important because it aims to evaluate the molecular and structural characteristics of the humic fractions in the region that is considered as one of the most important regions in the world, the Amazon.

***Keywords*—**Amazonian soil, characterization, fluorescence, humic acid, lifetime.

I. Introduction

THE Amazon forest provides important services to humanity, such as high biodiversity, climate regulation, carbon sequestration, and regulation of water cycles and nutrients. Therefore, Amazon has become an area for the research mainly related to the area of the soil in order to understand its soil development process in the region and possible consequences of land use change and occupation of soils. The main soil formations found in the Negro River basin are Oxisols, Podzols, and hydromorphic soils. Podzols are soils that have a strong vertical differentiation diagnosed by the presence of spodic horizon (Bh), which accumulates organic matter [1].

SOM plays an important role in environmental sustainability and participation in the formation and transformation processes of soil, especially in the carbon cycle. The main components of the SOM are humic substances (HS), having well defined physical and chemical characteristics and are composed of fractioned HA, FA, and HU according to their solubility. Thus, understanding the dynamics of SOM is essential for assessing the quality and capacity of the soil to resist the changes in their physical and chemical properties according to the weather conditions and the nature of the source material [2].

The amount of organic carbon stored in the surface layer (0 to 1.0 m) in hydromorphic podzols the upper Rio Negro is 87±7 kg m^{-2} for all soils, and corresponds to 14±1 Pg of the Carbon [3]. SOMs in these soils are not homogeneous, and studies to evaluate the dynamics of this matter are important mainly in this ecosystem that is considered one of the most important carbon sinks in the world [4].

The determination of the optical properties of SOM is an important process for understanding their structural fractions. The use of this technique can contribute to analyze the composition and interactions of HS which reflect on the future changes of these substances with changes in land uses. Fluorescence EEM is a selective and sensitive spectroscopic technique which allows a simple assembly or a mixture of fluorescent components present in humic fractions which can be measured, thereby providing a digital sample print [5], [6]. Furthermore, the EEM spectra can be used for the qualitative and quantitative characterization of fluorescent organic matter when combined with the advanced multivariate statistical techniques such as Parallel Factor Analysis (CP/PARAFAC), which can decompose the signal complex of fluorescence spectra into simple components.

EEM-CP/PARAFAC is a potentially useful technique in the evaluation of complex samples such as HS. This technique enables the evaluation of the decomposition of fluorescent components, regardless of complex formed in EEM, which represent groups called fluorescent components [5]-[8].

Suppression fluorescence, also known as quenching analysis, is a process which decreases the fluorescence intensity of a sample, which can result in decay due to molecular interactions [8]. These interactions include reactions in the excited state, molecular rearrangements, energy transfer, complex formation in the ground state and the collision energy

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[7], [8]. In organic matter, the fluorescence suppression is an essential characteristic verified in paramagnetic metal ions. Thus, the sensitivity and simplicity of quenching fluorescence makes it a viable technique to analyze these interactions [16].

TRFS is employed to assess the molecular interactions and movements of a sample occurring at the time interval peak and in nanoseconds. The TRFS is employed to measure the decay of the total fluorescence intensity of the molecule upon excitation or emission allowing the determination of fluorescence lifetime or it can be used to characterize the molecular motions of this fluorophore. So, this technique is useful because it can investigate the structure and dynamics of biological macromolecules [9]. According to [10], the TRFS is a new tool in the field of spectroscopy that allows a specific analysis of the structure and dynamics of biological macromolecules, and that will allow a breakthrough in many areas of biology research. Due to its high sensitivity, high selectivity, and non-destructive mechanism, it becomes an important technique to analyze metal ions [11], [12]. In literature, there are few studies reporting the use of this technique in HS. In this context, this study is aimed at the structural and molecular characterization of fractions of HA extracted from Spodosol of Amazonia through the techniques of fluorescence in the EEM and TRFS.

II. Material and Methods

A. Study Area

The study area is located in the city of Barcelos, in the basin of Demeni River, a tributary of the middle Rio Negro, Amazon, Brazil. The regional geology is represented by sediments of the Formation Içá, whose most recent sediments are found in the current floodplains. The climate is typically equatorial and is characterized by average annual temperature of 25 °C and high rainfall (about 3,000 mm), with no pronounced dry season.

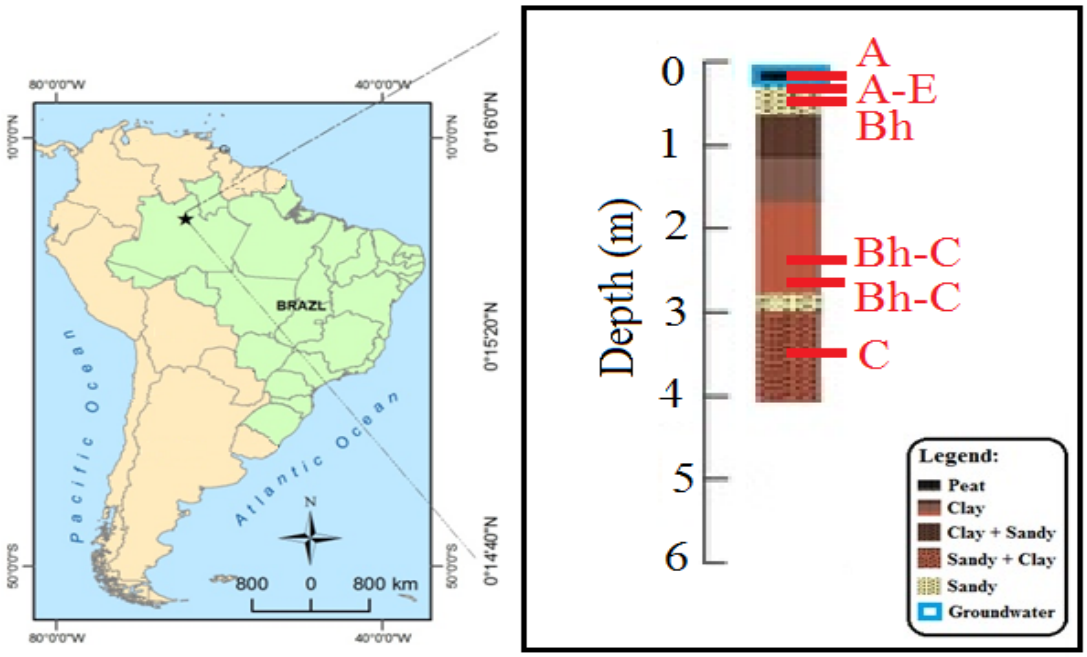

Fig. 1 Soil sampling site in the Amazon region, Brazil

B. Sample Preparation

Sampling was performed on representative horizons of each soil. Sampling procedures, preservation and preparation of samples followed the methods described in the literature [13], [14]. The extraction of HA, followed the recommendations suggested by the International Humic Substances Society (IHSS) and Swift [15]. Samples selected for analysis were: horizon A (0-15 cm); horizon A-E (15-30 cm), horizon Bh (40-50 cm), horizon Bh-C (240 cm), horizon Bh-C (260 cm) and horizon C (350 cm) as shown in Fig. 1.

C. Fluorescence Matrix Emission-Excitation Spectroscopy

To study the fluorescence mode in EEM, the concentration of solutions was 8.0 mg/L (pH=6.0), the spectra were obtained with scanning range between 240-700 nm for emission and 220-510 nm for excitation with an open filter and a step of 10 nm excitation totaling 30 scans. The spectra were processed by using mathematical method known as CP/PARAFAC.

D. TRFS

TRFS measurements were performed with a Hamamatsu Quantaurus-Tau system. The HA samples (8.0 mg/L, pH=8) were left for 10 minutes under an atmosphere of nitrogen gas to remove O_2 which induces photodegradation. Fluorescence spectra resolved in time were acquired in the scan range between 300-700 nm for emission and excitation at 280 nm. The same study was conducted with FA samples (8.0 mg/L, pH=8) extracted from Amazonian soil.

III. Results and Discussion

Fig. 2 shows the fluorescence spectra obtained in the EEM mode for HA samples extracted from Spodosol of Amazonia.

Spectra of 3D samples of HA were treated by mathematical method CP/PARAFAC. The samples obtained a concordia that ranged from 95.3% for HA, and the contribution of two components (fluorophores) in the course of the depth of the Amazon soil was found as shown in Fig. 3.

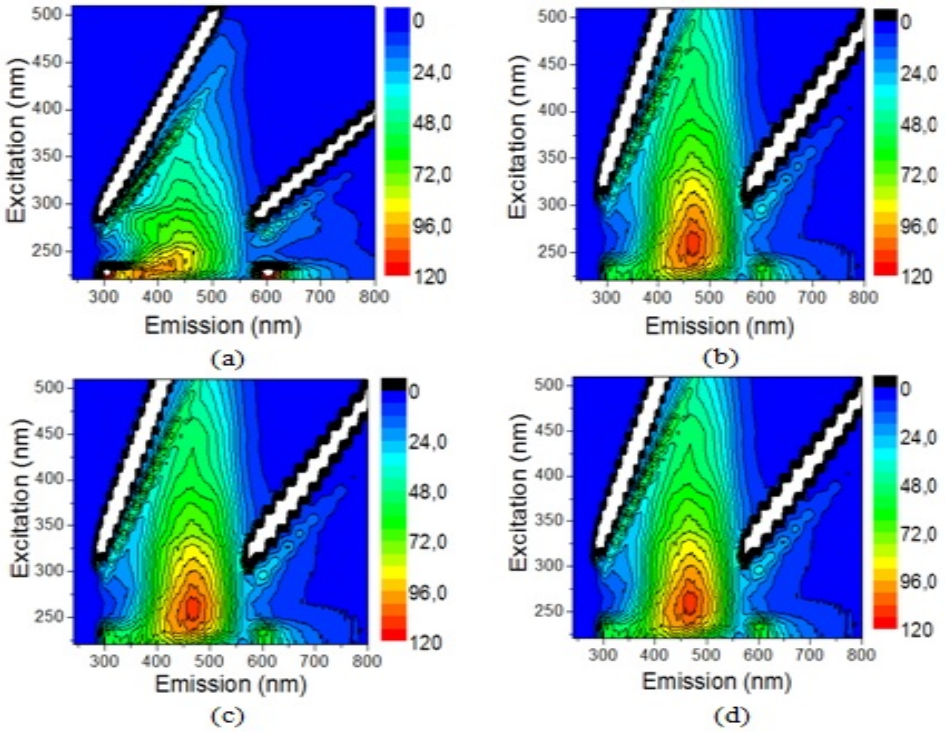

Fig. 2 Spectra in the mode emission-excitation for HA samples to the different depths: (a) horizon A, (b) horizon A-E, (c) horizon Bh and (d) horizon C

TABLE I
Lifetime and logKc (Cu^{+2}) of Values for Samples of HA Extracted from Amazonian Soil

Horizon (Depth cm)	τ1 (n seg)	$logK_c$ (Cu^{+2})	τ2 (n seg)	$logK_c$ (Cu^{+2})
A (0-15)	1.5	4.5	5.6	4.5
A-E (15-30)	1.4	5.3	5.2	4.6
Bh (40-30)	1.4	5.4	5.3	4.7
Bh-C (240-250)	1.4	5.5	5.1	4.5
Bh-C (260)	1.1	5.5	4.7	5.0
C (350)	1.3	5.0	5.1	4.5

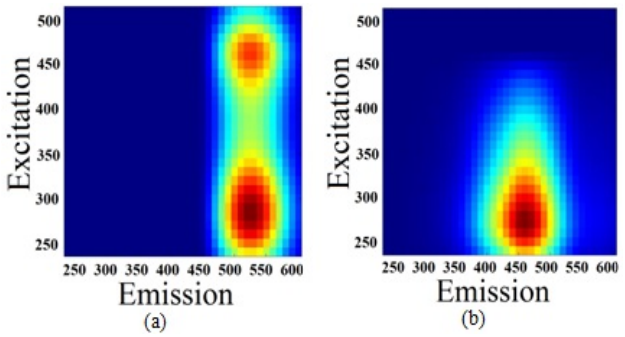

Fig. 3 Representation of Components 1 (a) and 2 (b) of the samples of HA in Amazonian soil

As shown in Fig. 3, Component 1 for HA, refers to low energy peaks which are known to be typically terrestrial HA with aromatic compounds having high molecular weight [6], [16], whereas Component 2 can be attributed to the peak A, which can be characterized by the presence of HS from the terrestrial environments.

According to [17], the HA of the soils are predominantly derived from lignin precursors, which have in its component structures of terrestrial plants. Table I shows the values of the lifetime employing TRFS for the samples of HA. Table I shows the presence of two lifetimes for HA samples, which are associated to Components 1 and 2 obtained by CP/PARAFAC.

From the data shown in Fig. 3 and Table I, different lifetimes for these samples can be suggested, and Components 1 (more complex structure) and Component 2 (simpler structure) are associated with lifetime τ1 and τ2, respectively. So, it can be seen that τ1 values for the HA were higher at the surface and lower at the horizon Bh-C (260 cm), which may be related to its interaction with metal ions such as Cu(II). The lowest lifetime may be associated with complexation of HA to metals, and thus the greater the capacity of the organic matter complexing the metal, the greater its stability constant and thereby lower the lifetime of that compound. Thus, this decay of the τ1 for horizon Bh-C (260 cm) may be associated with a

lower time for relaxation metal bound to HA, due to its interaction AH-Cu(II).

To decipher the relation between fluorescent component and organic matter dynamic in soils, further investigation needs to be done to characterize the moieties responsible for the fluorescence time decay diversity. Studies by Lukman et al. [12] evaluated the speciation of Eu^{+3} present in HS of various origins employing the TRFS. In this study, the authors applied the results of the different statistical methods, including the PARAFAC, and observed the presence of three different factors (A, B, C) corresponding to different species of Eu^{+3} as a function of pH. They obtained different lifetimes. Analyzing the results, the authors concluded that the A factor was free in solution, while the B factor was linked to the functional groups of HS, and the C factor is linked to the active sites of HS due to the abundance of this factor.

According to [18], the fluorescence decay rates are strongly dependent on the structure of the sample conformation, i.e. a protein. Its conformation characterizes its environment and inhibitory mechanisms. This protein can suffer and will affect the excited state of a molecule, and therefore its lifetime will change. Thus, the molecular dynamics is able to determine the rates of life structural conformations.

IV. Conclusion

The results show that the analysis of EEM-CP/PARAFAC was able to determine the presence of two components; more complex and simpler structure, which can be associated with both lifetimes obtained by the TRFS. The results showed that horizon Bh-C (260 cm) had two lower lifetimes (Ƭ1 and Ƭ2) which may be associated with a lower metal relaxation time bound to HA due to its interaction: HA-Cu(II). Therefore, studies of this nature are important because they aim at evaluating the molecular and structural characteristics, as well as the dynamic of the humics fractions of soil that is considered one of the world's largest carbon reservoirs, Amazonian soils.

Acknowledgment

The authors acknowledge the financial support for this work provided by the São Paulo Research Foundation (FAPESP) through the project (Process 2011/03250-2; 2012/51469-6; 2013/07276-1) and a scholarship (Process 2013/13013-3). National Counsel of Technological and Scientific Development (CNPq) through the project (303478/2011-0; 306674/2014-9) and the schorlaship (232225/2014-1-SWE). We also thank Embrapa Agricultural Instrumentation for supporting the development of the research. French ANR (Agence Nationale de la Recherche, Process number: ANR-12-IS06-0002 “C-PROFOR”).

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