Spectroscopic Characterization of Humic Substances Isolated from Sediment of an Area of Sugarcane Cultivation

G. Pantano, A. Santos, M.C. Bisinoti, and A.B. Moreira

Abstract Humic substances (HS) have an important environmental property, interacting with metal species and influencing their transport, accumulation, and bioavailability. In this study, the HS of sediment from an area of intensive sugarcane cultivation, considered the third largest in the state of SP, were characterized. This region accounts for about 60% of production in Brazil. The HS were extracted according to the method suggested by IHSS using an alkali extraction, followed by characterization by infrared spectroscopy (IR), ultraviolet/visible (UV/Vis) spectroscopy, and molecular fluorescence. The IR spectra showed characteristic bands of HS, and the E_4/E_6 ratio obtained in the UV/Vis analysis was <5, suggesting a more condensed structure. The results obtained in the emission molecular fluorescence spectra also showed characteristics of predominantly aromatic structures. In the synchronous molecular fluorescence, highest fluorescence intensity was in the region of 400 nm, which is assigned to aromatic polycyclic hydrocarbons with five aromatic rings. Thus, the results showed that the humic substances have a molecular structure with a higher degree of condensation, having thus more aromatic structures than aliphatics.

Keywords Humic substances • Characterization • Infrared • Molecular fluorescence • E_4/E_6 ratio

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Introduction

Humic substances (HS) have an important environmental property: the ability of complexing metals. Thus, HS can interfere in the transport, bioavailability, accumulation, and toxicity of metals in the environment. So, studies aimed at the characterization of HS are of great importance.

Studies about the characterization of HS extracted from sediments of rivers in the Turvo/Grande watershed (TGW) are practically nonexistent. This region has a drainage area of 15.925 km^2, and water bodies have been used for various purposes such as supply, irrigation, and power generation. Rural areas are impacted primarily by agricultural activity, which causes problems such as erosion and contamination by pesticides from the crops of sugarcane and orange. The state of São Paulo (SP) is the largest producer of cane sugar in the country (60% of national production), and the region of the TGW is the third largest in the state of SP, with a total planting area of 370.342 ha, accounting for a production of 18 million m^3 of vinasse per season. The cultivation of sugarcane growing in the TGW raises concerns, since studies have reported that the use of vinasse improperly can lead to soil salinization and contamination of groundwater and surface water by metals such as potassium, iron, and calcium (Bueno et al. 2009). It is thus essential to characterize HS and understand its behavior.

Materials and Methods

The study was conducted in the Turvo/Grande watershed. Surface sediments were collected with the aid of a Van Veen type dredger, with a total of four sampling points. Point 1 is an agricultural area (cane sugar and pasture) that can be considered the reference area. Point 2 is located in a dam used for public water supply in urban areas. Point 3 refers to a typical area of cultivation of sugarcane, and point 4 refers to a dam (hydroelectric) which receives all the pollution load of the watershed. The extraction of humic substances from sediments followed the recommendations suggested by the International Humic Substances Society. Using NaOH as extractant, to 150 g of sediment was added 1.5 L of NaOH 0.1 M, and the mixture was mechanically stirred for 4 h under nitrogen atmosphere in a jar test apparatus. The characterization was performed by spectroscopy in the infrared region and in the ultraviolet/visible region and by molecular fluorescence.

Results and Discussion

The molecular fluorescence spectra in emission mode are shown in Fig. 1. It is possible to verify maximum signal intensity of fluorescence at 450 nm for the HS from point 1 and 3 which can be attributed to the structure of humic acids. The HS
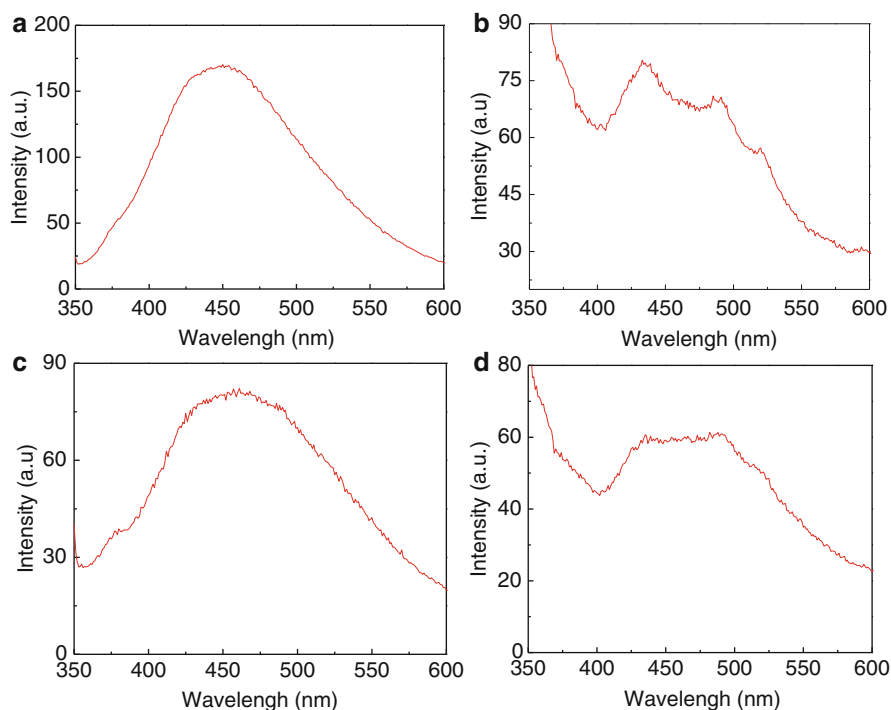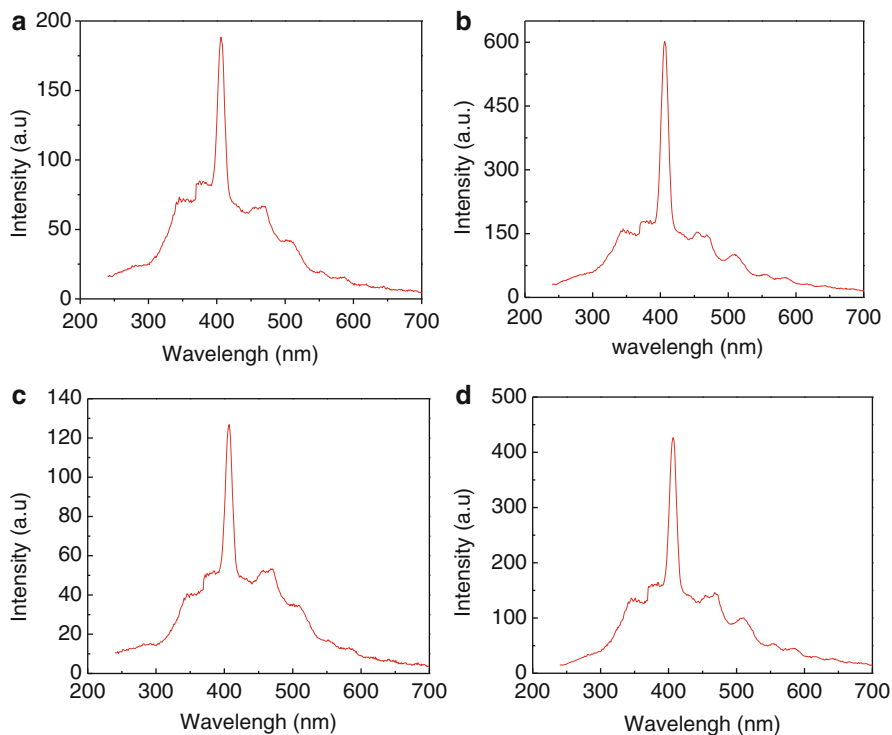
Fig. 1 Emission molecular fluorescence spectra (350–650, λ_{exc} 332 nm) of HS at 12.0 mg L^{-1} TOC from (**a**) Point 1, (**b**) Point 2, (**c**) Point 3, and (**d**) Point 4

extracted from points 2 and 4 showed two signal maxima at 430 and 480 nm. The first can be attributed to the presence of fulvic acid (FA), the second is attributed to humic acid (HÁ). Studies performed by Peuravuori et al. (2002) showed that FA and HÁ with excitation in the region of 250–260 nm and 300–340 nm show peaks with emission intensity in the region of 380–460 nm and 410–480 nm, respectively.

Figure 2 shows the spectra in synchronous mode, where the HS intensity showed maximum fluorescence at around 400 nm, which is associated with the presence of polycyclic aromatic hydrocarbons with five conjugated rings.

The infrared spectra shown in Fig. 3 revealed some characteristic bands of HS that have appeared in other studies (Khalaf 2003; Giovanela et al. 2010).

All the spectra show a wide and intense band around 3,400 cm^{-1}, which is assigned is the ν(O–H) stretching of carboxylic groups, phenol, and alcohols. The band in the region 1,660–1,630 cm^{-1} may be attributed to the ν(C=C) ring stretching and vibration of the aromatic ring. The band in the region of 1,400 cm^{-1} can be attributed to δ(OH) deformation and the ν(C–O) stretching of phenolic and carboxyl groups. This band is characteristic of humic acid molecules. Finally, the bands in the region 950–670 cm^{-1} can be attributed to deformation in the plane and out of the plane of the CH bound of the aromatic rings. Importantly, the band in the region 2,850 and 2,920 cm^{-1} that is not present indicates that the
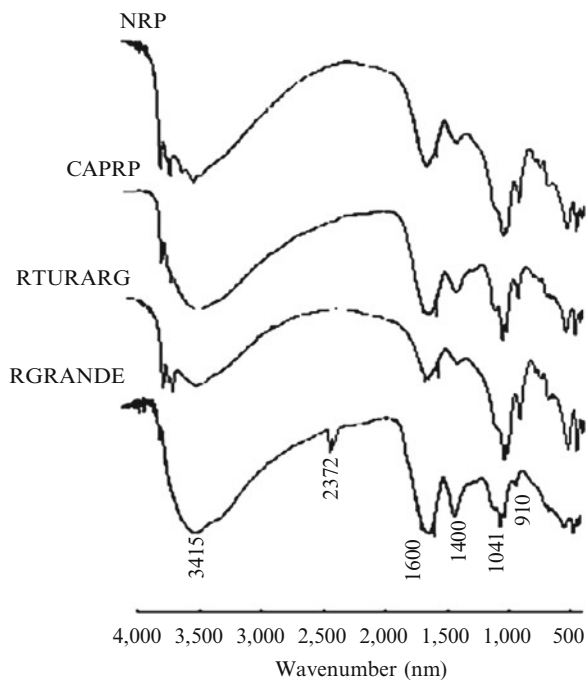
Fig. 2 Synchronous molecular fluorescence spectra (240–700, $\Delta\lambda$ 20 nm) to HS 12.0 mg L^{-1} TOC from (**a**) Point 1, (**b**) Point 2, (**c**) Point 3, and (**d**) Point 4

concentration of aliphatic groups is low, since these bands are related to the vibrations of symmetric and asymmetric stretching of CH and CH$_2$, respectively.

The E$_4$/E$_6$ ratio is used extensively in the literature to infer the aromaticity of the chemical structure of humic substances. Analyzing the results shown in Table 1 indicates that the ratios are less than five, suggesting that these HS have a molecular structure with a higher degree of condensation and thus more aromatic than aliphatic structures (Kononova 1966). The higher the value of the E_4/E_6 ratio, the lower the aromatic character of HS, and HS from point 3 thus have the least aromatic characteristic.

Conclusion

Based on the characterization by infrared, ultraviolet/visible, and molecular fluorescence spectroscopy of the HS from sediments of a region of predominance of the culture of sugarcane, it was concluded that HS have a chemical structure

Fig. 3 Infrared spectra
of the HS

NRP

CAPRP

RTURARG

RGRANDE

2372

3415   1600 1400 1041 910

4,000 3,500 3,000 2,500 2,000 1,500 1,000 500

Wavenumber (nm)

Table 1 Spectroscopic
characteristics of HS in
the UV/VIS region

Sampling site	Abs 465 nm	Abs 665 nm	E_4/E_6 ratio
1	2.00	0.57	3.51
2	0.52	0.18	2.88
3	1.81	0.41	4.41
4	0.89	0.28	3.07

predominantly aromatic, having a more condensed structure, probably because of the greater degree of humification and without too much variation from one location to another, for all regions receiving influences from agricultural activity.

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