Characterization of organic matter using fluorescence and absorbance spectroscopy: the case study of a Brazilian polluted river

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Abstract

This paper presents a critical analysis about the dynamic of the organic matter considering the complexity of the pollution sources matrix in a Brazilian critical basin characterized by a strong highly density with very low rate of wastewater treatment. The case study was the Iguaçu River in the Metropolitan Area of Curitiba, covering an area of 2,800 km², with 7 monitoring points in 107 km of river length. The Iguaçu River at the Upper Iguaçu Basin serves as an important water resource for the southeastern area of Parana State, Brazil. Currently, about 3 million people reside within the basin, and an additional number intake drinking water from the river. Major pollution sources include domestic sewage, industrial wastewater, and urban and agricultural runoff. For this investigation, directly influenced by anthropic activity, this study proposes to use fluorescence and visible ultraviolet spectroscopy, to better assess the origin and sources of organic matter. Additionally, a complementary monitoring plan is proposed to improve the assessment of the origin and dynamics of organic matter for the river system analyzed, based on conventional water quality parameters, biochemical oxygen demand, chemical oxygen demand, dissolved oxygen, total organic carbon, nitrogen, phosphorus and solids. The relationships between absorbance, fluorescence intensity and dissolved organic carbon, the fluorescence ratio and the peak wavelength with the highest intensity were used to characterize the origin of the organic carbon. The results indicate that the major pollution source is domestic sewage (allochthonous source), due to the high urban development associated with a low level of sanitary sewage treatment in Curitiba and Metropolitan Region. Complementary assays indicated low concentrations of chlorophyll–a, thus diminishing the participation of primary productivity as a source of organic carbon (autochthonous source). The high concentrations of BOD, COD, organic and ammoniacal nitrogen, also indicates the predominance of organic matter from domestic effluents. Thus, this paper highlights some aspects of a complementary monitoring plan that can be reproduced in other critical basins with strong organic water quality pollution to identify the potentials and weaknesses of each basin with a view to preserving the water body as well as developing society.

Keywords

Organic matter monitoring; Upper Iguaçu Basin – Brazil; water resources management.

1. Introduction

Water quality in rivers and lakes is related to the intrinsic physical, chemical and biological characteristics of each river basin. The configuration of the physical space and the form of human occupancy cause changes in the nutrient and organic matter dynamics that affect the water body. To determine these changes, it is necessary to perform a monitoring plan in the basin under study. However, the monitoring network that currently prevails in most of the main Brazilian river basins is lagging behind, sometimes spatially, with few sampling points, sometimes as to time, with long gaps. Another set of problems is the lack of joint data on water quality and quantity. Without a quantitative approach in terms of available flow, the
conceptual vision to estimate the self-depuration or assimilation of the water body is lost, and also the real picture of the water pollution. The reflex of these gaps in monitoring, and the way it is performed, ultimately influence the management of water resources, with late or inefficient actions in given regions (Knapik, 2009).

In recent years there has been considerable effort made to characterize natural organic matter (NOM) in the aquatic ecosystem. NOM is generally used to describe a broad group of organic compounds in all natural waters, and may also contain organic matter from domestic, industrial or agricultural sources (Page and Dillon, 2007). Organic matter is found in the form of dissolved organic carbon (DOC) and particulate organic carbon (POC), in sediments and aquatic biota. Different chemical, physical and biological processes determine the dynamics of organic matter in natural waters. The organic matter may change and give rise to different organic compounds, and interfere in nutrient availability, in solubility and toxicity of contaminants, or else it may change the acidity of natural waters by means of organic acids (Bowie et al., 1985).

Depending upon the source of organic matter, NOM can be characterized as allochthonous, i.e., from outside the system, through non-point contribution (surface runoff) and through direct anthropic action, by point discharges of pollutants. Besides the allochthonous sources, the production of organic matter by aquatic biota can contribute internally as a source of organic matter, in this case, autochthonous source. According to Zumstein and Buffle (1989), NOM can be divided into two large categories: labile organic matter (which can degrade further) and refractory organic matter (stable compounds). The refractory organic matter can be further divided into two classes, distinguished by chemical structure: pedogenic refractory organic matter (characterized by chemical structures with an aromatic functional group, usually originating in the soil) and aquagenic refractory organic matter (characterized by chemical structure with an aliphatic chain, originating in the aquatic biota).

The organic matter present in aquatic ecosystems may be determined quantitatively through biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total organic carbon (TOC) assays. Biochemical oxygen demand is the amount of oxygen dissolved in water, determined by an indirect estimate, needed for the biochemical decomposition of organic matter, through the metabolism of aquatic microorganisms. Factors such as the nature of the oxygen-demanding material, as well as water temperature, hydraulic factors and stream geometry, affects the rate at which BOD is removed from the water column (Bowie et al., 1985). Chemical oxygen demand is defined as the amount of a strong oxidant that reacts with a sample under specific conditions. The quantity of oxidant consumed is expressed in terms of equivalent oxygen (APHA, 1998). Due to the uncertainties and difficulties of the procedures in BOD and COD assays, in the seventies the determination of total organic carbon was proposed (Thomas et al., 1999). This assay came from the observation that the organic matter present in water and in effluents comprises several materials at different levels of oxidation, and in certain assays, such as BOD, it is not possible to measure all the fractions of the organic matter that contain carbon.

Although TOC assay do not result in the same type of information of BOD and COD assays, they can be empirically correlated for a given condition, and the TOC assay may then be used to estimate the values of BOD, COD and assimilable carbon fraction of the sample. The TOC assay does not replace the BOD and COD assays, but, different from them, it does not depend on the state of oxidation of organic matter, and it does not measure other organic compounds.
such as nitrogen and hydrogen, and inorganic compounds that may contribute to the oxygen demands measured by BOD and COD (APHA, 1998).

The BOD, COD and TOC analyses supply quantitative information on the organic matter present in the aquatic ecosystems. However, organic matter results from a complex mixture of substances with different structural compositions, indicating the need for a qualitative analysis which will provide information to identify their sources and transformation and degradation mechanisms. Both visible ultraviolet and fluorescence spectroscopy have been used by several researchers to characterize the structural composition of dissolved organic carbon and to identify its possible sources in the ecosystems studied (Cabaniss and Shuman, 1987; Senesi et al., 1989; Ahmad and Reynolds, 1995; Peuravuori and Pihlaja, 1997; Korshin et al., 1997; Frimmel, 1998; Westerhoft and Anning, 2000; Artinger et al., 2000; Chen et al., 2002; Pons et al., 2004; Azevedo et al., 2006; Spencer et al. 2007).

Substances commonly found in natural waters and effluents, such as tannins lignins, humic substances and several other aromatic compounds have a high capacity to absorb ultraviolet light (APHA, 1998). According to Korshin et al. (1997), the natural organic matter absorbs light over an extensive range of the spectrum, while inorganic substances, typical of natural waters, do not absorb light significantly at $\lambda >\sim 230$ nm. As a result, light absorbance by natural waters may be a semi-quantitative indicator of organic matter concentration in a water body. Beemster (2005) proposes that the energy of radiation, both in wave length in the ultraviolet range and in the visible range, will change due to absorption by certain molecules.

According to Beemster (2005), for certain conditions, absorbance and transmittance measurements at certain wavelengths of the ultraviolet range can be used to measure total organic carbon or the carbonaceous demand of oxygen. Thomas et al. (1999) cite the visible ultraviolet spectroscopy assay as a complement to the determination of the total organic carbon (TOC), since it provides qualitative information about organic matter. Others researchers use spectroscopy in the visible ultraviolet region to characterize the structural composition of dissolved organic carbon and to identify its possible sources in the ecosystems studied (Senesi et al., 1989; Peuravuori and Pihlaja, 1997; Artinger et al., 2000; Chen et al., 2002; Pons et al., 2004; Azevedo et al., 2006; Spencer et al. 2007).

Westerhoft and Anning (2000) indicate the fluorescence assay to identify allochthonous sources (sources outside the system) of the organic matter. One of the aims of using the fluorescence emission assay as a complement to other water quality parameters is the possibility of identifying the origin of organic matter, whether it be allochthonous (outside the system), or autochthonous (inside the system). Several authors performed studies to identify natural organic matter of different origins (Senesi et al., 1989; Frimmel, 1998; Peuravuori et al., 2002; Stedmon et al., 2003), or to distinguish the organic matter of natural origin from dissolved organic matter from domestic effluents (Ahmad e Reynolds, 1995; Galapate et al., 1998; Chen et al., 2002; Pons et al., 2004), by comparing the fluorescent fractions of dissolved organic matter that exhibits peaks at specific wavelengths.

In this research, emphasis is on consolidating physical, chemical and biological interactions associated with the dynamics of organic matter, considering the complexity of the pollution sources matrix in a Brazilian critical basin characterized by a strong highly density with very low rate of wastewater treatment. In this particular case the Iguaçu River in the Metropolitan Region of Curitiba, herein called Upper Iguacu Basin. For this investigation, directly
influenced by anthropic activity, this study proposes to use fluorescence and visible ultraviolet spectroscopy, to better assess the origin and sources of organic matter.

Commonly, according to studies reported, the fluorescence and visible ultraviolet spectroscopy, are used for natural water or effluents, possibly implying greater subjectivity of the results for this case. In this research too, the concept is advocated that water quality monitoring should include both conventional assays such as biochemical oxygen demand, chemical oxygen demand, dissolved oxygen and dissolved organic carbon, and spectroscopy assays, besides other parameters, since complementarily they enable the water resources manager to better characterize the organic matter. This highlights the main contribution of this paper.

2. Materials and Methods

2.1 Study Area

The case study was analyzed based upon the dynamics of soil use and land occupation in the Upper Iguaçu basin, southeastern area of Parana State, Brazil. The Iguaçu River drains an area about 2,800 km² and the main stream extends approximately 107 km to the boundary of the Metropolitan Area of Curitiba. In this research, 26 sub-basins were used to characterize the Upper Iguaçu Basin.

Currently, about 3 million people reside within the basin, which concentrates about 25% of the total population and 30% of the urban population of the state, with low rates of sewage treatment. Since it is a highly urbanized region, it has been undergoing a process of irregular occupation of flood plains and source areas, especially on the right bank of the Iguaçu River. As a consequence of this process, problems have been encountered in the water supply systems, the level of treatment of sanitary sewage, and urban drainage systems, which do not keep up with the growth of the cities, negatively affecting the environment and people’s quality of life.

As to the physical aspects, it is a predominantly flat region presenting a large area of natural flood plains on both banks. The sites are permanently wet soils, and the water table is close to the surface of the terrain, mostly covered by typical low vegetation. There is also intense sand mining activity in the trenches (digs) in the flatter areas of these flood-prone plains.

2.2 Sampling points and monitoring procedure

Seven stations were selected for field monitoring along the Iraí and Iguaçu Rivers, covering an extension of approximately 107 km, in an area of approximately 2,800 km², as presented in Figure 1 and Table 1. Two monitoring periods were analyzed: 20 campaigns performed between June 2005 and July 2006, and 5 campaigns performed during the period from March to August 2008.
Figure 1 Specification of the monitoring points in the Upper Iguaçu Basin

Table 1 – Location of the sites for samples collection in the Upper Iguaçu Basin

<table>
<thead>
<tr>
<th>Nº</th>
<th>Identification/Station</th>
<th>River</th>
<th>Municipality</th>
<th>Drainage Area (km²)</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Altitude (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0</td>
<td>Olaria do Estado</td>
<td>Iraí</td>
<td>Pinhais</td>
<td>182,00</td>
<td>25°26'27&quot;</td>
<td>49°07'06&quot;</td>
<td>876</td>
</tr>
<tr>
<td>P1</td>
<td>Water Clean Channel</td>
<td>Iraí</td>
<td>Piraquara</td>
<td>282,88</td>
<td>25°26'36&quot;</td>
<td>49°08'26&quot;</td>
<td>875</td>
</tr>
<tr>
<td>P2</td>
<td>BR 277 Bridge</td>
<td>Iguaçu</td>
<td>São José dos Pinhais</td>
<td>625,53</td>
<td>25°29'00&quot;</td>
<td>49°11'21&quot;</td>
<td>869</td>
</tr>
<tr>
<td>P3</td>
<td>Umbarazinho Bridge</td>
<td>Iguaçu</td>
<td>São José dos Pinhais</td>
<td>1283,65</td>
<td>25°35'56&quot;</td>
<td>49°15'39&quot;</td>
<td>865</td>
</tr>
<tr>
<td>P4</td>
<td>Cachoeira STP</td>
<td>Iguaçu</td>
<td>Araucária</td>
<td>2122,22</td>
<td>25°36'01&quot;</td>
<td>49°23'52&quot;</td>
<td>860</td>
</tr>
<tr>
<td>P5</td>
<td>Guajuvira Bridge</td>
<td>Iguaçu</td>
<td>Araucária</td>
<td>2577,76</td>
<td>25°36'01&quot;</td>
<td>49°30'48&quot;</td>
<td>858</td>
</tr>
<tr>
<td>P6</td>
<td>Balsa Nova</td>
<td>Iguaçu</td>
<td>Balsa Nova</td>
<td>3048,69</td>
<td>25°35'14&quot;</td>
<td>49°37'54&quot;</td>
<td>854</td>
</tr>
</tbody>
</table>
The physical-chemical parameters measured in situ were: dissolved oxygen (DO, mg/L), water temperature (ºC), electrical conductivity (µS/cm), pH, turbidity (NTU) and water transparency (Secchi disk). Water samples were collected for posterior analysis, according to the procedure of Standard Methods for the Examination of Water and Wastewater (APHA, 1998). The parameters determined in laboratory were: BOD$_5$ (Winkler method 5210B and 4500O-C, 5 day incubation and azide modification of the iodometric method), chemical oxygen demand (COD, open reflux method 5220B), organic and ammonia nitrogen (macro-Kjehdahl method 4500B), nitrite and nitrate (colorimetric methods 4500B and 4500E), total phosphorous (method 4500P B and D: sulfuric acid – nitric acid digestion followed by stannous chloride colorimetric method), solids (method 2540B for total solids dried at 103º-105ºC, and method 2540E for fixed and volatile solids ignited at 550ºC), chlorophyll-a (method 10200H, spectrophotometric determination) and dissolved organic carbon (DOC, 680ºC combustion catalytic oxidation and non-dispersive infra-red method), according to the procedure of Standard Methods for the Examination of Water and Wastewater (APHA, 1998).

For fluorescence measurements, the water samples were filtered with a 0.45 µm membrane, and the emission spectra from 300 to 600 were obtained applying excitation wavelength of 314 nm and 370 nm, according to Sierra et al. (2001) and Oliveira et al. (2006), performed with a Fluorescence Spectrophotometer F-4500 Hitachi. The synchronous spectra were obtained with excitation wavelength of 250 to 600 nm, with $\Delta \lambda = 18$ nm, often applied in the studies of humic substances or natural organic matter (Senesi et al., 1989 and Peuravuori et al., 2002). 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 a blank. Both these measurements for fluorescence and synchronous spectra were normalized by dissolved organic carbon (DOC in mg/L), according to Oliveira et al. (2006). Two indicators of the spectrum with excitation wavelength of 370 nm, were used to evidence the source of organic matter: wavelength of the peak of the spectrum (< 450 nm for an autochthonous source and > 450 nm for an allochthonous source); and fluorescence ratio between two emission wavelengths (FR) or inclination of the fluorescence spectrum between 450 and 500 nm wavelengths (FR values greater than 1.8 were indicative of an autochthonous source, while FR values less than 1.5 indicated allochthonous source), according to Westerhoft and Anning (2000).

The measurements of absorbance were performed with filtered water samples (0.45 µm membrane) in the range 200 to 700 nm, using a 10 mm quartz cell and Milli-Q water as a blank. In order to calculate the specific absorbance or absorptivity (SUVA, L/mg.m), the visible ultraviolet at 254 nm wavelength values were normalized by dissolved organic carbon (DOC mg/L) and by the optical path (m), according to Oliveira et al. (2006). Westerhoft and Anning (2000) used the SUVA values at the 254 nm wavelength to characterize organic matter, establishing two patterns: ~ 4.4 L/mg.m for fulvic acids and ~ 1.2 L/mg.m for organic matter from an autochthonous source. The absorbance spectra were also analyzed as a function of the absorbivity at 285 nm normalized by DOC ($A_{285}$/DOC in L/g.cm), according to Rostan and Celliot (1995). According to the authors, $A_{285}$/DOC values close to 20 L/g were considered DOC essentially formed by fulvic acids, while values below 10 L/g indicated the presence of aliphatic carbon (labile organic matter). The ratios E2/E3 (where E2 is the absorbance at the 250 nm wavelength and E3 is the absorbance at 365 nm wavelength), E3/E4 (absorbance at the 300 and 400 nm wavelengths) and E4/E6 (absorbance at the 465 and 665 nm wavelengths), were also analysed according to Senesi et al. (1989), Peuravuori and Pihlaja (1997), Artinger et al. (2000) and Chen et al. (2002).
### 3. Results and Discussion

#### 3.1 Water quality monitoring parameters

Table 2 shows a summary of the mean concentrations observed for the main parameters monitored and their fulfillment or not in the water quality classes according to the Brazilian standard classification CONAMA Resolution 357/05: class 1 represents the best condition of the water quality; class 2, that represents a good water quality; class 3, where the water body are considered polluted and inappropriate for some uses; and class 4, with an advanced stage of water quality degradation.

Table 2 – Summary of the main parameters analyzed during the monitoring period (Mean ± sd and number of samples)

<table>
<thead>
<tr>
<th>Variables</th>
<th>P0</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
<th>P6</th>
<th>P7</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOD (mg/L)</td>
<td>3.0±1.1</td>
<td>11.5±13.9</td>
<td>19.5±16.9</td>
<td>23.3±23.1</td>
<td>20.9±17.4</td>
<td>18.2±20.9</td>
<td>9.2±7.7</td>
<td></td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>19.3±4.1</td>
<td>18.9±11.5</td>
<td>29.5±14.1</td>
<td>29.0±10.1</td>
<td>32.7±13.8</td>
<td>26.4±12.2</td>
<td>19.3±7.6</td>
<td></td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>5.4±1.5</td>
<td>5.7±1.4</td>
<td>2.8±1.5</td>
<td>2.2±1.8</td>
<td>1.6±1.3</td>
<td>1.7±0.8</td>
<td>2.5±0.9</td>
<td></td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>6.9±1.8</td>
<td>7.9±4.9</td>
<td>13.3±9.0</td>
<td>12.5±8.3</td>
<td>12.3±7.2</td>
<td>10.2±5.7</td>
<td>9.3±6.4</td>
<td></td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>30.5±32.7</td>
<td>18.7±21.1</td>
<td>38.2±45.0</td>
<td>30.8±31.7</td>
<td>46.0±50.5</td>
<td>40.0±49.5</td>
<td>28.9±25.4</td>
<td></td>
</tr>
<tr>
<td>Conductivity (µS/cm)</td>
<td>16.8±20.3</td>
<td>19.2±9.3</td>
<td>109.9±57.2</td>
<td>98.0±51.8</td>
<td>104.7±54.9</td>
<td>91.9±46.5</td>
<td>78.9±43.8</td>
<td></td>
</tr>
<tr>
<td>Organic nitrogen (mg/L)</td>
<td>1.5±0.8</td>
<td>0.8±0.8</td>
<td>3.1±4.8</td>
<td>3.0±5.2</td>
<td>3.7±7.1</td>
<td>2.8±4.3</td>
<td>1.5±1.9</td>
<td></td>
</tr>
<tr>
<td>Ammonia (mg/L)</td>
<td>0.5±0.5</td>
<td>0.5±0.7</td>
<td>10.7±13.8</td>
<td>9.0±12.6</td>
<td>9.0±12.2</td>
<td>10.5±18.2</td>
<td>5.1±3.9</td>
<td></td>
</tr>
<tr>
<td>Total phosphorous (mg/L)</td>
<td>0.115±0.052</td>
<td>0.042±0.044</td>
<td>0.558±0.448</td>
<td>0.576±0.514</td>
<td>0.484±0.437</td>
<td>0.407±0.311</td>
<td>0.312±0.270</td>
<td></td>
</tr>
<tr>
<td>Total Diss. Solids (mg/L)</td>
<td>77.5±25.4</td>
<td>67.2±44.5</td>
<td>141.0±53.4</td>
<td>133.0±66.8</td>
<td>162.1±88.3</td>
<td>135.6±51.3</td>
<td>138.1±64.0</td>
<td></td>
</tr>
<tr>
<td>Total Susp. Solids (mg/L)</td>
<td>18.5±16.5</td>
<td>15.8±8.0</td>
<td>31.1±24.9</td>
<td>29.8±10.8</td>
<td>57.1±61.1</td>
<td>45.8±49.1</td>
<td>30.3±22.9</td>
<td></td>
</tr>
<tr>
<td>pH(e)</td>
<td>6.2±0.6</td>
<td>6.3±0.5</td>
<td>6.9±0.4</td>
<td>6.8±0.4</td>
<td>6.8±0.4</td>
<td>6.8±0.3</td>
<td>6.8±0.5</td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>17.2±2.9</td>
<td>17.2±2.7</td>
<td>17.7±3.0</td>
<td>18.2±3.0</td>
<td>18.0±2.9</td>
<td>18.3±3.1</td>
<td>19.0±3.3</td>
<td></td>
</tr>
<tr>
<td>Secchi depth (cm)</td>
<td>77.5±35.9</td>
<td>66.3±22.4</td>
<td>34.5±19.5</td>
<td>34.0±12.6</td>
<td>25.7±11.0</td>
<td>46.4±20.1</td>
<td>41.6±19.6</td>
<td></td>
</tr>
<tr>
<td>Flow (m³/s)</td>
<td>3.7±1.9</td>
<td>5.6±4.2</td>
<td>19.9±21.5</td>
<td>29.8±27.3</td>
<td>53.8±45.5</td>
<td>63.3±53.4</td>
<td>73.1±59.3</td>
<td></td>
</tr>
</tbody>
</table>

sd – Standard deviation;  (a)Class 1,  (b)Class 2,  (c)Class 3,  (d)Class 4,  (e)standard value for all classes
It was generally possible to identify a different profile for most of the parameters regarding P0 and P1 sampling points, which are different from the others mainly due to the low anthropic occupation of the drainage areas, and, consequently, the low influence of domestic effluents. Like conductivity, these points presented values that were 80% lower than the other sampling points. As regards the presence of solids, both the dissolved and suspended fraction presented high values with predominance of the non-volatile dissolved fraction, indicating the presence of inorganic compounds. The presence of suspended solids can also contribute to diminish the dissolved oxygen levels, since the organic content present in the solids may decompose.

Monitoring points P0, P1 and P6 presented the lowest BOD values, confirming the low input of organic material at the first two points (domestic effluents) and indicating the dilution and decomposition condition of the organic material at point P6 (a point located further downstream in the basin studied). In terms of frequency, more than 60% of the BOD data observed for points P0, P1 and P6 presented concentrations lower than 10mg/L, the maximum limit for Class 3 according to Brazilian standard resolution. At the P0 sampling point, all the data, monitored only during the period of 2008, were lower than 5mg/L, the maximum limit for Class 2 (CONAMA 357/05). On the other hand, at points P2, P3 and P5, in which there is a high degree of water body pollution, according to variables presented earlier, more than 65% of the data observed, on average, had concentrations higher than 10 mg/L.

As to COD, the mean concentration was 25.9 ± 12.6 mg/L, varying from 18.9 ± 11.5 at P1 (an area with low anthropic activity) to 32.7 ± 13.8 at P4 (an area characterized by anthropic and industrial activity, located after the mouth of the Barigui river). Although the results presented a high coefficient of variation (mean 48.6%), they were more homogeneous than the other variables. In terms of frequency, more than 70% of the data remained in the 20 to 40 mg/L range, without presenting a linear correlation with the other variables monitored.

The concentration of total phosphorus also presented a significant difference between the P0 and P1 sampling points and the other points monitored, again evidencing the land use and occupation characteristic. The data frequency observed with total phosphorus concentration less than 0.15 mg/L, a class for a lotic environment according to CONAMA Resolution 357/05, was higher than 75% at point P0 and 100% at point P1. At the other points, however, only an average of 22% of the data sampled were less than 0.15 mg/L. Despite the high concentrations of total phosphorus, in the stretch between P2 and P6 low concentrations of chlorophyll-a were observed with values below 2 µg/L.

The ammoniacal form of nitrogen predominated in the stretch between stations P2 and P6, confirming the presence of untreated domestic effluents. In terms of frequency distribution, more than 70% of the ammoniacal nitrogen data were below 10 mg/L and, on average, they remained below the limit of class 3 of the Brazilian standard resolution, of 13.5 mg/L. At points P0 and P1, the mean value was below 3.7 mg/L, the maximum limit for class 1 and 2. For the organic fraction, more than 86% of the data were less than 5 mg/L, except for P0 and P1 points, whose organic nitrogen concentration was higher, on average, than the ammoniacal nitrogen concentration. This difference is due mainly to the physical characteristics and occupation characteristics of the drainage areas of the respective points, where there is a predominance of non-urbanized areas. More than 90% of the nitrate values observed, and 86% of nitrite were below 1.0 mg/L, the maximum nitrite concentration allowed for all classes of CONAMA Resolution 357/05.
3.2 Visible Ultraviolet and Fluorescence Spectroscopy

Spectroscopy in the visible ultraviolet region was used to characterize the structural composition of dissolved organic carbon, and to identify its possible sources at 7 monitoring points along the Iguaçu river during the period from March to August 2008.

The absorptivity spectra were similar to the spectra from environments with presence of sewage, whose energy absorption is less than in environments where the pedogenic allochthonous source (material from the soil and from higher plants) of organic carbon predominates (Westerhoff e Anning, 2000; Pons et al., 2004). The spectra of point P6, located downstream from study area, presented the smallest difference among the collections performed, indicating little influence, both of seasonality and of the other monitoring points and their respective areas of contribution.

The mean values of SUVA$_{254}$ and A$_{285}$/DOC (Table 3) indicate a probable mixture of an autochthonous source (the result of biological activity in the environment) and an allochthonous source in the composition of dissolved organic matter. When the SUVA$_{254}$ values are low, this may indicate that the dissolved organic matter may also be from anthropic activity (allochthonous), such as domestic effluents, which present the dissolution of organic carbon that has a low absorption in the UV region, thus diminishing both the SUVA$_{254}$ and the A$_{285}$/DOC ratio. According to a study by Ma, Allen and Yin (2001), the effluents are compounds formed mainly by substances with aliphatic functional groups, with a low concentration of dissolved humic substances. Musikavong and Wattanachira (2007) also added that the SUVA$_{254}$ values tend to increase due to the biological processes involved in effluent treatment (for instance, aerobic lagoons), and there is an increase of values close to 1.2 L/mg.m, in samples of untreated effluents to values close to 1.8 L/mg.m, in samples of treated effluents. According to the authors, biological activity tends to remove the fraction that is not sensitive to UV emission, i.e., the most labile one that does not absorb in the UV-Vis region, diminishing DOC and maintaining absorbance.

<table>
<thead>
<tr>
<th>Sampling points</th>
<th>DOC</th>
<th>SUVA$_{254}$</th>
<th>A$_{285}$/COD</th>
<th>Probable organic matter source</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0</td>
<td>6.87 ± 1.85</td>
<td>2.01 ± 0.76</td>
<td>13.94 ± 5.26</td>
<td>autochthonous / allochthonous anthropic (sewage)</td>
</tr>
<tr>
<td>P1</td>
<td>8.47 ± 3.95</td>
<td>1.82 ± 1.06</td>
<td>12.74 ± 7.52</td>
<td>autochthonous / allochthonous anthropic (sewage)</td>
</tr>
<tr>
<td>P2</td>
<td>8.68 ± 2.61</td>
<td>1.36 ± 0.55</td>
<td>9.26 ± 3.81</td>
<td>autochthonous / allochthonous anthropic (sewage)</td>
</tr>
<tr>
<td>P3</td>
<td>8.27 ± 3.70</td>
<td>1.81 ± 1.56</td>
<td>12.66 ± 10.87</td>
<td>autochthonous / allochthonous anthropic (sewage)</td>
</tr>
<tr>
<td>P4</td>
<td>8.42 ± 6.27</td>
<td>1.91 ± 1.74</td>
<td>13.31 ± 12.24</td>
<td>autochthonous / allochthonous anthropic (sewage)</td>
</tr>
<tr>
<td>P5</td>
<td>9.31 ± 5.76</td>
<td>1.33 ± 1.07</td>
<td>9.28 ± 7.50</td>
<td>autochthonous / allochthonous anthropic (sewage)</td>
</tr>
<tr>
<td>P6</td>
<td>8.01 ± 3.10</td>
<td>1.13 ± 0.20</td>
<td>7.79 ± 1.33</td>
<td>autochthonous / allochthonous anthropic (sewage)</td>
</tr>
</tbody>
</table>

DOC: concentration of dissolved organic carbon (mg/L); SUVA$_{254}$ (L/mg.m): specific ultraviolet absorbance in the wavelength 254 nm normalized by dissolved organic carbon (mg/L) and the optical path (m); A$_{285}$/COD (L/g.cm): specific ultraviolet absorbance in the wavelength 285 nm normalized by dissolved organic carbon (g/L) and the optical path (cm).

The fluorescence spectra (at 314 and 370 excitation wavelengths) and absorbance spectra were normalized by the concentration of dissolved organic carbon, DOC (Werterhoff and Anning, 2000). The fluorescence spectra for a 370 nm excitation wavelength were analyzed in terms of the peak of the spectrum (< or > than 450 nm), and of the ratio of fluorescence intensities at 450 and 500 nm emission wavelengths (Werterhoff and Anning, 2000). As to
the synchronized spectra, the intensities were analyzed at the 298 and 480 nm wavelengths according to Ahmad and Reynolds (1995), Ma, Allen and Yin (2001) and Peuravuori et al. (2002).

In order to characterize the source of organic matter Westerhoff and Anning (2000) consider the ratio of emission fluorescence intensities at the 450 and 500 nm wavelengths \((FR = \frac{\lambda_{450}}{\lambda_{500}})\), with an excitation of 370 nm as an autochthonous source for the FR values higher than 1.8 and allochthonous for values below 1.5. The FR values (Table 4) of the water samples from points P2 (FR = 1.80 ± 0.09), P3 (FR = 1.80 ± 0.08), P4 (FR = 1.83 ± 0.09) and P5 (FR = 1.79 ± 0.1) indicate that autochthonous DOC predominates, or DOC formed by compounds that do not present fluorescence emission in this region and with this excitation, such as organic substances dissolved in domestic effluents. On the other hand, the samples from P0 (FR = 1.56 ± 0.09), P1 (FR = 1.67 ± 0.23) and P6 (FR = 1.70 ± 0.05) points presented intermediary FR values which indicate a probable mixture of DOC from a pedogenic source (humic substances, allochthonous) and from primary productivity (autochthonous source) or anthropic influence (effluents, allochthonous) (Azvedo et al., 2008). Peaks \(S_{298}\) and \(S_{480}\) (Table 4) refer to the synchronized spectra and are related to primary productivity and/or sewage \((S_{298})\) and more complex substances, such as humic substances \((S_{480})\) (Ahmad and Reynolds, 1995; Ma, Allen and Yin, 2001; Peuravuori et al., 2002).

### Table 4 – Average relationship among fluorescence intensity and dissolved organic carbon

<table>
<thead>
<tr>
<th>Sampling point</th>
<th>DOC</th>
<th>FR</th>
<th>(IF_{370})</th>
<th>(S_{298})</th>
<th>(S_{480})</th>
<th>Probable organic matter source</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0</td>
<td>6.87 ± 1.85</td>
<td>1.56 ± 0.09</td>
<td>62.19 ± 13.85</td>
<td>7.41 ± 2.24</td>
<td>5.31 ± 1.11</td>
<td>autochthonous / allochthonous pedogenic and anthropic (sewage)</td>
</tr>
<tr>
<td>P1</td>
<td>8.47 ± 3.95</td>
<td>1.67 ± 0.23</td>
<td>57.42 ± 22.05</td>
<td>12.06 ± 18.51</td>
<td>4.24 ± 2.40</td>
<td>autochthonous / allochthonous pedogenic and anthropic (sewage)</td>
</tr>
<tr>
<td>P2</td>
<td>8.68 ± 2.61</td>
<td>1.80 ± 0.09</td>
<td>68.08 ± 16.90</td>
<td>9.67 ± 8.48</td>
<td>3.96 ± 0.94</td>
<td>autochthonous / allochthonous anthropic (sewage)</td>
</tr>
<tr>
<td>P3</td>
<td>8.27 ± 3.70</td>
<td>1.80 ± 0.08</td>
<td>64.78 ± 49.07</td>
<td>11.23 ± 6.98</td>
<td>5.54 ± 2.94</td>
<td>autochthonous / allochthonous anthropic (sewage)</td>
</tr>
<tr>
<td>P4</td>
<td>8.42 ± 6.27</td>
<td>1.83 ± 0.09</td>
<td>91.84 ± 48.89</td>
<td>14.88 ± 6.25</td>
<td>6.00 ± 3.09</td>
<td>autochthonous / allochthonous anthropic (sewage)</td>
</tr>
<tr>
<td>P5</td>
<td>9.31 ± 5.76</td>
<td>1.79 ± 0.10</td>
<td>81.84 ± 53.45</td>
<td>11.73 ± 5.06</td>
<td>4.81 ± 3.04</td>
<td>autochthonous / allochthonous anthropic (sewage)</td>
</tr>
<tr>
<td>P6</td>
<td>8.01 ± 3.10</td>
<td>1.70 ± 0.05</td>
<td>76.55 ± 45.38</td>
<td>8.74 ± 2.23</td>
<td>4.22 ± 1.90</td>
<td>autochthonous / allochthonous pedogenic and anthropic (sewage)</td>
</tr>
</tbody>
</table>

DOC: concentration of dissolved organic carbon (mg/L); FR: ratio between the intensities of fluorescence emitted at 450 and 500 nm wavelengths, with an excitation of 370 nm; \(IF_{370/450}\): intensity of fluorescence emitted at 450 nm, with an excitation of 370 nm; \(S_{298}\): intensity of fluorescence emitted at 298 nm (synchronized spectrum, \(\Delta \lambda = 18 \text{ nm}\)); \(S_{480}\): intensity of fluorescence emitted at 480 nm (synchronized spectrum, \(\Delta \lambda = 18 \text{ nm}\)).

The increased FR ratio (Table 4) comes from the increase in domestic discharges, because since it is a lotic system, the tendency is for productivity to be small, and considering that FR increases with increased DOC, especially at the most critical points that are more greatly influenced by this type of effluent.

Analyzing the wavelength of the peak \((IF_{370/450})\) in the fluorescence spectra \((\lambda_{exc} = 370 \text{ nm})\), normalized by DOC, Table 4, and by Figure 2, it is observed that in most campaigns the curvature band of the peak predominated in the region below 450 nm, indicating little influence from allochthonous organic matter, according to Westerhoff and Anning (2000),
who classified the band between pedogenic allochthonous organic carbon, for a spectrum peak greater than 450 nm and autochthonous organic carbon for a peak below 450 nm. In the fluorescence spectra for excitation of 314 nm, according to a study by Frimmel (1998), who also classifies the standard length $\lambda_{em} = 450$ nm as the maximum emission of natural humic substances, the spectra peaks are located at wavelengths below 450 nm, confirming the low influence of pedogenic allochthonous organic matter.

In their studies, authors like Frimmel (1998), Sierra et al. (2005), Oliveira et al. (2006) and Azevedo et al. (2008) demonstrated a good correlation between DOC and absorbance and DOC and emitted fluorescence. Figure 2 shows the relations between DOC and absorbance (254 nm) and between DOC and fluorescence intensity (excitation at 370 nm), considering all the samples ($n = 34$). Between DOC and fluorescence intensity a linear correlation can be observed in the data sampled ($r = -0.7293$), but this does not occur between DOC and absorbance ($r = 0.0187$).

![Figure 2](image1.png)

Figure 2 – Fluorescence intensity (FI) spectra of emission with 314 and 370 nm of excitation, normalized by DOC (in mg/L) of samples related to monitoring point P4.

![Figure 3](image2.png)

Figure 3 – (a) Correlation between DOC and absorbance (254 nm), and (b) correlation between DOC and relative fluorescence intensity (excitation/emission = 370/450 nm), considering all sampling points from March to August 2008.
Figure 3 (a) indicates that DOC does not come from a pedogenic allochthonous source (humic substances), since absorbance at 254 nm increases as the concentration of dissolved humic substances increases, thus indicating that the dissolved material is either from sewage (allochthonous) or from primary productivity (autochthonous). In Figure 3 (b), the reduction of fluorescence intensity as a function of DOC indicates two hypotheses: either the increase of dissolved humic substances, which diminishes the fluorescence intensity; or the increase of DOC which does not emit fluorescence in this region, as DOC from sewage. Considering Azevedo et al. (2006), the humic substances present a linear fluorescence intensity with DOC up to the concentration of 25 mg/L, i.e., in the range obtained in Figure 3 (b) the signal should have been linear if the organic carbon increase had been due to the increase in humic substances. The chlorophyll-a concentrations in the environment studied were low, which indicates little influence of DOC of autochthonous origin (primary productivity). On the other hand, the high concentrations of ammonia (mean 4.23 ± 3.03 mg/L at P2 and 3.94 ± 1.96 mg/L at P6) and phosphorus (mean 0.40 ± 0.17 mg/L at P2 and 0.27 ± 0.10 mg/L at P6) during the 2008 period confirm the hypothesis that DOC is from sewage, especially at monitoring points P2 to P6. Moreover, at these monitoring points, absorbance at 254 nm presented a linear correlation between turbidity (r=-0.5616) and nitrate (r=-0.6403).

Senesi et al. (1989), Peuravuori et al. (2002) and Chen et al. (2003) also use the synchronized spectrum of fluorescence to study DOC. The peaks for the humic substances are generally located close to 450 nm for the fulvic acids, and between 465 and 500 nm for humic acids. Peuravuori et al. (2002) consider the peak between 270 and 370 nm, centralized at \( \lambda_{ex}/\lambda_{em} \) around 280/298 nm, as coming from the emission of fluorescence of aromatic aminoacids or volatile acids, containing aliphatic structures with conjugated bonds. Ahmad and Reynolds (1995) and Ma, Allen and Yin (2001) consider that peaks in this region are characteristic of domestic sewage.

In this study, according to Figure 4 and Table 4, it was possible to identify marked peaks in the 280-298 nm region, indicating the influence of domestic effluents. The emission at S298 presented a linear correlation with ammoniacal nitrogen (r=0.7718), organic nitrogen (r=0.6585) and conductivity (r=0.7615) for monitoring points P2 to P6, confirming the presence of sewage and the influence of the latter on dissolved organic carbon.
4. Conclusions

In a river with a significant presence of domestic and industrial effluents, whose basin presents many different uses, as in the case of Upper Iguaçu Basin, this research advanced when it studied the dynamics of organic matter using spectroscopy and visible ultraviolet assays associated with parameters such as BOD, COD, TOC. The results indicate that dissolved organic carbon originates from two main sources: anthropic allochthonous, i.e., domestic effluents; and in a smaller proportion, autochthonous. Complementary assays indicated low concentrations of chlorophyll–a, thus diminishing the participation of primary productivity as a source of organic carbon (autochthonous source). The high concentrations of BOD, COD, organic and ammoniacal nitrogen, especially at points P2 to P6, confirm the predominance of organic matter from domestic effluents. This stretch drain the most urbanized part of Curitiba and the Metropolitan Region, thus reflecting the poor collection and treatment of the existing sewage. Also, the low flow condition (headwater rivers), contributes to the low dilution of pollutants, a scenario which improves at the points located further downstream from the study area, Guajuvia Station (P5) and Balsa Nova Station (P6), located respectively 91 and 106 kilometers from the source of Iraí river.

ACKNOWLEDGMENTS

This research has the financial support of CNPq, FINEP/CNPq/CT-HIDRO n° 40/2006 and the PPGERHA–Graduate Program in Water Resources and Environmental Engineering of UFPR, and was developed in partnership with the Critical Basins Project: Technical Bases to define Progressive Goals for their Classification and Integration with the other Management Instruments, with financial support of FINEP/CNPq/CT-Hidro 01/2004.

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