



ESPECTROSCOPIA DE FLUORESCÊNCIA PARA DIFERENCIAÇÃO DE MATÉRIA ORGÂNICA EM EVENTOS DE POLUIÇÃO DIFUSA

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Resumo – Os eventos de poluição difusa são aqueles induzidos principalmente pela chuva, carreando poluição para dentro do rio. Um dos desafios envolvidos é entender quais são os tipos de compostos que estão sendo trazidos para o corpo hídrico, qual o seu comportamento durante os episódios de chuva e seu impacto. Neste sentido, a espectroscopia de fluorescência é uma técnica qualitativa que pode ser usada na diferenciação da matéria orgânica. Portanto, o objetivo deste trabalho foi usar a técnica de fluorescência para identificar qualitativamente os compostos orgânicos em um evento de chuva. Foi possível observar que as primeiras amostras do evento receberam um aporte de material refratário, com maiores picos nas bandas A e C da matriz de excitação-emissão (MEE). Em seguida, houve o recebimento de carga lábil devido ao aparecimento das bandas B, T₁ e T₂ e ao maior valor de carbono orgânico dissolvido (COD) (16.77 mg L⁻¹). Em seguida, o efeito da diluição torna-se perceptível devido ao decréscimo dos valores de COD e a atenuação das intensidades nas MEEs. Foi possível concluir que a técnica de fluorescência é capaz de indicar as características da matéria orgânica em eventos de poluição difusa.

Palavras-Chave – Rio Barigui, compostos lábeis e refratários, análise quali-quantitativa

FLUORESCENCE SPECTROSCOPY FOR ORGANIC MATTER DIFFERENTIATION IN DIFFUSE POLLUTION EVENTS

Abstract – Diffuse pollution events are induced, mainly by rainfall, carrying pollution into the river. One of the challenges involved is to understand what kind of compound is being carried, their behavior and impact. In this sense, fluorescence spectroscopy is a qualitative technique used for organic matter differentiation. The aim of this paper was to use fluorescence technique to evaluate organic compounds during a rainfall event. It was possible to observe that in the beginning of the event there was refractory compound input, with higher peak in regions A and C in excitation-emission matrix (EEM). Subsequently, a labile contribution occurred due to shown of the regions B, T₁ and T₂ in EEM, besides the biggest value of dissolved organic carbon (DOC) (16.77 mg L⁻¹). Thereafter, dilution effect is perceptible due to DOC decrease and EEM intensities attenuation. It was possible to conclude that fluorescence spectroscopy is capable of indicating organic matter characteristics in diffuse pollution events.

Keywords - Barigui River, labile and refractory compounds, quali-quantitative analysis

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INTRODUCTION

By definition, diffuse pollution (or non-point sources), in general, is caused by rainfall episodes carrying pollution over and through soil (USEPA, 1984). Diffuse sources can be originated from agricultural and urban runoff, water pollutants and sediment transport, atmospheric deposition, and soil erosion (USEPA, 1984; Novotny, 2002). Thus, randomness and unpredictability are caused by rainfall and quali-quantity strategies are necessary in order to quantify pollutant load into the river. One of them is using intelligent automatic sampling (Kozak, 2016).

Once technical and logistics difficulties are overcome, the next step is to assess physical, chemical and biological transformations. In this sense, to observe the organic contribution during rainfall events can be an alternative. Because of the organic matter excess in water bodies dissolved oxygen concentration can be reduced, and consequently its quality (Westerhoff e Anning, 2000). Therefore, a direct and widely measurement used to quantify organic carbon atoms present in water is total organic carbon (TOC), and their fractions, dissolved (DOC) and particulate organic carbon (POC) (Bridgeman *et al.* (2015). Additionally, UV-visible and fluorescence spectrophotometry can be applied for organic matter characterization (compounds and sources identification) (Knapik, 2014). Some of the advantages are that this technique is fast and simple, and requires small volumes of sample (Bridgeman *et al.*, 2015).

Fluorescence spectrophotometry is widely used for dissolved organic matter characterization, and pollution sources and compounds identification in surface waters (Sierra et al., 1996; Carstea, 2012). It is used because the fluorescence principle in water is induced by DOM availability (Sierra *et al.*, 1996). Thus, this measurement consists of a special luminescence to describe the light emission from electronically excited molecules, called fluorophores. They absorb and release (emission) energy at light form in a specific wavelength (Carstea, 2012).

In natural samples, organic matter is a complex and heterogeneous mixture from different sources, which implies some difficulties to identify each individual compound. Thus, for a better understanding, two main groups of fluorophores are usually separated: i) proteins, which include tryptophan, tyrosine and phenylalanine compounds, and ii) humic substances, which include humic and fulvic acids (Carstea, 2012; Hudson *et al.*, 2007). That occurs in the same area of optical space. Additionally, Coble (1996) proposed another nomenclature to represent the common areas in excitation-emission matrix (EEM): humic substances in peak A ($\lambda_{ex} = 230nm/\lambda_{em} = 400 - 500nm$) and peak C ($\lambda_{ex} = 300 - 500nm/\lambda_{em} = 400 - 500nm$), tryptophan as peak T₁ ($\lambda_{ex} = 290nm/\lambda_{em} = 350nm$) and T₂ ($\lambda_{ex} = 230nm/\lambda_{em} = 350nm$) tyrosine as peak B ($\lambda_{ex} = 230 - 275nm/\lambda_{em} = 310nm$). Peaks A and C represent refractory fraction, and peaks T₁, T₂ and B represent labile fraction of the carbon compounds.

Thus, this paper aims to identity the main organic matter characteristics in a diffuse pollution event through fluorescence spectrophotometry analysis, and, consequently, understand its behavior during a rainfall event in an urban basin. At the same time, explore how feasible and consistent this strategy can be for improving water quality dynamics in rivers.

MATERIALS AND METHODS

This study was carried out in Barigui River Watershed, which is a sub-basin located in the





central part of the Upper Iguassu Watershed at Paraná State (southern Brazil), as shown in Figure 1. The basin drains an area of 267 km², and Barigui River has 67 km of extension (SUDERHSA, 2002). The monitoring site (called BA01) is located at watershed upstream in urban area of Almirante Tamandaré city (25°18,7720 S; 49°17,7412 O) draining an area about 60.9 km².

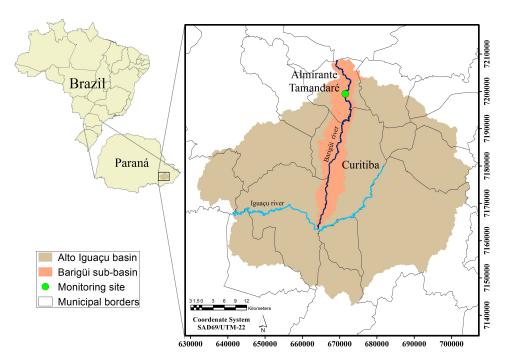


Figure 1: Monitoring site location at Barigui River Source: Kozak (2016)

At the local, there is a monitoring pluviometric station commanded by a waterlog H500-XL Logger. The samples were collected with an ISCO automatic sampler adapted to an intelligent automatic programming interface, developed by Braga (2013) (called SBN). SBN schedule consists of collecting a sample considering water level variations, following hydrograph behavior.

Data presented was collected during a rainfall occurred in March 15^{th} , 2015. Samples were collected in polyethylene bottles with one liter capacity. For DOC, UV-Visible and fluorescence analysis samples were filtered with a 0.45 μ m pre-washed cellulose acetate membrane. DOC was measured using TOC-V_{CPH} analyzer (Shimadzu). UV-Vis absorbance was measured using a UV-1601 PC spectrometer (Shimadzu), with a 1 cm quartz cells and distilled water as a blank, in the range of 200 to 600 nm. Fluorescence specters were performed in a fluorescence spectrometer Cary Eclipse (Varian Inc.) with a 1 cm quartz cells. Excitation wavelength ranged between 200 – 600 nm, in 10 nm intervals and with emission wavelength ranged between 200-600 nm in 3 nm intervals. For this measurements ultrapure water was used as a blank for equipment control. Fluorescence EEMs were corrected for inner filter effects (McKnight *et al.*, 2001; Carstea, 2012) and normalized in Raman Units (r.u.) (excited at 350 nm with voltage PMT 950V) using a program named FEEMC1.0 and evolution of the routine presented in Leithold (2017).





RESULTS

Diffuse pollution event occurred between 15^{th} March at 11:40 p.m. and 17^{th} March at 6:00 a.m., resulting in an event of 30.3 hours. During this time, 11 bottles have been collected. The rain started on 15^{th} March at 11:27 p.m. (with duration of 12.4 hours), and 13 minutes later SBN equipment did the first sampling. Before rainfall, initial flow was approximately 1.90 m³ s⁻¹ and increased until 4.48 m³ s⁻¹ at the peak (bottle 6). Event had an accumulated precipitation of 14.73 mm. Figure 2 shows corresponding hydrograph with respective excitation-emission matrix for each sample collected (bottle).

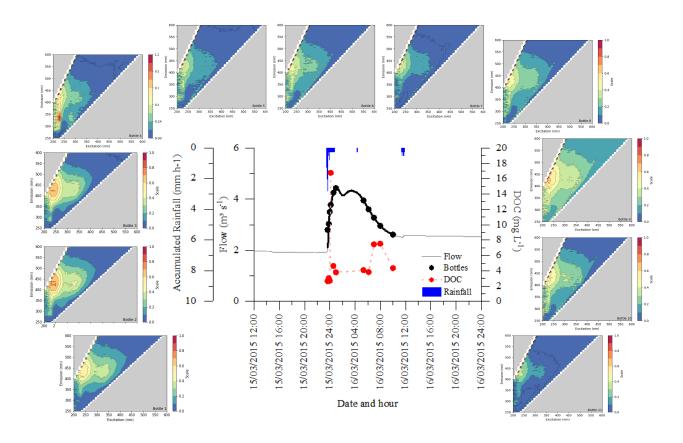


Figure 2: Diffuse pollution event (hydrograph) performed with respective three-dimensional excitation-emission matrices (EEMS) for water samples and DOC concentrations along the event (red points)

According to the results, it is possible to observe that accumulated rainfall occurred strongly producing a fast flow increase. Bottles 1 to 6 represents hydrograph rise, and theoretically receiving the biggest pollutant load. EEM for the first bottle (DOC of 2.62 mg L^{-1}) indicates a tendency of high intensities in peak A and C, despite the low values (0.86 and 0.38 r.u. respectively). Bottles 2 and 3 (with DOC of 3.01 and 2.67 mg L^{-1} , respectively), in sequence, also represent this tendency, increasing r.u. value for peak A. It can be assumed the first bottles were receiving the first-flush





phenomenon, which represents the initial wash off around, carrying refractory fractions of organic compounds into the river. Probably, the biggest fluorescence in A and C regions is due to an organic matter already degraded in soil.

On Nguyen *et al.*, (2013) study analyzed two storm episodes, occurred in summer (S) and fall (F) seasons. DOC concentrations showed a relatively narrow range in event F, from 1.3 to 1.6 mg C/L without any prominent peak. However, in event S, DOC varied from 1.6 to 2.8 mg/L, increasing by 75% from the initial concentration. The maximum value was observed several hours after the peak water level. In comparison with this paper, different conditions were observed, as: i) the DOC concentrations presented bigger values, increasing until 97% from the initial concentrations, and ii) the maximum value observed happened faster during the rainfall episode (bottle 4).

During hydrograph ascension, organic matter concentration remained stable, without significant ranges in EEM regions. This can indicate that organic load was bigger at the beginning of rainfall episode, due to fast water dynamic associated to diffuse substance drained. In the other hand, despite some similarities, in Nguyen *et al.*, (2013) showed higher values from FI value measured on EEM (correspond to peak C in this paper), 1.59 to event S and 1.85 to event F. Authors suggesting that the storm samples may contain the mixed sources of allochthonous and autochthonous. Finally, were highlighted that values tended to decrease with the time, which suggest that the variation may depend on storm characteristics.

However, in bottle 4 an intense peak at region T_2 and a signal in region T_1 showed up, representing labile contribution. Additionally, DOC concentrations was the biggest one in event, with 16.77 mg L⁻¹. This scenario can indicate some unexpected residual compound without intense degradation that had been washed by rainfall. Furthermore, rain intensity can increase effluent available in drainage network input or, even individual treatment systems (septic tank) overflow, and therefore, carrying into the river more labile and concentrated organic compounds.

Stedmon *et al.*, (2007) EEM from sea ice sample observed the occurrence in peak B, corresponding to the protein-like fluorescence component. Likewise, in Meng *et al.*, (2013) study were observed presence of the tryptophan-like and tyrosine-like proteins peak as an indicator of the anthropogenic inputs or the microbial products of the anthropogenic organics in Zhujiang River

In sequence, other bottles (5 to 8) showed all peaks attenuation in EEM and DOC concentrations decrease (4.60, 3.78, 4.06 and 3.78 mg L^{-1}), probably due to large water quantity introduced into the river (approximately 1.48 x 10⁵ m³) causing dilution. In bottle 9 and 10, there was increase in organic matter concentrations another time (also shown in DOC of 7.45 ad 7.51 mg L^{-1} respectively), both labile and refractory compounds. This situation may be related to increase in water body turbulence and water assimilation (hydrograph fall), causing organic matter resuspension.

In the end of the diffuse pollution episode, the last sample showed decrease on regions intensity in EEM and DOC concentration (4.32 mg L^{-1}). Therefore, it is possible that suspended substance had been dragged downstream without a bigger allochthonous organic matter coming up by urban drainage. In general, the organic compounds input during rainfall episodes was short and quickly, at most because of first-flush phenomenon washing-off pollutant and causing dilution effect by water quantity.

In Leithold (2017) study developed at Alto Iguassu Basin involving Iguassu River and four tributaries (*Atuba*, *Palmital*, *Belém* and *Barigui* Rivers) it was performed fluorescence spectrophotometry analysis associated with other parameters to evaluate organic matter. One of the monitoring sites stud-





ied was BA01 without rainfall episodes. EEM of BA01 showed low contribution of labile compounds. The author affirms that BA01 indicated the best environmental conditions, with low concentrations of labile and refractory organic matter, in comparison with other sites monitored in the study. Therefore, the EEM analysis during rainfall episodes was relevant considering diffuse pollution sources contribution which can decrease water body quality.

Some research used EEMs analysis to characterize the aquatic organic matter in different water resources or purpose, as oceans (Stedmon *et al.*, 2007); natural waters (Kalbitz *et al.*, 2000); rivers (Meng *et al.*, 2013; Leithold, 2017; Knapik, 2014), or even than to understand differences between pedogenic and aquagenic organic matter (Mobed *et al.*, 1996) and to evaluate effects of storm events (Nguyen *et al.*, 2013)

In this sense, it is relevant to highlight that EEM demonstrated an important instrument to evaluate (and suggest) organic matter contribution qualitatively. It was possible to recognize labile and refractory fractions. Complementarity, DOC values support the quantitative interpretations. Figure 3 illustrates two EEM presented previously, bottles 2 and 11 respectively.

At first, it is important to observe differences in colors between EEM. Color scale represents the fluorescence intensities (Raman units). Therefore, bottle 2 represents the beginning of the event, where there were refractory compounds input and, because of this, warm colors appeared (close to red in region A and C). On the other hand, bottle 11 represents the end of the event, where dilution effect were observed, and then showing cool colors (shades of blue) at EEM.

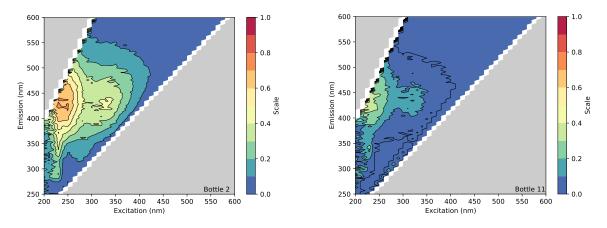


Figure 3: Three-dimensional excitation-emission matrices (EEMS) for water samples in bottle 2 and 11. Color scale represents the fluorescence intensities (Raman units)

Colors in EEM presented here have low intensity. However, in Leithold (2017) study, for a monitoring site at *Atuba* River upstream a sewage treatment station presented an EEM with high concentrations of labile organic matter. Scale ranged from 0 to 3 r.u. indicating higher color intensities in regions B, T_1 and T_2 . Besides, there are other ways to evaluate what kind of organic compound is predominant, as for example, SUVA₂₅₄ and A₂₈₅ relationships which support fluorescence analysis, also investigated in Leithold (2017) and Knapik (2014) studies.

CONCLUSION





Quali-quantity organic matter analysis through fluorescence spectrophotometry and DOC concentrations allowed a deep verification in type and behavior of the compound during diffuse pollution events. Thus, within this study, it was possible to conclude that:

- At begin of the event, first wash-off carried more refractory compounds into the river (bottles 1, 2 and 3).
- Unexpected residual compound had been washed and measured in bottle 4. Possible sources are drainage network input because of rain intensity and/or individual treatment systems, as septic tank.
- The biggest DOC concentrations was of 16.77 mg L⁻¹ in bottle 4, indicating probably a transport of some organic matter residual from upstream sites and/or urban drainage system.
- In the end of the event, dilution effects produced decrease in DOC concentrations and fluorescence intensities.
- Fluorescence spectrophotometry showed a great instrument to evaluate qualitatively organic matter contribution during diffuse pollution episodes.

Therefore, future studies can help evaluate, compare and understand diversity among different compounds and organic matter sources associated to distinct hydrological episodes which produce particular river responses. Finally, it is suggested the use of other relationship, as SUVA₂₅₄ and A₂₈₅ from UV-visible spectrophotometric techniques, to complement classification and indicate organic matter differentiation.

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