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Research Paper

Spectroscopic investigation of humic substances in a tropical lake during a complete hydrological cycle

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Fluorescence and UV-VIS techniques were employed for the investigation of natural organic matter (NOM) of a tropical lake. The relationships of absorbance/dissolved organic carbon (*A*/DOC), fluorescence intensity/dissolved organic carbon (FI/DOC), fluorescence ratio (FR), and peak wavelength with the highest intensity (PW) were used to distinguish the pedogenic or aquagenic origin of NOM. The values of FR, PW and A_{285}/DOC of high waters (HW) or flooded period samples and of low waters (LW) period samples of the dry season, except for September 2002, confirm the predominance of pedogenic material. The spectra of water were similar to the standard fulvic acid (FA), and the spectra of FA from the lake were similar to the nearby soils, indicative of pedogenic predominance. The results confirm that the dissolved NOM of Patos Lagoon – MS (Brazil), in all sampling periods, predominantly consisted of humic substances (FA) of pedogenic origin.

Keywords: Natural organic matter / dissolved organic carbon / absorbance / wind / seasonal

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1 Introduction

Natural organic matter (NOM) is distributed in soils, natural water, and sediments. Changes of soil composition or hydrological regime will influence the organic matter profile in aquatic environments, and may produce different geochemical effects in the ecosystem [1]. The chemical characteristics of NOM are influenced by material source and by biogeochemical processes involved in the carbon cycle in terrestrial and aquatic system. Such factors include the flux of allochthonous organic carbon coming from the hydrographic system, and production of autochthonous organic carbon by phytoplankton and aquatic plants. The biological activity, the photodegradation, the transportation of particulate organic matter to the sediment or remobilization, change NOM chemically by different ways according to their intensity and time of action [1, 2].

Several studies have been conducted about the importance of NOM in aquatic environments, sometimes working as a buffer in water of low alkalinity, transportation and degradation of essential or toxic organic and inorganic substances, mobilization and transportation of hydrophobic chemicals, heavy metals and radionuclides [3, 4].

The determination of dissolved organic carbon (DOC) does not give enough information about the nature of organic matter, because the composition of NOM depends on allochthonous source (mainly pedogenic) or autochthonous (aquagenic) source [5]. NOM constitutes of proteins, peptides, polysaccharides, and pedogenic or aquagenic refractory compounds. The proportion of proteins and polysaccharides in NOM can amount up to 20 to 30% (eutrophic lake), and the refractory compounds contribute at least 70 to 80%. The pedogenic refractory organic matter (PROM) possesses a carbon skeleton mainly composed by aromatic rings, with hydroxyl and

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carbonyl groups linked to them. The aquatic refractory organic matter (AROM) possesses a carbon chain constituting mainly of aliphatic organic carbon [5, 6]. These structural differences of the carbon skeleton are decisive to characterize which source (allochthonous or autochthonous) prevails in the composition of the natural organic matter [7].

The concentrations of pedogenic macromolecules such as humic acid (HA), lipids, and polysaccharides are low in aquatic environments, due to the retention by soil particles, while amino acid and proteins may be completely degraded. Consequently, the NOM chemical composition changes from lake to lake. However, in several cases fulvic acids (FA) predominate the pedogenic fraction, and peptides and polysaccharides the aquatic fraction. The origin of PROM is generally from soil erosion and the drainage of the hydrographic basin, mainly of fulvic acid molecules [7]. The AROM is produced by macrophytes and plankton decomposition, and includes substances with high molecular weight, such as proteins and carbohydrates, originating from detritus or the cellular walls [6].

The determination of the DOC concentration includes organics of both, autochthonous and allochthonous sources, while the absorbance (A) and the fluorescence (F) are more specific for refractory substances (PROM and AROM), mainly of pedogenic source. One of the advantages of the application of the technical spectroscopy is the fact of not needing to separate or pre-concentrate the dissolved natural organic matter (NOM) [8, 9]. This is possible because phenol compounds and other aromatics coming from terrestrial humic substance from aquatic plants may constitute up to 80% of dissolved organic matter (DOM), and of these, 30 to 40% is aromatic carbon [8, 10]. The variation of NOM (PROM and AROM) could be observed by the fact that PROM presents, mainly, aromatic structures with absorption of UV light and emission of fluorescence. The UV absorption and emission of fluorescence by AROM are 10 times lower than the PROM, due to its longer aliphatic chains [6, 7].

Westerhoff and Anning [11] verified that the FI can indicate the predominance of allochthonous or autochthonous DOC: allochthonous sources are considered if the maximum fluorescence intensity (FI) is observed at $\lambda > 450$ nm in the emission spectra (PW); autochthonous sources are considered if the maximum fluorescence intensity is observed at $\lambda < 450$ nm. They also considered the ratio of the emission intensity of the wavelength $\lambda = 450$ nm to the emission intensity of the wavelength $\lambda = 500$ nm ($FR = \lambda_{450}/\lambda_{500}$), with excitation wavelength $\lambda_{Ex} = 370$ nm. Values of $FR > 1.8$ indicate autochthonous sources, and $FR \leq 1.5$ indicate allochthonous sources of humic substance.

Natural waters present different chromophores derived from protein/amino acids and humic substances. Changes in the quantities of these chromophores will change the absorbance and fluorescence intensity of emission and excitation spectra and shift of wavelengths [12]. Fluorescence of low intensity in larger wavelengths is normally ascribed to humic acids [13–15]. According to Stewart and Wetzel [16], the humic substance fractions of low molecular mass shows higher fluorescence intensity and lower absorbance compared to the fractions of higher molecular mass that exhibits higher absorbance and lower fluorescence intensity.

Environmental factors could also affect the fluorescence intensity, as increase of pH decreases the FI of dissolved FA and HA, changes the acidic groups, and changes inter and intramolecules bonds [16–18]. Increasing of ionic strength will decrease the FI by changes of molecular structures and structural conformations of HS. The presence of heavy metals also decreases FI [13, 14,].

The high Paraná River in Brazil supports a great wealth of biological diversity. An extensive region of floodplain habitat remains within the high Paraná River between the Porto Primavera and Itaipu reservoirs, but it now experiences a modified flood regime due to the influence of the upstream reservoirs. Annual flooding and drying of the floodplain occurs, and this regime is greatly influenced by tributary rivers, especially the Ivinheima River which enters from the west. Most groups of aquatic organisms show highly specified diversity and strong patterns of ecological dynamics in response to habitat heterogeneity and water level fluctuations. Physico-chemical attributes and primary production show high temporal and spatial variation in both river and floodplain habitats. In general, the floodpulse reduces phytoplankton densities, promotes periphyton and aquatic invertebrate production in the floodplain, which supports juvenile fish feeding, growth, and development. Despite intensive agriculture and destruction of riparian vegetation, especially along the tributaries, the ecosystem still supports biotic diversity in this region of the continent. Several conservation areas have been established to protect the biological diversity supported by the floodplain region that remains in a relatively natural condition [19, 20].

The objectives of the current study were to identify which source (allochthonous or autochthonous) of the dissolved organic carbon (DOC) prevails, as well as to predict DOC concentration, through the determination of the relationships between fluorescence intensity, absorbance and DOC.

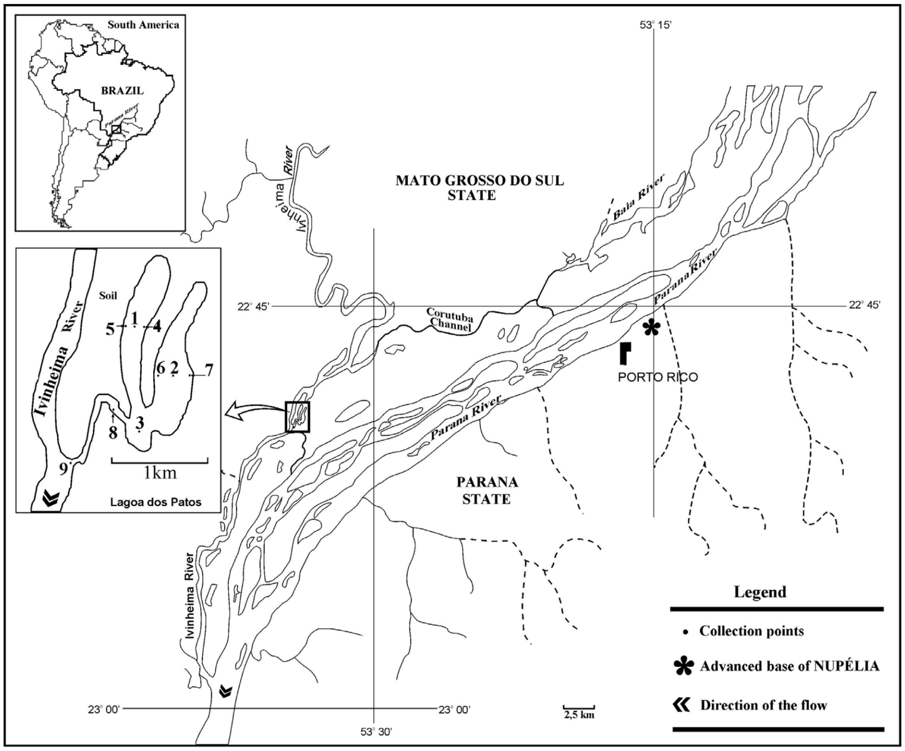

Figure 1. Localization of Patos Lagoon/MS, Ivinheima River, Parana River basin, and the sampling points (Brazil).

2 Experimental

2.1 Area of investigation

The upper Paraná hydrographic basin is located in South America and drains a vast area, over 802 150 km^2, in the Brazilian territory. Paraná River, lakes, secondary channels and tributaries constitute the drainage network in the region. Paraná River is the main active channel responsible for the drainage of the entire basin, and the Curutuba Channel, Ivinheima and Baia Rivers are tributaries of Paraná River, and besides innumerable lakes, form water bodies that complete the drainage of the region. Paraná River is important in making the frontier of Brazil-Paraguay and Paraguay-Argentina, and the local environment is a floodplain area that was modified after the construction of Itaipu Reservoir for generation of electric power [19, 21]. The Ivinheima River runs perpendicularly to the Paraná River until entering the plain, where it bends 90 degrees and comes to run parallel to the main channel. Patos Lagoon is a composite of at least three overflow lagoons (Fig. 1). Its maximum depth is slightly greater, varying between 2.8 m and 4.8 m, like its area, which exceeds 1.2 km^2 [19].

Concerning the flora, phytoplankton, macrophytes, and periphytic algae of the high Paraná River floodplain, the main source of information is an inventory performed by the NUPELIA/Universidade Estadual de Maringa research group. The Upper Paraná River floodplain presents notable complex composition of phytoplanktonic community, with pronounced seasonal variation. It also presents different macrophyte life forms (i.e., submerged, free-floating, emergent, and rooted with floating leaves), being *Eichhornia azurea*, *Eichhornia crassipes*, *Polygonum ferrugineum*, *Polygonum stelligerum* and *Utricularia* sp. among the most frequent species in lagoons and river shores. The riparian vegetation is in the submontane semideciduous seasonal forest domain, with two basic types of vegetation, one forest and one field (“várzea”, dry field and pasture). The riparian vegetation comprises 742 species (*Cecropia pachystachya*, *Inga vera* and *Triplaris americana*), from 409 genera and 119 families distributed between pteridophytes and phanerogams. The ten families with the highest species richness are Leguminosae, Asteraceae, Poaceae, Euphorbiaceae, Rubiaceae, Solanaceae, Myrtaceae, Cyperaceae, Sapindadaceae and Amaranthaceae. The riparian ecotones are fragile habitats, which, in despite of being located in a legally protected zone, are under the constant influence of disturbances of different intensities [20]. The investigated area presents the soil type *nitossolos vermelhos* ("terra rossa soil"), rich in organic matter, coming of decomposed vegetation ("humus"), and is used for the production of soy, maize and wheat.

2.2 Sampling points

The samplings of water and sediments were performed at Patos Lagoon every two months from May 2002 until March 2004, in nine superficial points and other three points with the maximum depth. Patos Lagoon is located at the right margin of Ivinheima River, MS, Brazil, and localized at 22049′33.66″S; 53033″9.9″W. The area has approximately 114 ha, average depth of 3.50 m, and length of 2065 m. The small channel of 10 m length and 8 m width connects the Patos Lagoon to Ivinheima River.

The physico-chemical parameters measured *in situ* were: dissolved oxygen (DO) and temperature with the oxygen meter (YSI-55, USA); electrical conductivity (µS cm^{-1}), pH, and redox potential (mV) with the digital potentiometer DIGIMED (DM2 and DM3 with platinum electrode); international unity of turbidity (IUT) was measured with the apparatus LaMote-2008 (USA); alkalinity (mmol L^{-1}) through the method of Gran, and water transparency with 0.30 m diameter Secchi disk. Iron was determined by atomic absorption spectrometry (Varian, Spectra AA-10 plus).

Water was collected using Van Dorn bottle, and aliquots of 500 mL were transferred to polyethylene flasks, previously washed with HCl and cooled to −20°C, for chemical oxygen demand (COD), nitrogen, and phosphorus determinations. Other aliquots were filtered, as soon as collected, using 0.45 µm of cellulose ester Millipore membranes that were previously washed according to the procedure of Rostan and Cellot [22]. After filtration, sub-samples were kept in polyethylene flasks and cooled at 4°C until chemical analysis. Other aliquots were filtered using fiber-filtering material (Whitman GF/C). The material retained was cooled, the chlorophyll-a determined, and the dissolved organic carbon (DOC) was determined with TOC 5000-A, Shimadzu. The measurements of absorbance in the range 200…700 nm were performed with Cary 50-Varian spectrophotometer, using a 10 mm quartz cell and Milli-Q water as a blank.

Fluorescence reading was performed with a Fluorescence Spectrophotometer F-4500 Hitachi. The emission spectra from 300 to 600 nm were obtained applying λ_{Exc} of 314 nm and 370 nm for being considered more effective in the study of HS [12].

The synchronous spectra were obtained with excitation wavelength (λ_{Ex}) of 250 to 600 nm, using $\Delta\lambda = 18$ nm, this variant being one often applied in the studies of humic substances or NOM [13, 17, 23–25]. The spectra were obtained applying the following conditions: 240 nm min^{-1}, slit of 5 nm, 10 mm quartz cell and Milli-Q water as blank.

The HS (HA and FA) were extracted according to Thurman and Malcolm [26], from 150 L of filtered water collected in January 2003 from point P-01. The sample of water was filtered (0.45 µm) acidified with HCl to pH 2.0. Afterwards, it was passed through a column containing XAD8, where FA and HA were retained and eluted with NaOH. The separation of the HA and FA was achieved through precipitation with addition of HCl (pH < 2.0). The separation and purification of HA and FA were achieved using the method of the International Society of Humic Substances (IHSS).

3 Results and discussion

Table 1 shows the physico-chemical characteristics of water collected from Patos Lagoon. The data are related to the period of low waters (LW) or dry season in September 2002, and high water (HW) or the flooded period of January 2004. The results indicate a seasonal variation of the limnological characteristics caused mostly in two periods: during high waters a decrease of dissolved oxygen, increase of inorganic carbon species (alkalinity), increase of nitrogen compounds, dissolved and suspended solids (conductivity and turbidity), and allochthonous (pedogenic) organic carbon change the lake characteristics (period of allochthonous inputs); in the period of low waters, the phytoplankton biomass increases (Table 2) (period of autochthonous primary productivity).

According to Thomaz et al. [19], the limnological characteristics of the floodplain habitats are greatly affected by the hydrologic regime of the Paraná and Ivinheima Rivers. High floods tend to homogenize river and floodplain habitats in terms of many physicochemical and biotic attributes. During low water periods, floodplain habitats are less connected with the river and subjected to local processes like wind turbulence and inputs from small tributaries. A peak of phosphate and total nitrogen in the floodplain lagoons usually occurs during the beginning of the floods, following the decomposition of the flooded biomass.

The period of low water occurs during winter and so coincides with lower temperatures and regional precipitation. During high water periods when the lagoons are deeper, thermal stratification may persist for periods longer than 24 hours, which leads to vertical stratification of nutrients and gases. During this phase, anoxic layers close to the sediments are frequently observed.

Table 1. Average of physico-chemical parameters of September 2002 and January 2004.

Parameter		Sept/02		Jan/04	
		Middle[a]	Margin[b]	Middle[a]	Margin[b]
DOC	mg L^{-1}	4.3 ± 1.4	5.6 ± 0.6	12.7 ± 2.8	14.7 ± 2.2
pH		6.1 ± 0.5	6.3 ± 0.1	6.5 ± 0.1	6.5 ± 0.1
Temp	°C	24.1 ± 0.2	24.6 ± 0.6	29.6 ± 0.2	29.6 ± 0.2
Conduct.	µS cm^{-1}	27.9 ± 2.9	27.5 ± 0.8	35.5 ± 1.2	34.6 ± 0.8
Turbidity	IUT	31.5 ± 6.5	35.1 ± 3.3	41.5 ± 10.6	47.8 ± 12.6
E_h	mV	161 ± 20	157 ± 9	123 ± 35	132 ± 35
DO	mg L^{-1}	7.0 ± 0.8	6.9 ± 0.9	4.8 ± 0.1	4.9 ± 0.4
Chl-a	µg L^{-1}	9.8 ± 2.1	11.7 ± 2.5	5.3 ± 1.0	5.4 ± 0.8
COD	mg L^{-1}	27.1 ± 4.2	22.9 ± 9.8	23.1 ± 9.9	66.6 ± 8.5
Alkalin.	mmol L^{-1}	53.1 ± 7.6	60.5 ± 3.2	120.1 ± 21.3	84.9 ± 8.9
P_{total}	µg L^{-1}	94.5 ± 13.2	133.5 ± 5.9	59.4 ± 15.1	74.7 ± 14.1
PO_4^{3-}	µg L^{-1}	7.2 ± 2.4	29.9 ± 2.7	5.3 ± 1.7	11.1 ± 3.9
Nitrate	µg L^{-1}	58.3 ± 11.6	55.9 ± 17.8	63.1 ± 15.8	23.2 ± 11,8
Nitrite	µg L^{-1}	3.4 ± 2.1	2.5 ± 0.4	7.6 ± 1.8	16.6 ± 1.6
NH_4^+	µg L^{-1}	1.5 ± 0.8	3.6 ± 0.4	31.7 ± 3.1	30.8 ± 6.8
N_{org}	µg L^{-1}	167.5 ± 27.9	245.5 ± 22.1	214.3 ± 19.7	228.1 ± 13.9
N_{total}	µg L^{-1}	0.183 ± 0.09	0.26 ± 0.08	0.257 ± 0.03	0.264 ± 0.02
Fe	mg L^{-1}	0.306 ± 0.03	0.43 ± 0.04	0.800 ± 0.11	1.303 ± 0.13

[a] Middle points (P-01 to P-03, *N* = 9).
[b] Margin (P-04 to P-08, *N* = 15).

Table 2. Average relationship among absorbance, DOC, and FI.

Parameter		Sept/02		Jan/04	
		Middle[a]	Margin[b]	Middle[a]	Margin[b]
DOC	mg L^{-1}	4.3 ± 1.4	5.6 ± 0.6	12.7 ± 2.8	14.7 ± 2.2
Chl-a	µg L^{-1}	9.8 ± 2.1	11.7 ± 2.5	5.3 ± 1.0	5.4 ± 0.8
A_{285}/DOC	L g^{-1} cm^{-1}	35.6 ± 6.8	93.5 ± 9.6	30.9 ± 0.9	39.1 ± 4.4
A_{254}/A_{203}		0.4 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
E_{300}/E_{400}	$E_3/E_4^{\triangledown}$	3.4 ± 0.2	2.6 ± 0.1	4.4 ± 0.2	3.8 ± 0.2
E_{250}/E_{365}	$E_2/E_3^{\triangledown}$	3.9 ± 0.2	2.7 ± 0.1	4.3 ± 0.3	3.9 ± 0.1
FI_{314}	$Em_{450}/DOC^{\oplus}$	11.9 ± 1.5	8.5 ± 0.8	8.4 ± 0.9	7.9 ± 1.0
FI_{370}	$Em_{450}/DOC^{\oplus}$	6.6 ± 0.7	4.9 ± 0.4	5.6 ± 0.4	5.6 ± 0.6
$FR_{450/500}$		1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1
PW	nm	451.5 ± 3.3	453.5 ± 3.7	452.9 ± 3.9	454.3 ± 3.9

[a] Middle points (P-01 to P-03, *N* = 9).
[b] Margin (P-04 to P-08, *N* = 15); FI: fluorescence intensity; FR: fluorescence ratio; PW: peak wavelength with the highest intensity; Em: emission wavelength; $^{\triangledown}$ in L g^{-1} cm^{-1}; $^{\oplus}$ in arbitrary unit L mg^{-1}.

During the low water phase, complete mixing of the water column occurs during the night or morning when the lagoons are usually shallower than 2 m. A second peak of nutrients in the lagoons is usually observed during low water (from July to October), and this peak is due mainly to sediment resuspension derived from the wind action. During this phase, the autochthonous input of nutrients, together with the diel mixing of the water column, leads to greater phytoplankton production in lagoons and chlorophyll-a concentrations [20].

3.1 UV-VIS spectroscopy: absorbance variation

The UV-VIS spectra of absorbance from the point P-04 were normalized in relation to the DOC and the optical length (m) as shown in Figure 2. The other points presented similar results, varying only in intensity. The UV-VIS spectra are also similar to the standard FA [13, 23, 27]. Despite the spectra being similar to FA, it is not possible to affirm that only FA constitute NOM, because humic acids (HA) absorb in the same region of the spectra. The absorptivity variation (in L mg^{-1} m^{-1}) according to the

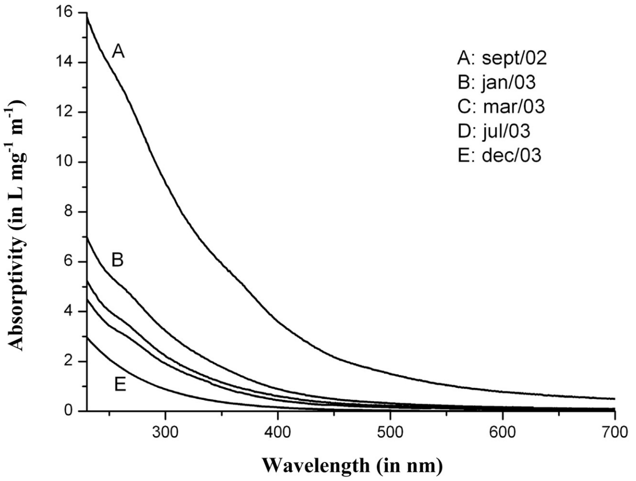

Figure 2. Absorptivity spectra of Patos Lagoon/MS (Brazil) water related to the sampling point P-04.

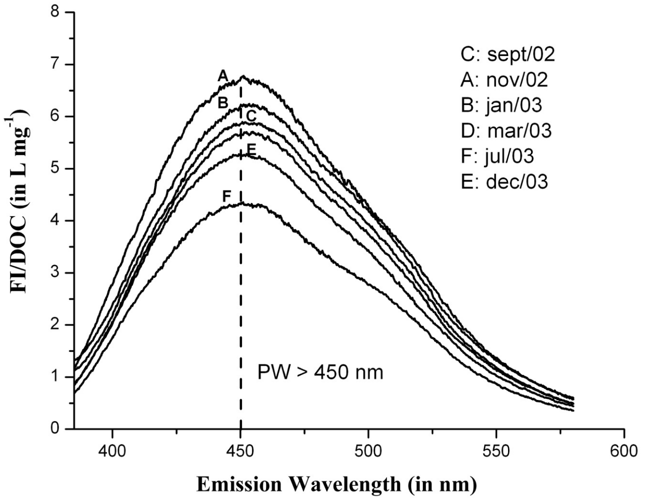

Figure 3. Fluorescence intensity (FI) spectra of emission with 370 nm excitation (λ_{Ex}), with arbitrary unity (a.u), normalized by DOC (in mg L^{-1}) of samples of Patos Lagoon related to the point P-04.

periods investigated shows the effect of seasonality. The spectra of July 2002, January 2004, and March 2004 were similar to the July 2003, January 2003, and March 2003, respectively. The spectra of September 2002 were unusual and a particular case. The alterations in the spectra of absorbance (Sept/02) probably resulted from the action of winds, which occurred during collection and in preceding days. This alteration was observed mainly in the points located in the margins, where small waves caused sediment suspension, dissolving mainly humic acid or substance with higher molecular weight (MW).

According to Rostan and Cellot [22], the ratio A_{285}/DOC (in L g^{-1} cm^{-1}) may be used as an indication of the principal DOC composition. When the ratio is about 20 L g^{-1} cm^{-1}, the DOC may be considered essentially formed by FA. If the ratio is smaller than 10 L g^{-1} cm^{-1}, it could be due to the presence of aliphatic carbon coming from the primary production. The A_{285}/DOC obtained in this study was (32.11 ± 10.04) L g^{-1} cm^{-1}. Westerhoff and Anning [11] consider the specific ultraviolet absorbance (SUVA) in the wavelength 254 nm as data to characterize the source of DOC (allochthonous or autochthonous), where the $SUVA_{254}$ of standard FA solution was 4.4 L mg^{-1} m^{-1} and the autochthonous source is 1.2 L mg^{-1} m^{-1}. The average value (N = 144) of the samples (surface and bottom) in this study was $SUVA_{254}$ = (4.4 ± 0.8) L mg^{-1} m^{-1}. Thus, the results of $SUVA_{254}$ and A_{285}/DOC indicate that in the lake of Patos-MS, allochthonous DOC prevails.

To characterize NOM or HS, relationships among the absorbance in different wavelengths are used. The quotients of the specific absorbance (in L g^{-1} cm^{-1}), E_3/E_4 (300 nm/400 nm) or E_2/E_3 (250 nm/365 nm), are inversely proportional to the molecular size and the aromaticity, being quite significant for aquatic samples [28, 29]. Artinger *et al.* [28] determined the values of the ratio E_3/E_4 (300 nm/400 nm) of HS, obtaining for HA < 3.5 and FA > 3.5, in terms of specific absorption at 300 (E_3) and 400 nm (E_4) in L g^{-1} cm^{-1}. They observed that the humic substances present high specific absorbance and lower ratios of E_3/E_4. Peuravuori and Pihlaja [29] consider more effective the ratio E_2/E_3 (250 nm/365 nm) to determine the aromaticity degree. In Table 2, in September 2002, it is observed that the samples of water presented lower ratios E_3/E_4 or E_2/E_3, indicating increase in the aromaticity, mainly in the margins, confirming the increase of the proportion of humic acid or higher molecular mass in the DOC.

3.2 Fluorescence variation

The emission spectra with λ_{Ex} at 370 nm (Fig. 3) were normalized in relation to the DOC (mg L^{-1}), and these spectra are similar to the other points. For this figure, it is possible to verify that all samples presented FI maximum in PW > 450 nm, indicating allochthonous source of DOC, with average values (N = 144) of samples (surface and bottom) which were: FR of 1.49 ± 0.02 and PW 452.5 ± 1.8 (Table 2).

The variation of the FI (Fig. 3) with seasonality can be related to two factors: i) the lesser intensity in July 2003 is due to the AROM increase, where there is minor amount of chromophores that absorb in the region of UV-VIS or emit fluorescence; ii) the increase of the absorbance in September 2002 and January 2003 (Fig. 2) and minor variation of the FI (Fig. 3) is related to the increase

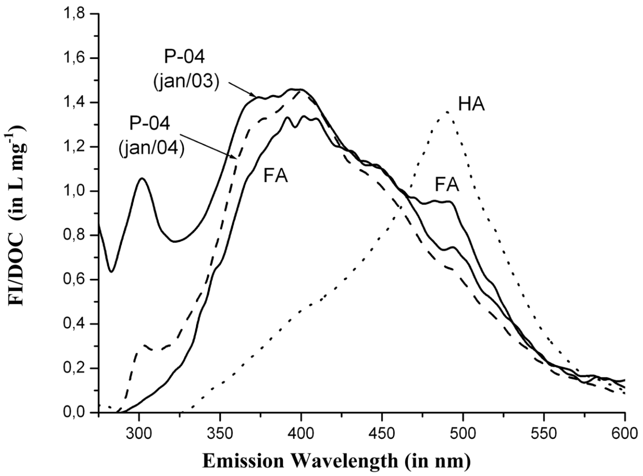

Figure 4. Synchronous spectra of humic and fulvic acid ($\Delta\lambda$ = 18 nm) of Patos Lagoon/MS (Brazil) water samples (P04).

of the molecular mass, as the dissolution of humic acid or increase of ratio PROM in relation to AROM, as decrease of fluorescence emission occurs with increase of the molecular weight [13].

The results of the absorbance and fluorescence indicate the predominance of allochthonous organic carbon in the investigated periods. The predominance of humic substance is also suggested by a comparison of the synchronous spectra (Fig. 4) of the water sample from the lake (January 2003 and January 2004), and of the fulvic acids and humic acids of the water extracted. The spectra of water sample (Patos Lagoon) were similar to the FA extracted, and this was similar to standard FA (Aldrich-USA). The spectra of water FA extracted, from Patos Lagoon, were similar to the soils of the investigated area, confirming the predominance of pedogenic substances in the NOM, mainly FA. According to Ferrari and Mingazzini [8], the peak of 340 to 400 nm in the synchronous spectra is related to two aromatic rings; of 400 to 430 nm, to the fulvic acid and 500 nm or more, to the humic acid. Lu *et al.* [9] divide the spectrum into four parts: band in $\lambda_{295...302}$ refers to protein-like substances; in λ_{380} and λ_{410} to FA and peak in λ_{488} to the humic acid, confirming the results obtained in the Patos Lagoon.

The next peak at 500 nm (Fig. 4) obtained in the water samples of Jan/03 and Jan/04 can be related to the fluorescence emission of FA or HA. Due to low concentration normally found for HA in water probably this peak is related to FA. The peak at 300 nm is related to NOM originating from the decomposition of aquatic macrophythe *Eichhornia azurea* (datum still not published), also confirming that in this period decomposition of NOM occurs.

The results of September 2002 (LW) and January 2004 (HW) of the middle and margin points (Table 2) confirm that NOM, dissolved in Patos Lagoon, is formed mainly by HS (pedogenic). The samples collected in the margin points of September 2002 were an exception and a great variation was observed if compared with other samples. In this period, an increase of primary productivity (chlorophyll-a), lower values of A_{285}/DOC and PW, and an increase of FR, A_3/A_4 and A_2/A_3 is expected. The increase of the absorbance values (Fig. 2), and the decrease of FI (Fig. 3) indicate HS with higher molecular weight (MW), probably HA, originating from the suspension of the sediment due to the action of winds, in this sample.

Pagioro *et al.* [30] observed in the Patos Lagoon strong interactions with sediments at depth lower than 2.5 m, and the entrance of suspended materials in the windy days, with fertilization by phosphorous and nitrogen. July, August, and September are the periods of LW and the dry season in the area. Specifically in September 2002 the samplings were performed in very strong windy days. As a consequence, the investigated proportion of high molecular mass of HS increased, due to the redissolution of sediment materials.

Aromatic rings with different substituents, including mono and polysubstituents are the responsible chromophores for the UV energy absorption of NOM humic fraction. Some compounds of lower molecular weight such as amino acids and alcohol may also contribute to absorbance, but such compounds are usually consumed by the biota. The NOM fraction produced by phytoplankton that shows fluorescence emission is easily distinguished by the lower wavelength of maximum emission. These compounds are released especially in the growth phase of algae as lipids, organic acids, aminoacids, peptides, carbohydrates and proteins. Algae also excrete phenols that could be transformed in polyphenols, and through the reactions with carbohydrates and proteins gave complex structures with fluorescence in the blue region of the spectra, giving higher FR and lower PW values [8, 24].

Environmental factors such as temperature, solvent, heavy metals, pH and salinity can affect the fluorescence intensity. The results of water analysis (Table 1) did not indicate these factors as responsible by *A* and FI variations. Small pH variations were observed, which could not influence FI. In relation to the pH, Miano and Senesi [14], verified that the pH variation doesn't alter FI of FA from red latosoils ("terra rossa soil"). The same results concerning pH influence were obtained for FA and HA from water extracted of Patos Lagoon, where the area presents soil of the type "terra rossa soil" ("*red nitossoils*"). In terms of ionic strength, in September 2002 samples the conductivity decreased, indicating that the FI should

Table 3. Correlation values among DOC, absorbance (254 nm), and between FI (λ_{ex} = 314 nm, λ_{em} = 450 nm) in the middle points (surface and bottom), margins, channel and the river of the Patos Lagoon – Ivinheima River (Brazil) ($P < 0.0001$).

Sampling		Absorbance (254 nm)		FI (E_{ex}/E_{em} = 314 nm/ 450 nm)	
		R	*N*	*R*	*N*
Middle	surface	0.903	36	0.962	36
	bottom	0.791	36	0.951	36
Margin		0.666	48	0.948	48
Channel		0.883	12[a]	0.945	12
River		0.951	12	0.935	12
Total		0.783	144	0.953	144

[a] $P = 0.0014$

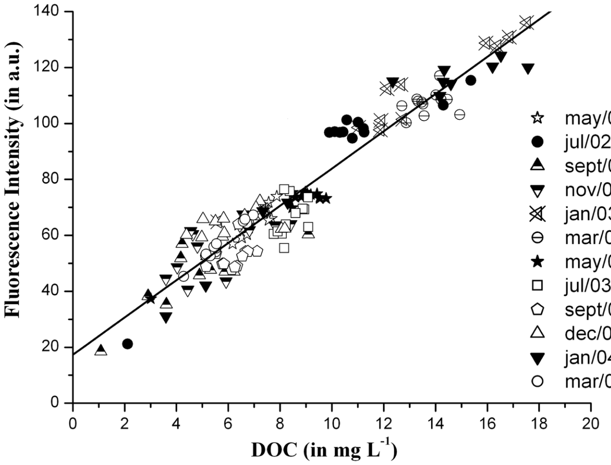

Figure 5. Correlations between DOC and relative fluorescence intensity (Ex/Em = 314 nm/450 nm) considering all sampling points from May 2002 until March 2004; *N* = 144; *P* < 0.0001; FI (a.u.), equation: FI = 17.33 + 6.65 · (DOC/mg L^{-1}); *R* = 0.953.

increase and not decrease. In various heavy metals (Cu, Ni, Co, Cr, Cd, Zn), larger variations did not to justify the decrease of FI in September 2002. The dissolved iron concentration changed significantly among sampling dates, but in September 2002 presented the lowest concentration of dissolved iron. In this case, these factors are not responsible for the variations of absorbance or fluorescence, it is the composition of NOM, confirming that the dissolved NOM, of Patos Lagoon, in the sampling periods, were humic substances of pedogenic origin.

3.3 Correlations between DOC and absorbance or fluorescence intensity

Table 3 shows the correlation values between DOC and relative fluorescence intensity (λ_{Ex} = 314 nm and λ_{Em} = 450 nm), and DOC with absorbance (λ = 254 nm). The fluorescence technique suffers the influence of several environmental factors (pH, ionic strength, solvent, heavy metals) [13]. However, a good correlation between DOC and FI has been observed. Frimmel [3] reported the correlation with DOC at emission wavelengths (λ_{Em}) of 350 nm and 550 nm and excitation wavelength (λ_{Ex}) of 314 nm. The ratio $\lambda_{Ex}/\lambda_{Em}$ was used by several authors: $\lambda_{Ex}/\lambda_{Em}$ = 310 nm/420 nm and $\lambda_{Ex}/\lambda_{Em}$ = 308 nm/420 nm [31]; $\lambda_{Ex}/\lambda_{Em}$ = 313 nm/410 nm [12], and Lombardi and Jardim [32] obtained good correlation through the synchronized spectrum with $\Delta\lambda$ of 18 nm.

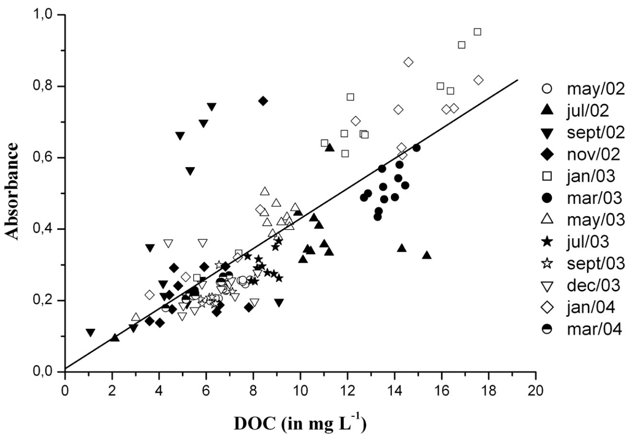

Figure 6. Correlation between DOC and absorbance (254 nm) considering all sampling points from May 2002 until March 2004. Optical pathlength of 1.0 cm; *N* = 144; *P* < 0.0001; *R* = 0.7835; equation: $A_{254} = 9.16 \cdot 10^{-3} + 4.21 \cdot 10^{-2} \cdot$ (DOC/mg L^{-1}).

Figures 5 and 6 show the relationships of DOC and FI, and of DOC and absorbance, respectively, considering all samples (*N* = 144), independent of depth and sampling points. The FI is more significant than absorbance to predict the DOC concentration.

The increase of the proportion of humic substances increased molecular weight, consequently, it increased the amount of aromatic rings. This increase of the aromaticity caused an increasing of the absorbance that dislocated the data of the periods of September 2002 (winds) and January 2003 and January 2004 (rains), altering the correlation between absorbance and DOC (Fig. 6). The increase of the aromaticity tends to decrease the fluorescence intensity emitted, not affecting the correlation between DOC and FI (Fig. 5).

4 Conclusion

The UV-VIS and fluorescence analysis show the predominance of humic substances (pedogenic) in the NOM dissolved of Patos Lagoon – MS (Brazil). The relationship of A_{285}/DOC, FI/DOC, FR and PW confirm this prevalence. In September 2002 the increase of molecular weight of the dissolved compound, probably indicates humic acid from sediments, caused by strong winds.

The values of FR, PW and A_{285}/DOC of the September 2003 samples, a period of low water and practically no wind, confirm the predominance of pedogenic material, however, without increasing the molecular weight of the dissolved compounds coming of the sediment.

The influence of the environment could be explained by the great variety of macrophytes and riparian vegetation that contribute to the aquatic NOM. The seasonality also has influences, as in the period of high water (HW) erosion and lixiviation of pedogenic material from the hydrographic basin into the lake occurs, and in the period of low water the presence of humic substances from the sediments suspension occurs, caused by the action of winds.

In order to predict the DOC concentration of Patos Lagoon – MS (Brazil), the F technique (R = 0.9531) was more significant than the absorbance measurements (λ = 254 nm, R = 0.7835), due to the increase of humic substances or of higher molecular weight in the NOM proportion that occurred in September 2002 (winds) and in the rainy months.

List of symbols and abbreviations

A:	absorbance
A_{254}/A_{365}:	ratio of absorptivities at 254 nm and 365 nm, indicates increase (in %) of small compounds with photodegradation
A_{285}/DOC:	ratio of absorptivity and DOC at 285 nm (in L g^{-1} cm^{-1})
AROM:	aquatic refractory organic matter
COD:	chemical oxygen demand
DO:	dissolved oxygen
DOC:	dissolved organic carbon
DOM:	dissolved organic matter
Ex/Em(275nm/303 nm):	ratio of the excitation at 275 nm to the emission at 303 nm
F:	fluorescence
FA:	fulvic acids
FI:	fluorescence intensity
FR:	FI ratio at λ (450 nm and 500 nm) with excitation at 370 nm
HA:	humic acids
HS:	humic substances
HW:	period of high water, from November until March: flooded period
IUT:	international unity of turbidity
LW:	period of low water, from April until September
MW:	molecular weight or molecular mass
NOM:	natural organic matter
PROM:	pedogenic refractory organic matter
PW:	wavelength peak with the highest intensity
SUVA$_{254}$:	specific UV absorbance (in L mg^{-1} m^{-1}) at 254 nm
UV:	ultraviolet radiation
λ:	wavelength
λ_{Ex}:	wavelength of excitation
λ_{Em}:	wavelength of emission

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