HELOISE GARCIA KNAPIK

ORGANIC MATTER CHARACTERIZATION AND MODELING IN POLLUTED RIVERS FOR WATER QUALITY PLANNING AND MANAGEMENT

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"ORGANIC MATTER CHARACTERIZATION AND MODELING IN POLLUTED RIVERS FOR WATER QUALITY PLANNING AND MANAGEMENT"

Tese aprovada como requisito parcial à obtenção do grau de Doutor, pelo Programa de Pós-Graduação em Engenharia de Recursos Hídricos e Ambiental do Setor de Tecnologia da Universidade Federal do Paraná, pela comissão formada pelos professores:

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We think too much and feel too little.
More than machinery we need humanity.
More than cleverness we need kindness and gentleness.
Without these qualities, life will be violent and all will be lost
Charlie Chaplin, 1889-1977

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If you want to build a ship, don't drum up people together to collect wood and don't assign them tasks and work, but rather teach them to long for the endless immensity of the sea.

Antoine de Saint-Exupéry, 1900-1944

To my parents, Bernardo and Terezinha Knapik,

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and all the truly passionate people that believe and fight for a better world

Abstract

In the aquatic environment, the organic matter plays an important role in the production and consumption cycles. However, in urbanized and polluted rivers, the allochthonous anthropogenic organic matter can enhance the water quality deterioration. Thus, this thesis aimed to propose a new strategy for monitoring and modeling the water quality through a quantitative analysis and qualitative characterization and differentiation of the organic matte in a polluted river. The reason for a more comprehensive approach is that the process relating organic matter dynamics in the aquatic ecosystem are not properly represented by the conventional set of parameters commonly used in monitoring plans. The problem relies in the identification of a parameter or group of parameters feasible enough to be used in water quality planning and management, considering monitoring and modeling approaches. The hypothesis is that a better representation and characterization of the organic matter in polluted rivers, based on distinct organic carbon fractions, can improve water quality planning and management actions. Thus, this thesis focused in the characterization, differentiation, and modeling of organic matter.

The methodological approach to achieve the hypothesis aforementioned consisted in the water quality monitoring and modeling in a polluted and urban river. The Upper Iguassu Watershed, located at Curitiba and Metropolitan Region, was used as the case study and a database thorough six monitoring sites. While a large group of conventional parameters were measured, efforts have being made to the measurement of the dissolved, particulate, and total organic carbon (DOC, POC, and TOC, respectively). At the Iguassu River, TOC, DOC, BOD, and COD concentration in a non-impacted site (IG01) was, respectively, 6.3 mgC/L, 4.6 mgC/L, 3.0 mgO_2/L, and 15.0 mgO_2/L. For a human impacted site, IG02, the median observed for the same parameter change to 10.9 mgC/L, 5.7 mgC/L, 17.0 mgO_2/L, and 38.4 mgO_2/L, respectively. Furthermore, site IG01 showed the higher percentage of refractory organic carbon (86% and 84%), and thus, the lower ratios of BOD_5/COD and BOD_5/TOC (0.14 and 0.16, respectively) when comparing molar concentration. For the intermediate sites, the percentage of refractory organic carbon ranged from 40% to 61%.

In addition, UV-visible and fluorescence spectrophotometric techniques were applied to the identification and differentiation of the organic matter properties and sources. The results of fluorescence intensity peaks B, T_2, and T_1, commonly related to the properties of the labile organic matter clearly indicate the presence of anthropogenic allochthonous

organic matter in the intermediate sites (IG02 to IG04). Fluorescence spectroscopy showed to be more representative than other techniques for the organic matter differentiation.

Complementarily, the organic carbon biodegradation was evaluated through an experimental procedure. Both dissolved, particulate, and total organic carbon were continuously measured to identify the biodegradation kinetics rates. Sites IG02 and IG05 presented high values of biodegradable DOC, POC, and TOC consumption during the experimental test. The higher decrease of humic-like fluorescence peak intensity at the polluted sites, compared to site IG01, may indicate that as the labile organic matter is assimilated, the energy released may facilitate the degradation of complex molecules by biological activity.

The results indicated that the integrated analysis of these methods can provide a better understanding in both quantitative and qualitative terms of the organic content, their origin, and how it will be degraded in the aquatic ecosystem. In addition, the measurement of TOC, POC, and DOC were less susceptible to interferences rather than other commonly used parameters in water quality monitoring and modeling. The evaluation of the biodegradation kinectics and the evolution of fluorescence excitation-emission matrix throughout the biodegradation experiment showed relevant considerations about the labile and refractory percentages and decomposition properties.

Complementarily, the proposed modeling approach considered the compartmentalization of the organic carbon primarily in dissolved and particulate fractions, and complementarily in labile and dissolved compounds. The results of the organic carbon modeling indicated that before urbanization (site IG01), the proportion of refractory organic carbon was higher, with RTOC about 39%, while LTOC was 61%, in average. Downstream site IG02, the proportion changed to 22% of RTOC and 78% of LTOC. The process based on labile and refractory organic carbon provided a gain in terms of quantification of the potential compounds that have a direct impact in oxygen depletion and water quality deterioration. In addition, the model structure, process considered, and the automatic calibration algorithm, provided interesting results for the case study analyzed.

Finally, in terms of water quality planning and management, this thesis provided distinct insights for a new set of parameters focusing in monitoring and modeling approaches for urban watersheds.

Key-words: Organic matter; Water quality modeling; Polluted Rivers; Upper Iguassu Watershed.

Resumo

No ecossistema aquático, a matéria orgânica exerce uma importante função no processo de ciclagem de nutrientes. No entanto, em rios urbanizados e poluídos, a qualidade da água pode sofrer ainda mais impactos negativos em função de fontes antrópicas de matéria orgânica. Dentro deste contexto, a presente tese propõe uma nova estratégia para o monitoramento e modelagem da qualidade da água com base em análises quantitativas e qualitativas para a caracterização e diferenciação da matéria orgânica em rios poluídos.

A razão para esta abordagem diferenciada é a de que os processos que regem a dinâmica da matéria orgânica no ecossistema aquático não são adequadamente representados apenas por parâmetros tradicionalmente utilizados no monitoramento da qualidade da água. Faz-se necessário a identificação de um parâmetro ou conjunto de parâmetros que sejam potencialmente aplicáveis tanto no monitoramento, como na modelagem matemática, tendo como foco o planejamento e a gestão integrada e sustentável da qualidade da água. Desta forma, a hipótese da presente tese é a de que uma melhor caracterização e quantificação do teor de matéria orgânica em rios poluídos, tendo como base as distintas frações de concentração de carbono orgânico, podem contribuir positivamente em novas estratégias de planejamento e gerenciamento da qualidade das águas. Assim, esta tese teve como enfoque o monitoramento, a caracterização, a diferenciação e a modelagem dos teores de matéria orgânica em rios poluídos.

A abordagem metodológica teve como principais atividades o monitoramento e a modelagem da qualidade da água em um rio urbanizado e poluído. A Bacia do Alto Iguaçu, localizada na cidade de Curitiba e Região Metropolitana, foi utilizada como estudo de caso. Esta bacia configura como uma importante área de desenvolvimento urbano em uma região com características hidrográficas importantes para a região sul do Brasil. O monitoramento foi realizado no rio Iguaçu, em seis estações de monitoramento abrangendo áreas preservadas, áreas com elevado desenvolvimento urbano, e áreas em recuperação.

A base de dados contemplou tanto parâmetros tradicionalmente utilizados no monitoramento de campo, como parâmetros específicos para a determinação da matéria orgânica. Assim, foram determinadas as frações de carbono orgânico dissolvido (DOC), carbono orgânico particulado (POC) e carbono orgânico total (TOC). A concentração de TOC, DOC, demanda bioquímica de oxigênio (BOD) e demanda química de oxigênio (COD) para o trecho com menos impactos devido à urbanização (IG01), apresentaram uma

concentração média de 6.3 mgC/L, 4.6 mgC/L, 3.0 mgO_2/L e 15.0 mgO_2/L, respectivamente. Para o ponto IG02, cuja área de drenagem engloba regiões com alta densidade demográfica, a concentração média foi, respectivamente, igual a 10.9 mgC/L, 5.7 mgC/L, 17.0 mgO_2/L e 38.4 mgO_2/L. Adicionalmente, o ponto IG01 apresentou as menores razões entre as concentrações molares de BOD_5/COD e BOD_5/TOC (0.14 e 0.16, respectivamente), e, consequentemente, as maiores frações de carbono orgânico refratário (86% e 84%). Para o trecho intermediário, a porcentagem de carbono orgânico refratário variou de 40% a 61%.

Complementarmente, com o objetivo de avaliar qualitativamente características sobre o teor lábil e refratário, e identificar possíveis fontes de matéria orgânica, foram também utilizadas técnicas de espectroscopia de ultravioleta visível e de fluorescência. Os resultados das intensidades de fluorescência dos picos relacionados à presença de matéria orgânica lábil, B, T_2 e T_1, indicaram uma maior presença de matéria orgânica alóctone no trecho intermediário do rio (IG02 a IG04). Comparando com os resultados de absorbância no ultravioleta visível, os resultados da espectroscopia de fluorescência foram mais representativos para a diferenciação qualitativa da composição da matéria orgânica.

Em três pontos de monitoramento (IG01, IG02 e IG05) foram também estimadas as taxas de decomposição biológica das frações de carbono orgânico. Para esta estimativa, foi proposto e avaliado um procedimento experimental, com incubação de amostras em condições controladas de temperatura e luminosidade. Os pontos de monitoramento IG02 e IG05 apresentaram os valores mais elevados de carbono biodegradável, com maior consumo da concentração inicial de DOC, POC e TOC durante o período de incubação. Ainda, houve um decréscimo mais acentuado da intensidade de fluorescência dos picos referentes a substâncias húmicas (A e C) nos pontos com maior teor de poluição orgânica, indicando, provavelmente, que à medida que a matéria orgânica lábil é assimilada, a energia liberada pode facilitar a degradação biológica de moléculas mais complexas.

Os resultados do monitoramento e do experimento de biodegradação do carbono orgânico confirmam que a abordagem integrada de diferentes parâmetros quanti-qualitativos é eficaz para avaliar os teores de matéria orgânica, sua origem e os processos de decomposição no ecossistema aquático. Complementarmente, é importante destacar que a determinação do DOC, POC e TOC é menos susceptível a interferências quando comparado com outros parâmetros tradicionalmente utilizados, como a demanda bioquímica de oxigênio. Ainda, com a avaliação da cinética de biodegradação e a respectiva evolução nos espectros de excitação-emissão de fluorescência durante o procedimento experimental foi possível

identificar as porcentagens lábil e refratária, além dos perfis de decomposição em função das frações de carbono orgânico.

Como terceira parte do desenvolvimento desta tese, foi proposto e implementado um modelo para a simulação do transporte e decaimento da matéria orgânica em rios. O modelo proposto teve como base a segmentação do carbono orgânico nas frações dissolvida e particulada, considerando compostos lábeis e refratários. No trecho a montante do ponto de monitoramento IG01, caracterizado pela baixa ocupação urbana, a proporção de carbono orgânico refratário e lábil foi, respectivamente, 39% e 61%. No trecho a jusante do ponto IG02, o teor da fração lábil foi superior, com uma média de LTOC igual a 78% e RTOC de 22%. Os resultados indicam que a hipótese adotada em relação às frações lábil e refratária proporcionou um ganho em termos da quantificação dos compostos com potencial para impactar diretamente a concentração de oxigênio dissolvido através da decomposição da matéria orgânica. É importante destacar que a plataforma gráfica desenvolvida, os processos considerados e o algoritmo de calibração automática com base em processos de otimização forneceram resultados interessantes e construtivos para o estudo de caso avaliado.

Finalmente, em termos de planejamento de gestão da qualidade da água, esta tese tem como contribuições a avaliação do uso de diferentes parâmetros focando em melhores estratégias para o monitoramento e modelagem da qualidade da água em bacias urbanas.

Palavras - chave: Matéria orgânica; Modelagem da qualidade da água; Rios poluídos; Bacia do Alto Iguaçu

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Abbreviations

A_{285}/COD: Specific ultraviolet absorbance in the wavelength 285 nm normalized by dissolved organic carbon (g/L).

AOC: Assimilable organic carbon

AROM: Aquagenic refractory organic matter

BDOC: Biodegradable dissolved organic carbon

BOD: Biological oxygen demand

BOD_5: Five day biological oxygen demand

BOM: Biodegradable organic matter

BPOC: Biodegradable particulate organic carbon

BTOC: Biodegradable total organic carbon

CBOD: Carbonaceous biological oxygen demand

CDOM: Chromophoric dissolved organic matter

CE: Computational element

COD: Chemical oxygen demand

DIC: Dissolved inorganic carbon

DO: Dissolved oxygen

DOC: Dissolved organic carbon

DOM: Dissolved organic matter

EEM: Excitation-emission matrix

EfOM: Effluent organic matter

FDOM: Fluorescence dissolved organic matter

FR: Fluorescence ratio. Ratio between the intensities of fluorescence emitted at 450 nm and 500 nm wavelengths, with an excitation of 370 nm.

LC-OCD: Liquid chromatography with on-line carbon detection

LDOC: Labile dissolved organic carbon

LMWDOM: Low molecular weight dissolved organic matter

LPOC: Labile particulate organic carbon

NDIR: Nondispersive infrared detector

NMR: Nuclear magnetic resonance

NOM: Natural organic matter

PARAFAC: Parallel Factor Analysis

POC: Particulate organic carbon

POM: Particulate organic matter

PROM: Pedogenic refractory organic matter

PSO: Particle Swarm Optimization

RDOC: Refractory dissolved organic carbon

ROCS-Model: River Organic Carbon Simulation Model

RPOC: Refractory particulate organic carbon

SEC-DOC: Size-exclusion chromatography with DOC detection

SOD: Sediment oxygen demand

$SUVA_{254}$: Specific ultraviolet absorbance at 254 nm wavelength normalized by dissolved organic carbon (mg/L) and corrected by optical path (m). Unit: L/mg.m.

TC: Total carbon

TIC: Total inorganic carbon

TN: Total nitrogen

TOC: Total organic carbon

UASB: Upflow anaerobic sludge blanket digestion reactor

UV-Vis: Ultra violet visible absorbance

WWTP: Wastewater Sewage Treatment Plant

List of Symbols

A_c	Cross-sectional area of the reactor, m^2
A_x	Cross-section area, m^2
B_0	Bottom width, m
O_s	Saturation concentration of dissolved oxygen, mg/L
$Q_{in,i}$	Total inflow to the CE i, m^3/s
$Q_{nps,i,j}$	The jth non-point source inflow to the CE i, m^3/s
$Q_{out,i}$	The total outflow from the CE i, m^3/s
$Q_{ps,i,j}$	The jth point source inflow to the CE i, m^3/s
$Q_{ps\_out,i,j}$	The jth point source outflows from the CE i, m^3/s
R_h	Channel hydraulic radius, m
S_e	Slope of the channel, m/m
K_1	Deoxygenation rate coefficient, d^{-1}
K_2	Reaeration rate, d^{-1}
K_3	Rate of loss of carbonaceous BOD due to settling, d^{-1}
K_4	Sediment oxygen demand rate, gm^{-2} d^{-1}
K_{ai}	Ammonia oxidation rate coefficient, d^{-1}
K_h	Hydrolysis of DOC, d^{-1}
K_{in}	Nitrite oxidation rate coefficient, d^{-1}
K_{L1}	Settling of LPOC, d^{-1}
K_{L2}	Resuspension of LPOC, gm^{-2} d^{-1}
K_{L3}	Mineralization of LPOC, d^{-1}
K_{L4}	Decay of LPOC to RPOC, d^{-1}

K_{L5}	Decay of LPOC to LDOC, d^{-1}
K_{L6}	Decay of LPOC to RDOC, d^{-1}
K_{L7}	Decay of LDOC to RDOC, d^{-1}
K_{L8}	Mineralization of LDOC, d^{-1}
K_{oa}	Organic nitrogen hydrolysis rate, d^{-1}
K_{os}	Organic nitrogen settling rate, d^{-1}
K_p	Particulate organic carbon dissolution rate, d^{-1}
K_{pd}	Organic phosphorus decay rate, d^{-1}
K_{ps}	Organic phosphorus settling rate, d^{-1}
K_{R1}	Settling of RPOC, d^{-1}
K_{R2}	Resuspension of RPOC, gm^{-2} d^{-1}
K_{R3}	Mineralization of RPOC, d^{-1}
K_{R4}	Decay of RPOC to RDOC, d^{-1}
K_{R5}	Mineralization for RDOC, d^{-1}
K_s	Particulate organic carbon settling rate, d^{-1}
L_i	Concentration of carbonaceous BOD at time *i*, mg/L
J_{in}, J_{out}	Flux of mass in and out the element due transport, $ML^{-2}T^{-1}$
$Nin, Nout$	Total number of inflows and outflows for each CE
n	Manning's roughness coefficient.
NH_3, N_a	Ammonia concentration, mgN/L
NO_2^-, N_n	Nitrite concentration, mgN/L
NO_3^-, N_1	Nitrate concentration, mgN/L
N_{org}	Organic Nitrogen
P_{diss}	Inorganic dissolved phosphorous concentration, mgP/L

P_{org}	Organic phosphorous concentration, mgP/L
Pop_{reach}	Population from each reach
QPC	Per capita flow, L/inhab.d
Q_{dom}	Domestic effluent flow, L/s
R	Ccoefficient of sewage return
S	Side slope
TD	Temperature dependent
U	Velocity, LT^{-1}
W_{capita}	Per capita load, g/inhab.d
W_{dom}	Load from non-treated effluent, kg/d
α_5	Rate of oxygen uptake per unit of ammonia nitrogen oxidation, mg-O/mg-N
α_6	Rate of oxygen uptake per unit of nitrite nitrogen oxidation, mg-O/mg-N

Chapter 1

Introduction

"Determining the ecosystem health and water quality of a river requires identification of both natural and anthropogenic influences on aquatic biogeochemistry. Organic matter is a critical component of many nutrient and trace metal biogeochemical cycles, and can significantly affect ecosystem biological processes and water quality."

Goldmand, J H. et al., 2012. Applications of fluorescence spectroscopy for predicting percent wastewater in an urban stream.
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1.1 Introduction

The organic matter is a complex mixture of organic compounds present in all surface, ground, and soil waters. It occurs naturally as part of the food web and in the process of nutrient cycling. Both the particulate and dissolved fractions are important components in the organic matter dynamics and act as a source of energy in aquatic ecosystems (Thurman, 1985). The concentration of organic matter influences the structure

of aquatic communities and characteristics such as pH-buffering, alkalinity, light attenuation, distribution of ions between aqueous and solid phases, and formation of colloidal particles (Mulholland, 1997; Thurman, 1985; Krusche et al., 2002; Nebbioso and Piccolo, 2012).

The decomposition of organic matter can decrease significantly the concentration of dissolved oxygen, and, consequently, increase the water quality deterioration problems. In addition, dissolved organic matter (DOM) can enhance the solubility and mobility of metals and other organic compounds (Perdue et al., 1979; Thurman, 1985) and interfere in the pollutant transport and micronutrient availability.

Anthropogenic inputs such as sewage discharge and non-point sources may affect the quantity and quality of DOM, and thus, the organic carbon concentration in rivers. Besides an overall broad concern and a recurrent focus of investigation (Kalscheur et al., 2012; Meng et al., 2013), it is still unclear the ecological consequences due to the different sources and amount of organic matter released in polluted rivers, and how it impacts the biogeochemical cycling of organic carbon and other nutrients in the aquatic environment.

The presence of organic matter in the aquatic environment is also important for the carbon flux between the air-water interface. When the respiration rate exceeds CO_2 fixation by phytoplankton, aquatic systems, especially lakes and oceans, become sources of CO_2, contributing to the increase of this constituent in the atmosphere and possible effects of global climate change (Fisher and Likens, 1972; Pers, 2000). Rivers constitute an important transport of organic matter to the oceans, especially those with overexploitation of resources and major releases of effluents. Additionally, another carbon flux observed in rivers can be accounted by the transport of sediments, and, in the presence of industrial activity or areas of mineralization, it can also transport adsorbed heavy metals.

An interesting strategy to study the organic matter dynamics in aquatic ecosystem is through water quality mathematical modeling. Through the representation of the streamflow characteristics and the mass transport mechanism, it is possible to identify and evaluate the behavior and the interactions between different compartments and the spatial and temporal variation in the river. However, most part of water quality models do not consider the organic carbon as a state variable (Shanahan et al., 1998; Somlyódy et al., 1998; Chapra; 1999). This lack of representation between the different fractions of organic carbon may influence the way that the decomposition process are calculated, with direct or indirect impact in overall understanding of the organic matter dynamics in the water column.

Additionally, the existence of incomplete data base and an effective monitoring plan of organic matter in aquatic ecosystems can also interfere the understanding of its dynamics and the development and validation of water quality simulation models (Pers, 2000). Different authors studied the organic matter characterization in the aquatic ecosystem (Wangersky, 1992; Chair et al., 1993; Mantovani and Novo, 1996; Imai et al., 2001; Krusche et al., 2002; Carstea et al., 2010). However, there still no consensus on the relationship between organic carbon and other traditional parameters currently used in monitoring strategies, especially considering a high urbanized basin. This analysis is generally presented in a simplified manner in the literature (Sharp, 1993; Chapra, 1999), with few studies that dedicates efforts to define what, why, and how the organic carbon dynamics can be applied on water quality planning and management.

1.2 Significance of Thesis

The organic matter is a complex mixture of compounds originated from different sources. Once in the aquatic environment, and considering the interactions between physical, chemical, and biological processes, different degradation mechanism affects the consumption and production rates. If the composition of the organic content is refractory, a slow impact may be observed in terms of oxygen consumption. Contrarily, if the composition of the organic content is labile, the decomposition rates increases, which implies in a rapid consumption of the dissolved oxygen and consequent water quality deterioration.

In terms of surface water quality modeling, these implications are particularly relevant to the selection of the variable used to describe the system. However, currently worldwide models used in water quality planning and management are mainly based on Biochemical Oxygen Demand (BOD) and Dissolved Oxygen (DO) mass balance, which, according to this thesis hypothesis, do not represent adequately the organic matter dynamics in the aquatic ecosystem.

Thus, this thesis establishes a starting point for a better understanding of how the organic carbon can be used to represent the overall process that may occur in a polluted river. Additionally, how do human activities affect the quantity and quality of organic carbon in rivers, and what are the ecological consequences due to different sources and amount of organic carbon released to the aquatic ecosystem. A strategy to this evaluation

lead to questions such as: "What is the relative importance of allochthonous organic carbon in aquatic ecosystem"? "What is the meaning of organic carbon as a monitoring parameter in the water quality characterization"? "What is the proportion of organic carbon considering the anthropogenic allochthonous organic matter"? "How to characterize and differentiate between autochthonous and allochthonous organic matter in human dominated basins"?

Complementarily, to achieve the causes and consequences of the natural and anthropogenic sources of organic carbon in urbanized and polluted rivers, and to investigate its overall dynamics, a mathematical model is also an important methodological approach. The advantage of using mathematical models to represent the transport mechanism and biochemical process is to analyze in an integrated manner the effects of different scenarios and evaluate better actions for water resources planning and management.

In the context of this thesis, a mathematical model is a fundamental step to advance in the water quality evaluation in polluted basins. Consequently, some questions that arise from this strategy are: "What is the difference between the dynamics of organic matter in rivers and other aquatic environments"? "Is the current database sufficient to model the processes involved in organic matter dynamics consistently"? "Is organic carbon a better variable to model and to represent the organic matter dynamics in a river"?

Thus, this thesis focus on answer these questions in order to better understand the organic matter dynamics in rivers and to develop, implement, and evaluate the advantages and limitations of a mathematical model to simulated the related physical, chemical, and biological processes.

1.3 Thesis Objectives

The overarching goal of this thesis is to propose a strategy for monitoring and modeling the river water quality through a quantitative analysis and qualitative characteristics of the organic matter in a polluted river. The hypothesis is that a better representation and characterization of the organic matter present in the water column, based on distinct organic carbon fractions and the respective identification as function of its origin, can produce changes in water quality planning and management actions. Thus, the original contribution of this study is to understand, identify, quantify, and utilize the

different fractions of organic carbon as state variables in a surface water quality model considering the transport, decay, and interaction with other variables.

In order to achieve this main goal, two complementary groups of specific goals were established. The first group is related to the study and quantification of the organic matter in an urbanized river. In this research, different procedures are proposed to evaluate the water quality and to identify the organic matter origin, composition, and degradation mechanisms in the water column. This includes the determination of the particulate and dissolved fractions of organic carbon, the main fluorescence and absorbance characteristics, and the biodegradation rates. The spatial and temporal variability and the relationship with conventional water quality parameters used in monitoring programs will be further evaluated.

The second group of specific goals of this thesis is to develop a mathematical algorithm considering different fractions of organic carbon as the main state variable in a surface water quality model. This approach implies considering the transport, decay and chemical process. The results from the monitoring strategy will be used to evaluate the model functionality. Additionally, an adapted particle swarm optimization algorithm is proposed as the automatic calibration tool for the model developed.

Finally, this thesis aims to consolidate a strategy to define why and how the organic carbon dynamics can be applied on water quality planning and management. The motivation is to evaluate the possible implications, limitations, and the potential of the use of organic carbon as the main parameter in a water quality monitoring strategy and as a state variable in a water quality simulation model and if it is the right parameter to promote a change in water resources planning, management and legislation in high urbanized basins.

1.4 Thesis Methodological Approach

The methodological approach of this thesis consists in three interconnected branches (Figure 1): (i) urban basin management; (ii) field monitoring; and (iii) mathematical modeling. In a broader sense, these three branches consolidate the basis of the water quality planning and management strategies proposed by this thesis.

The questions originated from currently urban basin management experiences represent the initial motivation for this study. Thus, the methodological approach

considered for this thesis aims to first evaluate the weaknesses and the potentialities of a different parameter to represent and evaluate the organic pollution in an urban basin.

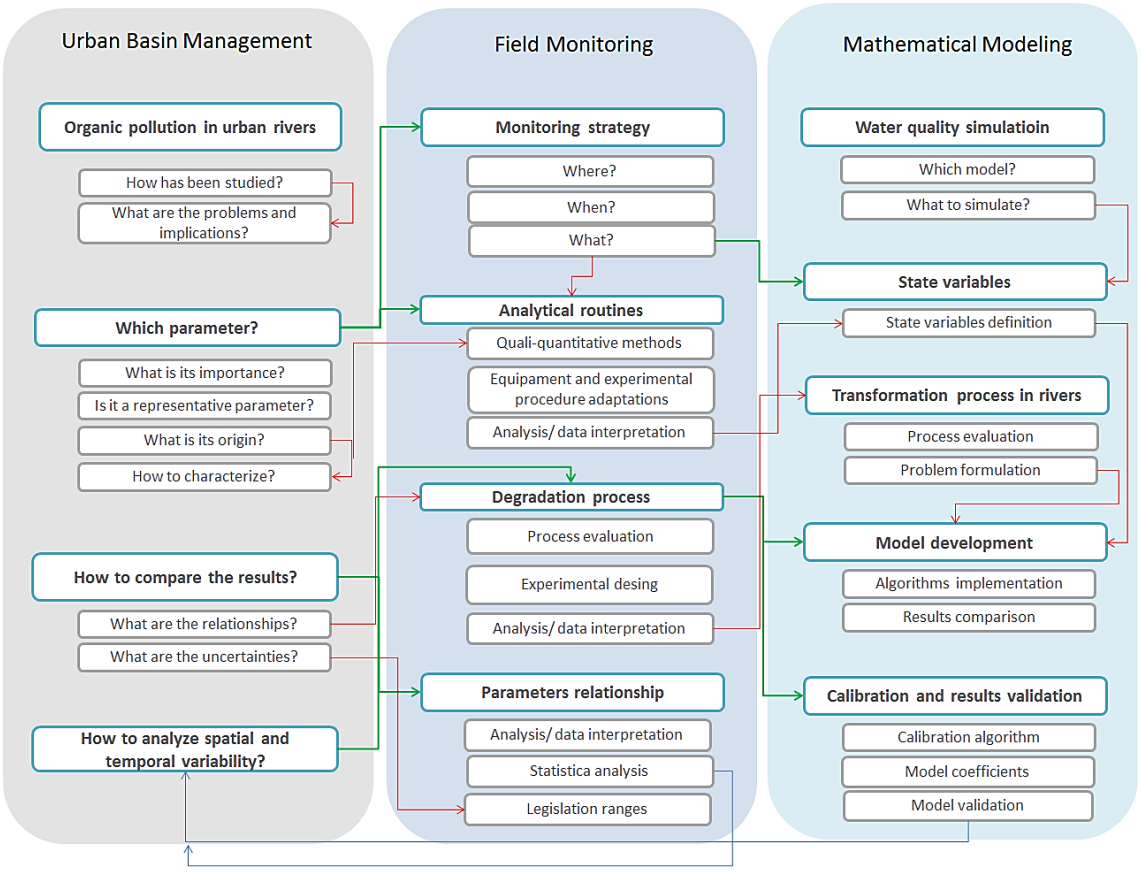


Figure 1: Schematic representation of thesis methodological approach

To support this investigation, a field monitoring was planned and executed in the Upper Iguassu Watershed, the case study proposed for this analysis. The monitoring strategy contemplated four main activities: (i) definition of the monitoring strategy; (ii) implementation of analytical routines; (iii) determination of degradation process; and (iv) evaluation of parameters relationship.

The monitoring strategy focused on criteria for monitoring sites location, frequency of analysis and the parameters analyzed. Considering this thesis hypothesis, different analyses for organic matter determination were conducted. Thus, new analytical routines were necessary to be implemented in order to quantify and characterize the organic matter content, such as the determination of organic carbon fractions (total, particulate, and dissolved), spectroscopic analysis and biodegradability tests. In addition, a

conventional set of parameters were also monitored for the overall classification of the river water quality.

Considering the evaluation of organic matter degradation process, a biodegradability experiment was planned for three sites monitored. For this analysis, an adapted method based on BDOC tests (Biodegradable Dissolved Organic Carbon) was tested and implemented for organic matter degradation.

The integrated analysis of the data interpretation, parameters relationship, and statistical evaluation is the last part of the field monitoring activity. The results of this part of the thesis are essential for the understanding the organic matter dynamics in a high polluted basin, as is the case of Iguassu River.

The mathematical modeling is the third part of this thesis. The definition and implementation of a simulation model is a key issue to integrate the urban basin management problem and the new strategies for field monitoring. It implies a methodological approach related to model formulation, hypothesis testing and considerations about the transformation mechanism, algorithms development and implementation, calibration, simulation, and validation of the proposed model.

1.5 Thesis Organization

The structure of this document is composed by a theoretical consideration on organic matter concepts and dynamics in aquatic systems and by the model developed and the results for the monitored data. In terms on organization, 8 chapters were defined to support the thesis evolution.

A brief introduction for the problem considered by this thesis, its significance, objectives, and methodological approach are presented in chapter 1. The motivation and the thesis main contribution are highlighted within this chapter. In addition, the following chapter introduces the Upper Iguassu Watershed, the case study proposed by this study. This basin has a complex problem of organic pollution, and, consequently, is a classic example of a challenging urban planning and management.

Chapters 3 and 4 present the theoretical basis for the organic matter characterization, transport mechanisms, sources evaluation and the modeling approach. A detailed description about the analytical methods for organic matter characterization, the

challenges in water quality planning and management, and the surface water quality modeling historical approach are analyzed.

Chapter 5 introduces the proposed model. It is presented a description of the model main features, interface, process formulation, and the algorithms implemented. Additional material about the proposed model is described in appendix 1.

The results for the monitored and experimental data and the proposed model are summarized in the Chapter 6. This chapter is composed by three main sections: (i) Water quality assessment; (ii) Biodegradability of organic carbon; and (iii) The modeling approach. The first part focused in an overall description and discussion about the water quality in the Iguassu River, considering the data from an extended monitoring performed at six sites along the main river. The second part summarized the experimental procedure and the main results of the biodegradation experiment. Finally, the last part of the chapter presents the model simulations, the tests to evaluate model performance, the calibration results and final analysis in the overall strategy considered in this thesis development.

Chapter 7 summarizes the final conclusion and this thesis main contribution. Complementarily, issues for further work and research are briefly discussed.

Chapter 2

The Urban Problem and Water Resources Planning and Management Strategies: The Upper Iguassu Watershed

"We emphasize that given our poor understanding of the ecological consequences of these ongoing DOC changes in human-dominated basins, substantially more research and management attention needs to be directed towards this ongoing environmental transformation".

Stanley, E. H. et al. 2012 Contemporary changes in dissolved organic carbon (DOC) in human-dominated rivers: is there a role for DOC management?
Freshwater Biology, v. 57 (1), 26-42.

2.1 Overview

Water pollution problems, in special those encountered in high urbanized watersheds, are substantially magnified in Brazil during the last decades. Efforts are needed to improve the environmental policies and strategies for planning and management actions. Thus, to support a

different approach that effectively will be applied to a sustainable development of a watershed, improved methods for investigation and evaluation of river water quality are required.

In the context of water quality planning and management, the fast urban development has not being followed by sustainable actions. Problems have been encountered in sewage collection and treatment, urban drainage systems, land use delineation, and maintenance of conservation areas.

Additionally, considering the database used for water quality planning and management strategies, some problems can be highlighted. First, there is a lack of reliable data for both quantitative and qualitative information. A second problem is related to the area covered by the monitoring and the frequency in which it is performed. In addition, the group of parameters used for water quality evaluation is, in some cases, not representative of the main causes of the pollution.

The Upper Iguassu Watershed is a classic example of these problems. The river water quality deterioration has reached a critical level. Significant amounts of financial resources are necessary to recover the system and to provide a long term sustainable improvement. In addition, besides, there is a long history of quantitative and qualitative monitoring programs at the Upper Iguassu Watershed, it is still a challenge to integrate all these data to evaluate and understand the dynamics of organic pollution in an urban river.

Thus, the main goal of this chapter is to present the Upper Iguassu Watershed as the main protagonist for the water quality problem considered by this thesis. This watershed has a complex and multivariate pollution sources matrix, and, consequently, an interesting and challenging water quality dynamics. This implies and demands in a new strategy of monitoring and evaluation of data base.

To support this strategy, this chapter is organized with the aim to highlight the watershed main characteristics related to the pollution sources matrix, considering land use and demographic aspects and the historical monitoring approach that have been realized at the Upper Iguassu Watershed. It is also presented an overview of the sites along the Iguassu River selected for the water quality evaluation.

Finally, the motivation for this approach, and to use the Upper Iguassu Watershed as a case study, is to analyze the impacts of organic pollution in urban rivers. It is expected that this strategy could provide new guidelines and the development of applicable tools for a sustainable watershed planning and management.

2.2 The Upper Iguassu Watershed

The Upper Iguassu Watershed is located in the East part of Iguassu Watershed, an important watershed at Parana State, Southern Brazil. The main river, Iguassu, has a total extension of about 1,275 km (approximately area of 68,700 km²), being 107 km located in the Upper Iguassu Watershed (approximately area of 2,700 km²) (Porto et al., 2007). Within its boundaries are located Curitiba (State capital) and other 14 municipalities. Figure 1 presents its location including the hydrographic representation and the municipality's segmentation. Complementarily, Table 1 summarizes the total number of inhabitants and the area of each municipality located within the Upper Iguassu Watershed boundaries.

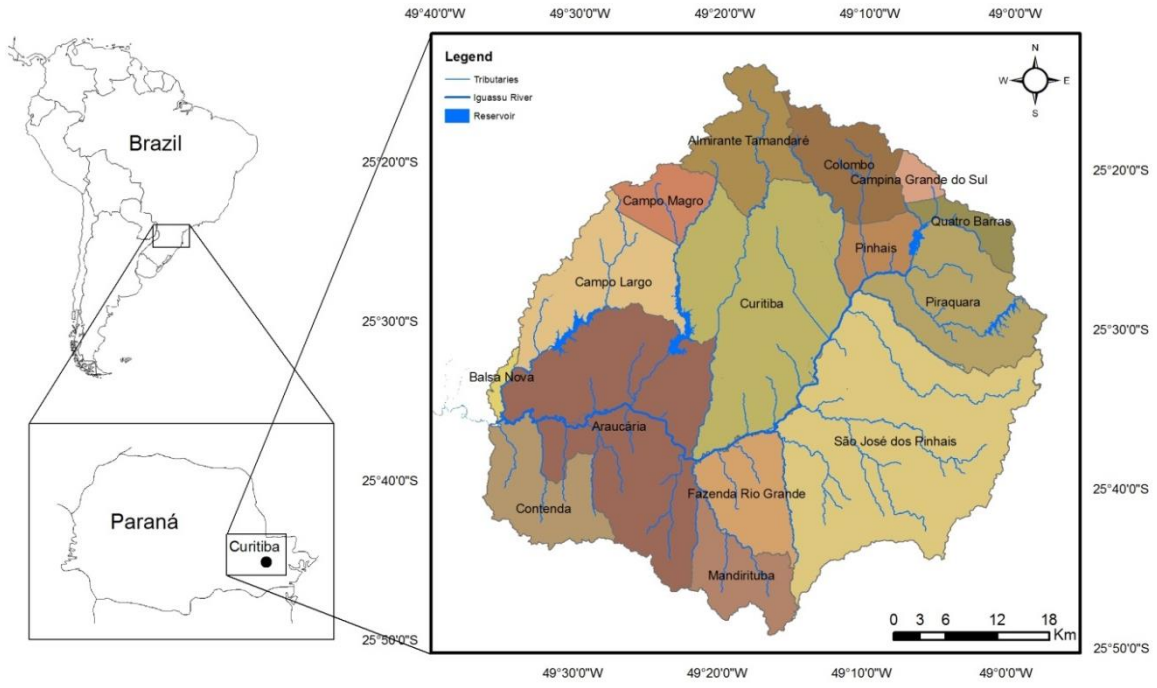


Figure 1: Location of Upper Iguassu Watershed and the 15 cities located within its boundaries.
(Source: Adapted from Águas Paraná, 2014)

Currently, about 2.8 million people reside within the watershed. The demographic density is not uniform, being a result from the hydrographic configuration of the watershed. The watersheds located at the right side of Iguassu River were first colonized, and represents nowadays the most part of the watershed urbanized areas. Atuba, Belém, and Barigui watersheds represent three important drainage area of Curitiba, and together comprise about

63% of the total number of inhabitants. Figure 2 presents the location of the main watersheds (Color scale indicates the crescent number of inhabitants). Table 2 summarizes the main watersheds and the respective drainage area and population.

Table 1: Number of inhabitants and area of each municipality located at Upper Iguassu Watershed

Municipality	Inhabitants[(a)]	Area (km²)[(b)]	Municipality	Inhabitants[(a)]	Area (km²)[(b)]
Almirante Tamandaré	108,168	130	Curitiba	1,735,401	435
Araucária	107,926	469	Fazenda Rio Grande	83,800	115
Campina Grande do Sul	36,291	21	Mandirituba	7,491	103
Campo Largo	90,044	250	Pinhais	116,824	61
Campo Magro	25,885	70	Piraquara	80,390	208
Colombo	215,955	118	Quatro Barras	18,995	40
Contenda	7,256	147	São José dos Pinhais	230,144	601
Total				*2,864,570*	*2,768*

[(a)] Total inhabitants for 2005 for each municipality; [(b)] Area within the Upper Iguassu Watershed boundaries. Source: Porto et al., (2007).

Table 2: Number of inhabitants and area of each municipality located at Upper Iguassu Watershed

Watershed	Inhabitants[(a)]	Area (km²)	Watershed	Inhabitants[(a)]	Area (km²)
Atuba	496,554	144.7	Mascate	52,198	25.9
Barigui	645,142	267.35	Mauricio	9,583	138.3
Belem	690,684	89.51	Miringuava Mirim	3,432	113.7
Cambuí	87,450	33.73	Miringuava	20,208	162.9
Canal Paralelo	50,614	21.91	Padilha	205,311	31.17
Cotia	5,765	152.5	Palmital	219,264	95.27
Despique	2,184	71.43	Passaúna	106,853	217.4
Divisa	58,745	20.47	Pequeno	123,890	137.7
Faxinal	1,329	60.09	Pianduva	780	30.8
Iraí	42,687	145.03	Piraquara	0	102.2
Iraizinho	66,792	52.81	Ressaca	92,588	15.04
Isabel Alves	6,401	64.23	Rio das Onças	1,562	76.5
Itaqui	41,136	45.08	Verde	18,165	173.06
Iguassu	172,315	241.58			
Total				*3,221,631*	*2,730*

[(a)] Total inhabitants for 2010 for each watershed. (Source: Porto et al., 2007)

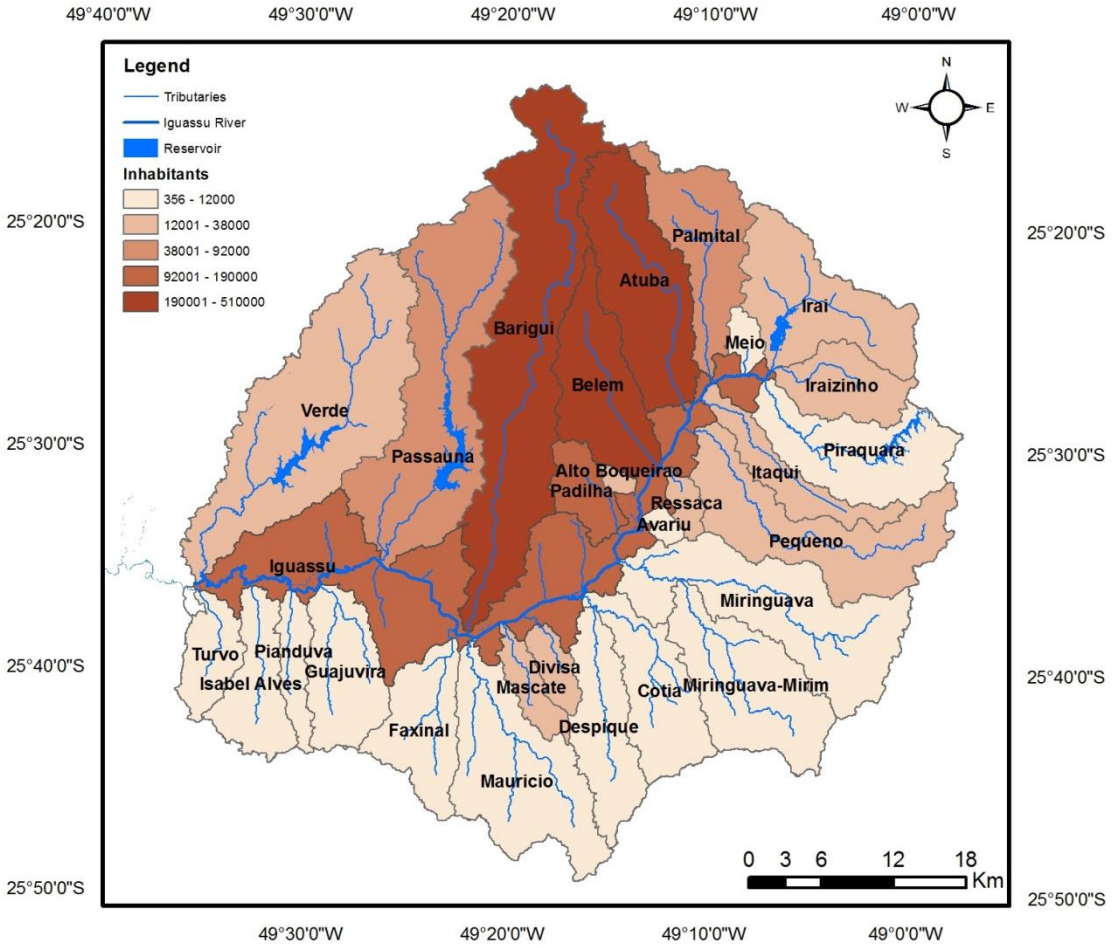


Figure 2: Location of the main watersheds in the Upper Iguassu Watershed. Color scale indicates the crescent number of inhabitants.

(Source: Adapted from Águas Paraná, 2014)

The demand for wastewater treatment is higher than the currently capacity of the 19 municipal waste water treatment plant (WWTP) in operation at the Upper Iguassu Watershed. The Upflow Anaerobic Sludge Blanket digestion Reactor (UASB) is the most common treatment (17 WWTP), followed by lagoon (2 WWTP), drying bed, flotation, and activated sludge (1 WWTP of each). Table 2 and Figure 3 summarize the main WWTP located at the watershed and the release site of the treated effluent (Iguassu River or a tributary). An average of 60% of the daily wastewater (daily production about 168 tBOD/day) is collected. From the total that is collected, 89% of the wastewater is treated, with an average of 70% of organic loads removal efficiency (Porto et al., 2007).

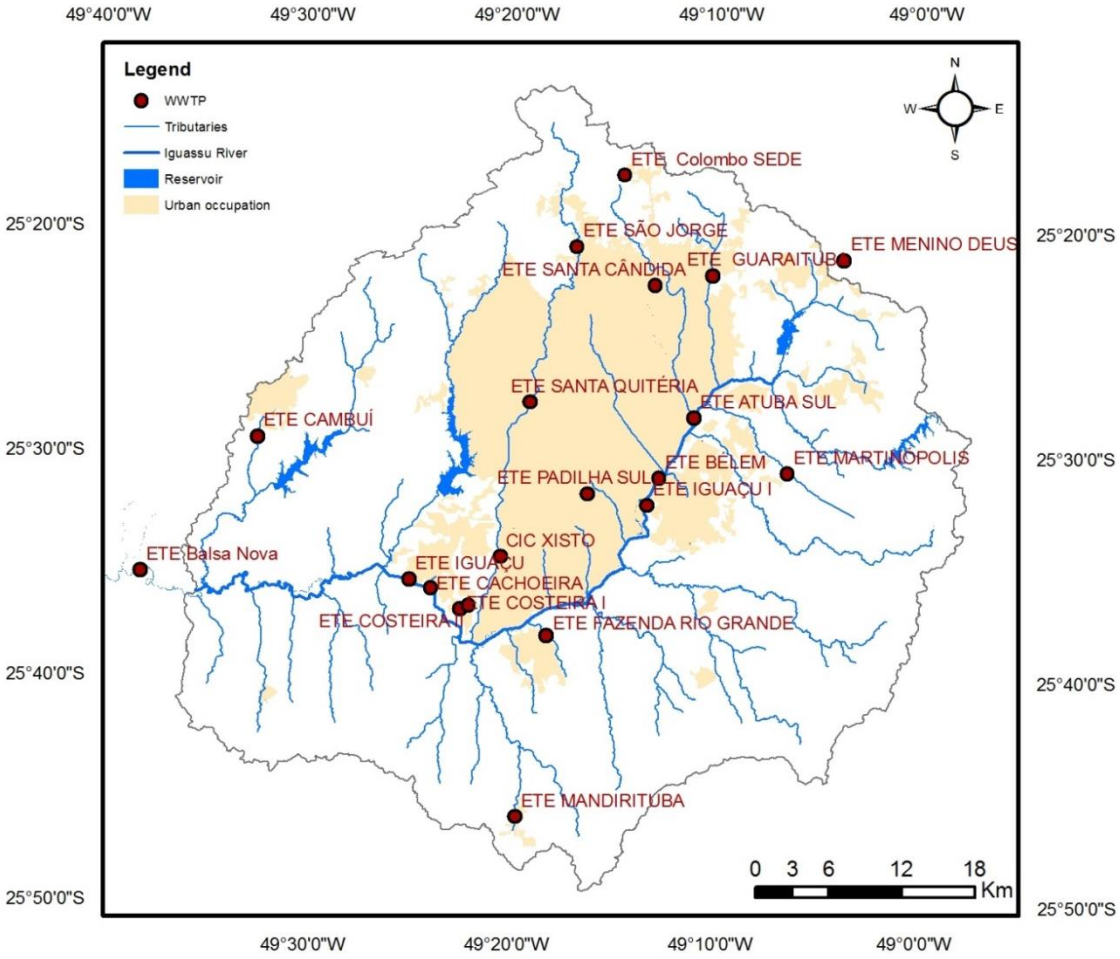


Figure 3: Location of the main WWTP in the Upper Iguassu Watershed.

(Source: Adapted from Águas Paraná, 2014)

The most part of the industries are located at Barigui, Belém, Padilha, Passaúna, and Atuba Watersheds. Although there is an important industrial development, the effluent in terms of organic loads (Biochemical Oxygen Demand), is significantly lower than the amount of domestic sources. For example, according to an estimative by Porto et al. (2007), the total load of industrial BOD is equivalent to a population of 35,000 inhabitants (57 tBOD/month). Thus, the domestic organic pollution, as a result of the low rates of wastewater collection and treatment, can be considered as the predominantly source of anthropogenic organic matter through Iguassu River and its main tributaries.

Table 3: Summary of main WWTP located within the Upper Iguassu Watershed

Municipality	River	WWTP Identification	Treatment Type
Almirante Tamandaré	Barigui	ETE São Jorge	UASB
Aracária	Iguassu	ETE Cachoeira	UASB/ Drying bed/ Lagoon
Araucária	Iguassu	ETE Costeira I	UASB
Araucária	Iguassu	ETE Costeira II	UASB
Araucária	Iguassu	ETE Iguassu	UASB
Balsa Nova	Iguassu	ETE Balsa Nova	UASB
Campo Largo	Cambuí	ETE Cambuí	UASB/ Flotation
Colombo	Palmital	ETE Guaraituba	UASB
Curitiba	Atuba	ETE Atuba Sul	UASB
Curitiba	Iguassu	ETE Belém	Activated Sludge
Curitiba	Barigui	ETE Santa Quitéria	UASB
Curitiba	Atuba	ETE Santa Cândida	UASB
Curitiba	Padilha	ETE Padilha Sul	UASB/Lagoon
Curitiba	Barigui	ETE Cic Xisto	UASB
Fazenda Rio Grande	Iguassu	ETE Fazenda Rio Grade	UASB
Madirituba	Mauricio	ETE Mandirituba	UASB
Quatro Barras	Iraí	ETE Menino Deus	UASB
São José dos Pinhais	Itaqui	ETE Martinópolis	Lagoon
São José dos Pinhais	Iguassu	ETE Iguassu I	UASB

UASB: Upflow anaerobic sludge blanket digestion. (Source: Adapted from Porto et al., 2007).

About 26.3% of the territory can be considered as a highly urbanized region, 62.4% of the catchment area is composed by agriculture and other land use (grass field, floodplains, swamp, lower anthropogenic occupation), and 9.6% by forest (estimated by the pollution sources matrix adapted from Porto et al., 2007), as can be summarized in Figure 4. The Upper Iguassu Watershed is a predominantly plain region, with important natural floodplains and wetlands along the Iguassu River. The climate is classified as a subtropical highland climate (Cfb - Köppen climate classification), with average annual temperature about 16 ºC (average during winter about 12ºC, and 20ºC during summer). The average annual precipitation is about 1,400 mm, being regularly distributed along the four seasons (Porto et al., 2007).

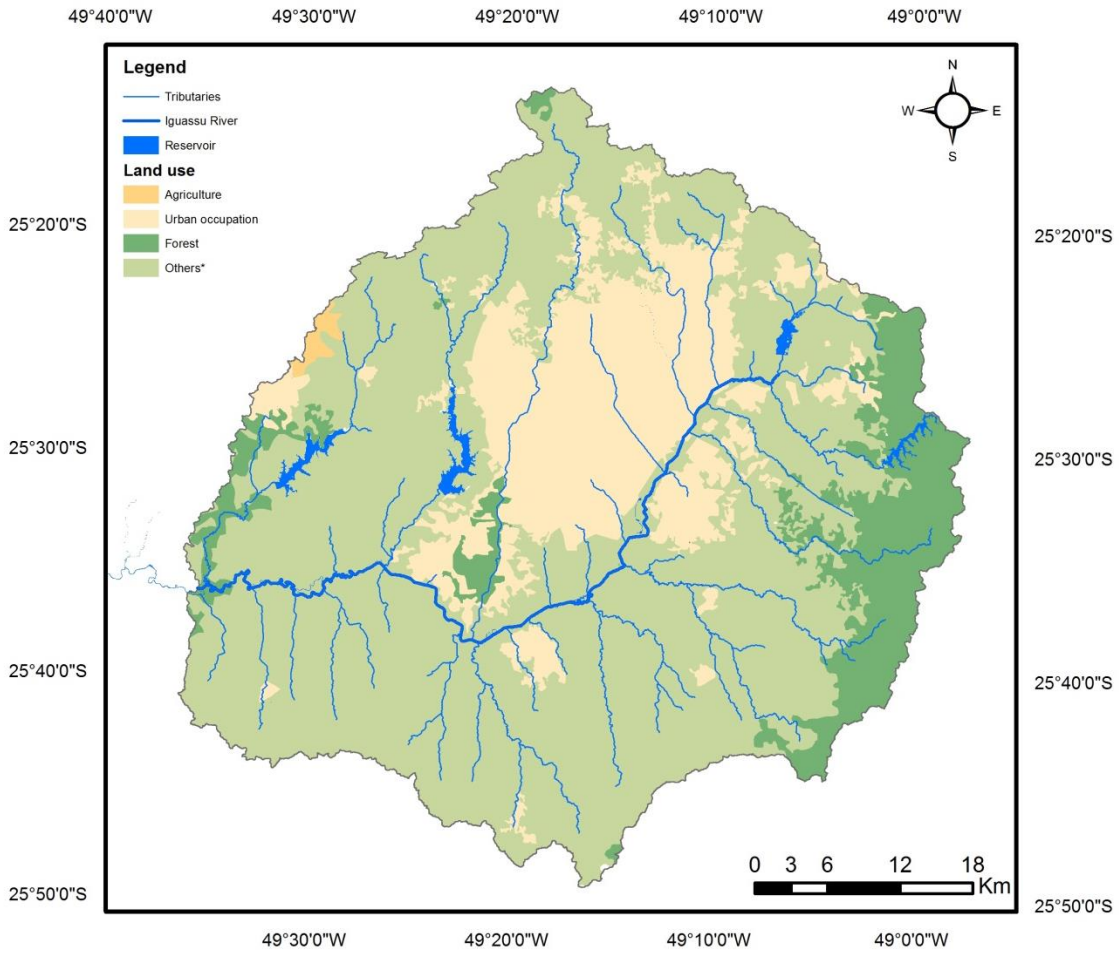


Figure 4: Land use and occupation in the Upper Iguassu Watershed.

* Other: agriculture, grass field, floodplains, swamp, lower anthropogenic occupation.

(Source: Adapted from Águas Paraná, 2014)

2.3 The Iguassu River Water Quality

2.3.1 Historical Approach for Water Quality Monitoring at Iguassu River

A mentioned in this chapter introductory description, the Upper Iguassu Watershed has a complex and multivariate pollution sources matrix. Within the watershed boundaries, the fast and unplanned urban development is, in the last decades, negatively affecting the quality of water at Iguassu River and in its main tributaries. The evolution of water quality deterioration either spatially, from the conserved headwaters to reaches and tributaries similar to wastewater

loads characteristics, or through years of continuous monitoring, several researches were conducted at the Upper Iguassu Watershed.

The history of studies contemplates small and located investigations, academic studies in specific problems, and in-depth projects involving academic efforts, regulatory agencies, and water sector companies. A mutual characteristic of all studies is the motivation to evaluate a real case of water quality problem, with all the particularities and challenging difficulties involving the complex and heterogeneous dynamic of land use and occupation of an urban basin.

In terms of academic investigations, different approaches have being taken in the last decades. Fields that has being studied can be summarized in monitoring (Gonçalves, 2011; Coelho, 2013), statistical modeling (Villa, 2008; França, 2009; Almeida et al., 2013), legal and financial concepts (Brites, 2011), water quality modeling (Bizzoni, 2000; Bäumle, 2004; Knapik, 2009), decision support and calibration tools (Nahon, 2009; Kondageski, 2009), and water resources planning and management strategies (Przybysz, 2007).

In reference to the monitoring activities developed at Iguassu River and/or its tributaries (Palmital, Atuba, Belém, Barigui), different categories of parameters or spatial and temporal frequency have been investigated. The water quality has being evaluated through biological indicators (Azevedo et al., 2010; Bem et al., 2013), limnology and eutrophication indicators (Mizukawa et al., 2009; Dombroski et al., 2009; Bem, 2009); identification of emerging contaminants (Osawa et al., 2013; Ide et al., 2013), analytical methods (Gonçalves et al., 2009), automatic instrumentation (Braga et al., 2003; Braga et al., 2013), spectroscopic techniques (Knapik et al., 2011; Bem et al., 2013; Knapik et al., 2013),

The characterization of sediment quality and transport dynamics has also been focus of studies. The Barigui and Iguassu River's sediments were monitored since 2006. Azevedo and Teixeira (2006) determined the particle size and the presence of heavy metals at Barigui River. Froehner et al. (2009) continued the previous research and evaluated the use of sediment physical and ecotoxicology characteristics as a potential tool for assessment of sewage pollution at Barigui River. At the Iguassu River, Dombroski et al. (2012) also evaluated the sediment chemical and physical characteristics focusing in alternatives for water quality planning and management guidelines at the Iguassu River. The sediment transport at the Barigui and Iguassu River has also being evaluated (Scuissiato et al., 2009).

Four main research projects developed by the Water Resources and Environmental Engineering Graduate Program (PPGERHA/UFPR) have historically a great impact in the conceptual advances and in the systematically monitoring and maintenance of the Upper

Iguassu Watershed database. During 2003 and 2005, significant efforts have being applied to the organization of the basis of the pollution sources matrix for the entire Watershed. The project (Iguassu Project) focused on sustainability and economic issues for water quality recovery at the Iguassu River (Fernandes et al., 2005).

The subsequent project was realized with a partnership between Federal University of Parana (UFPR) and Sao Paulo University (USP) during 2005 and 2007. This project (Enquadramento Project) was the starting point of the currently database of water quality parameters in six sites along the Iguassu River, covering an extension of 107 km in an area of 2,700 km². During the first two years of the project, more than 30 distinct parameters were systematically monitored through 20 samplings. In addition, the project advanced in the proposition of progressive water quality goals and financial issues according the Brazilian legal standards (Porto et al., 2007).

A continuity of this project during 2008 and 2012 (Integra Project) advanced in the optimization of the analytical methods used in the water quality monitoring. Another 10 samplings were performed, considering both water and sediment analysis along the Iguassu River and tributaries (Fernandes et al., 2012). The last research project started in 2014 (Integra 2 Project), and has as a primarily objective the evaluation of emerging contaminants at the Upper Iguassu Watershed, considering 20 different sites for a continuous monitoring.

However, although several improvements have been achieved in the hydrological concepts, the organic pollution sources matrix, the spatial and temporal statistical relationship between parameters and tributaries, the water quality modeling, the legal standards and financial strategies for water quality planning and management, there is still a challenge to integrate and apply all these knowledge. The common conclusion that can be taken from all the mentioned studies is that a new monitoring and data evaluation strategy is necessary to proper understand and advance in new actions for water quality planning and management, especially in a critical basin as is the Upper Iguassu Watershed.

2.3.2 Monitoring Strategy and the Pollution Sources Matrix: Integration of Spatial and Temporal Variability

The methodological approach of this thesis, to answer the main questions established in the previous chapter, consists in three interconnected branches: (i) urban basin management; (ii) field monitoring; and (iii) mathematical modeling. Clearly, the Upper Iguassu Watershed

figures as a classical example of fast urban development, and consequently, a direct and continued deterioration of water resources. New strategies considering distinct parameters, temporal and spatial variability could contribute to identify and highlight the key issues regarding water quality sustainability and water quality planning and management effective actions. Thus, a systematic monitoring plan is fundamental to better understand the overall aspects related to the use of the natural resources and the ecological consequences in the aquatic environment.

To support these thesis objectives, six sites were selected for the field monitoring. These sites were previous monitored since 2005 by distinct research projects, as described before (Porto et al., 2007; Fernandes et al., 2012). Figure 4 presents the location of the Iguassu River and the monitoring sites IG01 (headwater) to IG06. The sites selected covers an extension of 107 km of the Iguassu River, from less impacted watersheds (Iraí, Iraizinho, and Piraquara watershed), through the high urbanized region (Palmital, Atuba, Belém, Padilha, and Barigui watersheds), and downstream to areas under recovery (Faxinal, Passaúna, and Verde watershed). Table 3 presents a summary of main information about population, land occupation and density for each monitoring site.

Table 4: Summary of main information about population, land occupation and density for each monitoring site.

Monitoring site	Inhabitants[(a)]	Incremental Area (km^2)[(a)]	% Land occupation			Density (hab/km^2)
			Urban	Agricultural	Forest	
IG01	109,479	321.4	16.0	65.4	18.5	364.9
IG02	751,341	220.2	57.2	42.8	0.0	3,001.8
IG03	1,130,605	666.2	28.6	63.4	8.0	1,811.5
IG04	854,031	778.3	30.7	63.1	6.2	1,039.9
IG05	109,195	410.5	19.6	68.0	12.4	249.4
IG06	185,392	268.7	9.7	72.5	17.8	665.4

[(a)] Inhabitants and drainage area of each monitoring site. Note: total inhabitants differ from data presented in Table 1 since part of some municipalities is not located within the boundaries of Upper Iguassu Watershed. (Source: adapted from Porto et al., 2007).

The first site proposed for water quality monitoring, IG01, is strategically located in an area with low urban development, and, consequently, before the negative effects from the anthropogenic activities (domestic and industrial effluents, urban runoff). Sites IG02, IG03, and IG04 represents the intermediate sites of the watershed. These three sites are located in the most urbanized area of the Upper Iguassu Watershed, with significant inputs of domestic and industrial effluents, and urban runoff. Complementarily, important WWTP are located in

tributaries (or directly in the Iguassu River), and represents important organic pollution loads (Figure 3). Two sites, IG02 and IG04, are located a few meters downstream two important WWTP, Atuba Sul (Curitiba) and Cachoeira (Araucária), respectively. In addition, site IG02 has also been monitored for both river sides in recent researches (IG02A being the left side, and IG02B being the right side) due to interesting hydrodynamics, visual discrepancies in color alteration, and other field observations (Fernandes et al., 2012).

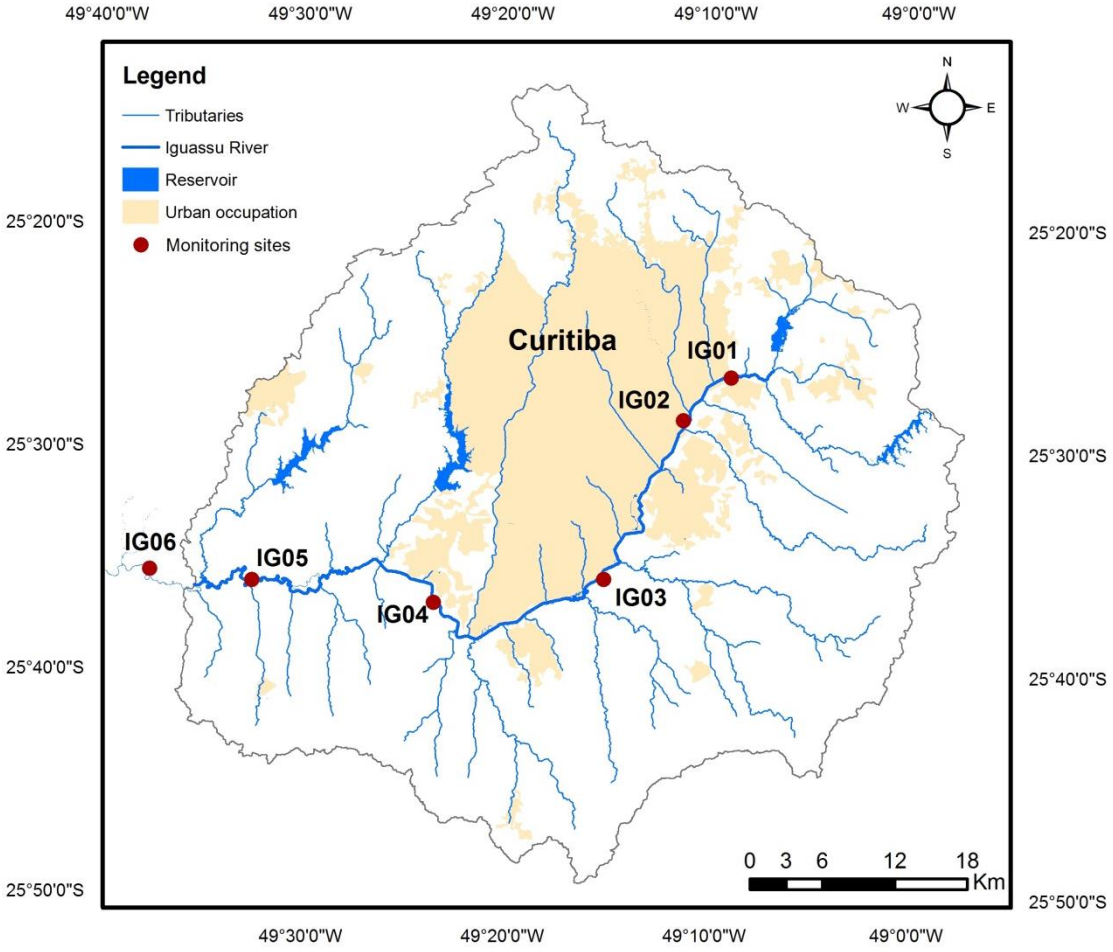


Figure 5: Location of the Iguassu River and the monitoring sites IG01 (headwater) to IG06.
(Source: Adapted from Águas Paraná, 2014)

Downstream the watershed, two sites were selected to evaluate the spatial influence of the anthropogenic organic pollution at the Iguassu River. Sites IG05 and IG06 drains an area predominantly with agriculture occupation (Table 3), and receives, beyond the remaining organic pollution from upstream, a significant contribution of flow from less impacted tributaries. Thus, the expectation is that these six sites could provide interesting data about the organic matter dynamics in urban basins. Further details about the monitoring sites are presented in Appendix 1.

2.3.3 Overview of Main Parameters Monitored: Water Quality Database of Iguassu River

Considering the monitoring activities developed in different research projects and academic activities since 2005, there are a total of 48 samplings performed at the Iguassu River and some tributaries (Palmital, Atuba, Belem, and Barigui Rivers). The first period of monitoring was performed during 2005 and 2006, with a total of 20 samplings in the six sites mentioned before (Iguassu River). The set of parameters monitored during this period was: chemical oxygen demand (COD), biochemical oxygen demand (BOD), dissolved organic carbon (DOC), solids (fix, suspended and volatile), nitrogen (organic, ammonia, nitrite, and nitrate), total phosphorous, turbidity, conductivity, temperature, pH, dissolved oxygen, and water transparency.

During 2008, 5 samplings were conducted considering an additional site located upstream site IG01. This year also was implemented the first spectroscopic analysis for the monitoring of water quality at the Iguassu River. Chlorophyll-*a* and the kinetics rates for BOD decomposition had bein also measured.

The next period of samplings, 2009-2010, 10 samplings were performed. An additional site located downstream site IG06 was included in the monitoring strategy. The number of parameters monitored also increased: phosphorous (total, orthophosphate, dissolved, particulate), coliforms, caffeine, and analyses of sediment (particle size, organic carbon, organic dry mass, nitrogen, and phosphorous).

During 2012 and 2013, two changes were implemented in the monitoring strategy. The first one considers the monitoring of four important tributaries in the most urbanized part of the watershed: Palmital, Atuba, Belém, and Barigui Rivers. The second change considers the measurement of parameters such as total and particulate organic carbon and alkalinity.

Considering the number of parameters monitored, this set of data has heterogeneous characteristics and reflects the evolution of the parameters selected for the continuous evaluation and the different objectives throughout some research projects. Table 5 presents a summary with the number of analyses for each parameter monitored at the Iguassu River since 2005. More important, this database represents 10 years of monitoring at the Iguassu River. The database have being used for distinct objectives, such as water quality evaluation (Coelho 2013), calibration of water quality models (Knapik, 2009), statistical modeling (França, 2009; Almeida, 2013), and evaluation of management strategies (Brites, 2010).

Table 5: Number of analysis for each parameter monitored at Iguassu River since 2005

Parameter	IG01	IG02A	IG02B[1]	IG03	IG04	IG05	IG06
BOD (mgO_2/L)	44	45	14	44	43	44	41
COD (mgO_2/L)	46	46	18	46	44	45	44
TOC (mgC/L)	8	8	8	8	7	8	8
POC (mgC/L)	8	8	8	8	7	8	8
DOC (mgC/L)	46	46	17	46	44	45	42
DO (mgO_2/L)	42	42	16	43	41	40	36
Fluorescence spectra	25	25	15	25	23	25	23
UV-visible absorbance	25	25	15	25	23	25	23
Conductivity (µS/cm)	47	47	18	47	44	45	42
Turbidity (NTU)	46	46	18	46	44	45	41
pH	47	47	18	47	45	45	43
Temperature (°C)	47	47	18	47	45	46	43
Secchi depth (cm)	37	30	4	36	28	37	32
Chlorophyll-*a* (µg/L)	9	9	4	9	8	9	9
Alkalinity (mg/L)	5	5	5	5	4	5	5
Total phosphorous (mgP/L)	44	46	17	46	44	45	42
Orthophosphate (mgP/L)	18	22	17	22	20	21	20
Dissolved phosphorous (mgP/L)	20	21	16	21	19	20	19
Particulate phosphorous (mgP/L)	20	21	14	21	18	21	20
Diss. Org. phosphorous (mgP/L)	16	20	15	19	18	17	18
Ammonia (mgN/L)	46	46	18	46	44	45	41
Organic nitrogen (mgN/L)	44	43	15	44	40	43	39
Kjehdahl nitrogen (mgN/L)	43	44	16	44	42	43	39
Nitrite (mgN/L)	44	45	17	45	43	44	40
Nitrate (mgN/L)	43	41	17	41	39	41	38
Total nitrogen (mgN/L)	42	40	16	40	38	40	36
Settling solids	47	47	18	47	45	46	43
Solids[2] (mg/L)	46	47	18	47	44	46	43
Total coliform (NMP/100ml)	18	19	16	19	18	19	16
Fecal coliform (NMP/100ml)	18	19	16	19	17	19	15
Total data	**1359**	**1373**	**586**	**1379**	**1291**	**1350**	**1253**

[1] Site IG02B is located in the opposite margin of site IG02A, data started in 2010. [2] Analyses of total, dissolved and suspended fraction for fix, total and volatile solids.

2.4 The impact of organic pollution in urban rivers

In non-impacted watersheds, autochthonous processes are the main source of organic matter. However in urbanized and polluted rivers, effluents can be an important source of organic matter. The specific wastewater load per capita are, in average, 54 g/inh.d in terms of BOD (mgO_2/L) (WHO, 1982), 27.3 g/inh.d in terms of TOC, 12.98 g/inh.d in terms of total nitrogen, and 2.13 g/inh.d in terms of total phosphorous (Servais et al., 1999). Consequently, the combination of organic matter, nitrogen, phosphorous, and heterotrophic bacteria present in effluents, can cause a drastic depletion of dissolved oxygen in water downstream of the effluent discharge (Servais et al., 1999).

The fast and continuous urban development is also increasing the spatial and the intensity of the impact of organic pollution in surface waters. There is a constant pressure to advance in the urban areas to floodplains or protection areas. In most cases, the infrastructure does not follow the same growth rate. As a consequence, the low rates of wastewater collection and treatment, or even the lack of properly treatment or the low removal efficiency of some types of WWTP, aggravates the organic pollution problem in urban basins.

A key issue for water quality planning and management is the rapid and appropriate analytical methods for the properly identification and differentiation between anthropogenic and natural sources of organic matter. The identification is important to quantify and characterize point and non-point sources, and thus, set strategies to control this inputs, test better removal practices, evaluate the impact of water quality deterioration and find alternative sites for discharges.

The differentiation is also important since the nature of organic pollution (i.e., compound composition) can lead to distinct degradation pathways (Servais et al., 1999). Labile organic matter has a higher potential to negatively affect the quality of a water body than a refractory composition. Since effluent organic matter (EfOM), specially the untreated wastewater, are mostly composed by labile compounds, its impact can reduce significantly the oxygen in the water column.

There are some recent researches that focused on the identification and evaluation of the influences of EfOM in urban rivers. Different methods have being applied to differentiate natural organic matter from EfOM in urban basins, such as pyrolysis-GC/Ms (Kalscheur et al., 2012), fluorescence spectroscopy (Goldman et al., 2012; Meng et al., 2013). In terms of DOC, Stanley et al. (2012) recently questioned how human activities affect the quantity and quality in human-dominated rivers. The authors evidenced that besides its importance of DOC in an ecological point of view, changes in its load or concentration in streams and rivers rarely motivates management activities (Stanley et al., 2012).

The common conclusion is that there is a crescent alteration in the organic quality in stream water due to wastewater discharges (Kalscheur et al., 2012; Stanley et al., 2012), but the intrinsic and ecological consequences are not well understood. The properly quantification and characterization of the organic matter through different analytical methods is absolutely necessary to understand what the compounds are and how the degradation mechanism occurs. Another priority must be on the identification of the most degraded sites, and what actions should be taken to minimize the causes and consequences. In summary, EfOM in urban rivers

can be considered as a chemical, biological, and a management problem. Besides a complex and heterogeneous problem, these three issues cannot be evaluated separately.

2.5 Summary

This chapter briefly discussed about the urban problem and water resources planning and management strategies. As mentioned, the Upper Iguassu Watershed has a classic example a complex and heterogeneous pollution source matrix, and, consequently, a persistent deterioration of the quality of water resources.

The Upper Iguassu Watershed has an ever-growing history of monitoring and academic evaluation of distinct characteristics. Although well documented, there are still weaknesses on the monitoring and the proposition and implementation of water quality planning and management strategies. Issues that remain important for evaluation are the parameters monitored and the understanding of how these parameters interact with other compounds and vary according to system physical, biological, and chemical characteristics.

These studies have shown an interesting and profound capacity of producing monitoring results, most of them integrating water quantity and quality. But despite that, a more in depth analysis, as performed by Knapik (2009), França (2009) and Almeida (2013), have indicated that the dynamics of pollution through monitoring snapshots is still a challenge. There is no basic correlation among water quality variables, and the main component of correlation as presented by Almeida (2013), in not constant through spatial distribution, basically requiring new water quality approach or a distinct water quality monitoring condition to ensure fairness for planning and management purposes.

Thus, this chapter aimed to not only describes the Upper Iguassu Watershed as a case study, but to give an insight about how important and complex is the urban question in water resources planning and management and a set for a new water quality monitoring strategy for water resources planning and management.

Chapter 3

Organic Matter Characterization and Quantification in Rivers

"It is generally accepted that nothing is more important in science than good data. And an essential condition for having good data is the need to know what we are measuring."

Filella, Montserrat. 2014. Understanding what we are measuring: Standards and quantification of natural organic matter.
Water Research 50, 287-293.

3.1 Overview

The organic matter is a complex mixture of organic compounds present in all surface, ground, and soil waters. Considering a holistic view of the global carbon cycle, it occurs naturally in particulate and dissolved form as part of the food web and in the process of nutrient cycling, sediment composition, organic production, and decomposition.

Thus, a first goal of this chapter is to present an overall description about the dynamics of organic matter in aquatic systems. This is a starting point for the definition of an organic matter monitoring strategy. In such context, different approaches for its monitoring and measurement are briefly summarized. Important aspects related to sampling and sample preservation, errors in its analytical method, equipment performance, filter porosity and other experimental procedures are highlighted. In addition, it is also presented information about fluorescence and UV-visible spectrophotometry as complementary tests for a qualitatively analysis of the organic matter in surface waters.

Quality and thus, degradability of organic carbon, involves biological, physical, and chemical aspects. Biodegradation and transformation of organic matter are processes that influence the fate, transport, and distribution of chemicals and the mass balance in aquatic systems. Therefore, to understand and to characterize properly the organic matter dynamics in the aquatic system, quantitative data about biodegradability and other transformation pathways are important to support this thesis hypothesis.

This chapter also aims to describe and analyze the main sources and transformation mechanisms of organic matter in a polluted river. Some issues about the origin of organic matter will be discussed considering the natural processes related to its dynamics and the anthropogenic sources that are predominant in an urban environment like the Upper Iguassu Watershed. Complementarily, its transformation mechanisms will be discussed, highlighting some differences and similarities about these process for lotic and lentic environments.

The concepts herein described are important to establish the mathematical formulation for this thesis modeling approach and to summarize some insights to one of this thesis introductory question: "How to characterize and differentiate between autochthonous and allochthonous organic matter in human dominated basins?" Complementarily, examples of currently statistical analysis to the evaluation of organic matter dynamics and its application in different fields are briefly described.

Hence, with a strategy considering different approaches for organic matter monitoring, analytical experiments and statistical tools, the final objective of chapter 3 is to analyze the impacts of organic pollution in urban basins and the challenges in water quality planning and management.

3.2 Sources and Mechanisms of Organic Matter in Rivers

The organic matter is composed by different organic compounds derived from both natural and anthropogenic processes and activities. According to its sources in the environment, physical, chemical, and biological conditions, hours to several days can be necessary to an effective assimilation and degradation.

Besides the intrinsic compounds characteristics, the dynamics of organic matter in surface waters also depends on environmental factors. Temperature, solar radiation time, flow regimes, moisture, biological community and others components are factors that influences its assimilation and degradation. Some of them may facilitate decomposition process, such as an increase of temperature, while others can slow the reaction rates.

Complementarily, it is important to highlight that the transport mechanism observed in rivers are different from those controlling factors in lakes and oceans. As the main focus of this thesis is urban basins, a more detailed description about the organic matter sources and mechanisms of transformation and transport in rivers is presented in the next items.

3.2.1 Organic Matter in the Aquatic Environment: Natural and Anthropogenic Sources

The presence of organic matter influences the structure of aquatic communities and characteristics such as pH-buffering, alkalinity, light attenuation, distribution of ions between aqueous and solid phases, and formation of colloidal particles (Mulholland, 1997; Thurman, 1985; Krusche et al., 2002; Nebbioso and Piccolo, 2012). Dissolved organic matter (DOM) can enhance the solubility and mobility of metals and organic compounds (Perdue et al., 1979; Thurman, 1985) and thus interfere in the pollutant transport and micronutrient availability. In addition, the bioavailable organic carbon derived from dissolved and particulate organic matter through photolytically or biologically process can enhance biological productivity in waters (Mostofa et al., 2013a).

Two major categories commonly distinguishes the natural organic matter (NOM) as a function of its composition: (i) biochemically well-defined compounds (polysaccharides, proteins, peptides, and lipids); and (ii) humic substances, which normally accounts for 70-80% of the NOM in lakes (Zumstein and Buffle, 1989; Filella, 2009) and 40-70% of the dissolved organic carbon (DOC) in rivers (Benner, 2003). The first category can be easily

biodegraded, i.e., composed mainly of labile substances, while the second one has an important resistance to microbial degradation.

Zumstein and Buffle (1989) also consider a second division for the refractory organic matter (humic substances): (i) pedogenic refractory organic matter (PROM), originating from soils and being essentially aromatic; and (ii) aquagenic refractory organic matter (AROM), originating from aquatic media and being mainly aliphatic.

Organic matter can be originated by three major sources: (i) allochthonous, i.e., from outside of the system such as atmospheric deposition or soil material transported by runoff; (ii) autochthonous or surface water-derived from algal or phytoplankton; and (iii) allochthonous synthetic organic substances of anthropogenic sources, i.e., effluent organic matter (EfOM) (Filella, 2009; Sharma et al., 2011; Mostofa et al., 2013a). Figure 1 presents a schematic representation of organic matter sources and pathways in aquatic systems.

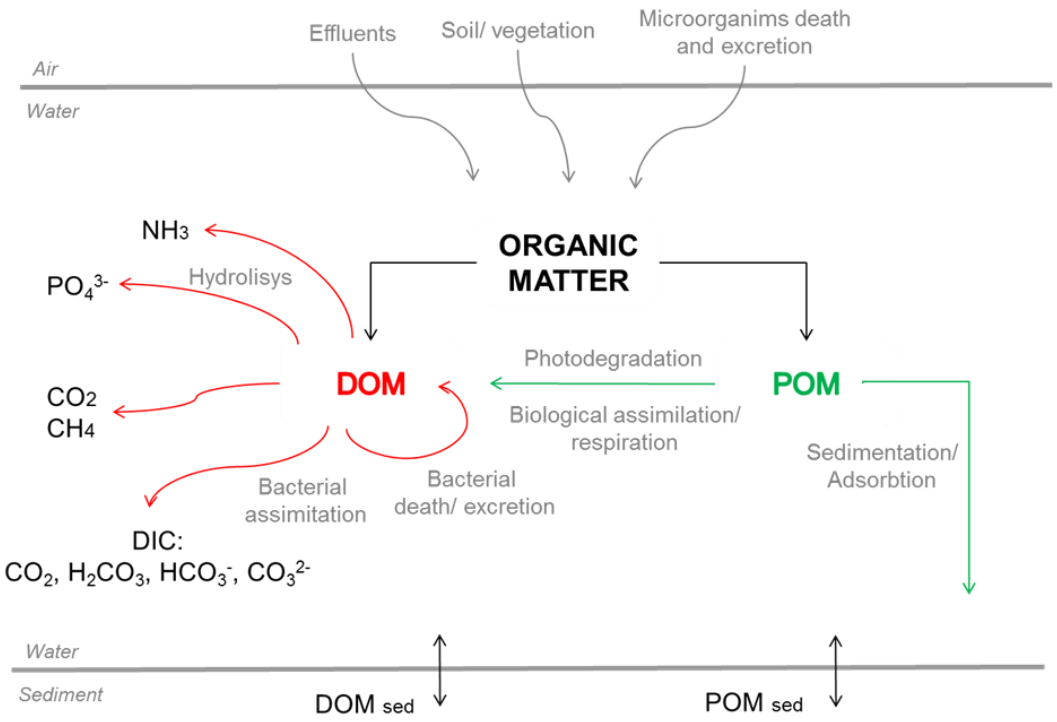


Figure 1: Schematic representation of organic matter sources and pathways in aquatic systems.

The process that originates plant and soil organic matter (fulvic and humic substances) presents variations in tropical, temperate and boreal regions, and are dependent of factors such as: (i) physical properties (temperature and moisture); (ii) chemical functions (nutrient availability, concentration of free oxygen and redox activity); and (iii) availability of microorganisms. In addition, allochthonous NOM has a large C/N ratio (near 100:1), is

highly colored, and has significant aromatic carbon content (Sharma et al., 2011; Jin et al., 2011, Mostofa et al., 2013a). Autochthonous NOM has relatively lower C/N ratio (near 10:1), is almost colorless and presents low aromatic carbon content. Photoinduced and microbial respiration (or assimilation), zooplankton, grazing, bacterial release and uptake, viral interactions, and microbial activities in sediment pore waters are the main process for the autochthonous organic matter production in surface waters (Sharma et al., 2011; Jin et al., 2011, Mostofa et al., 2013a).

Anthropogenic sources of organic matter represent a great contribution for the increasing of organic matter levels in surface waters, especially in developing countries. Effluents derived from agricultural, domestic, and industrial activities can alter not only the quality or water, but may cause impacts in the natural ecological succession. Agricultural practices can release significant amount of organic compounds derived from pesticides, herbicides and consequently, their degradation byproducts. Urban activities produce daily an elevated amount of sewage, with a variable composition that depends on levels and kinds of treatment applied. Problems can derive from the presence of emerging organic contaminants such as pharmaceuticals and personal care products, which ecological consequences are not well understood. In addition, wastewater derived organic compounds can be a precursor of toxic byproducts such as trihalomethanes, N-nitrosodimethylamine, and organic chloramines in water treatment process (Sharma et al., 2011; Jin et al., 2011, Mostofa et al., 2013a).

The occurrence of particulate and dissolved, and consequently, total organic carbon is significantly variable and depend on the type of aquatic system and sources. According to Leenheer and Croué (2003), particulate organic carbon (POC) generally represents a minor fraction (below 10%) of the total organic carbon (TOC), proportion that increases with a river's size and flow rate. Visco et al. (2005) show that TOC value is highly variable: from less than 1mg/L in underground or seawaters, to 2-10 mg/L in lake or river waters, up to 10 g/L in marshes and fens. Leenheer and Croué (2003) also indicate that dissolved organic carbon (DOC) concentration range from 0.1 mg/L in groundwater to 50 mg/L in swamps.

Considering domestic wastewater, it can be observed that the concentrations of TOC, POC, and DOC show variation according to the level and type of treatment. Sharma et al. (2011) found DOC concentrations of primary, secondary, and tertiary effluents from wastewater treatment plants used for soil aquifer treatment in the range of 9-35mg/L, 2-24 mg/L and 5-20 mg/L, respectively. Metcalf and Eddy (1991) indicates an average TOC concentration from weak, medium, and strong untreated domestic wastewater respectively 80 mgC/L, 160 mgC/L, and 290 mgC/L.

Complementarily, besides the importance of the specific load per capita, only few references had evaluated it in terms of organic carbon. BOD (mgO_2/L), for example, the estimated specific load worldwide used for raw wastewater is about 54 g/inh.d (WHO, 1982). According to Servais et al. (1999), for raw wastewater, the specific loads of TOC ranges between 26.4 and 28.3 gC/inh.d, with particulate organic matter constituting the main part, 19.0 and 20.7 gC/inh.d (70-76%), DOC from 6.5 to 8.5 gC/inh.d and the biodegradable fraction representing between 60 and 75%.

Depending on the type of treatment, the specific load of TOC in treated wastewater ranges between 3 and 10.8 gC/inh.d, from 2.1 and 4.9 gC/inh.d for DOC, and from 0.9 and 2.5 gC/inh.d for POC (Servais et al., 1999). The difference between the high and low removal of organic carbon in this case is the additional aerobic and anaerobic stage after the high load activated sludge process (Servais et al., 1999).

Another important consideration is about the organic carbon derived from allochthonous pedogenic sources. The annual export of organic from catchments varies from 100 up to 100.000 kg C/km²yr, according to a summary organized by Hope et al. (1994) using data from several studies considering different watershed scales and characteristics. Recent studies also indicated a wide range of organic carbon export rates (Mattsson et al., 2009; Graeber et al., 2012; Sandford et al., 2013; Stutter et al., 2013), as summarized in Table 1.

The values estimated does not show unique pattern, indicating that several factors may affect in an interrelated manner the export rates of organic carbon. For example, forested areas can release small amounts of DOC, in the range of 54±9 kg C/km².yr (Graeber et al., 2012), up to 5700 kg C/km².yr (Mattsson et al., 2009), considering an average annual runoff from 550 to 310 mm. Watershed size, land use and occupation, and annual runoff are important factors that affects the transport of organic carbon from soils to aquatic systems.

In addition, as highlighted and summarized by Hope et al. (1994), there are several sources of errors in estimating riverine carbon fluxes. Discharge, season, rainfall, watershed characteristics, percentage and type of land coverage, sampling and frequency of sampling, chemical and analytical methods, are examples of circumstances that may underestimate or overestimate the organic carbon export rates. Most part of studies relies only on DOC analysis to represent the overall loss of organic carbon. In small rivers, for example, POC are mostly affected by discharge and frequency of sampling, since the concentration of particulate compounds may fluctuate rapidly according to the watershed geomorphology. In large rivers, variations in POC can also be affected by the depth of water sampling (Thurman, 1985; Hope et al., 1994).

Table 1: Summary statistics for the export of organic carbon (kgC/km².yr) in distinct watersheds.

Watershed characteristics/ Location	Watershed size range (km²) [*average*]	% of land use and occupation				Annual runoff (mm/year) [average]	Loss of organic carbon (kg C/km².yr)			DOC/POC	Sampling	Reference
		Urban	Forest	Agriculture/ Pasture	Wetland		DOC	POC	TOC			
Forested boreal are (Finland, n=9 watersheds)	870 - 49400 [*11200*]	0.1 - 2.6 [0.5]	38 -54 [48]	1.0 - 42 [12]	1.9 - 48 [27]	250 - 400 [310]	2500 - 5700 [4000]	-	-	-	River water samples	Mattsson et al., 2009
Temperate agricultural area (Denmark, n= 10 watersheds)	1.8 - 180 [*72*]	0.5 - 100 [14]	0 - 45 [15]	0 - 86 [66]	0 - 5.8 [3.3]	260 - 590 [380]	1500 - 3500 [2500]	-	-	-		
Warm Mediterraneam catchment (France, n= 5 watersheds)	19 - 730 [*480*]	1.0 - 10 [4.3]	43 - 46 [44]	12- 15 [14]	0	430 - 660 [560]	670 - 1100 [870]	-	-	-		
Temperate forest (USA, Lawrence Lake)	0.3					1200	3020	600	3620	5	River water samples	Hope et al., 1994 [a]
Temperate forest (USA, Hubbard Brook)	0.1					700	1180	340	1520	3.5		
Temperate forest (USA, Bear Brook)	1.0					720	1780	170	1950	10.2		
Temperate forest (USA, Mirror Lake)	1.0					700	1820	70	1890	26		
Temperate forest (USA, Findlay Lake)	1.0					4480	5100	1040	6140	4.9		
Temperate forest (USA, Little Miami River, Ohio)	4545					340	2190	810	3000	2.7		
Temperate forest (USA, Neuse River, N.C.)	6694					360	2040	680	2720	3.0		
Temperate forest (Canada, Lake Marion)	13					2000	7260	840	8100	8.7		
Temperate forest (Canada, Canagagigue Creek)	25					150	1790	50	1840	35.8		
Temperate grasslands (USA, Brazos River)	113 968					55	200	210	410	0.9		
Temperate grasslands (USA, Missouri River)	1 084 545					35	160	510	670	0.3		
Temperate grasslands (USA, Mississippi River)	3 220 716					180	500	560	1060	0.9		
Agriculture (A1)	1.03		0.9	99.1		550	645 ± 111	-	-	-	River water samples	Graeber et al., 2012
Agriculture (A2)	0.44			100		550	394 ± 107	-	-	-		
Forested (F1)	0.07		100			550	68 ± 54	-	-	-		
Forested (F2)	3.29		100			550	295 ± 235	-	-	-		
Forested (F3)	4.36		75	25		550	54 ± 9	-	-	-		
Wetland (W2)	6.00	0.3	87.9	11.8		550	875 ± 140	-	-	-		
Wetland (W3)	43.30	2.5	70	27.4		550	344 ± 52	-	-	-		
Surface export	[b]			100		1100	1500	-	-	-	Runnoff from lysimeters	Sandford et al., 2013
Drainage export	[b]			100		1100	3500	-	-	-		
Nelka River (Sibéria)	30.8		90	10		-	4750	30	4780	158	River water samples	Suzuki et al., 2006
Surface export (Grazed grassland)	[b]			100 [c]		280	760 - 89900	-	-	-	Runnoff from lysimeters	McTiernan et al., 2001
Temperate grasslands (Cairn Burn, UK)	1.0			100 [c]		1115	77200	-	-	-	River water samples	Stutter et al., 2013

[a] Studies summarized by Hope et al., 1994. [b] Lysimeter area. [c] Grassland.

3.2.2 Transformation and Remineralization of Organic Matter in Rivers

Different chemical, physical, and biological process determines the dynamics of organic matter in natural waters. The decay of both particulate and dissolved organic matter is not simple, since the assimilation and decomposition process depend on the size of the organic materials (Thurman, 1985). While DOM is degraded mainly by microorganisms, both animal and microbial activities impact the decomposition rates of POM. Complementarily, the dynamics of decomposition and production of organic matter varies between types of ecosystems, such as lakes and streams.

In lakes, through photosynthesis, primary producers (phytoplankton) form both dissolved and particulate organic matter. The following steps of the food chain, with the consumption of algae by zooplankton and other aquatic organisms, and the predation by fish, quantifies the grazer pathway (Thurman, 1985). Complementarily, all organic matter produced in the different levels of the food chain is used by decomposers (bacteria and fungi). In the presence of oxygen, microbial activities accelerate the transformation of POM and DOM to carbon dioxide, water, and inorganic compounds.

In addition to microbial degradation, photoinduced process also acts in the breakdown of POM and DOM. Although grazer is a decomposition pathway, the microbial and photoinduced pathways contributes to the most part of the total organic matter assimilation and degradation in lakes (Thurman, 1985; Mostofa et al., 2013a). Moreover, according to the lake's hydrology, geomorphology, and the eutrophic state, POM may be deposited in the sediments and be further decomposed by microbial activities (Thurman, 1985). Figure 2 presents a simplified example of organic matter cycling (in terms of organic and inorganic carbon) in lakes through microbial action.

The differences on the transformation mechanism of organic matter when comparing lakes and rivers occurs not only due to physical and geomorphological characteristics, but also as a function of organic matter characteristics. Considering environments with low or without anthropogenic impacts, it can be considered that, in general, lakes are autotrophic systems (i.e., produces carbon through photosynthesis), while streams are heterotrophic systems (i.e., allochthonous source of organic matter such as terrestrial plants and runoff) (Thurman, 1985). Light penetration presents also different characteristics in the water column for lakes and streams, which may impact photoinduced process. In addition, the residence time and flow regimes change the overall dynamics for biodegradation and sedimentation in streams.

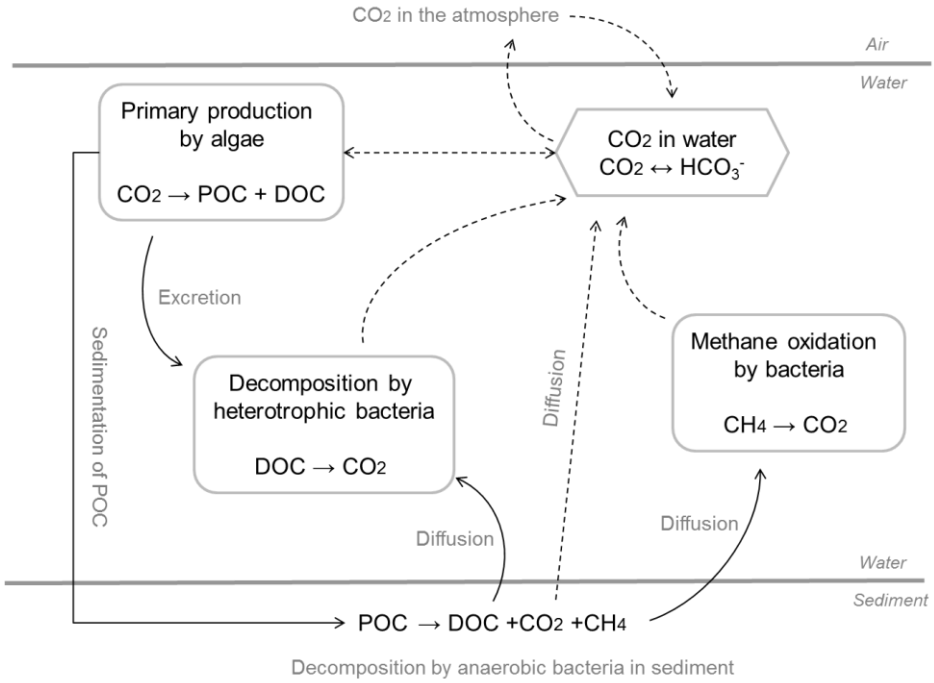


Figure 2: A simplified example of carbon cycling in lakes through microbial action.

(Source: Adapted from Thurman, 1985).

A schematic representation of the organic matter production and decomposition cycle in aquatic systems is summarized in Figure 3. Photosynthesis and respiration are reactions that complement each other in the environment. Through photosynthesis, autotrophs organism produces organic matter and oxygen converting inorganic nutrients and CO_2 using solar energy. Additionally, through a set of metabolic reactions, living organisms convert organic matter into energy.

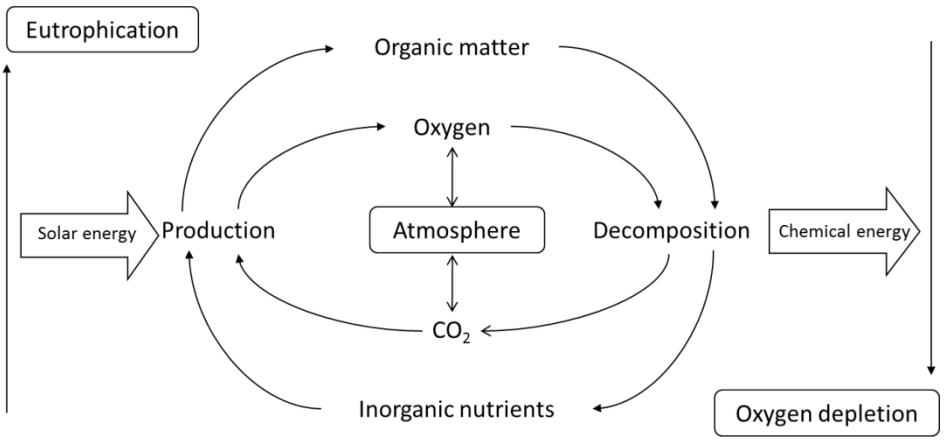


Figure 3: Schematic representation of the natural cycle of organic production and decomposition

(Source: Adapted from Chapra, 1999)

When these two processes are not in equilibrium, problems related to the excess of production and/or consumption can be encountered. Organic matter decomposition increases the oxygen depletion. The eutrophication process originates an excess of organic matter through algae biomass. Thus, oxygen depletion and eutrophication represents two extremes of the production/consumption cycle and, in a broader sense, represents a single problem.

In addition to production and decomposition processes, the organic matter interacts with the global carbon cycle through distribution and transportation or carbon compounds in the biosphere (Mostofa et al., 2013a). Biological and photoinduced degradation of organic matter, both DOM and POM fractions, can produce nutrients (NO_2^-, NO_3^-, and PO_4^{3-}) and other derived products such as H_2O_2, CO_2, CO, CH_4, DIC (CO_2, H_2CO_3, HCO_3^-, and CO_3^{2-}), low molecular weight DOM, autochthonous DOM and other compounds (Chapra, 1997; Mostofa et al., 2013a).

The organic matter dynamics in surface waters is a function of compounds sources (inputs) and mineralization (outputs). Several factors may affect the origin and dynamics of allochthonous and autochthonous organic matter in natural waters, such as: (i) types and nature of terrestrial plant material in soil; (ii) land management and natural effects (precipitation, flood, and drought); (iii) effect of temperature; (iv) photosynthesis in natural waters; (vi) metal ion complexation and salinity; (vii) microbial processes; and (viii) photoinduced processes (Evans et al., 2005; Mostofa et al., 2013a). These factors present different intensities according to the kind of aquatic environment, e. g., groundwater, rivers, lakes, and oceans. Besides some research studies show contradictory results about how each factor can increase or decrease the organic matter release to surface waters, the results are important to guide an overall understanding about the complexity of organic matter dynamics.

The type of soil and terrestrial plant communities affects the allochthonous organic matter derived from soil biodegradation. In addition, the watershed physical properties, such as altitude, slope, catchment area, percentage of natural cover, and other regional effects can either decrease or increase DOC levels in natural waters. Furthermore, alterations caused by land management and/or natural processes that changes the environment (precipitation, flood, and drought), may accelerate the rates in which organic matter is released and transported into rivers and lakes (Evans et al., 2005). These processes also depend on sazonality and anthropogenic actions (Mostofa et al., 2013a). In addition, factors such as plant production rates, plant physical structure, C/N ratio, grazing pressure, disturbance and plant

fiber content may affect the rate at which biodegradable organic carbon is leached from plants and decaying algae and became available in surface waters (Bachand and Horne, 2000; Lim et al., 2008).

Temperature also influences the production of autochthonous organic matter and the rates of DOC export from soil (Worrall et al., 2003; Evans et al., 2005). In the first case, the effects are related to solar radiation, and may affect the physical, photoinduced, microbial and ecological processes. As temperature or duration of solar radiation increases, more important are the above processes on organic matter dynamics. Considering the transport of allochthonous DOC, some authors suggests that an increase of temperature can enhance soil respiration and mineralization of plant organic matter (Mostofa et al., 2013a). In contrast, low water and air temperature may decrease the potential of photoproducts formation by photodegradation. Consequently, photosynthesis and primary production decreases in low temperatures, which may lead to a change in the autochthonous and allochthonous proportion.

The effect of metal ions is a result of complexation and adsorption processes. The complexation of metal ions can induce structural changes in organic matter and lead to the formation of aggregates compounds (Duan and Gregory, 2003). Thus, the resulting compounds present different optical properties, which may increase or decrease of absorbance and fluorescence intensities. Complementarily, salinity also promotes changes in optical characteristics by changing the molecules structures (Del Vecchio and Blough, 2002; Mostofa et al., 2013b).

Microbial and photoinduced processes play an important role in organic matter dynamics in surface waters. Both processes can release autochthonous dissolved organic matter (DOM) through respiration or assimilation of particulate organic matter (POM) in surface waters by algal or phytoplankton biomass assimilation or by photoreactions, respectively. Factors such as occurrence and nature of microbes, sources of organic matter, and the quantity of its fermentation products, temperature, pH, and sediment depth affects microbial activity. Complementarily, as biodegradation and photodegradation occurs simultaneously, some photoproducts may stimulate microbial activity by providing readily assimilable nitrogen compounds. The two processes also changes the molecular structure of chromophoric dissolved organic matter (CDOM) and fluorescent dissolved organic matter (FDOM), which can be evaluated by differences in the organic matter absorption and fluorescence properties (Mostofa et al., 2013a). A summary of main reactions for microbial

and photoinduced transformation of carbon/organic matter in aquatic systems is presented in Table 2.

Table 2: Summary of main reactions for microbial and photoinduced transformation of carbon/organic matter in aquatic systems

Microbial transformation	(1) Inorganic carbon assimilation from photosynthesis and chemosynthesis: **CO_2+H_2O +A (reduced: oxygen, sulfur, iron, or other reduced species) + Energy → (CH_2O) + A (oxidized)**
	(2) Aerobic decomposition of organic carbon: **(CH_2O) + O_2 → CO_2 + H_2O + Energy**
	(3) Anaerobic decomposition of organic carbon: **(CH_2O) + A (oxidized) → CO_2 + A (reduced)**
Photodegradation	**DOM +O_2+H_2O+H^+ $\xrightarrow{h\nu}$ H_2O_2 + $DOM^{\cdot+}$ + O_2 + OH^-** **H_2O_2 $\xrightarrow{h\nu}$ 2HO·** **$DOM^{\cdot+}$ + HO· $\xrightarrow{h\nu}$ [$DOM^{\cdot+}$HO·]*** **[$DOM^{\cdot+}$HO·]* $\xrightarrow{h\nu}$ LMWDOM+DIC+CO_2+other byproducts**

LMWDOM: Low molecular weight DOM;
(Source: Adapted from Thurman, 1985; Mostofa et al., 2013a)

Data from studies performed in different regions, and summarized by Mostofa et al., (2013b) suggests that biodegradation can decompose up to 85% of DOC, while photodegradation shows different rates according to the characteristics of aquatic environment and the hours of solar radiation. In rivers, for example, photodegradation is estimated to mineralize 1 % to 2 % in the surface layer during one day of solar radiation, and less than 1% in deeper layers. This leads to an average of 21-36% decrease in DOC concentration in stream waters along several days of evaluation. For lakes this percentage is higher, about 22-23% at the surface layer during one irradiation period, up to 41% in several days. As the solar radiation reduces in deeper layers, either the rates in which organic matter is mineralized by photoinduced process gradually decreases. Complementarily, the organic matter photodegradation is inversely proportional to DOC concentration. Waters with high DOC concentration commonly presents low rates of photodegradation. For unpolluted waters, photoinduced process are faster than microbial degradation, while in effluent-dominated river biological decomposition can act as the first degradation stage (Mostofa et al., 2013b).

In such a context, understanding the organic matter dynamics in aquatic systems is of great relevance and necessary to predict and control severe problems, especially in a high polluted environment. Chapra (1999) emphasize that the three major problems related to water quality and considered by mathematical models over the past 90 years are linked to the natural cycle of organic production and decomposition: (i) oxygen depletion, which the organic matter is modeled indirectly by BOD-DO mass balance; (ii) eutrophication, where the biomass is accounted by chlorophyll-a; and (iii) transport of toxic substances, that uses the simulation of dissolved solids and the adsorbed metals to represent the organic carbon content. The organic matter is, in fact, the heart of all these three problems, and, as a consequence, its evaluations and understanding is required.

According to Leenheer and Croué (2003), studies about the DOM can be divided into two categories: (i) whole water studies, in which DOM is characterized in water and its inorganic constituents; and (ii) studies of DOM fractions isolated from water and inorganic constituents. The scope of this research focuses on the first category, and, thus, different analytical methods for organic matter characterization will be presented as follows.

3.3 Analytical Methods for Organic Matter Characterization

The organic matter content in the aquatic system consists of a complex mixture of distinct chemical species. Therefore, its characterization based on analysis of individual compounds and their properties is still not possible (Frimmel, 1998; Leenheer and Croué, 2003; Sharma et al., 2011). The currently approach used in organic matter studies focus to characterize the presence of different group of compounds according to a set of fractions (Sharma et al., 2011). Figure 4 presents a schematic representation of different methods used for organic matter characterization.

Two indirect measurements are commonly used for a primary evaluation of the organic content in water samples: Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD). The main principle of both analyses is the measurement of the oxygen or oxidant, respectively, required for the oxidation of the organic matter. While BOD requires at least 5 days for its incubation and evaluation, COD uses a strong chemical oxidant which allows the quantification in several hours. Despite on this, COD test does not concern the same oxydisable compounds, since BOD can measure mostly the biodegradable fraction (Thomas and Theraulaz, 2007). The problem concerning this two analysis are related to the

subjectivity of the analytical methods, since the amount of the oxygen consumed will depend on several factors that are difficult to be rigorously controlled and compared.

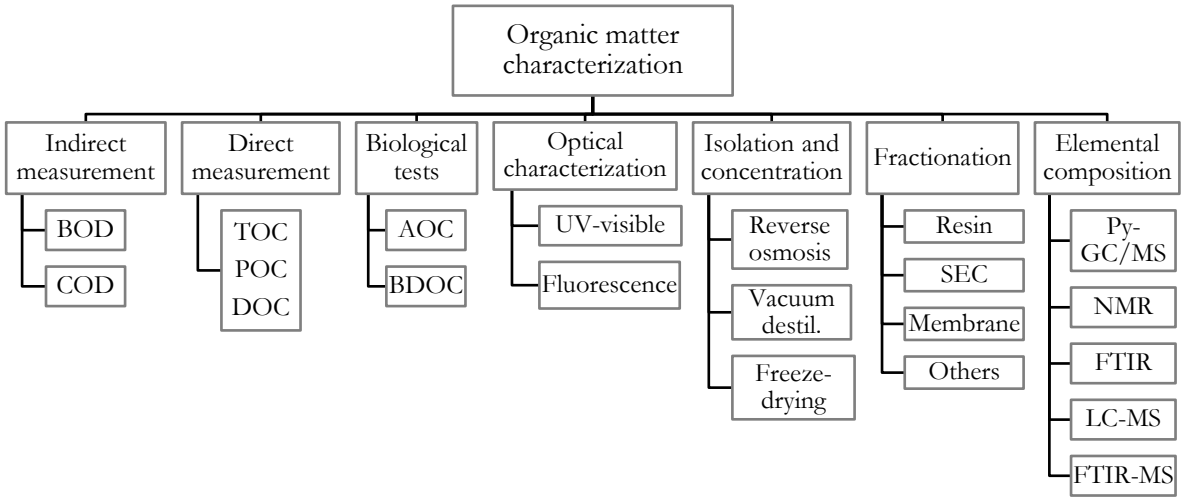


Figure 4: Schematic representation of different methods for organic matter characterisation

According to Leenheer and Croué (2003), TOC can be considered as the most comprehensive measurement that is widely used to quantify the presence of organic matter in aquatic systems. The organic carbon fractions can be operationally defined as dissolved organic carbon (DOC) and particulate organic carbon (POC). Most studies suggest that DOC is the organic carbon smaller than 0.45 μm diameter and POC is the fraction of TOC that is retained on a 0.45 μm porosity membrane. The determination involves the elimination of inorganic carbon by acidification, oxidation of the organic carbon (combustion or wet oxidation), and detection of the resulting CO_2 (Matilainen et al. 2011). Historically, its quantification has been proposed because of the problems related to BOD and COD tests (Thomas and Theraulaz, 2007).

Biodegradable organic matter (BOM) is also indicated as a complementary measurement of the organic matter characteristics. Several adapted methods can be used for its quantification, and are generally based on two concepts: (i) assimilable organic carbon (AOC); and (ii) biodegradable organic carbon (BDOC). Both experiments are based on the incubation of a sample in controlled temperature, in the dark, during several days (Van der Kooij, 1982; Servais et al., 1987). AOC measures the growth of bacterial inoculum according to the amount of biodegradable organic matter assimilated by microorganism and converted to biomass (Van der Kooij, 1982; Kaplan et al., 1993). BDOC test consists of measuring the

decay of DOC that is assimilated and mineralized by heterotrophic microorganisms (Escobar and Randall, 2001; Matilainen et al., 2011).

UV-visible and fluorescence spectrophotometry are two techniques mainly used to characterize and/or discriminate different compounds and its origins. These spectrophotometric analyses are based on intrinsic properties, such as absorption on both visible and UV light and the presence of fluorescence compounds in the aquatic system (Carstea, 2012). Due to its easy and fast analysis, these techniques are widely applied, with several indications about how are the most appropriate wavelengths to analyze, indexes to evaluate and compare the data, statistical modeling and other features.

According to Matilainen et al. (2011), there are a number of different methods for isolation and concentration, such as reverse osmosis (RO), evaporation under reduced pressure (vacuum distillation), freeze-drying, and sorption methods. The techniques basically consist to separate and concentrate the dissolved organic matter molecules from water for further characterization. One of the disadvantages with most of these methods is related to the concentration of salts, which may need to be removed prior to subsequent characterization and reactivity studies.

Another technique that can be applied for organic matter characterization is based on its fractioning into distinct categories with resin sorbents. This analysis consists in acidify the sample and pass it through a column with resin. Depending on the type of resin (sorbent), and due to the hydrophobic (humic and fulvid acids) and hydrophilic properties of organic matter, specific fractions of natural or effluent organic matter will be sorbed onto one type of resin (Leenheer and Croué, 2003; Sharma et al., 2011). Examples of commercial resins used to isolate different organic matter compounds from water are XAD-8 and XAD-4 (Leenheer and Croué, 2003; Labanowski and Feuillade, 2009).

In addition, size characterization of natural organic matter has also been applied for water quality monitoring. This analysis, also named as liquid chromatography with online organic carbon detection (LC-OCD) or size-exclusion chromatography with DOC detection (SEC-DOC), is based on molecular size determination with gel permeation chromatography (Sharma et al., 2011). Basically, the water sample is passed through a column of gel, and since the largest molecules have the shortest retention times, the extent to which fractions are retarded is a measure of their molecular size (Sharma et al., 2011).

The elemental composition is often used for calculation of the atomic ratios between organic carbon and oxygen, hydrogen and nitrogen. Different methods are used for its analysis (Matilainen, 2011): pyrolysis (Py-GC/MS), nuclear magnetic resonance (NMR),

fourier transform infrared spectroscopy (FTIR), liquid chromatography-mass spectrometry (LC-MS), fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS). According to Matilainen et al. (2011), these techniques, in most part of the methods, are complex and time consuming and require access to sophisticated analytical instrumentation. Mostly, for a better understanding about the organic matter quality and characteristics during different steps in water treatment, for example.

Sharma et al. (2011) emphasize that much of the currently knowledge on organic matter characterization protocols is based on drinking-water studies, i.e., mainly considering only natural organic matter. According to the authors, it is still necessary to demonstrate how to properly characterize effluent organic matter and test the applicability of the methods for this purpose, in order to better understand its fate, transport, and transformation.

Thomas and Theraulaz (2007) summarized different domains (families of organic and mineral compounds) and the applicable analytical method that can be used (Figure 5). The biodegradable organic matter can be analyzed through BOD, COD, and TOC, as UV spectroscopy. Other compounds, such as humic substances, are not suitable for detection by BOD, but can be estimated by COD or TOC analyses. Thus, complementary these methods can provide a better understanding for both quantitative and qualitative terms of the organic content, their origin and how it will be degraded in the aquatic ecosystem.

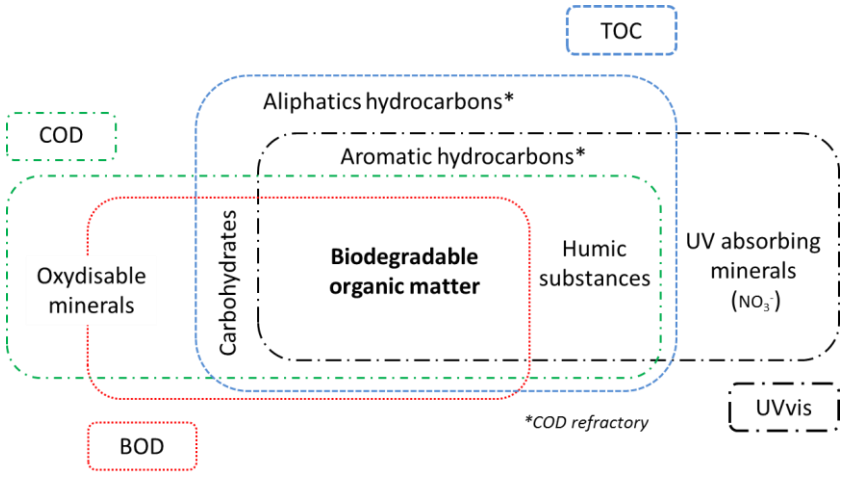


Figure 5: Comparison between different groups of compounds and methods for its analyses
(Source: Adapted from Thomas and Theraulaz, 2007)

In such context, the selection of the method for organic matter quantification and characterization depends up on the aims of the study. Some of the methods are more suitable for drinking water treatment analysis due to its low detection limits, while for effluent organic

matter evaluation other methods can be more easily applied in a routinely way. Thus, considering the purpose of this study, the methods selected for the evaluation of the organic matter content are: (i) BOD and COD; (ii) TOC, DOC, and POC; (iii) BDOC; and (iv) UV-visible and fluorescence spectroscopy. A more detailed description about these methods is presented in the next items.

3.3.1 Biochemical and Chemical Oxygen Demand

Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are two commonly analyses applied for wastewater control and water quality monitoring. The basic principle of BOD analysis is based on the measurement of the amount of oxygen necessary to the organic matter degradation in a specific volume. Considering the biodegradation process, the organic matter is assimilated by microorganisms under appropriate conditions (presence of nutrients such as nitrogen, phosphorous, and mineral nutrients), and converted into microbial biomass, eventual transformation products derived from the initial organic matter, CO_2 and H_2O (Davies, 2005; Jouanneau et al., 2014).

The BOD experimental test consists in incubating the sample in a specific flask, with controlled temperature (20 ± 1 ºC) in the dark during at least 5 days or more. Depending on the sample type, dilution with a solution composed with nutrient and saturated with oxygen may be required, addition of microorganisms (seed) and nitrification inhibitor (allythiourea) (APHA, 1998; Tomas and Theraulaz, 2007). Usually, BOD is expressed in terms of oxygen equivalent (mgO_2/L).

The oxygen demand is the amount of oxygen consumed during the incubation time, and can be measured with a manometric device or by direct oxygen measurement (potentiometric electrode or titration). Since several dilutions may be necessary in order to measure the concentration of oxygen at the end of the incubation time, a common reduction percentage between 40 and 60% of the initial concentration is indicated for BOD analysis (APHA, 1998; Tomas and Theraulaz, 2007).

The BOD test is an important and the most applied water quality parameter that has been used worldwide since it was first proposed in 1884, when it was observed that microorganisms caused a decrease in the dissolved oxygen levels of incubated water samples (Kumar and Kumar, 2005). Historically, the U.K. Royal Commission on River Pollution chose the five day period for BOD_5 tests to represent the travel time that the river water takes

from its source to its estuary in the U. K. (Jouanneau et al., 2014). In addition, the 5 day period is also refereed as the decomposition of carbonaceous BOD, since nitrogenous demand usually starts after the easily degradable organic matter is assimilated (APHA, 1998).

However, BOD is a time – consuming method to evaluate the biodegradable organic matter and the results may vary significantly (more than 20%) considering laboratory comparative measurements (APHA, 1998; Jouanneau et al., 2014). In addition, BOD is a subjective test that can be influenced by different factors, especially those that impose some important disadvantages that corroborates for the well-known subjectivity of this parameter (Comber et al, 1996): (i) since the test requires 5 days for completion, it is a time consuming analytical method and, therefore, is not suitable for real-time remedial action or efficiency control of wastewater treatment processes; (ii) considering relatively uncontaminated river samples, the detection limit of the method does not allow to have a precise result; (iii) since it is an indirect quantitative measurement of the organic content, it is not simple to interpret if low BOD values are due to low organic content or if it is due a small proportion of readily degradable or the presence of toxins that can inhibit biochemical oxidation; and (iv) the incubation is carried out in the dark, which does not represent the natural processes.

Despite the mentioned disadvantages, BOD is still one of the first and probably the most traditional parameter for water quality monitoring plan. Worldwide applications include its use for assessing the efficiency of wastewater treatment and can be considered as a key parameter for managing purposes, i.e., river classification, domestic and industrial effluents regulation. In wastewater treatment plants (WWTP), the associated analysis of BOD_5 and COD (ratio BOD_5/COD) can be used as an indication of the biodegradable fraction of the effluent (Metcalf and Eddy, 1991; Jouanneau et al., 2014). Complementarily, it is one of the parameters most used for mathematical simulations to evaluate the impacts of pollution in surface waters.

In addition to BOD analysis, COD is normally and systematically used for organic matter monitoring, especially in wastewater treatment plants. The COD analysis uses an oxidant solution for the mineralization of the organic content. The oxidant consumption is determined by a difference with a final redox titration or by UV-visible spectrophotometric methods. The amount of oxidant used is then correlated with the initial organic matter presented in the sample. Potassium dichromate ($K_2Cr_2O_7$) in concentrated sulphuric acid is usually applied as oxidant solution for mineralization of the organic content in hot acid during 2 hours (APHA, 1998; Tomas and Theraulaz, 2007). Problems related to COD analysis can

be due to the presence of suspended solids or occurrence of precipitation when using spectroscopy to quantify the amount of oxidant used.

3.3.2 Total, Particulate, and Dissolved Organic Carbon

As mentioned before, TOC is considered as the most relevant parameter for global determination of organic pollution in aquatic systems (Leenheer and Croué, 2003; Thomas and Theraulaz; 2007). Historically, it has been proposed in late 70's to overcome the subjectivities and problems related do BOD and COD analyses. Since then, improvements had been done in its instrumentation, collection and storage procedure, filters and/or membrane porosity standards. TOC analysis has the same basic principle of BOD and COD parameters, but instead of an indirect measure of the oxygen consumption due to the decomposition of organic matter, its determination involves the measurement of the carbon oxidized and converted to CO_2 (Thomas and Theraulaz, 2007). Figure 6 summarizes the typical definitions and their relations considering the carbon content in water. The major segmentation considers organic and inorganic carbon. Organic carbon bonds with hydrogen or oxygen to form organic compounds, while inorganic carbon is the structural basis for inorganic compounds such gas carbonates and carbonate ions (APHA, 1998).

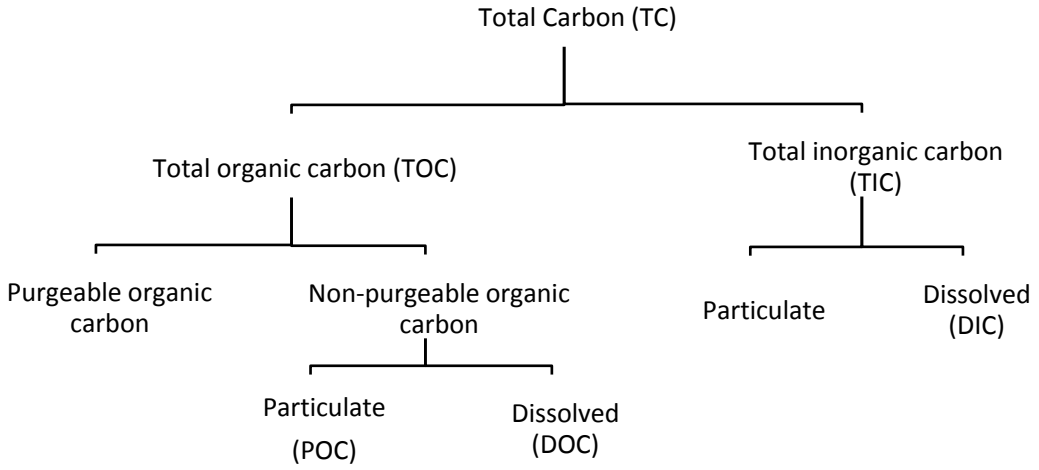


Figure 6: Summary of the typical definitions of carbon contend in water

The organic carbon can be also divided in non-purgeable and purgeable organic carbon (Figure 6). Purgeable or volatile organic carbon refers to the organic carbon that can be removed from sample after purging. According to APHA (1998), in most surface and groundwater waters the purgeable organic carbon fraction is negligible. Complementarily, purgeable organic carbon is sensitive to specific conditions and equipment employed in its measurement. For example, sample temperature and salinity, gas-flow rate, type of gas diffuser, purging-vessel dimensions, volume purged, and purging time may affect the measurement of purgeable organic carbon (APHA, 1998; Shimadzu, 2003).

Depending on the TOC instrument, the measurement of purgeable organic carbon can be done using a special device. After sparging the aqueous sample, the gas containing the volatilized CO_2 and the purgeable organic carbon is carried out in a specific lithium hydroxide-filled CO_2 absorber to eliminate the CO_2 originated from inorganic compounds. The remaining gas is than oxidized (e.g. in a combustion tube), and the converted CO_2 is measured (Shimadzu, 2003).

While purgeable organic carbon is always dissolved, the non-purgeable organic carbon fractions can be operationally defined as dissolved organic carbon (DOC) and particulate organic carbon (POC), and constitute the most relevant part of the organic carbon in natural environments (APHA, 1998).

One question that must be highlighted is that, according to the literature review in different applications of DOC and spectroscopy measurements, there is not a standard indication about the membrane or filter porosity used for samples pre-treatment. Some authors used porosity size of 1.0 μm for wastewater monitoring (Nataraja et al., 2006), 0.7 μm to analyze DOC in surface water and wastewater monitoring (Kaplan, 1994; Gergel et al., 1999; Saadi et al., 2006; Fellman et al., 2008) and 0.4 μm for treated and surface water analyses (Sugiyama et al., 2000; Potter, 2005). Other studies indicated porosity of 0.22 μm to analyze the dissolved fraction of organic matter without the interference of bacteria, which will be retained in the filter membrane (Kaplan, 1994; Mounier et al., 1998; Fellman et al., 2008; Bauer and Bianchi, 2011). However, this restrictive porosity is not often used for systematically analyses due to limitations in sample volume and throughout, especially in particle-rich freshwaters and/or estuarine waters (Bauer and Bianchi, 2011). On the other hand, higher-flow and capacity glass or quartz fiber filters (size porosity of 0.7 μm and 1.0 μm) allow the passage of a significant fraction of the free-living bacteria and viruses, which can interfere in two ways the DOC analysis: (i) if the sample is not immediately acidified, bacterial activity may alter the quantity and the characteristic of the DOC; and (ii) bacterial

biomass will be quantified as DOC, even though its components biomolecules may be significantly different from the ambient DOC. In this context, Bauer and Bianchi (2011) also highlighted that changes in DOC due to the inclusion of bacterial biomass in samples have not been rigorously addressed.

Thus, most studies suggest that DOC is the organic carbon smaller than 0.45 μm diameter and POC is the fraction of TOC that is retained on a 0.45 μm porosity membrane (Wangersky, 1993; Frimmel, 1998; Burkhardt e tal., 1999; Leenheer and Croué, 2003; Bayram et al., 2011; Matilainen et al. 2011). This size porosity is also indicated for spectroscopic analysis (Westerhoff e Anning, 2000; Ma et al., 2001; Reynolds, 2003). For the purposes of this research, DOC is the fraction of the organic carbon after a filtration through a 0,45 μm porosity size membrane filter.

Additionally, the type of filter or membrane is also important for dissolved organic analysis. A relevant concern is related to the release of organic carbon from the filter into the filtrate (Kaplan, 1994). Commonly used filter include glass fiber-filter (GF/F), silver membrane filters, nitrocellulose or cellulose acetate membrane. The type of filter or membrane depends upon the analysis, size porosity adopted for the study and costs. Pre-combusted glass-fiber filter does not release organic content, but the lower available porosity size is 0.7 μm. The advantage of this kind of filter is that it is possible to measure both dissolved and particulate fractions in the same sample, depending upon the equipment available. Another membrane that allows the analysis of POC and DOC in the same sample is the silver membrane filter. This kind of membrane has a nominal porosity of 0.45 μm, but its high cost is a disadvantage, especially for a systematic monitoring strategy. A relatively low cost option for DOC analysis is the acetate cellulose membrane. This membrane has a nominal porosity of 0.45 μm, but it needs to be washed before the use to eliminate possible sample contamination.

Considering the acid preservation, different acids had been tested. The addition of acid is indicated to stop the biological activity and to lower the pH for IC elimination. However, some interference may occur depending on the type of acid and equipment features used for the organic carbon analysis. According do Wallace (2003), in a test comparing sulfuric, phosphoric, nitric and hydrochloric acid, the most indicated acid for sample preservation is the phosphoric acid (H_3PO_4). The results indicated that H_3PO_4 is not as corrosive as the other one tested, and the combustion product is P_2O_5, which is readily taken up in water and thus will not interfere in the gas detector. Nitric and hydrochloric acid showed more potential of corrosion in some parts of the equipment due to the N_2O_4 and

HCl gas formed, respectively. Sulfuric acid will also produce SO_3 gas during combustion. The concern is that the detection properties of SO_3 are the same as CO_2, and thus it may interfere in the analysis if the instrument does not have an appropriate SO_3 scrubbing device. Besides that, sulfuric acid is the most used acid for preservation (APHA, 1998), and, in addition, Potter (2003) recommended that independent of the acid used for the analysis, it is important to keep the same one for both sample preservation and calibration standards.

Another kind of preservation that is still discussed is about the necessity to freeze the sample. Freezing is a common technique used in several studies, especially due to laboratory logistics or field distance. However, some recent publication (Spencer et al., 2007; Fellman et al., 2008) indicate some possible alteration in the results in DOC and spectroscopic analysis. Spencer et al. (2007) observed that after freezing, the concentration of DOC decreased 10% and, consequently, alterations where observed in spectroscopic analysis. Fellman et al. (2008) added that for concentrations higher than 5 mgC/L it is not indicated to freeze the sample, since this procedure will change de composition of the dissolved organic matter presented in the sample, and some aromatic fractions will precipitate. On the other hand, Potter (2003) indicates that the maximum period of freezing is 28 days if the sample pre-treatment (filtration and acidification) is done during the first 48 hours of collection.

Depending upon the equipment used, two general approaches are used to measure TOC. One method is based on the analysis of total carbon and total inorganic carbon. TOC is thus the difference between these two fractions. Another procedure is to first eliminate all the inorganic content of the sample and then analyze the organic carbon. In this second method, the elimination of inorganic carbon is done by acidification, reaching a pH bellow 2.0. This process converts inorganic carbon to dissolved CO_2, which then is purged from the sample. After this, the remaining organic carbon is oxidized (combustion or wet oxidation) and the resulting CO_2 is measured by a specific detector, as, for example, a nondispersive infrared detector (NDIR) (APHA, 1998; Matilainen et al. 2011). Figure 7 shows a schematic representation of these methods.

Once the IC content is eliminated, another consideration about organic carbon measurement is related to the total, particulate, and dissolved organic carbon. Different approaches are available for its quantification, and will depend on the type of filter/membrane and on the equipment features. For example, one way to quantify the TOC is by direct injecting the sample without filtering. The problem in this case is related to the tube and syringe diameter (some equipment have restrictions about this and depending on the sample characteristic, it may clog the system). Possibilities to overcome this problem is by

direct injecting the sample in the combustion tube (some equipment allow this procedure), by using a solid combustion or by reducing the size of the particles before the injection. For this case, a second analysis of the dissolved form will be necessary.

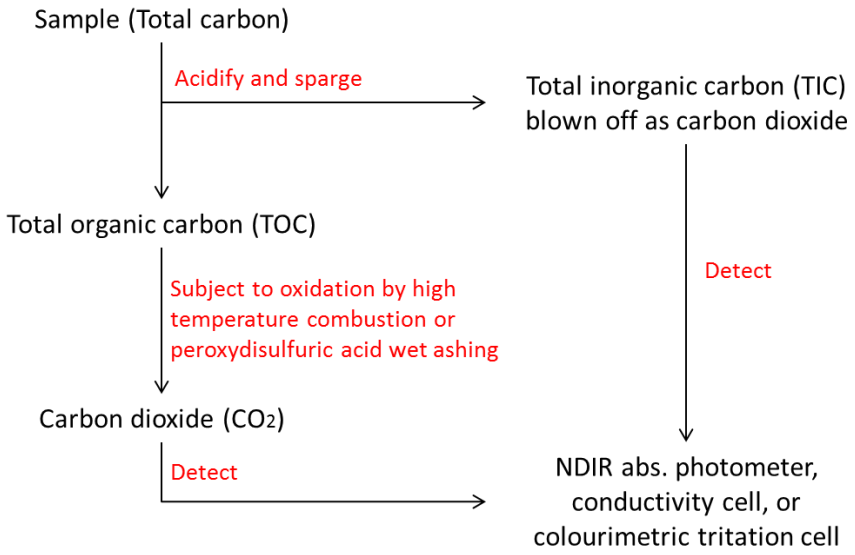


Figure 7: Schematic representation of the process for the determination of TOC and TIC for liquid samples.

(Source: Adapted from Urbansky, 2001)

POC can be than estimated by the difference between these two forms. When using a solid combustion module, the volume of sample for direct injection can be a limiting factor, since the detection limit of this type of equipment is normally not as sensitive as for liquid samples. Manual injection in the combustion tube can also show low repeatability due the low volume injected (normally less than 0.15 mL). For the third case, ultra sonication can be used to reduce the size of the particles. Tests may be necessary depending on the characteristic of the sample to avoid clogging the system when using the automatic sample injection, and, in addition, particles can settle during the analysis, which may alter the results.

Another possibility is by analyzing the DOC and POC in the same sample using a glass-fiber filter or silver membrane. In this case, the sample is filtered, and the DOC is analyzed. The filtrate retained in filter or membrane is burned in a solid combustion module do quantify the POC. TOC is than the sum of these two fractions. The problem related to the glass-fiber filter is related to the size porosity (0.7 μm) and the high cost of the silver membrane filter.

In addition, the sample can be oxidized before the analysis to generate CO_2 in a separated flask. The CO_2 can be analyzed in specific detectors (NDIR, gas chromatograph or

CO_2 sensor), both for TOC (raw sample), POC (oxidizing the particulate retained in a filter) or DOC (filtered sample). The problem of this procedure is related to the efficiency of oxidation, which have to be tested and evaluated. One example of this kind of method was proposed by Lampman et al. (2001). The authors adapted a method using wet-digestion with potassium persulfate ($K_2S_2O_8$) for measuring the carbon retained in glass fiber filter. After a digestion in autoclave (about 2.5 hours), the CO_2 is collected from the sealed 20 mL serum vials and the extract is injected in a GC equipment.

According to Visco et al. (2005), problems related to the TOC measurement in aqueous samples have been observed since 1970. Spencer et al. (2007) also emphasizes that to have a data base capable for comparison during time and space, is fundamental that the analysis does not have influence of environmental or storage variation. In international review, there are several guidelines concerning TOC measurement, but still with different and/or conflicting analytical methods. Factors that may affect the organic carbon analysis are temperature, salinity, pH, microbial activity, and surrounding vegetation (Visco et al., 2005).

In addition, according to Potter (2003), some of the equipment available for organic carbon analyses are not capable of completely eliminates the inorganic carbon from the sample. In this case, significant errors can be observed when the quantification of TOC or DOC is done by the difference from the total organic carbon and the dissolved fraction. The recommendation is to first eliminate the IC from the sample through external acidification and sparging.

Aiken et al. (2002) also emphasized about the ability to adequately measure POC. According to the authors, POC have been traditionally determined by directly measuring organic matter retained on a filter, followed by wet chemical or high temperature combustion techniques. Since historically, TOC data are, according to the authors, not as useful as DOC and POC data for understanding processes, environmental chemists and geochemists have not given much attention to problems associated with TOC analysis. The authors suggested that the problems associated with oxidation efficiency, since not all the particulate material will be oxidized depending on the combustion temperature, must be addressed in order to have reliable TOC data.

For the purpose of this thesis, the dissolved fraction is considered the sample that passes through a 0.45 μm porosity membrane filter. TOC and DOC samples will be directly injected in the instrument (TOC-V_{CPH} analyzer, Shimadzu) after IC removal (external sparging) using a gas tight micro syringe. POC is estimated by the difference between TOC and DOC.

3.3.3 Biodegradable Organic Matter

The experimental measurement of biodegradable organic matter differs by two different concepts: (i) biodegradable dissolved organic carbon (BDOC), and (ii) assimilable organic carbon (AOC). The first one, originally developed by Van der Kooij (1982) and co-workers, and later modified by authors such as Kaplan et al. (1993) and LeChevallier et al. (1993), measures the growth of bacterial inoculum according to the amount of biodegradable organic matter in the sample. The second method, developed by Servais et al. (1987), consists of measuring the DOC assimilated and mineralized by heterotrophic microorganisms (Escobar and Randall, 2001; Matilainen et al., 2011). BDOC is the difference between the initial DOC and the minimum reached during the incubation period (Frias et al., 1995), while in AOC methods, heterotrophic plate count, concentration of intracellular ATP or turbidity increase represents the biomass assimilated. Additionally, specific ultraviolet absorbance at 254 nm wavelength ($SUVA_{254}$) is also used to evaluate the biodegradability during BDOC tests (Trulleyová and Rulik, 2004; Saadi et al., 2006; Lim et al., 2008; Stutter et al., 2013).

BDOC has application in studies of biodegradability and transformation of effluent organic matter in surface waters (Namour and Mueller, 1998; Khan et al., 2003; Saadi et al., 2006; Lim et al., 2008; Labanowski and Feuillade, 2009), natural organic matter (Søndergaard and Worm, 2000; Stutter et al., 2013), water and reclaimed water distribution systems (Servais et al, 1987; Kaplan et al., 1992; Charnock and Kjonno, 2000; Escobar and Randall, 2001; Weinrich et al., 2010), and seawater (De Vittor et al., 2009; Lonborg et al., 2009).

From the pioneer BDOC proposition (Servais et al., 1987), authors have adapted the experimental procedure to optimize the incubation time and/or volume of sample analyzed (Frias et al., 1995; Escobar and Randall, 2001; Reuschenbach et al., 2003; Khan et al., 2003; Trulleyová and Rulik, 2004). Modifications and differences between BDOC methods are due to the reactor used for incubation, parameter measured, incubation time, nutrients and inoculum added and sample pre-treatment. One method for BDOC measurement involves the use of plug-flow biofilm reactors (Søndergaard and Worm, 2001; Khan et al., 2003). In this composition, the sample flows through a column filled with a material that allows the formation of biofilm. BDOC is the difference between DOC concentration in the inlet and outlet of the system after the stabilization of the bioreactor, which can take months to its colonization.

Another approach for BDOC analysis consists in the measurement of the DOC concentration in an incubated sample during a period of time. The sample can be stored in

individual bottles from 10 to 250 mL (Trulleyová and Rulik, 2004; Saadi et al., 2006; Labanowski and Feuillade, 2009; Wickland et al., 2012), or in large volumes (1 to 4 L glass bottles) with systematically collection of sub-samples (Lim et al., 2008; Reuschenbach et al., 2003; Namour and Mueller, 1998). The most common parameter measured for BDOC quantification is the DOC concentration, but some authors use complementarily parameters such as UV absorbance (Trulleyová and Rulik, 2004; Stutter et al., 2013), CO_2 detection (Wickland et al., 2012), and POC and TOC concentration (Servais et al., 1995).

The incubation time range between 2 to 10 days (Joret and Levi, 1986; Escobar and Randall, 2001), and 28 days (Kaplan et al., 1992; Charnock and Kjonno, 2000; Reuschenbach et al., 2003; Khan et al., 2005; Wickland et al., 2012) up to 60 days (Saadi et al., 2006), depending up on the source of sample and inoculum used. Some authors indicate the addition of nutrients (Reuschenbach et al., 2003; Labanowski and Feuillade, 2009; Stutter et al., 2013) and/or inoculum with indigenous bacteria (Servais et al., 1987; Namour and Mueller, 1998; Wickland et al., 2012), sand fixed bacteria (Escobar and Randall, 2001), suspended bacteria (Charnock and Kjonno, 2000), soil inocula (Saadi et al., 2006), and others. Additionally, some authors use a sample pre-treatment such as glass fiber or membrane filtration (Servais et al., 1987; Ribas et al., 1991; Kaplan et al., 1992; Trulleyová and Rulik, 2004; Wickland et al., 2012). Table 3 presents a summary of some adaptations tested for BDOC methods.

The motivation related to the analysis of the biodegradable fraction of the organic matter is to understand the transformation pathway in the cycling of biological and chemical elements. Thus, BDOC test will be used as a complement to the other analytical methods presented in this chapter, in order to estimate the rates in which the organic carbon is biologically decomposed and the fraction of biodegradable organic carbon in different sites of the study area

Table 3: Summary of BDOC adapted methods analysis and examples of application

N	Sample water pre-treatment	Sample type	Inoculumn	Nutrients	Shaking/ Aeration	Temperature (°C)	Culture type	Duration	Test parameter	Applicability	Reference
1	Membrane filtration	Drinking water	Indigenous bacteria			15-25	batch	10-30 days	DOC	Evaluate the effects of ozonation in drinking waters	Servais et al. (1987)
2			sand fixed bacteria			20	batch	2-7 days	DOC		Joret and Levi (1986)
3			Indigenous bacteria			20	Recirculating batch	3-5 days	DOC		Frias et al. (1992)
4	Glass fibre filtered		Indigenous bacteria			10-25	continuous	2 hours	DOC		Ribas et al. (1991)
5	Freeze-drying and reconstitution	Moorland stream	Indigenous bacteria/ without	N and P/ without	30 rpm	15	batch	41 days	DOC, UV absorbance	Analyze the fate of DOM in a moorland stream	Stutter et al. (2013)
6	Membrane filtration (0,45 µm)	Stream waters	Indigenous bacteria			5 and 15	batch (10 mL glass bottles)	28 days	DOC, UV absorbance, EEMs, XAD resin fractionation	Evaluate seasonality	Wickland et al. (2012)
7		Wastewater/ surface water			Aeration	20-22	batch (4 L glass bottles)	10 days	DOC, UV absorbance	Assess the potential for transformation of wastewater-derived contaminants in surface waters	Lim et al. (2008)
8		Raw and drinking water	sand fixed bacteria			20	batch	2-7 days	DOC	Test AOC and BDOC methods	Escobar and Randall (2001)
9	Glass fibre filtered	Drinking water				Room temperature	batch	28 days	DOC	Compare AOC and BDOC data for drinking water	Kaplan et al. (1992)
10		Raw and drinking water	Suspended inoculum bacteria			20	batch (40 mL tubes)	28 days	DOC	Test AOC and BDOC methods for drinking water	Charnock and Kjonno (2000)
11	Membrane filtration (0,45 µm)	River/ leachate	Bacteria from activated sludge	Ca, Fe, N, Mg, Na, K	50 rpm	20	batch (250 mL)	28 days	DOC	Biodegradability in environmental and anthropic samples	Labanowski and Feuillade (2009)
12		Stream water and samples taken in different steps in a water-treatment process					batch, recirculating batch	10 - 21 days	DOC, colony counts	Comparison of six different methods for AOC and BDOC	Frias et al. (1995)
13	Glass fibre filtered (0,4 µm)	Stream water and leaf leachate	Indigenous bacteria (free, suspended and attached to artificial substracta)		ocasional shaking	20 ± 0,5	batch (100 mL glass bottles with aluminium caps)	42 days	DOC, UV absorbance (254 and 400 nm)	Investigate the effect of different types of bacterial inocula	Trulleyová and Rulík (2004)
14		Organic compounds	Municipal and industrial inoculum	Ca, Fe, N, Mg, Na, K	150 rpm	22	batch (1L glass bottles)	28 days	DOC/ BOD	Comparison of respirometric biodegradation tests	Reuschenbach et al. (2003)
15		Effluent	Soil inocula			25	batch (20 mL glass vials)	60 days	DOC	Evaluate effluent DOM	Saadi et al. (2006)
16		Tap water and unchlorinated secondary effluent	Indigenous bacteria/ activated sludge inocolum/entrapped microbial cells	BOD nutrients buffer pillows - Hach	Aeration	20 ± 0,5	bioreactor/ batch	3 hours	DOC	Comparison of a bioreactor and the traditional batch system using entrapped microbial cells	Khan et al. (2003)
17	Glass fibre filtered	Lake water	Indigenous bacteria		plu-flow (3.5 h of residence time)	15 - 20	continuous bioreactor/ batch	3/4 hour for bioreactor and 28 days for batch	DOC	Test of bioreactor do determine BDOC	Søndergaard and Worm (2001)
18	Glass fibre filtered	Effluent from sewage treatment plant	Indigenous bacteria		Slow agitation/ aeration	20	reactor (3 L)	21 days	DOC	Evaluate the refractory fraction of effluents	Namour and Mueller (1998)

3.3.4 Fluorescence Spectroscopy

Fluorescence spectroscopy is an optical technique that has been applied for organic matter characterization in aquatic environments. The main principle of the analysis is based on the emission of light from molecules (named fluorophore) when they are excited by a special type of luminescence. The fluorophore absorbs energy (in the form of light) at a specific wavelength and releases it with lower energy in the form of emission of light at a specific higher wavelength (Carstea, 2012).

A schematically representation of the dissolved organic matter fluorophore and its respective excitation/emission wavelengths domains are represented in Figure 8. In the aquatic environment there is a complex mixture of different substances, thus the identification of each individual fluorescent compounds is not easy. For this reason, specific groups of fluorophore that occurs in the same area of optical space are commonly named as humic-like, fulvic-like, protein-like, tryptophan or tyrosine-like (Hudson et al., 2007; Carstea, 2012). Additionally, there is another nomenclature to represent these common areas proposed by Coble (1996): humic substances in peak **A** (λ_{ex}= 230 nm/ λ_{em}= 400-500 nm) and peak **C** (λ_{ex}= 300-500 nm/ λ_{em}= 400-500 nm), tryptophan as peak $\mathbf{T_1}$ (λ_{ex}= 290 nm/ λ_{em}= 350 nm) and $\mathbf{T_2}$ (λ_{ex}= 230 nm/ λ_{em}= 350 nm), tyrosine as peak **B** (λ_{ex}= 230-275 nm/ λ_{em}= 310 nm), and marine humic acids as peak **M** (λ_{ex}= 312 nm/ λ_{em}= 420-480 nm . This peak identification is schematically represented in an example of excitation-emission matrix as shown in Figure 9.

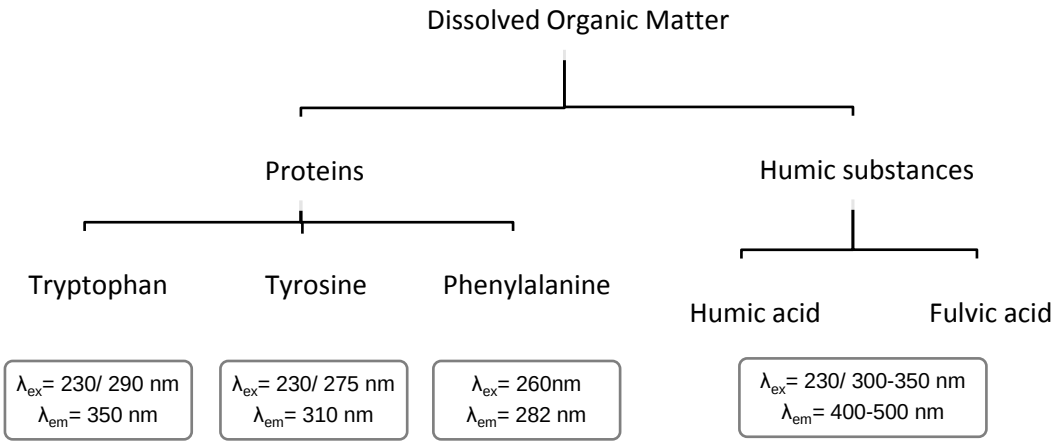


Figure 8: Schematic representation of DOM fluorescent fractions and its excitation/emission wavelengths.

(Source: Adapted from Carstea, 2012)

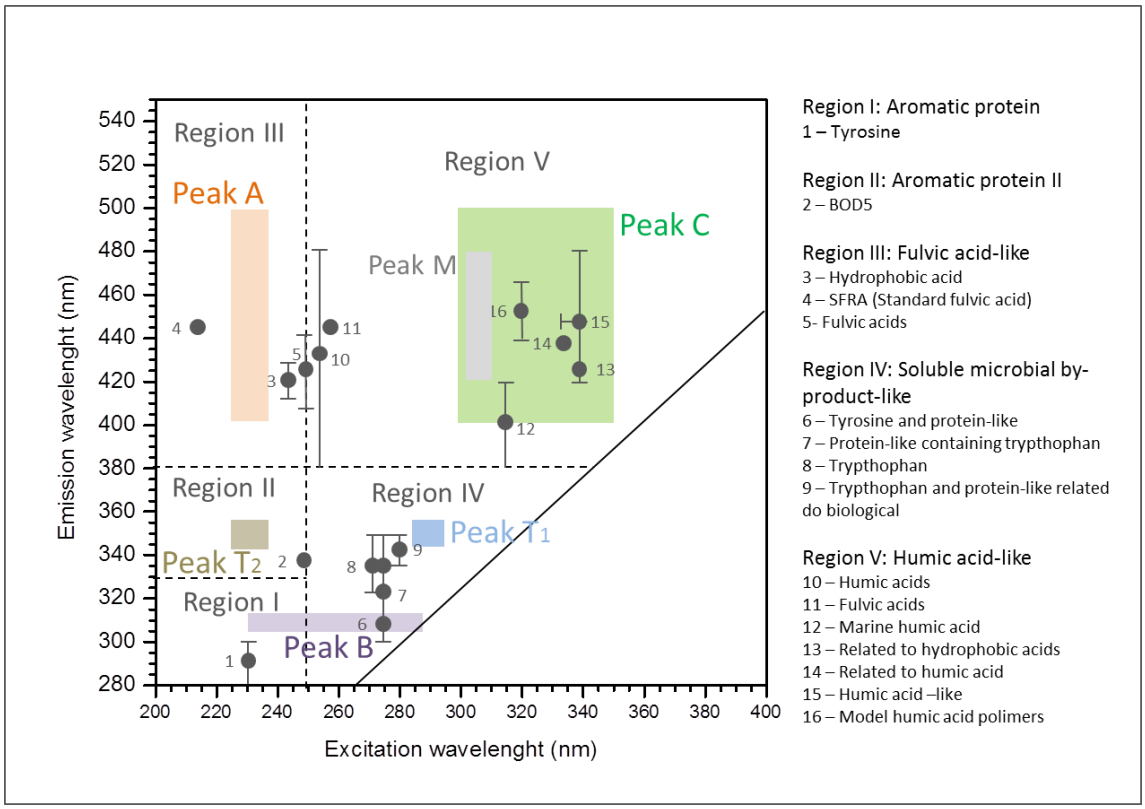


Figure 9: Peaks identification and excitation-emission fluorescence regions

(Source: Regions and specific points adapted from Chen et al., 2003; Peaks identification adapted from Coble, 1996)

These fluorescence peaks have also been applied to provide more information about the dissolved organic matter characteristics by correlating it to other water quality parameters. According to some studies, tryptophan-like fluorescence (peak T) correlates with BOD and has been used as a surrogate for this parameter (Hudson et al., 2008; Nataraja et al., 2006; Hur et al., 2008). Cumberland and Baker (2007) and Hudson et al. (2008) also indicate good correlation between peak T (tryptophan-like fluorescence) and/or peak C (humic-like fluorescence) and total organic carbon. But, as will be presented in the next items, these correlations must be investigated and evaluated with caution, since it appears to be site specific and depending up on the type of sample analyzes, i. e., wastewater, river, raw or treated water.

The fluorescence analysis can be performed by applying different emission and excitation wavelengths, excitation-emission matrix and/or synchronous fluorescence. Table 4 presents a summary of common wavelengths used for organic matter characterization related to water quality evaluation.

Table 4: Common wavelenghts used for organic matter characterization

Wavelength (nm)	Application	Reference
Synchronous Fluorescence ($\Delta\lambda=\lambda_{em}-\lambda_{ex}$)		
$\Delta\lambda$=18 nm	Aquatic humic matter	Peuravuori et al. (2002)
$\Delta\lambda$=20 nm	Treated wastewater	Ahmad and Reynolds (1995)
$\Delta\lambda$=25 nm	Surface water and plant extracts	Cabaniss and Shuman (1987)
$\Delta\lambda$=40 nm	Natural organic matter and wastewater differentiation	Galapate et al. (1998)
$\Delta\lambda$=60 nm	Detection of domestic and industrial effluents	Galapate et al. (1998), Reynolds (2002)
Fluorescence (excitation/emission wavelength)		
220/350 nm	Tyrosine in surface water	Baker e Spencer (2004)
248/340 nm	Tryptophan peak, NOM and domestic effluents characterization, BOD correlation	Ahmad and Reynolds (1999)
280/350 nm	Tryptophan-like fluorescence and sewage evaluation, BOD correlation, biodegradable organic matter differentiation	Pons et al. (2004), Reynolds (2002), Hudson et al. (2007)
280/440 nm	Non- biodegradable organic matter differentiation	Reynolds (2002)
314 nm	Natural organic matter and DOC characterization	Frimmel (1998), Westerhoff e Anning (2000)
340 nm	Properties of natural organic matter	Galapate et al. (1998), Chen et al. (2002), Pons et al. (2004)
360 nm	Humic and fulvic compounds	Senesi et al. (1989), Mafra et al. (2007)
370 nm	DOC and humic content characterization in water, soil and sediments	Westerhoff e Anning (2000)

Additionally, there are some indexes based on fluorescence intensities proposed by some authors in order to evaluate and compare the data. Westerhoff and Anning (2000) indicate the analysis of the emission spectra obtained by the 370 nm excitation wavelength for organic matter characterization. According to the authors, if the peak is located bellow 450 nm indicates the predomination of autochthonous organic matter, and peaks above 450 nm are an indication that there is more allochthonous organic matter. Another index proposed by these authors is calculated by the ratio of the emission intensity at λ=450 nm to the emission intensity at λ=500 nm ($FR=\lambda_{450}/\lambda_{500}$), considering the excitation wavelength λ_{ex}=370 nm. Values of FR > 1.8 indicate autochthonous sources, and FR ≤ 1.5 indicate allochthonous sources of humic substance.

However, besides fluorescence analysis is a rapid and direct measurement, there are some issues that must be taken into account. Carstea (2012) summarized some environmental factors that may affect the fluorescence intensity, such as solution temperature, composition, concentration, pH, and salinity. For an accurate result, these factors must be analyzed, especially in highly polluted samples, where the high concentration of contaminants may produce different signals and induce erroneous

interpretations due to these interferences. Furthermore, the relationship between fluorescence intensity and these environmental factors are not necessary linear, depending also on the origin and chemical composition (Hautala et al., 2000).

High temperatures, for example, decrease the fluorescence signal of the sample. Baker (2005) found that the most affected fluorophore is tryptophan in a comparative study with fulvic acid. According to Carstea (2012), no data correction is needed if the sample temperature, at the time of measurement, is between 20°C and 25°C.

The molecular structure, also presents an inverse relationship with fluorescence intensity. According to Senesi (1990) and Hautala (2000), the fluorescence intensity decreases with the increase of molecular size of humic compounds. This relationship is normally used to evaluate decomposition process through the fluorescence signal of the same sample through time, since it indicates differences in the molecular size of the compounds.

The fluorescence intensity can also be attenuated by the inner filtering effect (IFE). The inner filtering effect is a distortion and/or decrease in emission as a result of the absorption and emitted radiation by the sample matrix (Carstea, 2012). Various authors have shown that is essential for the correction of both primary and secondary inner filtering effect for data comparison and accurate representation of the fluorescence signal (Ohno, 2002). To make this correction, different approaches have been suggested by several authors, such as an empirical correction based on the Raman scatter peak and a mathematical correction based on absorbance profile of the same sample (McKnight et al., 2001; Lakowicz, J. R., 2006). This correction is also necessary in order to evaluate data from different equipment and from different data base.

Another thing that may affect the fluorescence of a fluorophore is the pH and the salinity of the sample that is being analyzed. According to Carstea (2012), intensities increase with higher pH until pH reaches the value of 10, and a slight decrease of its intensity occurs at pH 12 (Patel-Sorrentino, 2002). The authors indicated that no correction is needed if the sample pH is between 6 and 8. Additionally, certain fluorescence compounds may have its photoreactivity increased by high values of salinity (Chen et al., 2002; Carstea, 2012)

There are several examples of application of fluorescence technique in organic matter characterization highlighting its potential as a tool for water quality evaluation (Yan et al., 2000; Hudson et al., 2008; Henderson et al., 2009; Carstea, 2012). Some authors focused in the identification of autochthonous and allochthonous organic matter (Frimmel,

1998; Sugiyama et al., 2000; Westerhoff and Anning, 2000; Peuravuori et al., 2002; Chen et al., 2002). Other studies present different approaches for fluorescence analysis (Stedmon et al., 2003; Felipe-Soleto et al., 2007; Hur and Cho, 2012), the relationship between river, lake, and marine environments (Coble, 1996; Esteves and Duarte, 2000), and photodegradation process (Mostofa et al., 2007; Teixeira et al., 2013).

In such a context, this research also intends to use the fluorescence technique as a complementary tool to evaluate the organic matter dynamics considering a high polluted river as a case study. The motivation is that, in addition to other analytical methods, fluorescence analysis could provide a more detailed and rapid information about the organic matter characteristics even for a complex mixture of pollutants in an urban river.

3.3.5 UV-visible Spectrophotometry

The principle for the use of UV-visible spectrophotometry for organic pollution characterization and estimation is based on the absorbing property that several organic compounds show in the UV-visible region. According to Thomas and Theraulaz (2007), the presence of a lot of absorbing compounds in water and wastewater allows the use of this technique through the estimation of common patterns found by studies in different fields. In addition, it also provides a more detailed qualitative approach about the organic matter content.

In this context, examples of studies using UV-visible spectrophotometry in water quality monitoring are quite broad: (i) characterization of organic matter (Gallot and Thomas, 1993; Mounier et al., 1998; Ma et al., 2001; Imai et al., 2002); (ii) identification of organic matter sources in surface waters (Senesi et al., 1989; Peuravuori e Pihlaja, 1997; Khorassani et al., 1998; Artinger et al., 2000; Chen et al, 2002; Pons et al., 2004; Spencer et al., 2007); (iii) use of UV-visible index as surrogates for water quality parameters (Dobbs et al., 1972; Comber et al., 1996; Chevalier et al., 2002; Westphal et al., 2004; Nataraja et al., 2006; Hur and Cho, 2012); (iv) complementary analysis to verify the evolution of biodegradability tests (Saadi et al., 2006; Thomas and Theraulaz, 2007; Lesteur et al., 2010). Additionally, UV-visible data are also used to correct fluorescence spectroscopy data (inner-filtering effect correction).

Table 5 presents a summary of common wavelengths used for organic matter characterization related to water quality evaluation and its respective reference.

Table 5: Summary of main wavelenghts used for surface water quality monitoring

Wavelength (nm)	Application	Reference
254 nm	Organic matter characterization and source identification, DOC and conductivity correlation	Dobbs et al. (1972), Korshin et al. (1997), Westerhoff and Anning (2000), Pons et al. (2004)
280 nm	Domestic effluent characterization, BOD and DOC correlation	Nataraja et al. (2006)
285 nm	DOC composition, fulvic acids and labile organic matter	Rostan e Cellot (1995);
250 and 465 nm	pH effect on fluorescence signal	Hautala et al. (2000)
250/ 365	Aromaticity of organic compounds and molecular weight	Peuravuori and Pihlaja (1997)
300/ 400 (E3/E4)	Aromaticity and molecular weight of humic compounds	Artinger et al. (2000), Oliveira et al. (2006)
465/ 665 (E4/E6)	Aromaticity of organic compounds, molecular weight and particle size.	Senesi et al. (1989), Chen et al. (2002), Oliveira et al. (2006).

Similarly to fluorescence analysis, there are some indexes proposed by some authors to evaluate specific correlations and/or characterization of organic matter. One broadly applied index is the $SUVA_{254}$, normally used for organic matter characterization and source identification (Westerhoff and Anning, 2000). This index is calculated by the ratio between the absorbance at 254 nm wavelength and the concentration of dissolved organic carbon, correcting the value with the optical path. According to Westerhoff and Anning (2000), $SUVA_{254}$ values close to 4.4 L/mg.m indicate the presence of fulvic acid, and values close to 1.2 L/mg.m indicate that autochthonous organic matter predominates. Musikavong and Wattanachira (2007) also added that the $SUVA_{254}$ values tend to increase due to the biological processes involved in effluent treatment, 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. Biological activity tends to remove the fraction that is not sensitive to UV emission, i.e., the most labile compound that does not absorb in the UV-visible region, diminishing DOC and maintaining absorbance (Musikavong and Wattanachira, 2007).

Rostan and Cellot (1995) used the specific absorptivity at 285 nm (ratio between absorbance value and dissolved organic carbon concentration) to differentiate fulvic acid and labile organic matter. According to their study, A_{285}/DOC close to 20 L/g indicate that the COD are mainly due to fulvic acid, while values lower than 10 L/g indicate the presence of labile organic matter (aliphatic carbon). According to a study by Ma et al. (2001), this index can be used for sewage identification, since effluents are formed mainly by compounds with aliphatic functional groups, with low concentration of dissolved humic substances.

Comparing with other analytical methods generally used for organic matter estimation, as BOD, COD, and TOC, it is a fast and easy method and basically depends on sample filtration (0.45 μm) as a pre-treatment. However, UV-visible spectrophotometry is also influenced by pH, temperature, ionic strength, solvent purity and polarity and solute concentration (Burgess, 2007).

In this thesis, fluorescence and absorbance measurements will be performed in filtered samples (0.45 μm porosity size membrane), without other chemical pre-treatments (e.g., acidification).

3.4 Statistical Approach for an Organic Matter Monitoring Plan

In recent years, spectrophotometric monitoring has been proposed as an alternative to the use of traditional parameters. UV-visible and fluorescence spectrophotometry are two techniques used to characterize and/or discriminate different organic compounds and its origins. These spectrophotometric analyses are based on intrinsic properties, such as absorption on both visible and ultraviolet light and the presence of fluorescence compounds in the aquatic system (Carstea, 2012). Due to its easy and fast analyses, these techniques are widely applied, with several indications about how are the appropriate wavelengths and indexes to evaluate and differentiate compounds (Westerhoff and Anning, 2000; Peuravuori et al., 2002; Pons *et al.*, 2004; Henderson et al., 2009; Quaranta et al., 2012), statistical modeling (Murphy et al., 2010; Hur and Cho, 2012; Carter et al., 2012; Cohen et al., 2014), in-situ monitoring tools (Kowalczuk et al., 2010; Carstea, 2012; Shutova et al., 2014), identification of pollution sources (Goldman et al., 2012; Meng et al., 2013).

Fluorescence and absorbance spectroscopy has already been applied as surrogate method for surface waters characterization and organic matter sources identification (Thomas et al., 2005; Hur and Cho, 2012; Kwak et al., 2013), wastewater characterization (Reynolds and Ahmad, 1997; Escalas et al., 2003; Nataraja et al., 2006; Hur et al., 2010; Melendez-Pastor et al., 2013; Yang et al., 2014), industrial effluents (Chevakidagarn, 2007), and for organic matter identification in reservoirs (Westphal et al., 2004; Nguyen et al., 2011).

In such a context, a set of known data of BOD, COD, TOC or DOC are compared to a complementarily set of absorbance and/or fluorescence intensities. Table 6

presents a summary of previous work relating different samples sources and the respective correlation between parameters.

For example, the specific absorbance at 200 nm wavelength (UV_{200}), 254 nm (UV_{254}), and 280 nm (UV_{280}) have been analyzed with BOD, DOC, and TN concentration considering wastewater samples (Nataraja et al., 2006) and surface waters affected by sewage (Hur et al., 2008; Hur and Cho, 2012). The results of Nataraja et al. (2006) indicated that UV_{280} could be useful to estimate the BOD concentration, with good correlations for raw non-filtrate effluent. However, the results did not presented a unique pattern of correlation (Table 6), indicating that the relationship between absorbance and BOD may be wastewater and treatment plant specific and variable with time and treatment (Nataraja et al., 2006). Hur and Cho (2012) found good correlation between UV_{200} and UV_{254} and TN (*r=0.911* and *r=0.914*, respectively) and BOD (*r=0.706* and *r=0.892*, respectively). However, these results considered only two samplings with a short time interval in a river affected by sewage. Consequently, the good correlations may not be representative to extrapolate as a surrogate method for other flow conditions.

Based upon the same principles, tryptophan – like fluorescence peak and humic-like fluorescence peak are often tested with BOD, TOC, and DOC concentration (Baker, 2002; Hudson et al., 2007; Cumberland and Baker, 2007; Hur et al., 2008; Hur and Cho, 2012). In such cases, PARAFAC (Parallel Factor Analysis) is complementary used to separate and identify different areas of the EEM data (components), and has been successfully applied in several on-going investigations related to water quality (Felipe-Sotelo et al., 2007; Stedmon and Bro, 2008; Hur and Cho, 2010; Cid et al., 2011).

In a study to evaluate the possibility of using fluorescence spectrometry as a substitute for BOD testing, Hudson et al. (2007) found high correlations for a widely variety of samples. The authors emphasize, however, that the potential use of the technique is not as a surrogate, but as an independent indicator test for the presence of bio-available organic matter, associated biological activity and oxidizing potential with probable associated impacts on water quality. In addition, studies that explored such correlations found that sewage samples presents a better relationship between spectroscopic data and BOD or other parameter than samples from river (Nataraja et al., 2006; Cumberland and Baker, 2007; Hudson et al., 2007).

Complementarily, most of studies found in the literature do not present an overall analysis toward more efficient water resources planning and management research. The

challenge is to establish a strategy that combines and compares the water quality database considering its interpretation and application by regulatory agencies.

Table 6: Examples of correlations relating absorbance and fluorescence peak intensities to traditional water quality parameters for river, sewage and sewage impacted waters.

Sample	Number of samples	Parameter	Optical parameter	Correlation coeff. (Person's r)	Reference
River	124 (river) and 141 (sewage)	BOD	Tryptophan like fluorescence - T1	0.612	Hudson et al. (2007)
Effluent[1]				0.714	
River		TOC		0.457	
Effluent				0.714	
River	64	TOC[2]	Fluorescence-like peak and humic-like peak	0.409 / 0.463	Cumberland and Baker (2007)
Bog	49			0.756 / 0.504	
Groundwater	16			0.631 / 0.63	
Pond	14			0.657 / 0.609	
Treated final effluent	16			0.167 / 0.277	
Raw wastewater [4]	24	BOD	UV_{280}	0.73	Nataraja et al. (2006)
Filtered raw wastewater	12			0.63	
Primary wastewater [4]	34			0.49	
Filtered primary wastewater	17			0.24	
NSB [4]	19			0.11	
Raw + primary	29			0.95	
Filtered raw + primary	29			0.79	
River (non-affected by sewage) [5]	55	BOD	Peak I and A[3]	0.892 / 0.901 [6]	Hur et al. (2008)
			UV_{254}	0.778 [6]	
			Conductivity	0.684 [6]	
			EEM peak	0.91 [6]	
River (non-affected by sewage) [5]	31		Peak I and A[3]	0.615 / 0.591 [6]	
			UV_{254}	0.249 [6]	
			Conductivity	0.102 [6]	
			EEM peak	0.654 [6]	
River (affected by sewage) [5]	24		Peak I and A[3]	0.627 / 0.755 [6]	
			UV_{254}	0.545 [6]	
			Conductivity	0.706 [6]	
			EEM peak	0.737 [6]	
River	91	BOD	T peak	0.2 [6]	Baker (2002)
			F peak	0.68 [6]	
River (affected by sewage) [7]	35	TN	UV_{200} and UV_{254}	0.911 / 0.914	Hur and Cho (2012)
			C1, C2, and C3[8]	0.951 / 0.927 / 0.950	
		BOD	UV_{200} and UV_{254}	0.706 – 0.892	
			C1, C2, and C3[8]	0.948 / 0.938 / 0.948	
		DOC	UV_{200} and UV_{254}	0.770 – 0.973	
			C1, C2, and C3[8]	0.977 / 0.967 / 0.977	

[1] Effluent derived from domestic and industrial effluent; [2] Filtered samples were considered as TOC. [3] Peak I corresponding to $\Delta\lambda$ = 30 nm at 285nm, and Peak A corresponding to $\Delta\lambda$ = 60 nm at 285nm. [4] Raw: inlet wastewater; Primary: outlet of the primary settling basin; NSB: nitrification settling basin. [5] Sampling in three different times. [6] Sperman's rho coefficient. [7] Sampling in 18 sites along the main river in two days. [8] PARAFAC components: C1 and C2 being humic-like substances and C3 being related to tryptophan-like fluorescence (Acidified samples used for fluorescence analysis).

Additionally, considering the reasons indicated in the previous works, if a surrogate parameter is used to estimate the organic content in terms of BOD_5, for example, it is important that the results should retain a degree of comparability with historical data

(Comber et al., 1996). Other aspect that is important to highlight is that there are several indications, according to previous works cited, that exists a relationship between organic matter and fluorescence in water. However, this relationship is variable and may be site specific, indicating that if a surrogate is the final product, it must be used in accordance of the hypothesis adopted.

3.5 Challenges in Water Quality Planning and Management

Nowadays, it is well known that water quality deterioration is a multivariate problem derived from various causes, such as anthropogenic activities and modifications on land use, with multiple consequences. Thus, the identification of the pollution sources and the respective action to minimize its impacts on the water body depend on in-depth studies and monitoring programs. Up until now, however, management and restoration efforts focus on a traditional set of variables that typically includes biological oxygen demand, nitrogen, phosphorous, sediment or flow.

Historically, the motivation for choosing these variables was related to the impact they cause or the process they may indicate that is occurring in aquatic ecosystem, such as problems in depuration, eutrophication, and transportation or presence of metals in sediments. But, despite the organic carbon is directly related to all of these problems (Chapra, 1999), changes in its load or concentration in streams and rivers rarely motivates management activities (Stanley et al., 2012).

According to Stanley et al. (2012), some exception includes policies and activities intended to reduce organic matter loading from sewage effluents, prevent manure inputs during periods of intense runoff, increase carbon sequestration or minimize costs associated with dissolved organic carbon removal from drinking water sources. Even though, organic carbon monitoring is not included in a systematic way in planning and management strategies, especially in developing countries. In fact, first are necessary more studies to better understand the role of organic matter dynamics in a watershed and improve its analytical techniques.

Prairie (2008), in a review about the past, on-going, and future steps on studies about organic matter dynamics in lakes, its accumulation and transformation process in the water column, emphasis that DOC can be considered as a great modulator in the aquatic ecosystem. According to the author, it means that DOC is a variable that can modify the

influence of other variables due to the many constraints that DOC exerts on physical, chemical, and biological process and properties of aquatic ecosystems.

Considering physical process, DOC has an important property of light-absorbing characteristic, which influences and controls the depth light penetration and consequently the light-derived process used by live organism (Prairie, 2008). Additionally, according to Prairie (2008), and considering author's studies in Canadian lakes, most of the light absorbed by the chromophoric portion of the DOC is transformed as heat and complementary to other variables will influence the thermal regime, vertical structure, and duration of stratification of a lake.

The strong light-absorbing property are intrinsic to the DOC, which means that in other aquatic environments it is also an important mechanism of transformation, even though the hydrodynamics characteristics of a river, for example, does not allow the same time scale that the organic carbon will interact with light in the water column. Even for lakes and reservoirs, there are still unanswered questions about this kind of mechanisms. In an urban river, and considering the complex mixture of different pollutants, understand this properties and process is also a challenge.

The influence of DOC in the chemical properties of an aquatic ecosystem is related to different aspects such as adsorption, transport, and bioavailability of metals and organic contaminants (Haitzer et al., 1998; Winch et al., 2002), fate of nutrients and other elements by the C:N:P ratio that can be used by the phytoplankton (Prairie, 2008), among other process. In terms of biological aspects, DOC is a source of food for microorganism, directing influencing lake and rivers metabolism. In the presence of other nutrients, heterotrophic bacteria utilize DOC to convert it to biomass as part of the food web.

Besides these relationships are well documented for lakes and reservoirs (Westphal et al., 2004; Mostofa et al., 2007; Weissenberger et al., 2010), ocean ecosystems (Mopper et al., 1991), groundwater dynamics (Barcelona, 1984, Mostofa et al., 2007), in environments with low or no anthropogenic influence (Kiffney, Richardson & Feller, 2000; Futter et al., 2008) and water treatment plants (Baghoth, Sharma & Amy, 2011; Matilainen et al., 2011), changes in natural organic carbon dynamics in streams and rivers in human-dominated basins was not an object of study in the past years. As such, understanding how are the natural process and the ecological consequences due anthropogenic activities in streams and rivers needs to be part of an overall approach to a long-term management and the sustainability of these environments.

Stanley et al. (2012) recently questioned how human activities affect the quantity and quality of dissolved organic carbon in streams and rivers. The authors evidenced that besides its importance in an ecological point of view, the organic carbon is not a parameter commonly used for management purposes. The case presented by the authors focused mainly in impacts due land-use changes and how restoration/management actions could potentially be used to minimize some problems considering DOC as the main water quality indicator. They also emphasized that the currently understanding of the ecological consequences of DOC changes in human-dominated basins need to be overcome through more applied research and management attention towards this ongoing environmental transformation.

Thus, based on previous studies, and the motivation concerning the use of organic carbon in a new strategy for monitoring, planning and management actions, understand the organic matter sources, mechanism, and pathways in urban dominated rivers are still a wide field of research. Mostofa et al. (2013a) also highlighted that the scope for future research includes the determination of concentration levels of dissolved organic matter in important rivers and lakes in developing countries, in order to improve the information available and identify either if the concentration increased or decreased over the last few decades.

3.6 Summary

This chapter summarizes some important aspects related to the organic matter in aquatic systems. Organic matter plays an important role in aquatic dynamics, and its understanding is fundamental for monitoring strategies and management actions. Additionally, this chapter describes some of the analytical methods applied for organic matter quantification and characterization in surface waters. Aspects related to pre-treatment, storage, equipment features, and data analysis are highlighted based on previous studies in surface and wastewater monitoring.

Furthermore, a brief description about the statistical approach for organic matter monitoring is presented. This step is important for a better understanding of the organic matter variability during time and space. In addition, how the organic matter parameters can be correlated to other traditionally parameters used in surface water quality monitoring. A critical analysis about the use of surrogates for its monitoring is also presented.

One point that is important to highlight is that, considering a high polluted basin, it is a challenge to integrate all this quantitative and qualitative data. As stated in the introductory chapter, this thesis aims to establish a starting point for a better understanding of how the organic carbon can be used to represent the overall process that may occur in a polluted river. Thus, the combination of the different analytical methods described within this conceptual chapter is the main basis to characterize and differentiate between autochthonous and allochthonous organic matter in human dominated basins. To complement this strategy, a mathematical formulation of the transport mechanisms and the biogeochemical process is fundamental. The conceptual basis for a mathematical modeling approach and the proposed model to integrate this analysis are presented in the next two following chapters.

Chapter 4

Water Quality Modeling in Surface Waters

"Ironically, although organic carbon is at the heart of all three problems (dissolve oxygen depletion, eutrophication, and toxics), it has never been among the key model variables".

Chapra, Steve C. 1999. Organic carbon and surface water quality modeling. Progress in Environmental Science v. 1, n. 1, p. 49-70.

4.1 Overview

The quality of surface water is related to the physical, chemical, and biological characteristics of each watershed. The configuration of the physical space and characteristics of human occupancy cause changes in nutrient and organic matter dynamics that may affect the water body. The challenge of using mathematical modeling as a supporting tool to evaluate water quality planning and management strategies is to integrate both monitoring and modeling, especially in a watershed where fast urban development occurs.

Mathematical models represents, in a simplified way, different systems and the interactions between a set of variables, based on hypothesis about its structure and behavior. It is based on two basic components that represent the streamflow characteristics and the mechanisms of mass transport. In this context, to understand the dynamics of organic matter in a river, it is important to analyze both the system characteristics and the transformation mechanisms of the pollutants.

The challenge of using mathematical modeling as a support tool to evaluate water quality planning and management options is to integrate both monitoring and modeling, especially in a basin where fast urban development occurs. Additionally, the reliability of the model predictions also depends on accuracy of input data. Although the values of model coefficients in surface water quality models can be found in the literature, these parameters need to be adjusted to the physical, chemical, and biological conditions of the basins studied.

Thus, this chapter aims to provide the conceptual basis for the development of a mathematical model to simulate the transport mechanism and the process related to the organic carbon mass balance in a river. The first part presents a brief description about concepts in water quality modelling and the evolution of surface water quality models. From the pioneer formulation of Streeter and Phelps BOD-DO mass balance, a lot of improvements have been applied in the development of mathematical models. Different state variables and process had been tested to represent either a natural cycle of elements, or the impacts of a hazardous compound.

Thus, a review about the main models used worldwide for water quality planning and management is highlighted, focusing on its characteristics, similarities, and differences. Peterson Matrix representation is used to summarize the main equations, process, coefficients, and variables.

In addition, it is discussed the use of optimization techniques for water quality calibration. A simulation-optimization algorithm based on Particle Swarm Optimization (PSO) process is proposed as a calibration tool. The objective of an automatic calibration tool is to find the most appropriate group of values for a set of selected coefficients for a water quality model simulation that can adjust the model to the observed data.

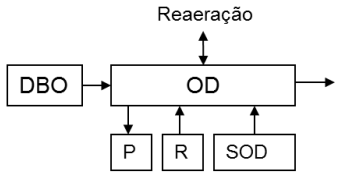
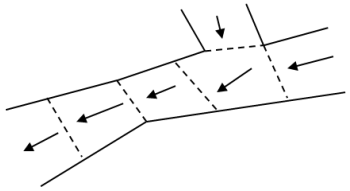
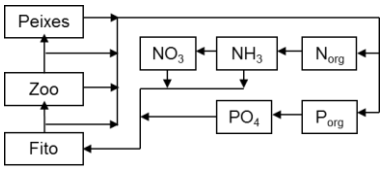
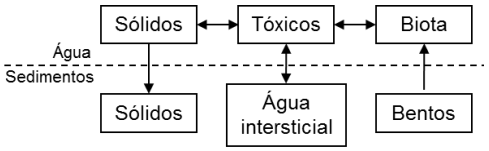
Finally, a critical analysis about organic matter modeling is presented at the end of the chapter.

4.2 Water Quality Modeling: Historical Approach

In the last decades, several researchers worldwide developed or improved mathematical models to evaluate the mechanisms of transport and degradation of pollutants in rivers, lakes, reservoir and oceans. The Streeter-Phelps model, dated from 1925, which proposes a first order decay for biochemical oxygen demand (BOD) and dissolved oxygen (DO), was the pioneer representation of this combination of processes.

The most recent computational models allow the simulation of several variables and its interaction with physical, chemical and biological characteristics. Table 1 presents a representation proposed by Chapra (1997), indicating four main changing points in the history of water quality modeling.

Table 1: Summary of four changing points in water quality modeling approach

1925-1960 (Streeter-Phelps) Problem: primary and raw effluents; Pollutants: BOD and DO; System: river and estuary (1D); Kinetics: linear; Solution: analytic	
1960-1970 (Computational) Problem: primary and raw effluents; Pollutants: BOD and DO; System: river and estuary (1D/2D); Kinetics: linear; Solution: analytic and numeric schemes.	
1970-1977 (Biology) Problem: eutrophication; Pollutants: nutrients; System: river, lake, and estuary (1D/2D/3D); Kinetics: nonlinear; Solution: numeric schemes.	
1977- today (Toxics) Problem: toxics; Pollutants: organic and metals; System: water-sediment interface, food web (1D/2D); Kinetics: nonlinear; Solution: analytic and numeric schemes.	

(Source: Adapted from Chapra, 1997)

Until the 60s, the main water quality issues were associated to the presence of raw and primary effluent. As a consequence, several studies focused to evaluate the dissolved oxygen levels in the water column. Due to the computational limitation, the first mathematical models developed in considered simple geometry, linear solutions and steady state flow (Chapra, 1997). With the improvement of computational techniques, more complex problems concerning water pollution and the consequent decrease of dissolved oxygen in rivers and estuaries were performed during the next decade. In terms of concept, analytical and numerical schemes were used to solve the transport and mass balance equations.

Since 1970, another environmental issue was also considered in the algorithms: eutrophication. To simulate the eutrophication process, nutrients like nitrogen and phosphorous were introduced as state variables, considering also the relationship with algae and the food web dynamics.

Additionally, according to Chapra (1997), a fourth step in the evolution of water quality modeling development was characterized by the consideration of toxic substances, such as heavy metals, both in the water column and sediments. Fragoso Jr. et al. (2009) emphasize that problems related to public health and politician considerations about the environmental degradation were the strongest motivation.

Thus, not only the focus of the problem has changed in recent decades, but also how the different processes are discussed. Table 2 shows a relationship between the pollution problem, state variables, processes and governing functions.

Table 2: Relationship between water quality problems, state variables, process and governing functions

Pollution problem	State variable	Main process	Governing functions
Dissolved oxygen demand	DO, BOD	Decay, oxygen consumption, reaeration	Water temperature, wind velocity, streamflow velocity
Eutrophication	Algae (chlorophyll-a), inorganic nutrients (ammonia, nitrate, phosphate, silica), particulate organic matter	Growth and mortality of algae, mineralization of particulate organic matter	Solar radiation, water temperature
Heavy metals	Inorganic suspended solids, heavy metals	Sedimentation, resuspension, partitioning	Streamflow velocity, wind and waves.

Chapra (1999) highlights that the three pollution problem presented in Table 2 are intrinsically connected to the organic carbon cycle. Consequently, the mathematical representation of this processes are also related to the mechanisms of production and consumption of organic carbon in the aquatic environment. Figure 1 shows a schematic representation of this relationship. The oxygen depletion is caused largely by the decomposition of organic matter. The eutrophication process originates an excess of organic matter through algae biomass. Thus, oxygen depletion and eutrophication represents two extremes of the production/consumption cycle and, in a broader sense, represents a single problem.

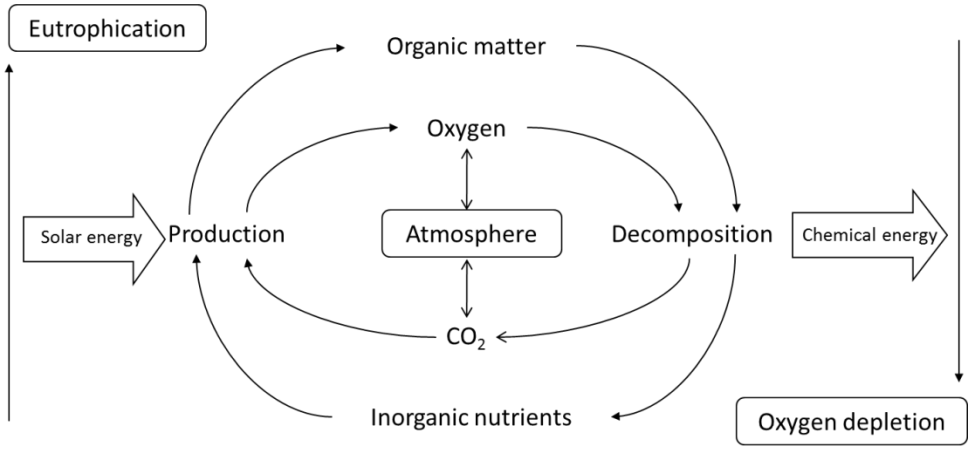


Figure 1: Production/consumption natural cycle

(Source: Adapted from Chapra, 1999)

In rivers, for example, the dissolved oxygen depletion zone is located downstream from an effluent discharge. The oxygen depletion occurs by the decomposition of the organic matter present in the effluents. The decomposition of the organic matter also produces nutrients that can originate excessive plant growth, resulting in eutrophication process if the hydraulic condition if favorable.

Also according to Chapra (1999), the association between organic carbon concentration and heavy metals are due to the adsorption mechanisms, and, consequently, in its transportation in the water column. Thus, understanding the mechanism of transport, sedimentation, decay, production, and consumption of organic carbon are essential for the modeling of heavy metals in both water and sediments.

However, as highlighted by Somlyódy et al. (1998) and Chapra (1999), although that the organic carbon plays a key role in all these process, it is still not considered as a state variable in the most water quality simulation models used worldwide. One reason for that is due to the uncertainty regarding to the analytical determination and interpretation of organic carbon as a water quality parameter.

Historically, and in an indirect way, the organic carbon is presented in the mass balance formulation of several simulated process (Chapra, 1999). In the first set of developed models, which addressed the oxygen depletion problem, BOD represents the organic carbon content in terms of equivalent oxygen consumed. When eutrophication is simulated, the organic carbon content is accounted by a stoichiometric fraction of the plant biomass, i.e, represented by chlorophyll-a. Models that simulate heavy metals are based on suspended solids transport, which, within this context, represents the particulate organic carbon.

However, these three examples of alternative variables have weaknesses. The biochemical oxygen demand (BOD), although traditionally used as an indirect indicator of the water quality organic content, is a time consuming test and has well known subjectivities and uncertainties (Kamiyama, 1988, Shanahan et al., 1998). Since it is a biological test, errors in the collection, storage and in the analytical procedure can hide or cause significant differences in oxygen consumption and, consequently, in the amount of organic matter determination. Shanahan et al. (1998) also emphasize that the mass balance if not fully accounted when using BOD as the key variable for the simulation, since its measurement does not include totally the biodegradable fraction of organic matter. The authors also point out that some peculiarities about the composition of the organic matter cannot be measured by the BOD analytical method.

The problem with the use of chlorophyll-a and suspended solids as surrogates of organic carbon is that, depending on its origin (autochthonous or allochthonous), the fraction of organic carbon is not constant (Thomann and Mueller, 1987). Allochthonous particles, for example, have a content of 5% of organic carbon in its composition, while the phytoplankton biomass has about 40% of organic carbon.

Thus, in the next items will be described discussed about some concepts in water quality modelling and how the currently worldwide models represents the mains parameters related to the organic pollution in rivers. Although the transport mechanism and chemical process are general for a broad range of parameters, emphasis is done to the organic matter simulation.

4.3 Concepts in Water Quality Modeling

The fate of inorganic and organic compounds in the aquatic environment is determined by physical, chemical, and biological processes. According to the compounds intrinsic characteristic and the environment analyzed, these process may include transport, sedimentation, resuspension, sorption, volatilization, hydrolysis, oxidation, photoinduced degradation, biological degradation, and bioconcentration. Some of the main process that is considered in this thesis development is described as follow.

4.3.1 Transport Mechanism

The transport of dissolved and particulate compounds in rivers depends on advection and diffusion mechanisms. The advective transport characterizes the physical transport (longitudinal, lateral, or vertical) of dissolved or particulate material at the current water velocity. Diffusion represents the mixture of different compounds within the water column and can be classified in two different scales: molecular and turbulent. Molecular diffusion is the mixing of matter that occurs by the random motion of molecules within the fluid. Turbulent diffusion (or eddy diffusion) refers to the mixing of matter due to turbulence. Turbulent diffusion is significantly larger than molecular diffusion, and, when this mixture is caused by the interaction with velocity gradients (rather than random motion) in the water column, it is named dispersion (Schnoor et al., 1987; Chapra, 1997).

In addition, velocity gradients will be affected by shear forces at the boundaries of the stream, such as wind shear at the air-water interface, shear stresses at the lateral and bottom interface. Consequently, the mass transport depends on characteristics such as channel slope, flow, section area, and roughness. The mass transport in streams and rivers is predominantly by advective mechanisms, while the transport in lakes and wide bodies (such as bay) is often dispersion-controlled (Schnoor et al., 1987; Chapra, 1997).

The advection-dispersion equation is the basic equation that describes the advection and dispersion of dissolved and particulate matter. The equation is based on the principle of conservation of mass and the Fick's law. The mass conservation principle states that the rate of change of mass in a control volume is equal to the difference between the rate of change of mass in the control volume due to advection and diffusion effects and the transformation (physical, chemical, or biological processes) reaction rates.

Equation 1 is the one-dimensional advection-dispersion mass transport equation. This equation considers the effects of advection, dispersion, dilution, constituent reactions and interactions (kinetics), and sources and sinks.

$$\underbrace{V\frac{\partial C}{\partial t}}_{Acumulation} = \underbrace{\underbrace{\frac{\partial\left(A_x D_L \frac{\partial c}{\partial x}\right)}{\partial x}dx}_{Dispersion} - \underbrace{\frac{\partial(A_x \bar{U} C)}{\partial x}dx}_{Advection}}_{Transport} + \underbrace{V\frac{dc}{dt}}_{Kinetics} \pm \underbrace{Es}_{External\ sources\ and\ sinks} \quad (1)$$

Where:

C: concentration (mg/L); t: time (s); A_x: cross section ara (m²); D_L: longitudinal dispersion coefficient (m²/s); $\bar{U}$: average velocity (m/s); x: distance (m), V: volume (m³); Es: external sources and sinks.

The term dC/dt refers to the constituent changes independent of advection, dispersion, and other inputs (sources or sinks) and is related to physical, chemical, and biological reactions, such as decomposition, reaeration, and sedimentation. Sources and sinks of pollutants include the input of mass by effluents or extraction of mass by withdrawals.

4.3.2 Sedimentation and Resuspension

Considering a river, suspended sediment particles and adsorbed compounds can be transported downstream with the water velocity (advective and dispersive mechanisms) and vertically downward according to the particle sedimentation velocity. In an opposite movement, the bottom sediment can be resuspended. In addition, an adsorbed compound can be released from the bottom sediment through desorption or diffusion. The difference between sedimentation and resuspension is the net sedimentation, and, thus, a single coefficient can be used to represent the two processes (Schnoor et al., 1987; Chapra, 1997).

4.3.3 Hydrolysis and Biological Transformation

Hydrolysis is a chemical reaction in which a molecule is split into two parts by the addition of a molecule of water. One part of the original molecule (parent molecule) gains hydrogen (H^+) from the water molecule, while the other part will form a new bond with the

remaining hydroxyl group (OH^-). Examples of compounds that are susceptible to be hydrolyzed in natural waters are organic esters, amides, amines, carbamates, and alkyl halides.

Complementarily, biological transformations are important degradation mechanism in surface waters and refer to any microbiologically mediated reaction that changes the organic chemical. Biodegradation may occur under aerobic and anaerobic conditions through bacteria, algae or fungi activities. During biodegradation, a series of metabolic or enzymatic processes transform the organic matter into energy, biomass, and products such CO_2 and H_2O (aerobic biodegradation) or compounds such as N_2, H_2S, CH_4, and reduced forms of metals (anaerobic biodegradation) (Davies, 2005).

Some authors suggest different denominations for biological reaction according to the final product. For example, mineralization if often used for reactions that leads to CO_2 and H_2O as products. Biodegradation is referred for oxidation reaction that eventually leads to CO_2 and H_2O as products (Schnoor et al., 1987).

Biodegradation rates may be affected by factors that influence or alters biological growth and stability, such as: temperature, nutrients availability, acclimation, population density or biomass concentration.

4.4 Review of Main Surface Water Quality Models Characteristics

In this item it is presented a brief review about the main features of different water quality models used for water resources planning and management in the last few decades. Currently, there are several models that have been developed by academic institutions, regulatory agencies, government and private companies. The main goal of this review is to identify the main characteristics, similarities, differences, and gaps that currently exist in the field of surface water quality modeling. Moreover, to present a critic perspective for identifying a water quality parameter suitable for a consistent evaluation of pollution processes in river, especially with strong urban influence, towards a sustainable water resources planning and management strategies.

To perform this review, a comparative table was elaborated to summarize three categories of data: (i) general aspects; (ii) hydraulic and temporal characteristics; and (iii) water quality parameters. This review was based on supplementary documents of the computational models investigated, and in some review papers. Some of these references focus more detailed on flow routing level (Zoppou, 2001), water quality modeling (Rauch et al., 1998;

Cox, 2003), differences between watershed models and stream water quality models (Horn et al., 2004), ecological modeling (Koelmans et al., 2001), applicability of different models (Park et a., 2008), and general issues about water quality models (Riechken, 1995; Ambrose et al., 1996; Shoemaker et al., 1997).

In the first part of the table (Table 3) is presented some general characteristics such as functionality, year that the model was developed, main authors, accessibility, and interface. The functionality is related to the use of the model for planning, operation or design purposes. The accessibility considers if a model has a public domain or if it is necessary to have a license to use the model platform.

The second part of this review (Table 4) focus on the hydraulic, temporal discretization, process formulation, and other characteristics of the models evaluated. Depending on the system simulated, the mechanisms of transport can be considered as 0D (zero dimensional, considering a control volume assuming completely and instantaneously mixed), 1D (e.g., the longitudinal advective and dispersive transport in a river, or the vertical process in a lake), 2D or 3D (e. g., when lateral and vertical mechanisms are relevant to the overall mass transport). A second division considers the spatial modeling scale: lumped, if the physical characteristics of the watershed are assumed to be homogeneous (constant), and distributed, if the parameters that represent the physical heterogeneities of a watershed are considered functions of time and space. In relation to the temporal scale, a model can be considered as a steady-state model when the variables defining the system are not dependent on time, or a dynamic model, when the mathematical formulation of fate and transport are a function of time.

Both empirical and mechanistic models were described in the review. An empirical model (or black-box model) does not account for causality between input disturbance effect and the output responses (e.g regression relationship between two variables). A mechanistic model considers that there are process that explain and changes the inputs, state variables, and the outputs. In this class of models, equations are used to represent the mass-balance budge, the transport mechanism, and the rates in which the compounds are consumed or produced within the system.

The review also considers classification about the routing level (simple storage, hydrologic and hydraulic), the pollution transport (advective diffusion equation, completely mixed reactor or plug and flow), the chemical processes on water column (first-order kinetics, processes kinetics, daughter products, sorption), model quantity component (rivers, lakes, reservoirs, pipes, rainfall runoff). In addition, aspects related to automatic calibration (genetic

algorithm, PSO), optimization (analytical, linear programming, non-linear programming), uncertainty analysis (dynamic analysis, sensitivity analysis, first-order second moment, Monte Carlo), and costs analysis is also compared.

The third part of this review (Table 5) presents the water quality parameters considered by the models evaluated. For this analysis, the most frequent parameters used in water quality simulation models were selected, as well as specific parameter considered by some models. The parameters analyzed were: BOD, DO, temperature, organic carbon (total-TOC, particulate - POC and dissolved - DOC), solids (inorganic, suspended, volatile, settleable and total dissolved), sediments (organic and inorganic), total coliform, total inorganic carbon, nitrogen (ammonia, organic nitrogen, nitrite and nitrate), phosphorous (organic, orthophosphate, dissolved), alkalinity, pH, phytoplankton, algae, and chlorophyll-*a*.

Table 3: Main characteristics of selected water quality models

Model			Qual2e-Uncas	Qual2k (versão 2.04, 2006)	Qual2kw 5.1	WQRRS	HEC-5Q v.8.0	Aquasim 2.0	ATV Model	CE-QUAL-ICM	CE-QUAL-W2 (v.3.6)	CE-QUAL-RIV1 (v.2.0)	DESERT 1.1	ISIS Water Quality (v3.5)	MIKE 11	RWQM1	SOBEK 2.12	DELFT-3D	WASP 7.3	Aquatox (3)	DR3M-QUAL
Main characteristics	Year		1987	2003	2008	1978	1986/ 1998	1994/ 1998	1996	1993/ 1995	2008	1995	1996	2003	2003	2001	2005	2003/ 2014	1983/ 2010	2009/2014	1982
	Functionality	Planning	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
		Operational					✔						✔				✔	✔			
		Design				✔		✔					✔				✔	✔			✔
	Accessibility	Public domain	✔	✔	✔	✔	✔	(a)		✔		✔	✔			✔		(a)	✔	✔	✔
		Commercial						✔						✔	✔		✔	✔			
		Interface/Programing language	Windows/ Fortran	Excell/ VBA/ Fortran	Excell/ VBA/ Fortran	DOS/ Fortran	DOS/ Windows/ Fortran	Windows/ C++		Fortran	Windows/ Fortran	Fortran	Windows / C++	Windows/ Fortran	Windows/ Pascal	(b)	Windows/ Fortran90, C++	Windows /Fortran90, C++	Windows	Windows/ Delphi	
	References		Brown and Barnwell, 1987	Chapra et al. 2003	Pelletier, G; Chapra, S. C., 2006	Hidrologic Engineering Center, 1978, Zoppou, 2001	Hidrologic Engineering Center, 1986/ 1998 ; Zoppou, 2001; Rauch et al., 1998	Reichert, 1995; Reichert, 1998; Horn et al 2004	Horn et al., 2004	Cerco e Cole, 1995; Shoemaker et al, 1997	Cole e Wells, 2008;	Riechken, 1995.; Environmental Laboratory, 1995; Ambrose et al., 1996	Ivanov et al., 1996; Horn et al., 2004	Cox, 2003	Ambrose, 1996; Cox, 2003; DHI, 2007	Shanahan et al., 1998; Reichert et al., 2001; Horn et al., 2004	SOBEK-RE Help Desk, 2005	Deltares, 2014	Ambrose et al, 1996; Wool et al., 2001	Cought, J. S., 2009; Park et al, 2008; Koelmas et al., 2001.	Zoppou, 2011

(a) Public domain for academic applications/ Educational package.

(b) The model can be implemented in different programing languages.

Table 4: Temporal scale and hydraulic features

	Model		Qual2e-Uncas	Qual2k (versão 2.04, 2006)	Qual2kw 5.1	WQRRS	HEC-5Q v.8.0	Aquasim 2.0	ATV Model	CE-QUAL-ICM	CE-QUAL-W2 (v.3.6)	CE-QUAL-RIV1 (v.2.0)	DESERT 1.1	ISIS Water Quality (v3.5)	MIKE 11	RWQM1	SOBEK 2.12	DELFT-3D	WASP 7.3	Aquatox (3)	DR3M-QUAL
Temporal and hidraulic characteristics	Space scale	0D																		✓	
		1D	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	(b)	✓	✓	✓	✓	
		2D								✓	✓					(b)	✓	✓	✓	✓	
		3D								✓						(b)		✓	✓	✓	
	Space modelling scale	Lumped	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			✓	✓	
		Distributed																			
	Time modelling scale	Continuous	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
		Event				✓															
	Temporal variation	Steady-state	✓	✓	✓				✓					✓		(b)					
		Quasi-dynamics	✓												✓				✓		
		Dynamic model				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	(b)	✓	✓	✓	✓	
	Routing level	Simple storage	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			✓	✓	✓
		Hydrologic				✓	✓								✓						✓
		Hydraulic				✓			✓		✓	✓		✓	✓		✓	✓			✓
	Pollution transport	Advective diffusion equation	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
		Completely mixed reactor				✓	✓	✓												✓	
		Plug-flow						✓													✓
	Pollutant predictive method	Empirical																			✓
		Mechanistic	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	Chemical processes on water colum	First-order kinetics	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
		Process kinectics		✓	✓			✓			✓					✓			✓	✓	
		Sorption													✓				✓		
	Chemical processes on sediment	Taxas de entrada					✓		✓	✓	✓	✓	✓		✓	✓	✓		✓	✓	
		Não coesivos		(a)	(a)						✓				✓						
		Coesivos		(a)	(a)						✓			✓	✓			✓			
	Model quantity component	River	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
		Lake				✓	✓	✓		✓	✓							✓	✓	✓	
		Reservoir				✓	✓			✓	✓							✓	✓	✓	
		Pipes																			✓
		Rainfall runoff				✓															✓
		Others				(c)		(d)			(e)	(c)			(g)			✓		(e)	(g)
	Authomatic Calibration	Genetic algorithm			✓																
		Particle Swarm Optimization																			
		Others						✓			(f)										
	Optimisation	Analytical																			
		Linear programing											✓								
		Non-linear programing																			✓
	Uncertainty analysis	Dynamic programing																			
		Sensitivity analysis	✓					✓					✓							✓	
		First-order second moment	✓																		
		Monte Carlo	✓																		
		Others	✓											(h)							
	Costs analysis	Life cycle																			
		Others																			

(a) Depends on the model version
(b) User defined
(c) Open channel and estuaries
(d) Biofilm reactors
(e) Estuaries
(f) Hydraulic calibration module
(g) Open channel
(h) Probability analysis

Table 5: Water quality parameters

	Model		Qual2e-Uncas	Qual2k (v. 2.04, 2006)	Qual2kw 5.1	WQRRS	HEC-5Q v.8.0	Aquasim 2.0	ATV Model	CE-QUAL-ICM	CE-QUAL-W2 (v.3.6)	CE-QUAL-RIV1 (v.2.0)	DESERT 1.1	ISIS Water Quality (v3.5)	MIKE 11	RWQM1	SOBEK 2.12	DELFT-3D	WASP 7.3	Aquatox (3)	DR3M-QUAL
Water quality parameters	Biochemical Oxygen Demand		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	Dissolved Oxygen		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	Temperature		✓	✓	✓	✓	✓		✓	✓	✓	✓		✓	✓	✓		✓	✓	✓	
	Organic Carbon	Dissolved									(a)							(a)			
		Particulate									(a)							(a)			
		Total									(a)										
	TIC- Total Inorganic Carbon			✓	✓	✓					✓					✓		✓			
	Solids	Inorganic suspended		✓	✓	✓				✓	✓										
		Suspended				✓			✓								✓	✓			✓
		Volatile																			
		Settleable																			✓
		Total dissolved				✓															
	Sediment	Organic				✓					✓					(c)		✓			
		Inorganic				✓									✓	(c)		✓		✓	
	Bacterial pollutants		✓			✓					✓	✓		✓	✓	✓	✓	✓			
	Nitrogen	Ammonia (NH_3)	✓	✓	✓	✓	✓		✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	
		Organic nitrogen	✓	✓	✓		✓		✓	✓	✓	✓		✓		✓	✓	✓	✓		
		Total nitrogen	✓	✓	✓	✓	✓		✓	✓	✓		✓			✓				✓	
		Nitrite	✓			✓	✓		✓	✓					✓	✓	✓	✓			
		Nitrate	✓	✓	✓	✓	✓		✓	✓	✓	✓			✓	✓	✓	✓	✓	✓	
	Phorphorous	Organic phosphorous	✓	✓	✓					✓	✓	✓				✓	✓	✓	✓		
		Orthofosphato								✓	✓	✓				✓	✓	✓	✓		
		Dissolved phosphorous	✓	✓	✓	✓				✓	✓					✓	✓	✓	✓	✓	
		Total phosphorous	✓	✓	✓		✓		✓	✓	✓		✓	✓	✓	✓			✓	✓	
	Alkalinity			✓	✓	✓					✓					✓					
	pH			✓	✓	✓			✓					✓		✓		✓		✓	
	Soil erosion																				
	Clorofila-a (phytoplankton)		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	
	Benthic algae			✓	✓	✓			✓		✓			✓	✓	✓		✓	✓	✓	
	Conservative substances		✓		✓		✓		✓		✓			✓		✓		✓			
	Zooplankton					✓	✓		✓	✓	✓				✓	✓				✓	
	Sediment Oxygen Demand			✓	✓		✓			✓	✓					✓					
	Others parameters			Pathogens, detritus, conductivity, nitrogen and phosphorous in the sediment.	Pathogens, detritus, conductivity, nitrogen and phosphorous in the sediment.	Detritus, CO_2, fish.		(b)	Bacteria, salts, heavy metals, organic contaminants	Salinity, silica, bacteria, sediment diagenesis.	Silia, iron, labile and refractory organic matter.	Dissolved iron and manganese, macrophytes.		Salts, solar radiation, silica.	Heavy metals, iron, nutrient transport and eutrophication. Sediment transport.	(c)	Chloride, heavy metals, organic micro-pollutants.	COD, heavy metals, organic micro-pollutants, iron, methane, CO_2, and others.	Heavy metals, bacteria.	Toxics in the sediment, detritus, sand, silt, caly, salinity, fish.	

(a) Organic carbon, nitrogen and phosphorous is simulated as a stoichiometric relationship with the organic matter (b)User defined (c) Non-conservative substances in the sediments

According to the tables presented, some characteristics can be highlighted. First of all, considering the parameters used for water quality simulation, it can be observed that there are specific groups that are largely used, such as BOD, DO, nitrogen, phosphorous, temperature, and algae. Sediments, solids, and organic matter are parameters that just few models consider as state variables, indicating that there is still a field for development in water quality simulation.

Another aspect that can be observed is about the use of algorithms for automatic calibration. According to the review presented, just two models have this kind of tool for model calibration, Qual2k (Chapra et al., 2003) and Ce-Qual-W2 (Cerco and Cole, 2005). However, only the first model utilizes the automatic calibration to adjust the coefficients related to the water quality simulation with the observed data.

Optimization processes and cost analyses are also two features covered by only few models. These tools can be used for water resources planning and management, such as treatment plant optimal location and costs related to different efficiencies and effluent treatment systems (Brites 2010; Garcia, 2011).

4.5 Peterson Matrix Analysis

The Peterson Matrix (Reichert et al., 2001) consists in a matrix that summarizes the stoichiometric coefficients and the relationship between the state variables and the process simulated by a mathematical model. In each line it is represented a different process, such as oxidation, hydrolysis, and aeration. The columns highlights, the state variables considered by the model. The stoichiometric relationship between a state variable and a process is set by a positive or negative (source or sink) signal, and in specific cases with the stoichiometric coefficient. Additionally, the last column of the matrix represents the process rates. The combination of the stoichiometric relationship (matrix cells) with the process rates (lines) gives the equation for a specific state variable (columns). An hypothetic example of a Peterson Matrix representation is presented in Table 6, considering C_i the state variable, $st_{(i,j)}$ are the stoichiometric coefficients, and r_j are the process rates. Considering this example, Equation 2 can represent the mass balance for the variable C_4.

Table 6: Peterson matrix representation

State variables (i)		1	2	3	4	...	*Process rates*
(j)	***Process***	C_1	C_2	C_3	C_4	...	
1	Oxidation	$st_{(1,2)}$			$st_{(1,4)}$	→	r_1
2	Hydrolysis				0	→	r_2
3	Settling		$st_{(3,2)}$		$st_{(3,4)}$	→	r_3
...	...						

$$\frac{dC_4}{dt} = st_{(1,4)}r_1 + 0r_2 + st_{(3,4)}r_3 \quad (2)$$

The purpose of using the Peterson Matrix to represent the mathematical processes of a model is to identify similar features between existent models as well as different process that can be formulated to simulate the organic matter dynamics in the water column.

In order to illustrate a detailed example of a Peterson Matrix representation for a water quality simulation model, in the next item is presented this formulation for the processes considered by Qual2e model (Brown and Barnwell, 1987). This computational model is widely used in simulation studies of water quality in different basins (Drolc and Konkan, 1996; Park and Lee, 2002; McAvoy, 2003; Palmieri and Carvalho, 2006; Porto et al., 2007; Knapik, 2009).

4.5.1 Peterson Matrix Representation for Qual2e Model

The computer based Qual2e model (Brown and Barnwell, 1987) is suitable for one dimensional well-mixed rivers with constant flow. The model considers the transport mechanism – dispersion and advection – significant only along the main direction of flow (longitudinal direction). Qual2e is considered a quasi-dynamic model because it can simulate steady and unsteady transport of pollutant (diurnal variations using meteorological data on water quality only can be studied). The model can simulate the interaction of up to 15 water quality constituents: dissolved oxygen (DO), BOD, temperature, algae as chlorophyll-*a*, organic nitrogen, ammonia, nitrite and nitrate, organic and dissolved

phosphorous, coliforms, arbitrary non-conservative constituents and three conservative constituents (Figure 2).

The river system to be modeled is divided into reaches, and each reach can be divided into a number of sub-reaches or computational elements of equal length. This model is capable of simulating multiple discharges (point sources), withdrawal, tributary flows, and incremental inflows (non-point sources). Additionally, the model includes a sensitivity analysis, first-order and Monte-Carlo analysis (Brown and Barnwell, 1987; Zoppou, 2001).

Although the Qual2e model has been developed by the U.S. Environmental Protection Agency in 1987, it is one of the most widely used computer programs for conventional pollutant impact evaluation (Ambrosse et al, 1996). Cox (2003), in a review of currently available in-stream water quality models, summarized several advantages of Qual2e model for simulating dissolved oxygen. The model is extensively documented in the user manual (Brown and Barnwell, 1987) which explains the theory behind the model. The Qual2e model state variables, stoichiometric coefficients and the kinetics coefficients are presented in Table 7. Peterson Matrix representation is presented in Table 8.

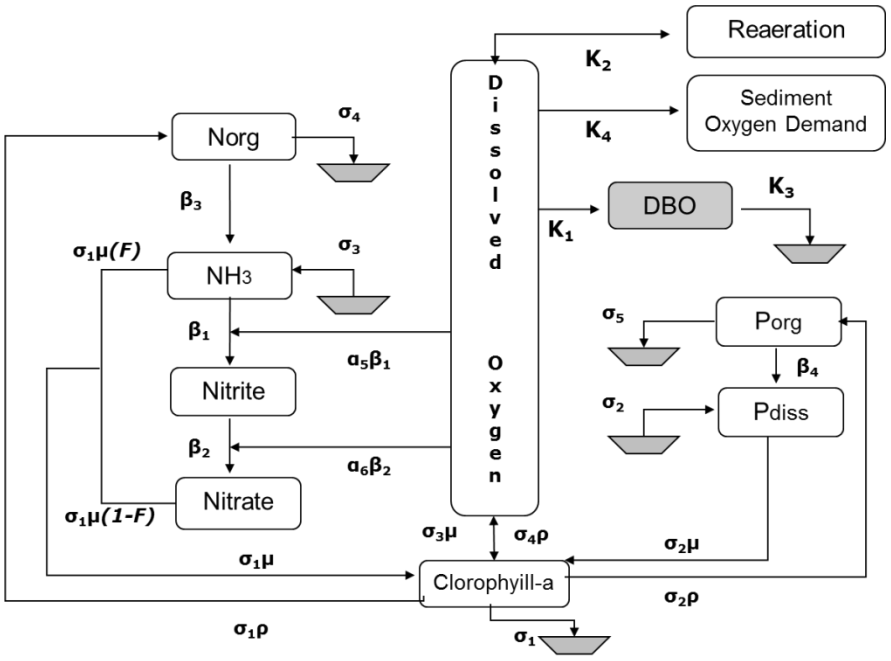


Figure 2: Schematic representation of the main processes considered by Qual2e model

(Source: Adapted from Brown and Barnwell, 1987)

Table 7: State variables, stoichiometric coefficients and kinetics coefficients for Qual2e Model

Symbol	State variable description
L	Concentration of ultimate carbonaceous BOD, mgO_2/L
O	Concentration of dissolved oxygen, mgO_2/L
N_1	Ammonia nitrogen concentration, mg-N/L
N_2	Nitrite nitrogen concentration, mg-N/L
N_3	Nitrate nitrogen concentration, mg-N/L
N_4	Organic nitrogen concentration, mg-N/L
P_1	Concentration of organic phosphorus, mg-P/L
P_2	Concentration of inorganic or dissolved phosphorus, mg-P/L
A	Algal biomass concentration, mg-A/L
E	Concentration of coliforms, colonies/100mL
R	Concentration of the non-conservative constituent, mg-ANC/L
Symbol	***Kinetic coeficients description***
K_1	Deoxygenation rate coefficient, TD, day^{-1}
K_2	Reaeration rate, day^{-1}
K_3	Rate of loss of carbonaceous BOD due to settling, TD, day^{-1}
K_4	Sediment oxygen demand rate, TD, g/ft^2-day
K_5	Coliform die-off rate, TD, day^{-1}
K_6	Decay rate for the constituent, TD, day^{-1}
β_1	Ammonia oxidation rate coefficient, TD, day^{-1}
β_2	Nitrite oxidation rate coefficient, TD, day^{-1}
β_3	Organic nitrogen hydrolysis rate, TD, day^{-1}
β_4	Organic phosphorus decay rate, TD, day^{-1}
σ_1	Settling rate for algae, TD, ft/day
σ_2	Benthic source rate for dissolved phosphorus, TD, $mg\text{-}P/ft^2$-day
σ_3	Benthic source rate for ammonia nitrogen, TD, $mg\text{-}N/ft^2$-day
σ_4	Organic nitrogen settling rate, TD, day^{-1}
σ_5	Organic phosphorus settling rate, TD, day^{-1}
σ_6	Rate coefficient for constituent settling, TD, day^{-1}
σ_7	Benthic source for constituent, TD, $mg\text{-}ANC/ft^2$-day
M	Algal growth rate, day^{-1}
P	Algal respiration rate, day^{-1}
Symbol	***Stoichiometric coefficients description***
α_1	Fraction of algal biomass that is nitrogen, mg-N/mg-A
α_2	Phosphorus content of algae, mg-P/mg-A
α_3	Rate of oxygen production per unit of algal photosynthesis, mg-O/mg-A
α_4	Rate of oxygen uptake per unit of algal respired, mg-O/mg-A
α_5	Rate of oxygen uptake per unit of ammonia nitrogen oxidation, mg-O/mg-N
α_6	Rate of oxygen uptake per unit of nitrite nitrogen oxidation, mg-O/mg-N
F	Fraction of algal nitrogen taken from ammonia pool

(Source: Adapted from Brown and Barnwell, 1987)

Table 8: State variables, process and rates of Qual2e model

State variables (i)		1	2	4	5	6	3	7	8	9	10	11	
(j)	***Process***	CBOD (L)	Dissolved oxygen (O)	Ammonia (N_1)	Nitrite (N_2)	Nitrate (N_3)	Organic Nitrogen (N_4)	Organic phosphorus (P_1)	Dissolved phosphorus (P_2)	Chorophyll-a (A)	Coliform (E)	ANC (R)	***Process rates***
1	CBOD decay - biodegradaton	(-)1	(-)1										K_1L
2	CBOD settling	(-)1											K_3L
3	Reaeration		(+)1										$K_2(Os-O)$
4	Sediment Oxigen Demand (SOD)		(-)1										K_4/H
5	Hydrolysis of organic nitrogen to ammonia			(+)1			(-)1						β_3N_4
6	Ammonia biological oxidation (nitrification)		(-)α_5	(-)1	(+)1								β_1N_1
7	Oxidation of nitrite		(-)α_6		(-)1	(+)1							β_2N_2
8	Nitrogen settling						(-)1						σ_4N_4
9	Hydrolysis of organic phosphorus							(-)1	(+)1				β_4P_1
10	Organic phosphorus settling							(-)1					σ_5P_1
11	Benthal source of ammonia nitrogen			(+)1									σ_3/H
12	Benthal source of dissolved phosphorus								(+)1				σ_2/H
13	Algae growth		(+)α_3	(-)Fα_1		(-)(1-F)α_1			(-)α_2	(+)1			μA
14	Algae respiration		(-)α_4				(+)α_1	(+)α_2		(-)1			ρA
15	Algae settling									(-)1			σ_1A/H
16	Coliform die-off										(-)1		K_5E
17	ANC decay											(-)1	K_6R
18	ANC settling											(-)1	σ_6R
19	ANC benthal source											(+)1	σ_7/H
ANC: Arbitrary nonconservative constituent													

4.6 Water Quality Model Calibration

In recent years, many calibration tools have been proposed to use as an alternative to the trial and error manual calibration. Kondageski (2008), Wang et al. (2008), Afshar et al. (2011), and Garcia (2011) applied evolutionary computation techniques for water quality simulation models calibration. In some cases, the calibration tool is already included on the model interface, such as Qual2kw (Pelletier et al. 2006).

Generally, for the parameters estimation problem, the solution consists in finding the global minimum of a function with several variables defined in suitable spaces (Samarano and Prado, 2005; Wang et al., 2008, Afshar et al., 2011). According to Samarano and Prado (2005), the use of automatic calibration based on optimization techniques allow a simultaneous search for a group of parameters, which can minimizes the effort compared to a manual calibration.

The Particle Swarm Optimization (PSO) has been applied as a calibration tool in distinct water resources management projects, such as reservoir operation (Baltar and Fontane, 2008; Reddy and Kumar, 2007), rainfall-runnoff correlation and urban storm water management models (Chau, 2004; Muleta et al., 2006; Zhang and Lai, 2011), water quality simulation models (Zhou et al., 2006; Wang et al., 2008; Afshar et al., 2011), and watershed planning and management (Baltar and Fontante, 2008; Shourian et al. 2008, Garcia, 2011).

The PSO was first developed by Kennedy and Eberhart in 1995 based on the behavior of swarm as a simplified social system, like a bird flocking. It comprises a very simple concept, has an easy implementation and is computationally inexpensive in terms of both memory requirements and speed (Kennedy and Eberhart, 1995).

Each individual in the particle swarm is identified by a velocity and position in the search space of a problem or function. The aim is to evaluate the objective function at each individual current location. The movement of each particle depends on its own position and velocity and by combining some aspect of the history of the others members of the swarm. According to Poli et al. (2007), a particle by itself has almost no power to solve any problem, and progress occurs only when particles interact. Eberhart and Shi (2001) and Poli et al. (2007) also emphasis that as opposed to genetic algorithms (GA) and other evolutionary algorithms (EVO), the evolutionary process in the PSO does not create new individuals. Instead, the population only evolves their social behavior accordingly to their movement towards a destination. Additionally, the optimization problem is always to minimize the

objective function, which can be, for example, the difference between observed and the predicted data.

In the original PSO technique each individual in the particle swarm is composed of three *D*-dimensional vectors, where *D* is the dimensionality of the search space, x_i is the current position, p_i is the previous best position and v_i is the velocity (Poli et al., 2007). The concept consists of, at each time step, changing the velocity (accelerating) each particle toward its *pbest* (previous best) and *gbest* (global best) locations (Eberhart and Shi, 2001). Figure 3 presents a schematic representation of PSO algorithm.

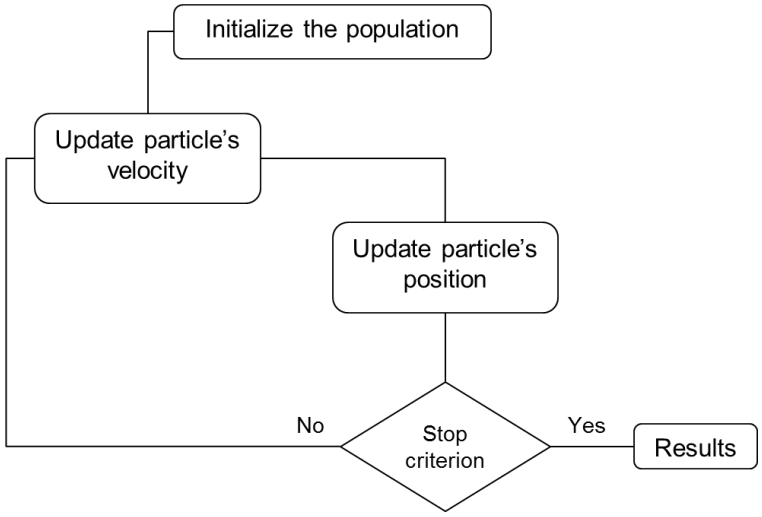


Figure 3: PSO algorithm

(Source: Adapted from Samarano and Prado, 2005)

The PSO algorithm can be described as follow (Poli et al., 2007):

- Initialize randomly the positions and velocities of N particles on *D* dimensions in the search space;
- Loop until a sufficiently good fitness or a maximum number of iterations is reached;
- Evaluate the desired optimization fitness function in D variables;
- If current value is better than $pbest_i$, then set $pbest_i$ equal to the current value;
- If $f(x_i) < f(pbest_i)$ then $pbest_i = x_i$;
- Identify the particle in the neighborhood with the best success so far, and assign it to *gbest*;
- If $pbest_i < f(gbest)$ then $gbest = pbest_i$;

- Change the velocity and position of the particle according to the following equation:

$$v_{k+1}^{in} = wv_k^{in} + c_1 r_1\left(pbest^{in} - x_k^{in}\right) + c_2 r_2\left(gbest^{n} - x_k^{in}\right) \quad (3)$$

$$x_{k+1}^{in} = x_k^{in} + v_{k+1}^{in} \quad (4)$$

Where:

$X_i = (x_{i1}, x_{i2}, \ldots, x_{iN})$: represents the position vector of the particle *i*;

$V_i = (v_{i1}, v_{i2}, \ldots, v_{iN})$: represents the velocity vector of the particle *i*;

w: represents the inertia weight;

$c_1\ e\ c_2$: represents the acceleration coefficients;

$r_1\ e\ r_2$: random numbers between zero and one;

$pbest_i$: previous best – represents the value of its own previous best function result;

$gbest_i$ global best – previous best solution of the entire swarm;

$k=1,2,\ldots,max$: maximum number of iterations;

i : particle's index in the swarm. Ranges from 1 to N;

N: number of particles;

According to Eberhart and Shi (2001), values of the inertia weight between 0.4 and 0.9 provides a balance between global and local exploration and exploitation, and in average, results in fewer iterations to find a sufficiently optimal solution. A large inertia weight facilitates a global search while a small inertia weight facilitates a local search (Wang et al., 2008)

Eberhart and Shi (2001) suggests that the sum of the acceleration constants, c_1 and c_2, should be between 0 and 4. These terms represents the magnitude of the random forces in the direction of personal best and neighborhood best. Low values allow particles to roam far from target regions before being tugged back, while high values result in abrupt movement toward, or past, target regions (Eberhart and Shi, 2001; Poli et al, 2007). Making $c_1 = c_2 = 2$ is an option to keep the balance between the particle and the group behavior (Garcia, 2011).

4.7 Critical Analysis on Organic Matter Modeling

Considering the models developed on the last decades and on its common characteristics, it can be observed that there is lack on the simulation of different fractions of organic carbon and its variation according to its source in the aquatic environment (Shanahan et al., 1998; Somlyódy et al., 1998; Chapra, 1999). Thus, the development of a mathematical model that considers both dissolved and particulate organic carbon, and its labile and refractory fractions, could enhance the understanding of the organic matter dynamics in rivers.

The consequence of this lack on representation is that the process and mechanism that control the transport and fate of organic matter in rivers depends on its origin and influences the way that the dissolved oxygen is consumed in the aquatic system. If the composition is more refractory, the impact will be slow due to the time it will take to decompose. If the composition is labile, the decomposition process will occur in a faster manner, which implies in a rapid consumption of the dissolved oxygen and consequently in deterioration of the water quality.

Currently worldwide models used in water quality planning and management are mainly based on BOD-DO mass balance. Models such as Qual2k (Chapra et al., 2003), Ce-Qual-W2 (Cole and Wells, 2008), Aquatox (Park and Clough, 2014), and DELFT 3D (Deltares, 2014) considers different fractions of organic matter related constituents instead of just BOD mass balance. In Qual2k model, for example, the simulation is based on fast and slow terms of BOD (assuming that the measurement is performed in filtered samples) and particulate organic matter (phytoplankton and detritus) (Knapik et al., 2011).

Ce-Qual-W2 (Cole and Wells, 2008) considers the simulation of carbonaceous BOD and organic carbon as fraction of organic matter contends in the water column and sediments. The model considers both dissolved and particulate fractions for labile and refractory organic matter. The labile organic matter includes the interaction with algae mortality (particulate and dissolved) and excretion (dissolved). The decomposition of labile organic matter leads to refractory organic matter and to the mineralization of ammonium, phosphorous, and inorganic carbon.

The models Aquatox (Park and Clough, 2014) and DELFT 3D (Deltares, 2014), recently incorporates in its process formulation and state variables the simulation of organic matter. Aquatox simulates the dissolved and suspended organic material (term named "detritus"), considering eight compartments in two main group: (i) refractory (resistant to

decomposition) dissolved, suspended, sedimented, and buried detritus; and (ii) labile (readily decomposed) dissolved, suspended, sediment, and buried detritus. The model also considers that the refractory detritus (dissolved, suspended, and in the sediments) not decompose directly, but is converted to labile detritus through microbial colonization. The model simulates detritus as the organic matter (dry mass), but, it can be used BOD or organic carbon values as input data. According to Park and Clough (2014), organic matter is assumed to be 1.90 times the organic carbon concentration (stoichiometry derived), and a more complex relationship for BOD, according to Equations 4 and 5. In Aquatox DOC and POC are only considered as state variables in the sediment compartment.

$$OM = CBOD \cdot \left(\frac{CBOD5\_CBODu}{O2Biomass}\right) \tag{5}$$

$$CBOD5\_CBODu = \left(\frac{1}{100\% - PercentRefr_{Time}}\right) \tag{6}$$

Where: OM: organic matter input as required by Aquatox (gOM/m³); $CBOD$: carbonaceous biochemical demand 5-day from uses input (g O_2/m³); $CBOD5\_CBODu$: CBOD5 to CBODu (ultimate CBOD) conversion factor; $PercentRefr_{Time}$: user-defined percent refractory matter for given source of organic matter; $O2Biomass$: ratio O_2 to organic matter (remineralization parameter).

As Aquatox model, DELFT 3D model also simulates the organic matter (detritus), considering stoichiometric relationship to estimate the proportion of carbon, nitrogen, and phosphorous content. The mineralization process is formulated as a first-order kinetic process, with 5 stages for the degradation of particulate and dissolved fractions (rapid to slow degradation). While Aquatox assumes that the decomposition of refractory OM will lead to labile OM through colonization, the hypothesis of DELFT 3D is that the less readily degradable detritus (refractory) is produced from the readily degradable fraction (labile) proportional to the mineralization rate.

Another example of a modeling approach considering DOC transport from soils and the variation in streams was proposed by Boyer et al. (1996). In this study, the focus was to develop a simple model to describe the variation of dissolved organic carbon in an upland catchment during snowmelt. The authors simulated the flow using TOPMODEL software. The respective patterns of DOC in soil and streamwater were analyzed for different discharges. The soil was considered as small reservoirs to apply the model developed by Grieve (1991). For the streamwater concentration, the model formulation considered a simplified mass balance calculated through a mixture of waters from direct snowmelt and

from the upper and lower soil reservoirs. In this study, the authors did not considered the transformation mechanism of DOC in the water column. Although the authors emphasized that the results did not perfectly represented the monitored data, the estimative for different discharges suggested that there is a strong relationship between the monitored peaks of DOC and the increased flows during snowmelt.

A recent study evaluated the transport of DOC in an estuary river system with DELFT 3D software (Brown et al., 2014). The main objective was to simulate the terrestrial derived DOC transport resulting from the impact of a hurricane. Besides the results were consistent with the monitored data, the authors did not considered biochemical process for its decay in the water column. The authors made the assumption that DOC is a conservative parameter. However, it is susceptible of photochemical and biological decomposition. Even though the terrestrial organic carbon presents a more refractory composition, the biochemical process may have a significant impact on DOC decomposition rates and, consequently, on decay patterns.

Different authors studied the organic matter characterization (Wangersky, 1992; Chair et al., 1993; Mantovani and Novo, 1996; Imai et al., 2001; Krusche et al., 2001; Carstea et al., 2010) and organic carbon flux in aquatic ecosystems (Hope et al., 1993; Tank et al. 2004; Karlsson et al., 2005; Neale et al., 2011; Futter et al., 2011; Kalscheur et al., 2012). Most part of the studies focuses on organic carbon flux from terrestrial sources (Boyer et al., 1996; Cole and Carraco, 2001; Ouyang, 2003; Evans, Monteith and Cooper, 2004) and on temporal dynamics on lakes and reservoirs (Westphal et al., 2004; Weissenberger et al., 2010), ocean (Mopper et al., 1991; Brown et al., 2014) and groundwater (Barcelona, 1984), with low or without anthropic influence (Kiffney et al., 2000; Futter et al., 2008) and in drink water treatment plans (Baghoth et al., 2011; Matilainen et al., 2011).

The hypothesis presented by Chapra (1999), and according to Figure 4, the organic carbon characterization as function of its source can cause changes in water quality planning and management strategies. According to the author, the lack that has been observed in the last few years related to the organic carbon modeling does not make sense nowadays since some difficulties on its analytical determination has been minimized.

Thus, the original contribution of this study is to understand and utilize the different fractions of organic carbon as a state variable in a surface water quality model considering the transport, decay and process with other variables. Additionally, analyze the possibility to correlate the organic carbon concentration with other common parameters used in water quality monitoring such as BOD and COD. This analysis is presented in a simplified manner

in the literature (Sharp, 1993; Chapra, 1999), with few studies that dedicates efforts to define what, why, and how the organic carbon can be applied on water quality planning and management.

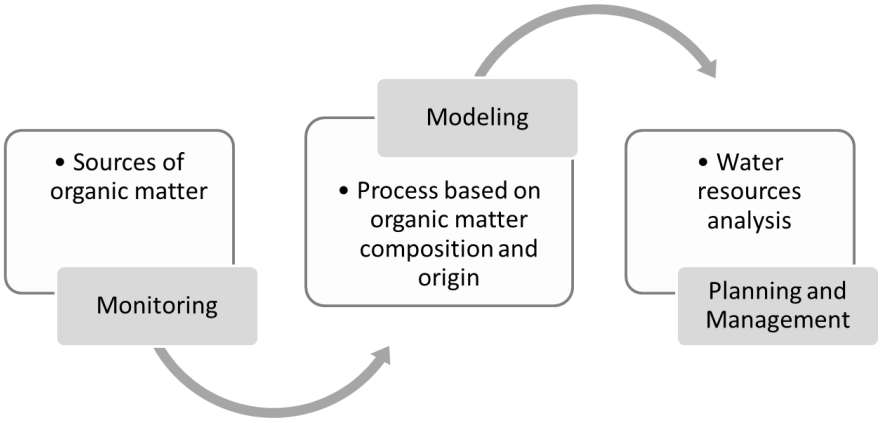


Figure 4: Contribution highlights on monitoring, modelling, and water quality planning and management

4.8 Summary

This chapter presented a review about the main characteristics of surface water quality models, highlighting the different approaches for organic matter simulation. In the context of this thesis development, this description is important to support the hypothesis that the organic carbon can be a better variable for organic matter modeling in rivers. In addition, it is introduced the Peterson matrix for a model analysis and representation. The use of this matrix configuration is interesting for a general analysis of the process and variables considered by a mathematical model, and, consequently, to identify gaps on its discretization.

An algorithm for automatic calibration is also presented within this chapter. PSO, due to its successfully application for surface water quality models calibration, has been selected for the calibration method. More detailed information and the tests for the algorithm implementation are presented in the chapter 6.

Finally, this chapter presents a critical analysis on organic matter modeling. This analysis establishes the fundamental basis for the proposed model development in the context of planning and management strategies. This chapter also closes the literature review necessary to support this thesis methodological approach. Based on the concepts herein described, and the characteristics of organic carbon fractions discussed at chapter 3, next chapter presents a detailed description about the model developed.

Chapter 5

ROCS Model – River Organic Carbon Simulation Model: Concepts, Process, and Structure

"A basic principle of stream water quality models is the conservation of mass. Thus, a very fundamental concern with the existing approach is the fact that using BOD as a state variable intrinsically means that mass balances cannot be closed because BOD is ill defined and does not account for all biodegradable organic matter. Rather than being a unique material, BOD is the result of a bioassay measurement, the yield of which changes with the type of substrate consumer.

Shanahan, P. et al. 1998. River Water Quality Modeling II: Problem of the art. Water Science and Technology 38(11), 245-252.

5.1 Overview

This chapter presents some features of the proposed model developed to simulate the organic carbon transport and decay in rivers: ROCS Model – River Organic Carbon Simulation Model. A one-dimensional, non-uniform, steady-state model is proposed to simulate the transport, decay, and conversion process of organic carbon in rivers. The first

part of this chapter summarizes some general concepts related to the development of an original mathematical approach for organic carbon simulation considering the particulate and dissolved fractions, and labile and refractory compounds. The process rates and kinetics involved are described by Peterson Matrix representation.

The second part summarizes the water quality state variables. In this part, it is detailed the assumption and equations used to represent the variables in the water column. For organic carbon simulation, the following variables were considered: labile particulate organic carbon (LPOC), refractory particulate organic carbon (RPOC), labile dissolved organic carbon (LDOC), and refractory dissolved organic carbon (RDOC). A second approach based only on DOC and POC compartmentalization is also implemented. Additionally, it is presented the algorithms implemented for BOD, DO, nitrogen (organic, ammonia, nitrite, and nitrate), and phosphorous (organic, and dissolved inorganic). These complementarily variables were tested and used to evaluate the model discretization, input data, and to compare the analytical solutions with a numerical model. A list of state variables and the corresponding chemical analysis necessary for model implementation is summarized at the end of this section.

A third part of this chapter describes the overall aspects related to the computational platform developed, the physical representation, and flow balance. The main model structure was implemented in Excel spreadsheets and Visual Basic for Application (VBA). This structure is organized in modules, each one with a specific function. Examples of this module are the data base module, hydraulic, point and non-point sources generator, statistical, and calibration module.

The concepts herein described complement the information about the organic carbon characterization and monitoring detailed in previous chapters. In this thesis it is proposed the use of mathematical models to analyze the combination of different chemical, physical, and biological processes and thus, understand causes and consequences.

5.2 Conceptual Kinetic Model Development

Differently from other models that simulate the concentration of organic matter and uses stoichiometric relationship to estimate the organic carbon, nitrogen, and phosphorous fractions, such as Aquatox (Park and Clough, 2014), Ce-Qual-W2 (Cole and

Wells, 2008), and Delft-3D (Deltares, 2014), the proposed model intend to simulate and evaluate the transport and decay of the organic carbon in rivers.

In other to evaluate probable differences in considering a reduced or expanded number of variables to represent the measured DOC, POC, and TOC, two model approaches were implemented in the ROCS main structure. The first one is based on DOC and POC relationship suggested by Chapra (1997), assuming only the transport and decay of organic carbon without the interactions with algae (internal production by excretion and/or death) and the consumption by phytoplankton and/or other living organisms.

In the second modeling approach, four variables were considered for the organic carbon mass balance: labile particulate organic carbon (LPOC), refractory particulate organic carbon (RPOC), labile dissolved organic carbon (LDOC), and refractory dissolved organic carbon (RDOC). The main processes simulated are settling and resuspension of particulate fractions, mineralization to inorganic forms (ammonia, phosphate, inorganic carbon), dissolution from particulate do dissolved fractions, and decay between labile and refractory organic carbon.

Complementarily, were also implemented the algorithms related to BOD, DO, organic nitrogen (N_{org}), ammonia (NH_3), nitrite (NO_2^-), nitrate (NO_3^-), organic phosphorous and inorganic phosphorous simulation. For this set of variables, the following processes were considered: biological decay and BOD settling, organic phosphorous decay and sedimentation, organic nitrogen decay and sedimentation, nitrification, sediment oxygen demand and atmospheric reaeration. Figure 1 shows the schematic representation of the state variables, coefficients, and process. The Peterson matrix for the proposed model is presented in Table 1. The state variables, stoichiometric, and kinetics coefficients are presented in Table 2.

Thus, two groups of state variables are representing the organic matter content: BOD and organic carbon as LPOC, RPOC, LDOC, and RDOC (or DOC and POC in the first modeling approach). The model considers these two groups of variables independently, and only BOD is interconnected with the DO model. The primarily objective of these two approaches is to evaluate the organic carbon as a monitoring parameter and modeling variable, by comparing the results with a commonly used parameter, i. e., BOD.

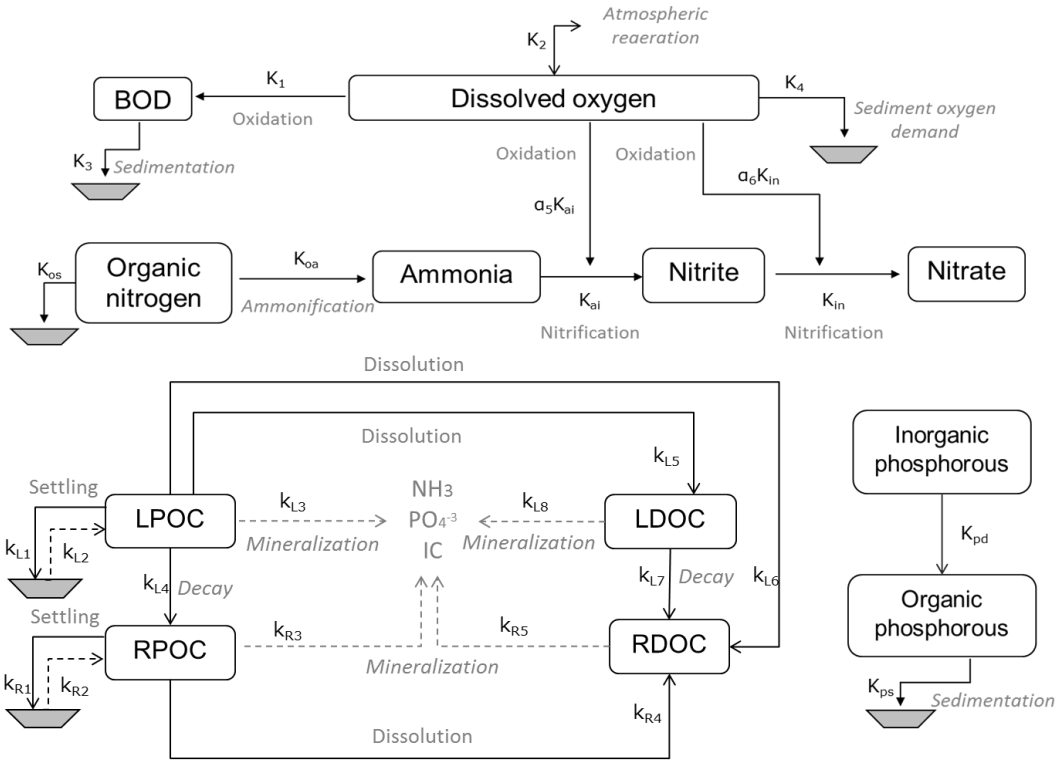


Figure 1: Conceptual representation of the proposed model

Table 1: Process rates, state variables and kinetics coefficients for ROCS Model in Peterson Matrix notation

State variables (i)		1	2	3	4	5	6	7	8	9	10	11	12	
(j)	Process	Labile POC (LPOC)	Refractory POC (RPOC)	Labile DOC (LDOC)	Refractory DOC (RDOC)	CBOD (L)	Dissolved oxygen (O)	Ammonia (Na)	Nitrite (N_i)	Nitrate (Nn)	Norg	Porg	Pdiss	Process rates (mg/L.d)
1	Settling of LPOC	(-)1												KL1 LPOC
2	Resuspension of LPOC	(+)1												KL2 /H
3	Mineralization of LPOC	(-)1												KL3 LPOC
4	Decay of LPOC to RPOC	(-)1	(+)1											KL4 LPOC
5	Decay of LPOC to LDOC	(-)1		(+)1										KL5 LPOC
6	Decay of LPOC to RDOC	(-)1			(+)1									KL6 LPOC
7	Settling of RPOC		(-)1											KR1 RPOC
8	Mineralization of RPOC		(-)1											KR3 RPOC
9	Decay of RPOC to RDOC		(-)1		(+)1									KR4 RPOC
10	Resuspension of RPOC		(+)1											KR2 /H
11	Decay of LDOC to RDOC			(-)1	(+)1									KL7 LDOC
12	Mineralization of LDOC			(-)1										KL8 LDOC
13	Mineralization of RDOC				(-)1									KR5 RDOC
14	CBOD decay - biodegradaton					(-)1	(-)1							K1L
15	CBOD settling					(-)1								K3L
16	Reaeration						(+)1							K2(Os-O)
17	Sediment Oxigen Demand (SOD)						(-)1							K4/H
18	Decay of Norg to ammonia							(+)1			(-)1			Koa Norg
19	Settling of Norg										(-)1			Kos Norg
20	Ammonia biological oxidation						(-)α_5	(-)1	(+)1					Kai Na
21	Oxidation of nitrite						(-)α_6		(-)1	(+)1				Kin Ni
22	Hydrolysis of organic phosphorus											(-)1	(+)1	Kpd Porg
23	Organic phosphorus settling											(-)1		Kps Porg

Table 2: State variables, stoichiometric coefficients and kinetics coefficients for ROCS Model

Symbol	State variable
LPOC	Concentration of labile particulate organic carbon, mg-C/L
RPOC	Concentration of refractory particulate organic carbon, mg-C/L
LDOC	Concentration of labile dissolved organic carbon, mg-C/L
RDOC	Concentration of refractory dissolved organic carbon, mg-C/L
L	Concentration of ultimate carbonaceous BOD, mg-O/L
O	Concentration of dissolved oxygen, mg-O/L
N_a	Ammonia nitrogen concentration, mg-N/L
N_n	Nitrite nitrogen concentration, mg-N/L
N_i	Nitrate nitrogen concentration, mg-N/L
N_{org}	Organic nitrogen concentration, mg-N/L
P_{org}	Concentration of organic phosphorus, mg-P/L
P_{diss}	Concentration of inorganic or dissolved phosphorus, mg-P/L
Symbol	**Stoichiometric coefficients**
α_5	Rate of oxygen uptake per unit of ammonia nitrogen oxidation, mg-O/mg-N
α_6	Rate of oxygen uptake per unit of nitrite nitrogen oxidation, mg-O/mg-N
Symbol	**Kinetic coefficients**
K_{L1}	Settling of LPOC, day^{-1}
K_{L2}	Resuspension of LPOC, gm^{-2} day^{-1}
K_{L3}	Mineralization of LPOC, TD, day^{-1}
K_{L4}	Decay of LPOC to RPOC, TD, day^{-1}
K_{L5}	Decay of LPOC to LDOC, TD, day^{-1}
K_{L6}	Decay of LPOC to RDOC, TD, day^{-1}
K_{R1}	Settling of RPOC, day^{-1}
K_{R3}	Mineralization of RPOC, TD, day^{-1}
K_{R4}	Decay of RPOC to RDOC, TD, day^{-1}
K_{R2}	Resuspension of RPOC, gm^{-2} day^{-1}
K_{L7}	Decay of LDOC to RDOC, TD, day^{-1}
K_{L8}	Mineralization of LDOC, TD, day^{-1}
K_{R5}	Mineralization for RDOC, TD, day^{-1}
K_1	Deoxygenation rate coefficient, TD, day^{-1}
K_2	Reaeration rate, day^{-1}
K_3	Rate of loss of carbonaceous BOD due to settling, TD, day^{-1}
K_4	Sediment oxygen demand rate, TD, gm^{-2} day^{-1}
K_{ai}	Ammonia oxidation rate coefficient, TD, day^{-1}
K_{in}	Nitrite oxidation rate coefficient, TD, day^{-1}
K_{oa}	Organic nitrogen hydrolysis rate, TD, day^{-1}
K_{pd}	Organic phosphorus decay rate, TD, day^{-1}
K_p	Particulate organic carbon dissolution rate, TD, day^{-1}
K_s	Particulate organic carbon settling rate, TD, day^{-1}
K_h	Hydrolysis of DOC, TD, day^{-1}
K_{os}	Organic nitrogen settling rate, TD, day^{-1}
K_{ps}	Organic phosphorus settling rate, TD, day^{-1}

(TD: temperature dependent)

The simulated river system is idealized as a series of well-mixed (laterally and vertically) reactors (control volume), considering that advection dominates. The external loads are assumed as inputs in the beginning of each control volume. For the variables simulated, the mass balance is solved using the respective analytical solution in each control volume (further details about the physical representation and flow balance are presented in item 5.3).

Considering the well-mixed series of reactors, and assuming first-order decay reaction, the mass balance for a differential element (Δx, extension of each computational element), can be represented through (Chapra, 1997):

$$\Delta V \frac{\partial C}{\partial t} = J_{in} A_c - J_{out} A_c - k \Delta V \bar{C} \quad (1)$$

Where ΔV is the volume of each element (L^3); A_c is the cross-sectional area of the reactor (L^2); k is the kinetic coefficient (T^{-1}); J_{in} and J_{out} are the flux of mass in and out the element due transport ($ML^{-2}T^{-1}$); $\bar{C}$ is the average concentration for each reactor (ML^{-1}). For the sequence of reactor representing the river reach, the flux into each element can be defined as:

$$J_{in} = UC \quad (2)$$

Where: J_{in} is the flux of mass into each control volume ($ML^{-2}T^{-1}$); U is the velocity (LT^{-1}); C is the concentration. The flux out each element can be estimated by a first-order Taylor-series expansion:

$$J_{out} = U\left(C + \frac{\delta C}{\delta x}\Delta x\right) \quad (3)$$

Substituting Equations 2 and 3 in 1 yields:

$$\Delta V \frac{\partial C}{\partial t} = UA_c C - UA_c\left(C + \frac{\delta C}{\delta x}\Delta x\right) - k \Delta V \bar{C} \quad (4)$$

Rearranging the terms:

$$\Delta V \frac{\partial C}{\partial t} = -UA_c \frac{\delta C}{\delta x}\Delta x - k \Delta V \bar{C} \quad (5)$$

Dividing by $\Delta V = A_c \Delta x$ and taking the limit $(\Delta x \rightarrow 0)$ yields:

$$\frac{\partial C}{\partial t} = -U \frac{\delta C}{\delta x} - kC \tag{6}$$

Considering steady-state $(\partial C/\partial t = 0)$ and assuming that $C = C_0$ at $x = 0$, Equation 6 can be solved for:

$$C = C_0 e^{-\frac{kx}{U}} \tag{7}$$

The term x/U can also be written in terms of travel time (time in each the compound will remain within each control volume and thus, susceptible for decay). Equation 7 represents generically the analytical solution for a variable simulated. A more detailed explanation about the process related to the mass balance for each of the state variable is presented in the following items.

5.2.1 Organic Carbon Simulation Model

As mentioned before, two approaches were considered for the organic carbon simulation: (i) a model based on DOC and POC mass balance; and (ii) a compartmentalization between labile and refractory organic carbon, for both dissolved and particulate fractions. The first modeling approach for the simulation of particulate and dissolved organic carbon was implemented based on an adapted model suggested by Chapra (1997). Originally, the model was proposed to simulate the nutrient/food-chain interactions for a stratified lake using 8 state variables. POC and DOC are considered as the non-living organic carbon, divided into particulate and dissolved fractions. Figure 2 shows a schematic representation of the kinetic segmentation, highlighting the POC and DOC relationship.

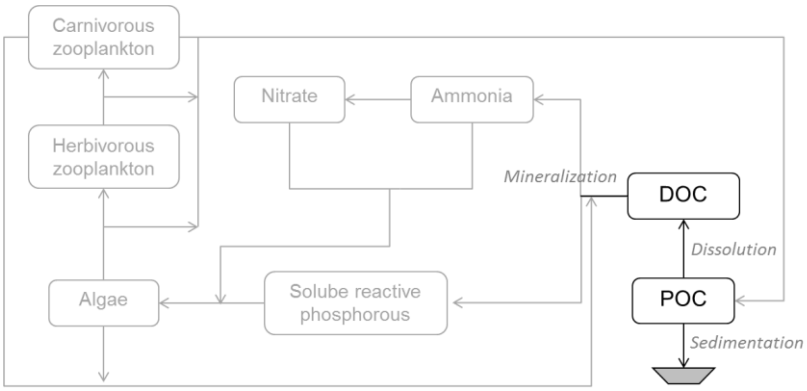


Figure 2: Kinetic sedimentation for nutrient cycling (Adapted from Chapra, 1997)

In this simplified model, the following processes were considered: sedimentation, and dissolution of POC, and mineralization of DOC. The equations and the respective analytical solution are presented below.

- Particulate organic carbon:

$$\frac{dPOC}{dt} = -\underbrace{k_p POC}_{dissolution} - \underbrace{k_s POC}_{settling} \tag{8}$$

$$POC = POC_0 e^{-(k_p + k_s)\cdot t} \tag{9}$$

- Dissolved organic carbon:

$$\frac{dDOC}{dt} = \underbrace{k_p POC}_{dissolution} - \underbrace{k_h DOC}_{hydrolysis} \tag{10}$$

$$DOC = DOC_0 e^{-k_h t} + \frac{k_p POC_0}{k_h - (k_p + k_s)}\left[e^{-(k_p + k_s)t} - e^{-k_h t}\right] \tag{11}$$

Where: POC: particulate organic carbon (mg/L); DOC: dissolved organic carbon (mg/L); k_p: particulate organic carbon dissolution rate (d^{-1}); k_h: dissolved organic carbon hydrolysis (mineralization) rate (d^{-1}); k_s: particulate organic carbon sedimentation rate (d^{-1});

For the second modeling approach, named ROCS-Model, four variables are considered to the organic carbon mass balance: labile particulate organic carbon (LPOC), refractory particulate organic carbon (RPOC), labile dissolved organic carbon (LDOC), and refractory dissolved organic carbon (RDOC). The process simulated considers settling and resuspension of particulate fractions, mineralization to inorganic forms (ammonia, phosphate, inorganic carbon), dissolution from particulate do dissolved fractions, and decay between labile and refractory organic carbon (Figure 3). According to the Peterson Matrix representation (Table 1), 13 coefficients were included in the kinetic model segmentation. Three auxiliary coefficients (K_{LPOC}, K_{RPOC}, K_{LDOC}) were introduced to facilitate the equations and solutions discretization. The equations and the respective analytical solution for LPOC, RPOC, LDOC, and RDOC are presented below.

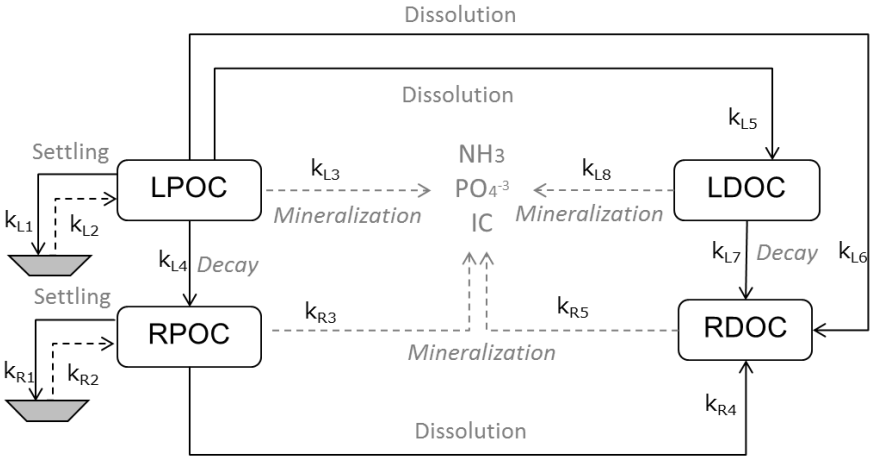


Figure 3: Conceptual kinetics segmentation for the proposed model

- Labile Particulate Organic Carbon (LPOC):

$$\frac{dLPOC}{dt} = -\underbrace{k_{L1}LPOC}_{Sedimentation} - \underbrace{k_{L3}LPOC}_{Mineralization} - \underbrace{k_{L4}LPOC}_{Dissolution} - \underbrace{k_{L5}LPOC}_{Dissolution} - \underbrace{k_{L6}LPOC}_{Dissolution} + \underbrace{\frac{k_{L2}}{H}}_{Ressuspension} \quad (12)$$

$$\frac{dLPOC}{dt} = -K_{LPOC} \cdot LPOC + \frac{k_{L2}}{H} \quad (13)$$

$$K_{LPOC} = k_{L1} + k_{L3} + k_{L4} + k_{L5} + k_{L6} \quad (14)$$

$$LPOC = LPOC_0 e^{-K_{LPOC} \cdot t} + \frac{k_{L2}}{K_{LPOC} \cdot H}(1 - e^{-K_{LPOC} \cdot t}) \quad (15)$$

Where: $LPOC$: labile particulate organic carbon (mgL^{-1}); H is the depth (m); K_{LPOC} is an auxiliary coefficient for model implementation; k_{L1}: Settling of LPOC (d^{-1}); k_{L2}: Resuspension of LPOC (gm^{-2} day^{-1}; k_{L3}: Mineralization of LPOC (d^{-1}); k_{L4}: Decay of LPOC to RPOC (d^{-1}); k_{L5}: Decay of LPOC to LDOC (d^{-1}); k_{L6}: Decay of LPOC to RDOC (d^{-1}).

- Refractory Particulate Organic Carbon (RPOC):

$$\frac{dRPOC}{dt} = + \underbrace{k_{L4} \cdot LPOC}_{Mineralization} - \underbrace{k_{R1} \cdot RPOC}_{Sedimentation} - \underbrace{k_{R3} \cdot RPOC}_{Mineralization} - \underbrace{k_{R4} \cdot RPOC}_{Decay} + \underbrace{\frac{k_{R2}}{H}}_{Ressuspension} \quad (17)$$

$$\frac{dRPOC}{dt} = +k_{L4} \cdot LPOC - k_{RPOC} \cdot RPOC + \frac{k_{R2}}{H} \quad (18)$$

$$K_{RPOC} = k_{R1} + k_{R3} + k_{R4} \quad (19)$$

$$RPOC = RPOC_0 e^{-K_{RPOC} \cdot t} + \frac{k_{L4}}{(K_{RPOC} - K_{LPOC})} LPOC_0 (e^{-K_{LPOC} \cdot t} - e^{-K_{RPOC} \cdot t}) - \frac{k_{L4} \cdot k_{L2}}{(K_{RPOC} - K_{LPOC})} LPOC_0 (e^{-K_{LPOC} \cdot t} - e^{-K_{RPOC} \cdot t}) + \frac{k_{L4} \cdot k_{L2}}{K_{LPOC} \cdot H}(1 - e^{-K_{RPOC} \cdot t}) + \frac{k_{R2}}{H}(1 - e^{-K_{RPOC} \cdot t}) \quad (20)$$

Where: $RPOC$: refractory particulate organic carbon (mgL^{-1}); H is the depth (m); K_{RPOC} is an auxiliary coefficient for model implementation; k_{R1}: Settling of RPOC (d^{-1}); k_{R3}: Mineralization of RPOC (d^{-1}); k_{R4}: Decay of RPOC to RDOC (d^{-1}); k_{R2}: Resuspension of RPOC (d^{-1}).

- Labile Dissolved Organic Carbon (LDOC):

$$\frac{dLDOC}{dt} = \underbrace{k_{L5} \cdot LPOC}_{Dissolution} - \underbrace{k_{L7} \cdot LDOC}_{Decay} - \underbrace{k_{L8} \cdot LDOC}_{Mineralization} \quad (21)$$

$$K_{LDOC} = k_{L7} + k_{L8} \tag{22}$$

$$LDOC = LDOC_0 e^{-K_{LDOC}\cdot t} + \frac{k_{L5}}{(K_{LDOC}-K_{LPOC})} LPOC_o (e^{-K_{LPOC}\cdot t} - e^{-K_{LDOC}\cdot t}) - \frac{k_{L5}\cdot k_{L2}}{K_{LPOC}\cdot H\cdot (K_{LDOC}-K_{LPOC})} LPOC_o (e^{-K_{LPOC}\cdot t} - e^{-K_{LDOC}\cdot t}) \tag{23}$$

Where: $LDOC$: labile dissolved organic carbon (mgL^{-1}); $LPOC$: labile particulate organic carbon (mgL^{-1}); H is the depth (m); K_{LPOC} and K_{LDOC} are two auxiliary coefficients for model implementation; k_{L2}: Resuspension of LPOC (gm^{-2} d^{-1}); k_{L3}: Mineralization of LPOC (d^{-1}); k_{L4}: Decay of LPOC to RPOC (d^{-1}); k_{L5}: Decay of LPOC to LDOC (d^{-1}); k_{L7}: Decay of LDOC to RDOC (d^{-1}); k_{L8}: Mineralization of LDOC (d^{-1});.

- Refractory Dissolved Organic Carbon (RDOC):

$$\frac{dRDOC}{dt} = \underbrace{k_{L6}\cdot LPOC}_{Dissolution} + \underbrace{k_{L7}\cdot LDOC}_{Dissolution} + \underbrace{k_{R4}\cdot RPOC}_{Dissolution} - \underbrace{k_{R5}\cdot RDOC}_{Mineralization} \tag{24}$$

$$K_{LPOC} = k_{L1} + k_{L3} + k_{L4} + k_{L5} + k_{L6} \tag{25}$$

$$K_{RPOC} = k_{R1} + k_{R3} + k_{R4} \tag{26}$$

$$K_{LDOC} = k_{L7} + k_{L8} \tag{27}$$

$$RDOC = RDOC_0 e^{-k_{R5}\cdot t} + \frac{k_{L6}}{(k_{R5} - K_{LPOC})} LPOC_o (e^{-K_{LPOC}\cdot t} - e^{-k_{R5}\cdot t}) + k_{L7}\left\{ \frac{LDOC_o}{(k_{R5} - K_{LDOC})} (e^{-K_{LDOC}\cdot t} - e^{-k_{R5}\cdot t}) + \frac{k_{L5}\cdot LPOC_o}{(K_{LDOC} - K_{LPOC})\cdot (k_{R5} - K_{LPOC})} (e^{-K_{LPOC}\cdot t} - e^{-k_{R5}\cdot t}) - \frac{k_{L5}\cdot LPOC_o}{(K_{LDOC} - K_{LPOC})\cdot (k_{R5} - K_{LDOC})} (e^{-K_{LDOC}\cdot t} - e^{-k_{R5}\cdot t}) \right\}$$

$$+k_{R4}\left\{\frac{RPOC_o}{(k_{R5}-K_{LPOC})}(e^{-K_{LPOC}\cdot t}-e^{-k_{R5}\cdot t})\right.$$
$$+\frac{k_{L4}\cdot LPOC_o}{(K_{RPOC}-K_{LPOC})\cdot(k_{R5}-K_{LPOC})}(e^{-K_{LPOC}\cdot t}-e^{-k_{R5}\cdot t})$$
$$-\frac{k_{L4}\cdot LPOC_o}{(K_{RDOC}-K_{LPOC})\cdot(k_{R5}-K_{RPOC})}(e^{-K_{RPOC}\cdot t}-e^{-k_{R5}\cdot t})$$
$$\left.-\frac{k_{L4}\cdot k_{L2}}{(K_{RPOC}-K_{LPOC})\cdot K_{LPOC}\cdot H\cdot(k_{R5}-K_{LPOC})}(e^{-K_{LPOC}\cdot t}-e^{-k_{R5}\cdot t})\right\}$$

$$+k_{R4}\cdot\left(\frac{k_{L4}\cdot k_{L2}+k_{R2}\cdot K_{LPOC}}{K_{LPOC}\cdot H}\right)\cdot\left[\frac{(1-e^{-k_{R5}\cdot t})}{k_{R5}}-\frac{(e^{-K_{RPOC}\cdot t}-e^{-k_{R5}\cdot t})}{(k_{R5}-K_{RPOC})}\right] \quad (28)$$

Where: $RDOC$: refractory dissolved organic carbon (mgL^{-1}); K_{RPOC}, K_{LPOC} and K_{LDOC} are auxiliary coefficients for model implementation; k_{R5}: Mineralization for RDOC (d^{-1}).

5.2.2 BOD Simulation Model

The mass balance for BOD is based on the amount of biodegradable organic matter in the water column and the process of decomposition (biological assimilation) and sedimentation. Figure 4 presents a schematic representation of these two processes. Equations 5 and 6 present the mass balance and the analytical solution.

Figure 4: schematic representation of process related to bod mass balance

$$\frac{dL}{dt}=-K_1L-K_3L \quad (29)$$

$$L=L_0e^{-(K_1+K_3)t} \quad (30)$$

Where: L: Concentration of carbonaceous BOD (mg/L); K_1: Deoxygenation rate coefficient (d^{-1}); K_3: Rate of loss of carbonaceous BOD due to settling (d^{-1});.

In addition, in the proposed model it is possible to simulate the BOD mass balance for anoxic conditions. Anoxic conditions occur when the deficit of oxygen is equal to the saturation concentration of oxygen, i. e., there is not oxygen available in the water column. Under these conditions, the rate of oxygen consumption is not exponential, since Equation 5 is limited by the rate in which the oxygen enters in the water column through reaeration, according to the following equation (Chapra, 1997):

$$\frac{dL}{dt} = -K_2 O_s \tag{31}$$

Thus, when an anoxic condition is observed in the simulation, the oxidation of BOD will be represented by:

$$L = L_i - K_2 O_s (t - t_i) \tag{32}$$

Where: L_i: Concentration of carbonaceous BOD at time *i* (mg/L); O_s: Saturation concentration of dissolved oxygen (mg/L); *i*: is the time when the anoxic condition starts.

When the concentration of dissolved oxygen is not equal to zero, the model uses Equation 30 do calculate the concentration of BOD.

5.2.3 Nitrogen Simulation Model

The mathematical simulation of nitrogen cycle in aquatic systems is based on the conversion between the different fractions and the removing from the water column. The first one is related to the ammonification and nitrification process that converts organic nitrogen to ammonia nitrogen, and the last one in nitrite and nitrate. The second process is related to the biological assimilation or sedimentation (Chapra, 1997). In the currently version of the proposed model, were implemented routines to simulate the concentration of organic nitrogen, ammonia, nitrite and nitrate, considering process such as sedimentation and oxidation (Figure 5). The equations were implemented according the mechanisms used in Qual2e model (Brown and Barnwell, 1987).

For organic nitrogen, the following process and equations were considered. The same simplification used by Qual2e model was adopted, since both particulate and

dissolved forms of organic nitrogen were considered as only one state variable. Particulate organic nitrogen can be removed from the water column by sedimentation, while dissolved organic nitrogen can be oxidized to ammonia (Chapra, 1997).

Figure 5: Schematic representation of process related to nitrogen mass balance

The equation for organic nitrogen mass balance and the respective analytical solution are:

$$\frac{dN_{org}}{dt} = -K_{oa}N_{org} - K_{os}N_{org} \tag{33}$$

$$N_{org} = N_{org\_0}e^{-(K_{oa}+K_{os})t} \tag{34}$$

Where: N_{org}: Organic nitrogen concentration (mg-N/L); K_{oa}: Organic nitrogen hydrolysis rate (d^{-1}); K_{os} : Organic nitrogen settling rate (d^{-1}).

For the simulation of ammonia, were considered the ammonification and nitrification process without the interaction with the sediment and biological assimilation. The equation for ammonia nitrogen mass balance and the respective analytical solution are presented in Equation 35 and 36.

$$\frac{dN_a}{dt} = K_{oa}N_{org} - K_{ai}N_a \tag{35}$$

$$N_a = N_{a\_0}e^{-K_{ai}t} + \frac{K_{os}N_{org\_0}}{K_{ai}-(K_{oa}+K_{os})}\left[e^{-(K_{oa}+K_{os})t} - e^{-K_{ai}t}\right] \tag{36}$$

Where: N_a: Ammonia nitrogen concentration (mg-N/L); N_{org}: Organic nitrogen concentration (mg-N/L); K_{oa}: Organic nitrogen hydrolysis rate (d^{-1}); K_{os} : Organic nitrogen settling rate (d^{-1}); K_{ai}: Ammonia oxidation rate coefficient (d^{-1}).

For the simulation of nitrite, were considered the two steps of nitrification. The equation for nitrite mass balance and the respective analytical solution are presented in Equation 37 and 38.

$$\frac{dN_i}{dt} = K_{ai}N_a - K_{in}N_i \tag{37}$$

$$N_i = N_{i\_0}e^{-K_{in}t} + \frac{K_{ai}N_{a\_0}}{K_{in} - K_{ai}}[e^{-K_{ai}t} - e^{-K_{in}t}] + \frac{K_{ai}K_{oa}N_{org\_0}}{K_{ai}-(K_{oa}+K_{os})}\left[\frac{e^{-K_{in}t}-e^{-K_{ai}t}}{K_{in}-K_{ai}} + \frac{e^{-(K_{oa}+K_{os})t}-e^{-K_{in}t}}{K_{in}-(K_{oa}+K_{os})}\right] \tag{38}$$

Where: N_i: Nitrite nitrogen concentration (mg-N/L); N_a: Ammonia nitrogen concentration (mg-N/L); N_{org}: Organic nitrogen concentration (mg-N/L); K_{oa}: Organic nitrogen hydrolysis rate (d^{-1}); K_{os} : Organic nitrogen settling rate (d^{-1}); K_{ai}: Ammonia oxidation rate coefficient (d^{-1}); K_{in}: Nitrite oxidation rate coefficient (d^{-1}).

Finally, the simulation of nitrate considers the nitrification process, in which nitrite is converted to nitrate. The equation for nitrite mass balance and the respective analytical solution are presented in Equation 39 and 40.

$$\frac{dN_n}{dt} = K_{in}N_i \tag{39}$$

$$N_n = N_{n_0} + N_{i_o} + N_{a_0} - N_{i_0}e^{-K_{in}t} + \frac{K_{oa}N_{org_o}}{K_{oa}+K_{os}} + \frac{N_{a_0}}{K_{in}-K_{ai}}[K_{ai}e^{-K_{in}t} - K_{in}e^{-K_{ai}t}] + \frac{K_{oa}N_{org_0}}{K_{ai}-(K_{oa}+K_{os})}\left[\frac{K_{in}e^{-K_{ai}t} - K_{ai}e^{-K_{in}t}}{K_{in}-K_{ai}}\right] + \frac{K_{ai}K_{oa}N_{org\_0}}{K_{ai}-(K_{oa}+K_{os})}\left[\frac{(K_{oa}+K_{os})e^{-K_{in}t}-K_{in}e^{-(K_{oa}+K_{os})t}}{(K_{oa}+K_{os})(K_{in}-(K_{oa}+K_{os}))}\right] \tag{40}$$

Where: N_n: Nitrate nitrogen concentration (mg-N/L); N_i: Nitrite nitrogen concentration (mg-N/L); N_a: Ammonia nitrogen concentration (mg-N/L); N_{org}: Organic nitrogen concentration (mg-N/L); K_{oa}: Organic nitrogen hydrolysis rate (d^{-1}); K_{os} : Organic nitrogen settling rate (d^{-1}); K_{ai}: Ammonia oxidation rate coefficient (d^{-1}); K_{in}: Nitrite oxidation rate coefficient (d^{-1}).

5.2.4 Dissolved Oxygen Simulation Model

The simulation of dissolved oxygen was implemented considering the processes related to biological oxygen demand, sediment oxygen demand, oxygen consumption due to the oxidation of ammonia to nitrate and this to nitrite, and atmospheric reaeration. The equation that represents these processes is based on the formulation of Qual2e model (Brown and Barnwell, 1987). Figure 6 shows a schematic representation for DO simulation model.

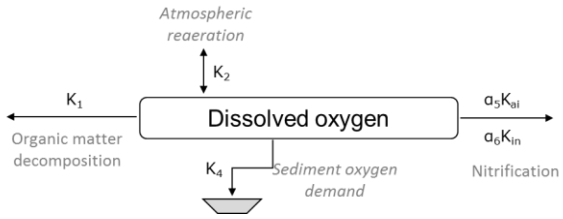


Figure 6: Schematic kinetic representation for DO modeling

The equation for the DO mass balance depends on the rate at which oxygen enters in the water column (reaeration), the initial concentration of BOD, organic nitrogen, ammonia and nitrite, as is presented in the following equation:

$$\frac{dO}{dt} = -K_2(O_S - O) + K_1 L \frac{K_4}{H} + \alpha_5 K_{ai} N_a + \alpha_6 K_{in} N_i \tag{41}$$

The concentration of DO can be analyzed by the oxygen deficit, calculated as the difference between the saturation concentration of oxygen and the concentration of DO in the water column:

$$D = O_s - O \tag{42}$$

$$\frac{dO}{dt} = -\frac{dD}{dt} \tag{43}$$

Where: O : Concentration of dissolved oxygen (mg/L); D : Dissolved oxygen deficit (mg/L); O_s: Saturate concentration of dissolved oxygen (mg/L);

The corresponding analytical solution for the deficit of dissolved oxygen can be represented by the following equation:

$$D = D_0 e^{-K_2 t} + \underbrace{\frac{K_1 L_0}{K_2 - (K_1 + K_3)}\left[e^{-(K_1+K_3)t} - e^{-K_2 t}\right]}_{Bichemical\ oxygen\ demand} + \underbrace{\frac{K_4}{K_2 H}\left[1 - e^{-K_2 t}\right]}_{Sediment\ oxygen\ demand}$$

$$\underbrace{+\frac{\alpha_5 K_{ai} N_{a\_0}}{K_2 - K_{ai}}\left[e^{-K_{ai}t} - e^{-K_2 t}\right] + \frac{K_{oa} N_{org\_0}}{K_{ai} - (K_{oa} + K_{os})}\left[\frac{e^{-(K_{oa}+K_{os})t}}{K_2 - (K_{oa} + K_{os})} - \frac{e^{-K_{ai}t} - e^{-K_2 t}}{K_2 - K_{ai}}\right]}_{Oxigen\ cunsumption\ due\ nitrification}$$

$$\underbrace{+\frac{\alpha_6 K_{in} N_{i\_0}}{K_2 - K_{in}}\left[e^{-K_{in}t} - e^{-K_2 t}\right] + \frac{\alpha_6 K_{in} N_{a\_0}}{K_{in} - K_{ai}}\left[\frac{e^{-K_{ai}t}}{K_2 - K_{ai}} - \frac{e^{-K_{in}t}}{K_2 - K_{in}} - \frac{(K_{ai} - K_{in})e^{-K_2 t}}{(K_2 - K_{ai})(K_2 - K_{in})}\right]}_{Oxigen\ cunsumption\ due\ nitrification}$$

$$\underbrace{+\frac{\alpha_6 K_{in} K_{ai} N_{org\_0}}{K_{ai} - (K_{oa} + K_{os})}\left[\frac{\frac{e^{-(K_{oa}+K_{os})t}}{K_2 - (K_{oa}+K_{os})} - \frac{e^{-K_{in}t}}{K_2 - K_{in}}}{K_{in} - (K_{oa} + K_{os})} - \frac{\frac{e^{-K_{in}t}}{K_2 - K_{in}} - \frac{e^{-K_{ai}t}}{K_2 - K_{ai}}}{K_{ai} - K_{in}}\right]}_{Oxigen\ cunsumption\ due\ nitrification} \quad (44)$$

Where: O : Concentration of dissolved oxygen (mg/L); D : Dissolved oxygen deficit (mg/L); O_s: Saturate concentration of dissolved oxygen (mg/L); L : Concentration of ultimate carbonaceous BOD (mg/L); K_1 : Deoxygenation rate coefficient (d^{-1}); K_2 : Reaeration rate (d^{-1}); K_3 : Rate of loss of carbonaceous BOD due to settling (d^{-1}); K_4 : Sediment oxygen demand ($g/m^2.d$); K_{oa}: Organic nitrogen hydrolysis rate (d^{-1}); K_{os} : Organic nitrogen settling rate (d^{-1}); K_{ai} : Ammonia oxidation rate coefficient (d^{-1}); K_{in} : Nitrite oxidation rate coefficient (d^{-1}); α_5: Rate of oxygen uptake per unit of ammonia nitrogen oxidation (mg_O/mg_N); α_6 : Rate of oxygen uptake per unit of nitrite nitrogen oxidation (mg_O/mg_N); N_{org}: Organic nitrogen concentration (mg-N/L); N_a : Ammonia nitrogen concentration (mg/L); N_i : Nitrite nitrogen concentration (mg/L); N_n: Nitrate nitrogen concentration (mg-N/L); H: depth (m) and t : time (d).

In addition, to calculate the saturation concentration of oxygen, it was implemented a routine based on altitude and water temperature. The altitude for each computational element is estimated based on the headwater data and the longitudinal declivity. The saturation concentration of oxygen is calculated by (Chapra et al., 2007):

$$lnO_{s(T,O)} = -139{,}34411 + \frac{1{,}575701 \cdot 10^5}{T_a} - \frac{6{,}642308 \cdot 10^7}{T_a^2} + \frac{1{,}23438 \cdot 10^{10}}{T_a^3} - \frac{8{,}621949 \cdot 10^{11}}{T_a^4} \quad (45)$$

$$O_{s(T,elev)} = e^{\ln O_{s(T,O)}} \cdot (1 - 0{,}1148 \cdot elev/1000) \quad (46)$$

Where: $O_{s(T,O)}$: saturation concentration of dissolved oxygen at 1 atm (mg/L); T_a: absolute water temperature (K). $T_a = T + 273{,}15ºC$; $elev$: elevation above sea level (m).

5.2.5 Phosphorous Simulation Model

The mathematical formulation considered for total organic phosphorous and dissolved inorganic phosphorous was adapted from Qual2e model (Brown and Barnwell, 1987). For the organic phosphorous mass balance, where considered the process related to the decomposition of organic phosphorous to inorganic phosphorous (dissolved) and sedimentation. Figure 7 shows a schematic representation of this configuration.

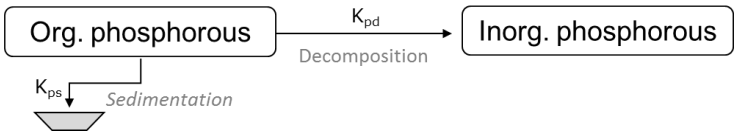


Figure 7: Schematic representation of organic phosphorous model process

The equation for total organic phosphorous mass balance and the respective analytical solution are:

$$\frac{dP_{org}}{dt} = -K_{pd}P_{org} - K_{ps}P_{org} \quad (47)$$

$$P_{org} = P_{org\_0}e^{-(K_{pd}+K_{ps})t} \quad (48)$$

Where: P_{org} : Organic phosphorous concentration (mg-P/L); K_{pd} : Organic phosphorus settling rate (d^{-1}); K_{ps} : Organic phosphorus decay rate (d^{-1}).

The concentration of inorganic phosphorous considers the decomposition of total organic phosphorous. The equation for inorganic dissolved phosphorous mass balance and the respective analytical solution are:

$$\frac{dP_{diss}}{dt} = K_{pd}P_{org} \quad (49)$$

$$P_{diss} = P_{diss} + \frac{K_{pd}P_{org\_0}}{K_{pd}+K_{ps}}\left[1 - e^{-(K_{pd}+K_{ps})t}\right] \quad (50)$$

Where: P_{diss}: Concentration of inorganic dissolved phosphorus (mg-P/L); P_{org}: Concentration of organic phosphorus (mg-P/L); K_{pd} : Organic phosphorus decay rate (d^{-1}); K_{ps} : Organic phosphorus settling rate (d^{-1}).

5.3 Model Main Structure and Modules

The proposed model is implemented using Excel spreadsheets as graphical interface. The algorithm is based on structured programing, using Visual Basic for Application (VBA). Figure 8 presents a schematic representation of the main structure of the model. There is a main spreadsheet ('Main menu'), were the user can set the initial data necessary to run the model. The secondary segmentation is based on 7 modules: Main data, Diffuse sources module ('DSM menu'), Point sources module ('PSM menu'), Hydraulic module ('HM menu'), Water quality module ('WQM menu'), Monitoring data module ('Monitoring') and Calibration module ('Calibration').

From the main data module, additional data are calculated and filled in the following modules. Figure 8 presents a general map of the model structure. Green and red sets with 'R' and CE' indicate, respectively, spreadsheet were data are recorded by Reach or by Computational Element. For each module there is a set of auxiliary spreadsheets, in a total of 52 model spreadsheets.

In the following items are presented the physical representation, system discretization, flow balance and hydraulic module, point and non-point sources module, graphic representation, monitoring data and statistical module, and automatic calibration module.

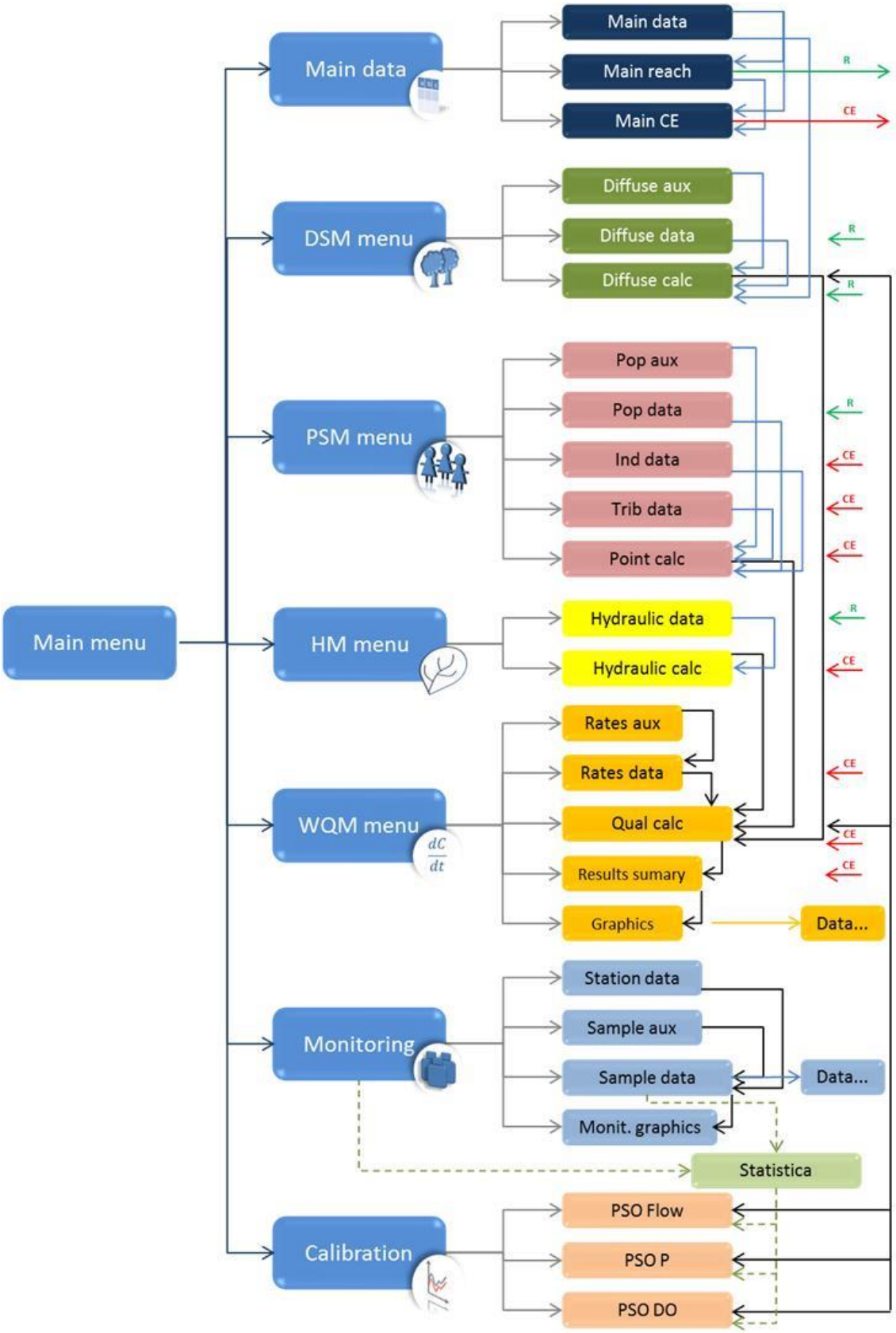


Figure 8: Schematic representation of the model main structure

5.3.1 Physical Representation

The physical representation for the watershed and river configuration is composed by reaches and computational elements (Figure 9). Each reach represents an incremental area of the watershed (constant hydrogeometric properties), and can be divided in one or more computational elements (control volume). All the computational elements need to have the same extension. The inputs of tributaries are added in the model through point sources in a specific section at the point source module.

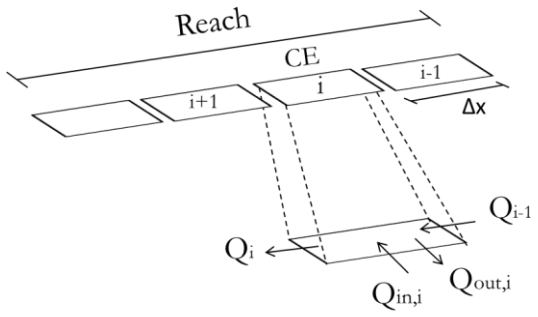


Figure 9: Reach and computational element schematic representation

The computational elements are the control volumes of the model discretization. Each compartment works as a well-mixed (laterally and vertically) reactor, in which the flow and mass balance is calculated. The number of computational elements depends on the river length and the extension of each computational element. Figure 10 presents a schematic representation of main data module and the respective modules that are coupled together.

At **"Main data"** spreadsheet, some input that are necessary to start the simulation, such as river length, computational elements segmentation, river and watershed name, total area, headwater data and initial temperature. These data are automatically added in the specific spreadsheets for the equation of other model functions.

At **"Reach data"** spreadsheet it is necessary to set the total area of the watershed and the number of computational elements. With this data, the model generates the segmentation for the other routines, and organizes the data to be used in the modules of point source and non-point source to generate these two groups of loads.

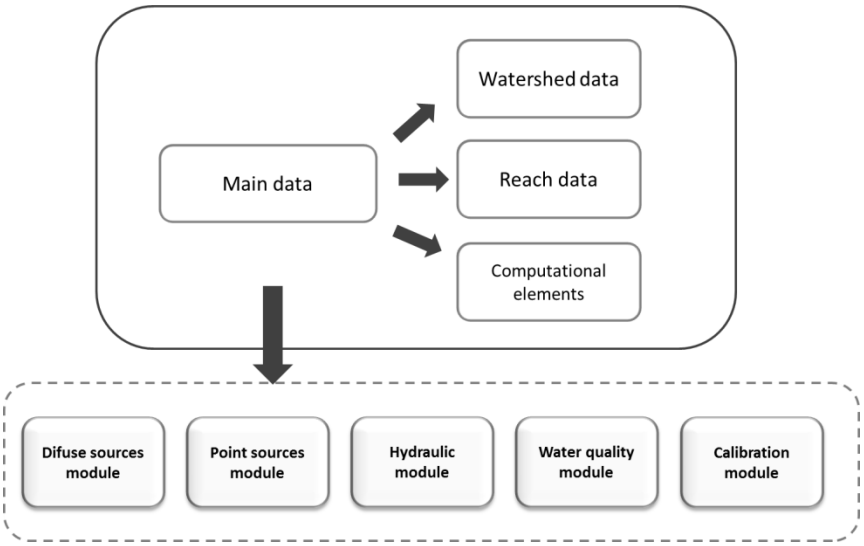


Figure 10: Schematic representation of the main data module

5.3.2 Flow Balance and Hydraulic Module

The model considers steady-state flow balance, implemented for each model computational element. The flow balance is calculated between the inflows and outflows (Figure 11).

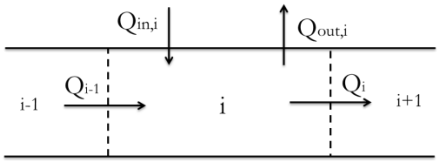


Figure 11: Model reaches flow balance

The total inflow is computed according to the non-point (incremental flow) and point sources (domestic, tributaries, and industrial/WWTP effluents). For the first computational element (CE), it is also considered the headwater flow. The incremental flow is estimated based on the relative area of each river reach, through the specific flow, and being subsequently linearly divided between each computational element from the river reach.

$$Q_{in,i} = \sum_{j=1}^{Nin} Q_{nps,i,j} + \sum_{j=1}^{Nin} Q_{ps,i,j} \quad (51)$$

$$Q_{out,i} = \sum_{j=1}^{Nout} Q_{ps\_out,i,j} \quad (52)$$

Where: $Q_{in,i}$ is the total inflow to the CE i (m^3/s); $Q_{nps,i,j}$ is the jth non-point source inflow to the CE i (m^3/s); $Q_{ps,i,j}$ is the jth point source inflow to the CE i (m^3/s); $Q_{out,i}$ is the total outflow from the CE i (m^3/s); $Q_{ps\_out,i,j}$ is the jth point source outflows from the CE i (m^3/s); Nin and $Nout$ is the total number of inflows and outflows for each CE.

Under the conditions of steady-flow, the hydrologic balance is estimated through Manning's equation (Chapra, 1997), assuming a trapezoidal channel:

$$Q = \frac{A_x \cdot R_h^{2/3} \cdot S_e^{1/2}}{n} \quad (53)$$

Where: A_x is the cross-section area (m^2), R_h is the channel hydraulic radius (m), S_e is the slope of the channel (m/m) and n is the Manning's roughness coefficient. Considering a trapezoidal channel, the cross-section area and the hydraulic radius can be expressed as:

$$A_x = (B_0 + Sh)h \quad (54)$$

$$R_h = \frac{(B_0+Sh)h}{B_0+2h\sqrt{S^2+1}} \quad (55)$$

Where B_0 is the bottom width (m), S is the side slope, R_h is the channel hydraulic radius and h is the depth. Equations 27 and 28 can be combined, resulting in:

$$Q = \frac{1}{n} \frac{[(B_0+Sh)h]^{5/3}}{(B_0+2h\sqrt{S^2+1})^{2/3}} S_e^{1/2} \quad (56)$$

If flow is given and the cross-section area and hydraulic radius can be expressed as function of depth. Thus, Equation 30 is solved by an iterative method (roots problem).

$$f(h) = \frac{1}{n} \frac{[(B_0+Sh)h]^{5/3}}{(B_0+2h\sqrt{S^2+1})^{2/3}} S_e^{1/2} - Q \quad (57)$$

The stream velocity ($\bar{U}$) is calculated with the cross-section area (A_x) and flow (Q). The travel time (d^{-1}) is calculated as a function of stream velocity and the length of each computational element. Figure 12 shows a schematic representation of the hydraulic module and the relationship with the other model modules.

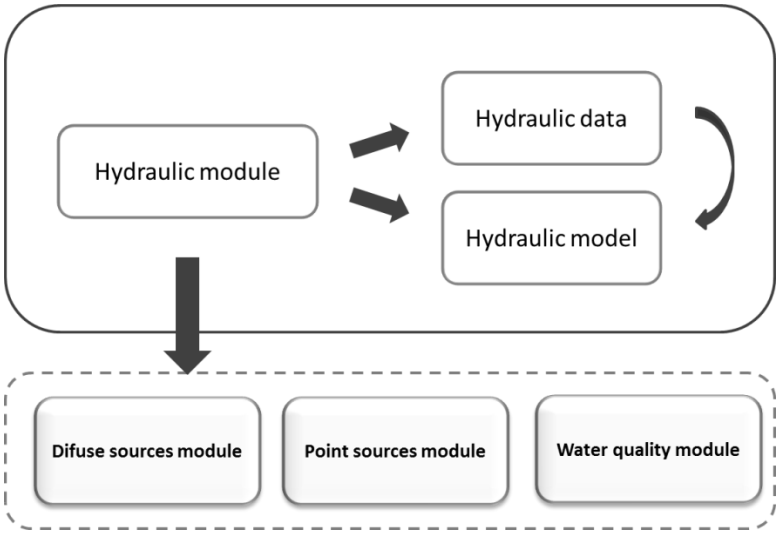


Figure 12: Schematic representation of the hydraulic module

Specific data that are needed within the hydraulic module are: bottom width (m), side slope, channel slope, Manning's roughness coefficient. Previous data that are automatic estimated for the hydraulic module are: reach segmentation and the respective areas (km^2), initial and final km of each computational element, flow (headwater, non-point and point sources), altitude (m).

5.3.3 Diffuse Sources Module

The diffuse sources module have two properties: (i) works as the database for use and land occupation; and (ii) generates from its input data the diffuse loads. The basic principle to calculate the diffuse loads are based on the incremental area of each reach according to the river segmentation. Auxiliary coefficients are used do estimate the loads according to different types of land use, such as agriculture, urban area or forests. The non-point flow is also estimated from the incremental area and specific flow. There is also a possibility to estimate the specific flow through the regionalization equation (permanence flow). Figure 13 presents a schematic representation of the diffuse sources module and the respective modules that are coupled together.

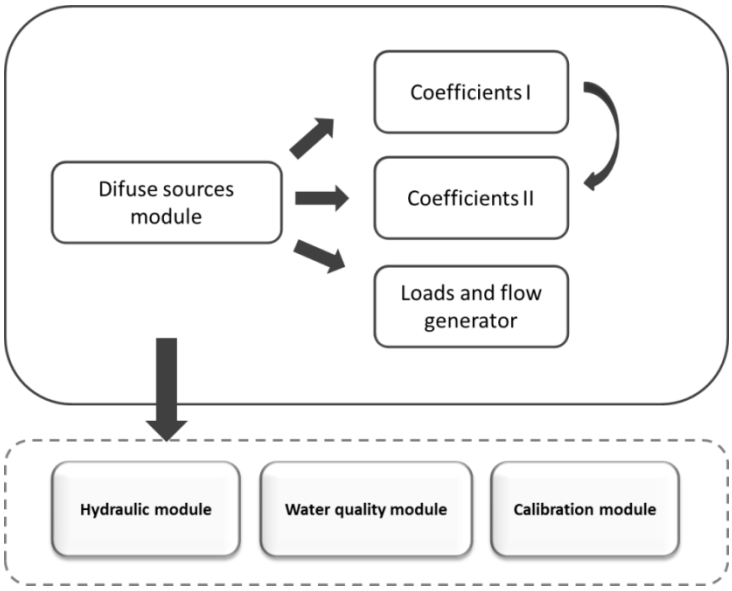


Figure 13: Schematic representation of the diffuse source module

5.3.4 Point Sources Module

The point sources module consists of three segments: (i) domestic effluents; (ii) industries and wastewater treatment plants (WWTP); and (iii) tributaries. Figure 14 shows a schematic representation of the point source module spreadsheets.

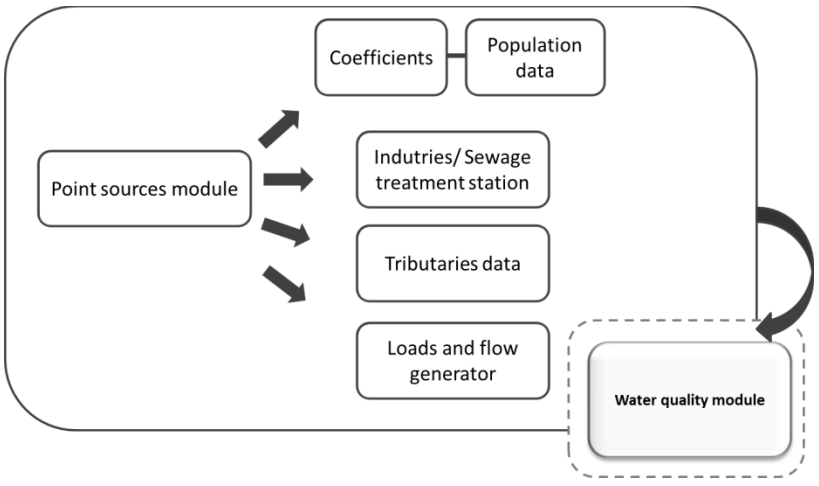


Figure 14: Schematic representation of the point sources module

The domestic effluent is estimated based on the population of each incremental area of the watershed, considering local rates of collection and treatment. An auxiliary

spreadsheet was implemented for the specific per capita loads. The total number of inhabitants can be divided into different levels of wastewater collection and treatment. The respective domestic wastewater flow is estimated based on the following equations:

$$Q_{dom} = \frac{Pop_{reach} \cdot QPC \cdot R}{86400} \tag{58}$$

$$W_{dom} = Pop_{reach} \cdot W_{capita} \tag{59}$$

Where: Q_{dom} is the domestic effluent flow (L/s); Pop_{reach} is the population from each reach (inhab); QPC is the per capita flow (L/inhab.d); R is the coefficient of sewage return; W_{dom} is the load from non-treated effluent (kg/d); W_{capita} is the per capita load (g/inhab.d).

Industries and WWTP loads have a specific spreadsheet to input data. The user needs to identify the distance from the headwater in which an industry or a WWTP is located. It is also possible to simulate withdrawal within this module. Tributaries are simulated as point sources, and, as the same as industries, the user need to locate appropriately the location flow and loads.

5.3.5 Water Quality Simulation Module and Graphic Representation

The data generated in the previous module are automatically allocated in the water quality simulation module. In this module, all the analytical equations for the variables simulated are solved, and the results are listed. There is the possibility also to view graphically the main results for the river length. Figure 15 presents a representation for this module and the relationship between the other modules. The user can modify the specific coefficients for the mass balance, or select the automatic calibration for model adjustment.

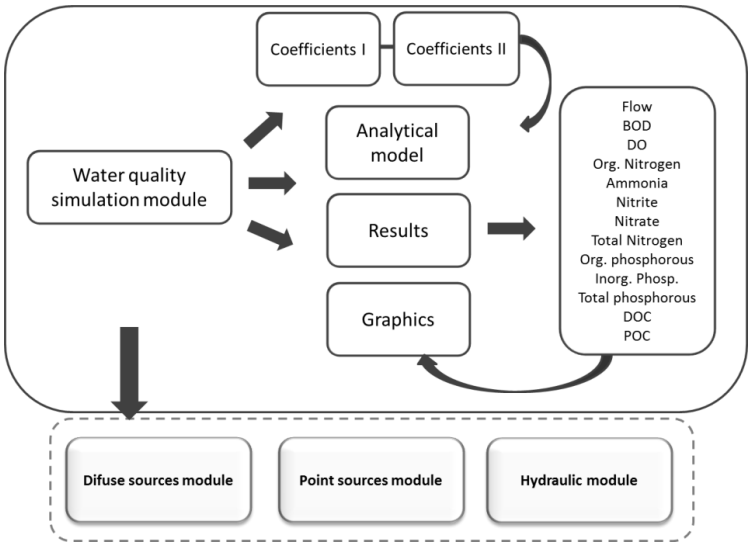


Figure 15: Schematic representation of the water quality simulation module

5.3.6 Monitoring Data and Statistical Module

The computational model also aims to be a database for the monitoring data of the watershed. This is important to summarize all the information that is necessary for monitoring evaluation and model calibration. Figure 16 shows a schematic representation of the monitoring data module and the relationship with the other model modules.

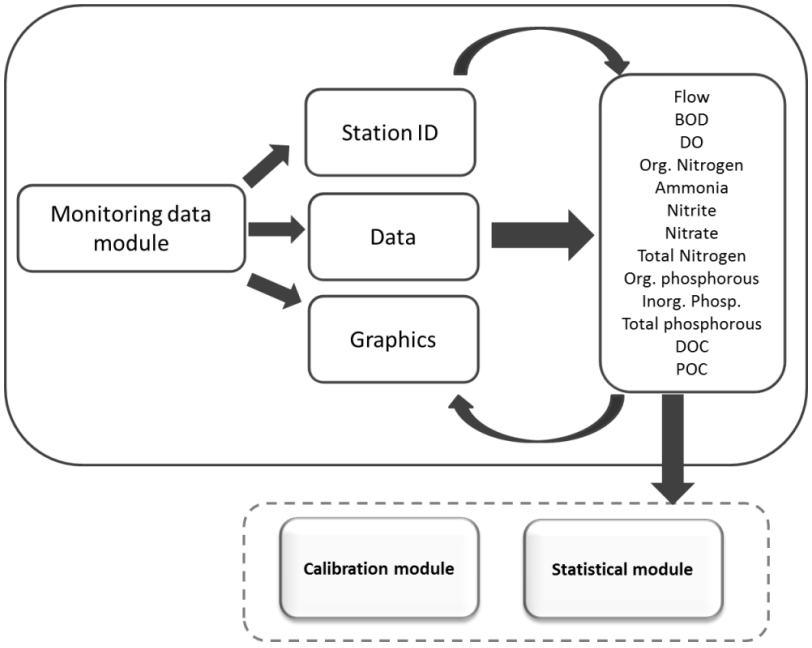


Figure 16: Schematic representation of the monitoring data module

5.3.7 Automatic Calibration Module

For the proposed model, an automatic calibration tool was implemented based on optimization routines. The algorithm is based on Particle Swarm Optimization (PSO). As described in Chapter 4, PSO has the capability to search for the best group of coefficients according to some restrictions by minimizing an objective function. In this case, the objective function to minimize is the quadratic sum of the observed and simulated data. The median of monitored data at selected control sites along the main river is used to estimate the quadratic difference between observed and simulated data. The maximum number of iterations is the stop criteria. Additionally, upper and lower boundaries were set to the coefficients. Figure 17 presents a representation of this module and the relationship with the other model modules.

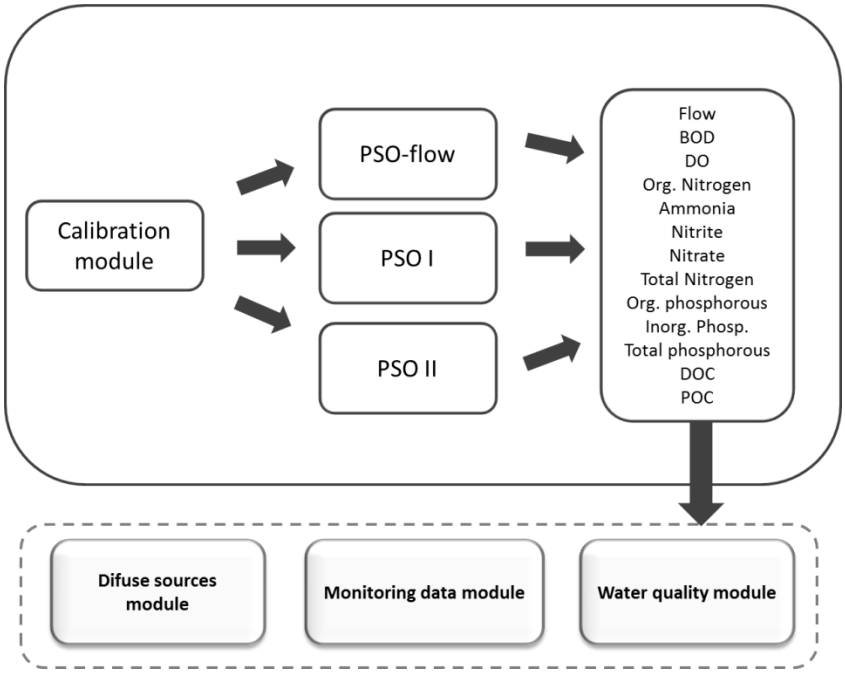


Figure 17: schematic representation of the calibration module

The calibration is performed separately considering: (i) first, the flow is calibrated based on an auxiliary coefficient applied in the specific flow at the diffuse sources module; (ii) second, organic carbon is calibrated according to the respective model simulated; (iii) the calibration of organic and inorganic phosphorous is based on the measured total phosphorous; and (iv) the remaining variables (BOD, nitrogen, and BOD) are calibrated according the respective coefficients.

(i) Flow calibration: the simulated flow is adjusted to the observed data in the control sites along the main reach through an auxiliary coefficient applied in the specific flow at the diffuse sources module. The objective function to be minimized is:

$$f_{flow} = min \sum_{i=1}^{N} \left(Q_i^{obs} - Q_i^{sim} \right)^2 \quad (60)$$

Where: f_{flow} is the flow objective function; Q_i^{obs} is the observed flow (m^3/s); Q_i^{sim} is the simulated flow (m^3/s); and N is the number of control points (e. g., number of sites with monitored data).

(ii) Organic carbon calibration: for the simplified model based on POC and DOC mass balance (Chapra, 1997), three coefficients are calibrated k_p (POC dissolution rate, d^{-1}), k_h (DOC hydrolysis rate, d^{-1}), and k_s (POC sedimentation rate, d^{-1}). In this case, the restriction considered in the PSO algorithm is that $k_h - (k_p + k_s) \neq 0$. The evaluation of simulated and measured data is based on POC and DOC directly. For the second modeling approach, ROCS-Model, 13 coefficients are calibrated (K_{L1}, K_{L2}, K_{L3}, K_{L4}, K_{L5}, K_{L6}, K_{L7}, K_{L8}, K_{R1}, K_{R2}, K_{R3} K_{R4}, K_{R5}). The observed POC concentration is used to evaluated the sum of LPOC and RPOC (considered as simulated POC), and the observed DOC concentration is used to evaluate the sum of LDOC and RDOC (considered as simulated DOC). Due to the analytical solution, five restrictions are considered: $k_{r5} \neq k_{LPOC} \neq 0, k_{r5} \neq k_{LDOC}, k_{r5} \neq k_{RPOC}, k_{RPOC} \neq k_{LPOC}$, and $k_{LDOC} \neq k_{LPOC}$. For both cases, the objective function to be minimized is:

$$f_{OC} = min \sum_{i=1}^{N} \left[w_{POC} \left(POC_i^{obs} - POC_i^{sim} \right)^2 + w_{DOC} \left(DOC_i^{obs} - DOC_i^{sim} \right)^2 \right] \quad (61)$$

Where: f_{OC} is the organic carbon objective function; POC_i^{obs} and POC_i^{sim} are the observed and simulated POC (mg/L), respectively; DOC_i^{obs} and DOC_i^{sim} are the observed and simulated DOC (mg/L), respectively; w_{POC} and w_{DOC} are the weight of the variables POC and DOC ($w_{POC} = 1 - w_{DOC}$), respectively; and N is the number of control points (e. g., number of sites with monitored data).

(iii) Total Phosphorous Calibration: for the phosphorous model calibration two coefficients were adjusted based on total phosphorous observed data and the sum of simulated organic and inorganic fractions: K_{pd} (organic phosphorus decay rate, d^{-1}), and K_{ps} (organic phosphorus settling rate, d^{-1}). The objective function to be minimized is:

$$f_p = min \sum_{i=1}^{N} \left(P_i^{obs} - P_i^{sim}\right)^2 \quad (62)$$

Where: f_p is the total phosphorous objective function; P_i^{obs} and P_i^{sim} is the observed and simulated total phosphorous concentration, respectively (mg/L); and N is the number of control points (e. g., number of sites with monitored data).

(iv) BOD, Nitrogen, and DO calibration: the last calibration step adjust the coefficients for the BOD, nitrogen (organic, ammonia, nitrite, and nitrate), and DO mass balance. These six variables involves 8 coefficients: K_1 (BOD deoxygenation rate), K_2 (reaeration rate); K_3 (BOD settling rate), K_4 (Sediment oxygen demand), K_{oa} (organic nitrogen hydrolysis rate), K_{os} (organic nitrogen settling rate[1]), K_{ai} (ammonia oxidation rate), and K_{in} (nitrite oxidation rate). The restrictions are: $K_2 \neq K_{ai} \neq K_{in}$, $K_{ai} \neq (K_{oa} + K_{os})$, $K_{in} \neq (K_{oa} + K_{os})$, $K_2 \neq (K_{oa} + K_{os})$, $K_2 \neq (K_1 + K_3)$. Due to the variables different scales, the objective function to be minimized in this case considers the normalization of the quadratic difference between observed and simulated data:

$$f_{DO} = min \sum_{i=1}^{N} \left\{ \frac{\left(DO_i^{obs} - DO_i^{sim}\right)^2}{\left|max\left(DO_i^{obs} - DO_i^{sim}\right)\right|} + \frac{\left(BOD_i^{obs} - BOD_i^{sim}\right)^2}{\left|max\left(BOD_i^{obs} - BOD_i^{sim}\right)\right|} + \frac{\left(Norg_i^{obs} - Norg_i^{sim}\right)^2}{\left|max\left(Norg_i^{obs} - Norg_i^{sim}\right)\right|} + \frac{\left(NH3_i^{obs} - NH3_i^{sim}\right)^2}{\left|max\left(NH3_i^{obs} - NH3_i^{sim}\right)\right|} + \frac{\left(NO2_i^{obs} - NO2_i^{sim}\right)^2}{\left|max\left(NO2_i^{obs} - NO2_i^{sim}\right)\right|} + \frac{\left(NO3_i^{obs} - NO3_i^{sim}\right)^2}{\left|max\left(NO3_i^{obs} - NO3_i^{sim}\right)\right|} \right\} \quad (63)$$

Where: f_{DO} is the objective function for BOD, nitrogen, and DO; DO_i^{obs} and DO_i^{sim} are the observed and simulated DO (mg/L), respectively; BOD_i^{obs} and BOD_i^{sim} are the observed and simulated BOD (mg/L), respectively; $Norg_i^{obs}$ and $Norg_i^{sim}$ are the observed and simulated organic nitrogen(mg/L), respectively; $NH3_i^{obs}$ and $NH3_i^{sim}$ are the observed and

simulated Ammonia (mg/L), respectively; $NO2_i^{obs}$ and $NO2_i^{sim}$ are the observed and simulated Nitrite (mg/L), respectively; $NO3_i^{obs}$ and $NO3_i^{sim}$ are the observed and simulated Nitrate (mg/L), respectively; and N is the number of control points (e. g., number of sites with monitored data).

5.4 Correspondence between State Variables and Measured Data

According to the model assumptions and features, there are some aspects about the correspondence between state variables and measured data that must be highlighted. For the organic carbon simulation, there is a need for different input data according to the kind of pollution source. The non-point sources consider two export rates for organic carbon based on POC and DOC (kg/km².year). An assumption is made that all the non-point sources are part of refractory pool of particulate and dissolved organic carbon (allochthonous pedogenic organic carbon). The point sources of organic carbon need a more detailed input data. For per capita loads (g/inh.d), the model considers a specific load for each variable simulated (LPOC, RPOC, LDOC, and RDOC). Since the inhabitants are classified according to the collection and treatment of the respective wastewater, the per capita loads are grouped as follow: (i) labile fractions refer to raw effluent and the collected effluent that is not treated; (ii) refractory fractions refer to the treated wastewater. With the specific per capita load and the number of inhabitants, the model estimates the domestic point sources and allocated in the specific module. The values of tributaries are also inserted in the model according to the four fractions of organic carbon or by adding the measured data of POC and DOC (filtered samples) and estimating a percentage or labile and refractory fractions. For model calibration, the same approach of percentages can be used to compare measured and simulated data.

BOD is considered as the 5 day biochemical oxygen demand (BOD_5), expressed in terms of oxygen equivalent. The analysis of BOD is considered for non-filtered samples. The export rates for non-point source loads estimation is expressed in terms of BOD (mgO_2/L), and the per capita loads for BOD are expressed in g/inh.d.

The nitrogen module is composed by four variables: organic nitrogen, ammonia, nitrite and nitrate. These variables correspond to the respective parameters measured in laboratory, considering non-filtered samples. All the export rates for non-point sources are

expressed in kg/km².year. The per capita loads are also considered for each fraction (g/inh.d.).

The simulation of phosphorous considers the total organic phosphorous (particulate and dissolved) and inorganic phosphorous (dissolved). Commonly, the inorganic phosphorous is only considered in its dissolved fraction since this is the available form to be assimilated. The measurement of the dissolved inorganic phosphorous is through the orthophosphate (or dissolved reactive phosphorous). Two export rates for non-point sources are expressed in kg/km².year. The per capita loads are also considered for each fraction (g/inh.d.).

5.5 Summary

In the context of this thesis, the development of a mathematical model aims to consolidate the third part of a multivariate problem that integrates what, why, and how to simulate the organic matter content in a river with overexploitation of resources. Thus, this chapter presented the proposed model main structure, process, and algorithms implemented.

The modeling concept and the computational tool herein presented, ROCS – Model, is based on a distinct discretization of the organic carbon fractions, particulate and dissolved, and in the compounds degradability, i.e. labile and refractory organic carbon.

In summary, ROCS Model has the following characteristics:

- One dimensional, well-mixed vertically and laterally control volume;
- Steady-state hydraulics, non-uniform, steady-flow is simulated;
- It is programed in the Windows macro language: Visual Basic for Applications (VBA), with Excel being the graphical user interface;
- The model is segmented in river reaches, with control volumes of equally spaced elements (computational elements);
- Multiple loads and abstractions can be input to any reach;
- The representation of organic carbon it through labile and refractory characteristics, in both particulate and dissolved fraction. This forms are intend to represent the slow (refractory) and fast (labile) organic carbon decomposition;

- BOD, DO, nitrogen (organic nitrogen, ammonia, nitrite, and nitrate), and phosphorous (organic, and dissolved) can also be simulated;
- For carbonaceous BOD, it is simulated anoxic conditions by limiting the oxidation reactions to the rate in which the oxygen enters in the water column through reaeration;
- The model generates the point and non-point loads according to land use and occupation and inhabitant's data of each drainage area. Point sources are segmented in population (raw and treated domestic effluents), tributaries, and industries;
- The user can add observed data in the river simulated;
- An automatic calibration tool based on PSO algorithm is used to calibrate model coefficients with the observed data;

Chapter 6

Organic Carbon Characterization and Modeling: Case Study of Upper Iguassu Watershed

"Theory has been useful in constructing a coherent picture of the data universe at our disposal, a picture which has all too often been demonstrated to have been over-simplified as we have invented new methods for data acquisition. Data of greater accuracy or precision, taken more densely in time or space, or data of an entirely new type, have all, at some time, forced us to reconsider our understanding of the way the world works."

Wangersky, Peter J. 1993. Dissolved organic carbon methods: a critical review. Marine Chemistry, v. 41, p. 61-74.

6.1 Overview

This chapter is divided in three sections: (i) the water quality assessment; (ii) results from the organic carbon biodegradation experiment; and (iii) the organic matter modeling

results applied to the Upper Iguassu River. The first part summarizes the water quality monitoring strategy for an urban basin, considering a total of 48 samplings performed at six monitoring sites along the main river. From the overall database, a subset of 8 samplings performed during 2012 and 2013 are the basis for the organic matter quantitative (TOC, POC, DOC, BOD, and COD) and qualitative analyses (absorbance and fluorescence spectroscopy).

A biodegradability test was introduced in the campaign number 45, for three sites monitored at Iguassu River (IG01, IG02, and IG05). The method developed and the results from the biodegradation experiment are presented in the second part of this chapter. The evaluation of the biodegradation was analyzed through the organic carbon decay (DOC, POC, and TOC), absorbance, and fluorescence intensity variation during the incubation time. A comparative analysis with the deoxygenation rates estimated by BOD tests is also presented.

The third part of this chapter focused on answering the question: "Considering that the characteristics, the sources and the biodegradation mechanisms of organic matter is known, how can organic carbon be used to represent the overall organic matter content in a water quality simulation model?" To answer this question, several tests were performed with the proposed model. A comparative analysis with other aggregated variables is also presented. Tests on model performance, sensitivity analysis and model calibration were performed to evaluate the model capabilities and its limitations.

Finally, this chapter presents a critical analysis about the monitoring strategy and the modeling approach. This analysis aims to evaluate the overall steps taken to consolidate the scientific contribution of this thesis. In addition, it aims to inquire about the water quality implications that arise as a consequence of the hypothesis considered.

6.2 Water Quality Assessment: Evidence of Anthropogenic Impacts

As detailed in Chapter 2, the Upper Iguassu Watershed has a complex and heterogeneous pollution sources matrix. Consequently, the deterioration of the water resources (tributaries and main river) can be observed through different parameters (Knapik, 2009). Some of the main tributaries drain the most urbanized part of Curitiba and Metropolitan Region, such as Palmital, Atuba, Belém, and Barigui Rivers. The pollution loads of these tributaries contribute for a continuous decay of water quality of the Iguassu River.

To evaluate this spatial evolution of water quality deterioration, and to identify where the river can assimilate and recover its equilibrium, a set of 6 sites were systematically monitored in the Iguassu River. The sites selected covers different parts of the watershed, from low anthropogenic influence (IG01), high impacted sites (IG02 to IG04) and sites with dilution effects (IG05 to IG06). For each site, conventional water quality and a specific set of parameters were selected for a better evaluation of the measured organic matter. The analyses herein included of the monitored data comprises four main steps or subgroups: (i) A summary of the main parameters and a brief diagnosis about the overall water quality of the Iguassu River; (ii) A specific analysis of the organic carbon fractions (DOC, POC, and TOC); (iii) A comparative analysis between all the quantitative parameters applied for organic matter measurement (COD, BOD, TOC, POC, and DOC); and (iv) A qualitative approach for the characterization of the organic matter through absorbance and fluorescence techniques. With this strategy, the final objective of this chapter first section is to summarize the main improvements in the understanding of the water quality considering conventional and additional parameters. The main results of each subgroup of analysis are presented in the next following items.

6.2.1 Overview of Sites Monitored, Analytical Methods, and Laboratory Procedures

6.2.1.1 Samplings Sites and Monitored Parameters Data Base

The experimental part of this thesis contemplates samplings from Iguassu River, located at Upper Iguassu Watershed, Parana State, Brazil. As previously mentioned, for the monitoring strategy, samples were collected at six monitoring sites along 107 km of the Iguassu River. As mentioned, the data base contemplates different periods of monitoring. For the purpose of this thesis contribution, and considering the analysis strategy defined before, the subsets of monitored data are:

- Group 1: All parameters monitored from 2005 to 2013, with a total of 48 samplings. This group contemplates more than 30 distinct parameters monitored systematically at the 6 sites along Iguassu River. This set of data is the basis for the diagnosis of the overall water quality of the Iguassu River (Table 1).

- Group 2: Specific parameters monitored from Aug/2012 to Dec/2013 (10 samplings, being 4 during Spring, 2 during Summer, 1 during Fall, and 3 during Winter). The parameters for this analysis are: DOC POC, TOC, BOD, COD, specific absorbance and fluorescence peaks intensity. This subset of data is the basis for the quantitative and qualitative analysis of organic matter at the Iguassu River. This group is also identified as samplings number C39 to C48.
- Group 3: Samples collected on July/2013 (C45), Sep/2013 (C46), Oct/2013 (C47), Dec/2013 (C48) on sites IG01, IG02, and IG05. These samples were used to conduct the biodegradability experiment. This group is also identified as Biodegradability Experiment Number 1, 2, 3, and 4, respectively.

Table 1: Number of analysis for each parameter monitored at Iguassu River since 2005

Parameter	IG01	IG02A	IG02B[1]	IG03	IG04	IG05	IG06
BOD (mgO_2/L)	44	45	14	44	43	44	41
COD (mgO_2/L)	46	46	18	46	44	45	44
TOC (mgC/L)	8	8	8	8	7	8	8
POC (mgC/L)	8	8	8	8	7	8	8
DOC (mgC/L)	46	46	17	46	44	45	42
DO (mgO_2/L)	42	42	16	43	41	40	36
Fluorescence spectra	25	25	15	25	23	25	23
UV-visible absorbance	25	25	15	25	23	25	23
Conductivity (µS/cm)	47	47	18	47	44	45	42
Turbidity (NTU)	46	46	18	46	44	45	41
pH	47	47	18	47	45	45	43
Temperature (°C)	47	47	18	47	45	46	43
Secchi depth (cm)	37	30	4	36	28	37	32
Chlorophyll-*a* (µg/L)	9	9	4	9	8	9	9
Alkalinity (mg/L)	5	5	5	5	4	5	5
Total phosphorous (mgP/L)	44	46	17	46	44	45	42
Orthophosphate (mgP/L)	18	22	17	22	20	21	20
Dissolved phosphorous (mgP/L)	20	21	16	21	19	20	19
Particulate phosphorous (mgP/L)	20	21	14	21	18	21	20
Diss. Org. phosphorous (mgP/L)	16	20	15	19	18	17	18
Ammonia (mgN/L)	46	46	18	46	44	45	41
Organic nitrogen (mgN/L)	44	43	15	44	40	43	39
Kjehdahl nitrogen (mgN/L)	43	44	16	44	42	43	39
Nitrite (mgN/L)	44	45	17	45	43	44	40
Nitrate (mgN/L)	43	41	17	41	39	41	38
Total nitrogen (mgN/L)	42	40	16	40	38	40	36
Settling solids	47	47	18	47	45	46	43
Solids[2] (mg/L)	46	47	18	47	44	46	43
Total coliform (NMP/100ml)	18	19	16	19	18	19	16
Fecal coliform (NMP/100ml)	18	19	16	19	17	19	15
Total data	**1359**	**1373**	**586**	**1379**	**1291**	**1350**	**1253**

[1] Site IG02B is located in the opposite margin of site IG02A, data started in 2010.
[2] Analysis of total, dissolved and suspended fraction for fix, total and volatile solids.

Considering the first group of data, a total of 30 main parameters were systematically monitored since 2005. This database has heterogeneous characteristics in terms of number of parameters monitored, but, as presented in Table 1, for the most part of the parameters there are enough data for the required analysis. Some of the parameters were just introduced in the monitoring activity in the last 8 campaigns, such as TOC and POC. In addition, an exploratory analysis of the bottom sediment was also implemented during two periods: samplings n. 26 to 35, and samplings n. 39 to 48. The characteristics of the bottom sediment were evaluated by total and organic carbon, organic matter dry mass, total nitrogen, and total phosphorous.

6.2.1.2 Chemical Analyses

The physical-chemical parameters measured in situ were: dissolved oxygen (DO, mg/L), water temperature (ºC), conductivity (µS/cm), pH, turbidiy (NTU), alkalinity (titration method), water transparency (Secchi disk) and flow. The parameters determined in laboratory were: BOD_5 (respirometric method), COD (closed reflux, titrimetric method), organic and ammonia nitrogen (phenate method), nitrite and nitrate (colorimetric methods 4500B and 4500E), total phosphorus (method 4500P B and D: sulphuric acid – nitric acid digestion followed by stannous chloride colorimetric method), orthophosphate (PO_4^{-3}, ascorbic acid method), solids (method 2540B for total solids dried at 103º-105ºC, and method 2540E for fixed and volatile solids ignited at 550ºC), according to the APHA (1998). All sampling bottles were acid washed and baked during 5 hours at 550°C. Samples were stored in the dark and refrigerated at 4ºC until analysis. For dissolved analysis, samples were filtered with a pre-washed 0.45 µm acetate membrane filter. Further information about the analytical methods is presented in Appendix 2.

The concentration of Dissolved (DOC) and Total Organic Carbon (TOC) were measured using a TOC-V_{CPH} analyzer (Shimadzu). The inorganic carbon was eliminated before the analysis, with a 10 min sparging time using Nitrogen as the carrier gas. For each sample, 100 µL was injected at least 6 times (a direct injection in the combustion tube was adapted using a gas tight micro syringe). For DOC concentrations, samples were filtered with a pre-washed 0.45 µm PVDF syringe filter. The particulate organic carbon (POC) was calculated by the difference between TOC and DOC. Further information about calibration procedures are presented in Appendix 2.

For bottom sediment analyses, samples were collected with a Peterson dredge. The dredge was launched until a representative amount of sediment was obtained. After collection, samples were thoroughly mixed in a plastic tray, and stored in plastic containers under refrigeration. A subsample of each collected material was used for grading tests. The remaining sediment samples were dried with forced air and heating (40 °C). Dried samples were ground and sieved (120 mesh). The parameters analyzed for bottom sediment samples were: total and organic carbon (high temperature combustion – Shimadzu), organic matter dry mass (loss on ignition at 550 °C, Heiri et al., 2001), total nitrogen (digestion with alkaline potassium persulfate followed by colorimetric method, Smart et al., 1983), and total phosphorous (loss on ignition and ascorbic acid method, Andersen, 1973).

6.2.1.3 Spectrophotometric Analyses

For fluorescence and absorbance measurements, samples were filtered with a pre-washed 0.45 μm PVDF syringe filter and/or acetate membrane filter. Samples were stored in free-carbon glass ampoules (acid washed and baked during 5 hours at 550°C) and refrigerated at 4ºC until analysis.

Fluorescence measurements were performed in a fluorescence spectrometer Cary Eclipse (Varian Inc.), with excitation wavelength in the range of 200 – 600 nm, in 10 nm intervals and with emission wavelength in the range of 200-600 nm in 5 nm intervals. The emission and excitation slits were fixed at 5 nm, the scanning speed was 3000 nm/min and the PMT voltage was set on 950 V. Ultrapure water was used as both reference and a blank, with a 1 cm quartz cells. The results of fluorescence excitation-emission matrices (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 emission monitored at 400 nm). The fluorescence peaks intensities identification was determined by a routine implemented in VBA. The range of emission and excitation wavelength used for fluorescence peak identification was based on a classification proposed by Coble (1996) as follow: humic substances in peak **A** (λ_{ex}= 230 nm/ λ_{em}= 400-500 nm) and peak **C** (λ_{ex}= 300-500 nm/ λ_{em}= 400-500 nm), tryptophan as peak $\mathbf{T_1}$ (λ_{ex}= 290 nm/ λ_{em}= 350 nm) and $\mathbf{T_2}$ (λ_{ex}= 230 nm/ λ_{em}= 350 nm) and tyrosine as peak **B** (λ_{ex}= 230-275 nm/ λ_{em}= 310 nm).

In addition, the fluorescence emission in single wavelengths and specific ratios were also analyzed. The fluorescence ratio (FR), calculated by the ratio of the emission intensity at

λ=450 nm and the emission intensity at λ=500 nm (FR=$\lambda_{450}/\lambda_{500}$) was used to evaluate the occurrence of autochthonous sources (FR > 1.8) and allochthonous source of humic substances (FR ≤ 1.5) (Westerhoff and Anning, 2000). A summary of fluorescence intensity peaks commonly used to evaluate the organic matter sources have been described in details in Chapter 3.

UV-Vis absorbance was measured using a UV-1601 PC spectrometer (Shimadzu), with a 1 cm quartz cells and ultrapure water as a blank, in the range of 200 to 600 nm. The specific absorbance in the 254 wavelength, $SUVA_{254}$ (Westerhoff and Anning, 2000), was calculated by dividing the UV_{254} (a.u) absorbance with the respective DOC concentration (mg/L) and corrected by the optical path (m). $SUVA_{254}$ values close to 4.4 L/mg.m indicate the presence of fulvic acid, and values close to 1.2 L/mg.m indicate that autochthonous organic matter predominates (Westerhoff and Anning, 2000). The ratio between absorbance at 285 nm and the DOC concentration, A_{285}/DOC (Rostan and Cellot, 1995), was used to differentiate fulvic acid (≈20 L/g) and labile organic matter (≈10 L/g). A summary of absorptivity in different wavelength ranges commonly used to evaluate the organic matter sources have been described in details in Chapter 3.

6.2.2 Overview of Main Results: Traditional Approach for Water Quality Assessment

Table 2 presents the chemical results for the water quality measurements. It is generally possible to identify a different pattern for the most of the parameters at the monitoring sites affected by urban development (IG02-IG06) and the one located in a not impacted area (IG01). The median of BOD_5, NH_3, and PO_4^{-3} from sites downstream of the urban development (IG02 to IG06) were significantly higher than the median of water samples from the site with low anthropogenic impact (IG01). For DO, the median downstream of the site IG02 were lower than site IG01 (Wilcox rank sum test, $p<0.05$). Other parameters such as turbidity, conductivity, and alkalinity also confirms the spatial tendency of the water quality deterioration of the Iguassu River.

Figure 1 presents box-plots variation of the results for BOD_5 (mgO_2/L) and DO (mgO_2/L) for the 6 sites monitored along the main river. The results contemplate 48 samplings. These two parameters illustrate clearly the spatial variation of pollution loads and the extension where dilution and decomposition becomes relevant to the water quality

recovery. The three most impacted sites, IG02 (margins A and B), IG03, and IG04, reflects the pollution loads of important tributaries that drains the most urbanized area of Upper Iguassu Watershed (Palmital, Atuba, and Belem Rivers), with low rates of sewage collection and treatment.

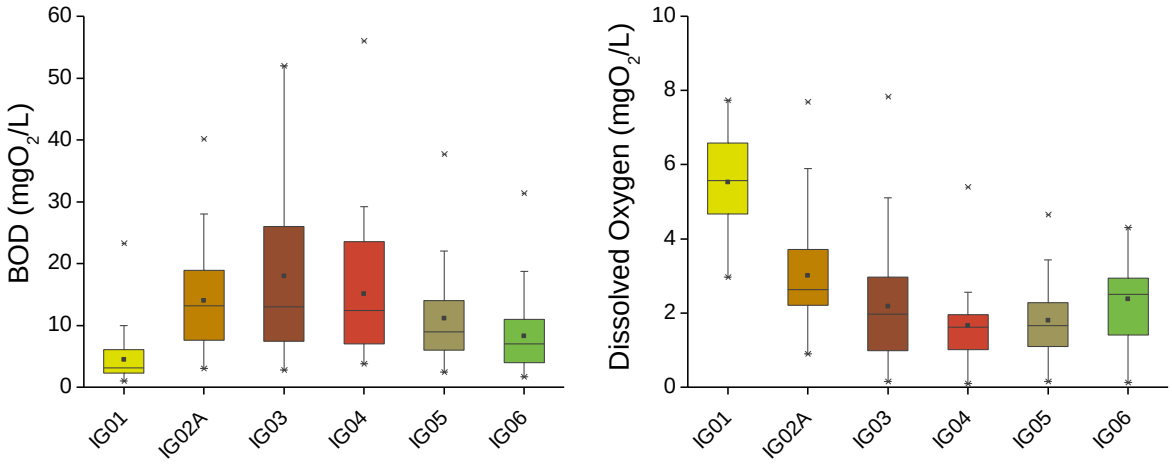


Figure 1: Variation of BOD (left) and DO (right) for the monitoring sites. Samplings C01 to C48.

As BOD_5 and DO, COD (mgO_2/L) and DOC (mgC/L) also showed variations between sites monitored, but the differences were not statistically significant for all sites monitored. Figure 2 presents the box-plot for COD and DOC concentration during 48 samplings.

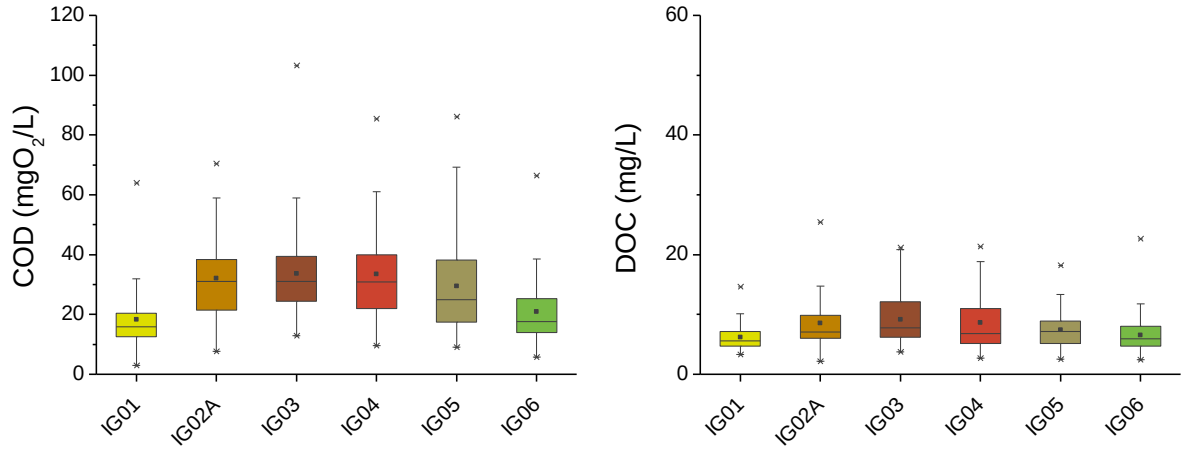


Figure 2: Variation of COD (left) and DOC (right) for the monitoring sites. Samplings C01 to C48.

Table 2: Maximum, minimum, and median concentration for some parameters monitored along the main river. (Samplings C01 to C48)

Parameter		BOD (mgO_2/L)	DO (mgO_2/L)	COD (mgO_2/L)	DOC (mgC/L)	PO_4^{-3} [a] (mgP/L)	NH_3 (mgO_2/L)	Alkalinity [b] (mg/L)	Cl-*a* [b] (µg/L)
IG01	*Median*	3.1 ± 2.5	5.6 ± 1.2	16.3 ± 6.7	5.6 ± 1.6	0.02 ± 0.03	0.16 ± 0.15	21.3 ± 1.0	0.004 ± 0.001
	Minimum	1.0	3.0	5.4	3.3	0.01	0.01	19.4	0.003
	Maximum	23.3	7.7	64.0	14.6	0.14	0.77	21.3	0.005
IG02A	*Median*	13.1 ± 6.4	2.6 ± 1.1	31.0 ± 10.0	7.1 ± 3.3	0.16 ± 0.21	6.32 ± 3.40	110.6 ± 19.7	0.005 ± 0.002
	Minimum	3.0	0.9	7.8	2.2	0.01	0.25	75.7	0.003
	Maximum	40.2	7.7	70.4	25.5	0.96	20.85	120.3	0.006
IG03	*Median*	13.0 ± 10.8	2.0 ± 1.1	31.0 ± 11.4	7.8 ± 3.4	0.22 ± 0.23	5.11 ± 3.53	98.0 ± 22.5	0.003 ± 0.001
	Minimum	2.8	0.2	12.8	3.7	0.02	0.88	79.5	0.003
	Maximum	52.0	7.8	103.2	21.2	1.10	19.35	131.9	0.006
IG04	*Median*	12.4 ± 8.0	1.6 ± 0.7	30.9 ± 12.2	6.8 ± 3.7	0.27 ± 0.21	4.90 ± 2.64	108.6 ± 15.7	0.005 ± 0.004
	Minimum	3.8	0.1	9.6	2.7	0.01	0.45	81.5	0.003
	Maximum	56.0	5.4	85.5	21.4	1.51	14.89	108.6	0.01
IG05	*Median*	8.9 ± 5.9	1.7 ± 0.7	24.9 ± 13.5	7.2 ± 2.4	0.12 ± 0.15	4.46 ± 2.64	102.8 ± 21.1	0.006 ± 0.013
	Minimum	2.4	0.2	9.0	2.6	0.01	0.45	71.8	0.001
	Maximum	37.7	4.7	86.2	18.2	0.64	28.68	122.2	0.03
IG06	*Median*	7.0 ± 3.8	2.5 ± 0.8	17.7 ± 8.1	5.9 ± 2.1	0.11 ± 0.12	3.73 ± 2.42	76.6 ± 10.4	0.006 ± 0.018
	Minimum	1.7	0.1	5.7	2.5	0.01	0.34	67.9	0.006
	Maximum	31.4	4.3	66.5	22.7	0.62	17.11	91.2	0.042

[a] Parameter monitored during samplings C26 to C48.
[b] Parameters monitored during samplings C45 to C48.

Accordingly to previous results, the concentrations of phosphorous (orthophosphate and dissolved) also indicates the impact of pollution at the Iguassu River. Figure 3 presents the results for samplings n. C26 to C48 along the Iguassu River (orthophosphate was included in the monitoring activities only at sampling n. C26). The median of PO_4^{-3} and dissolved phosphorous from sites downstream of the urban development (IG02 to IG06) were significantly higher than the median of water samples from the site with low anthropogenic impact (IG01) (Wilcox rank sum test, $p<0.05$).

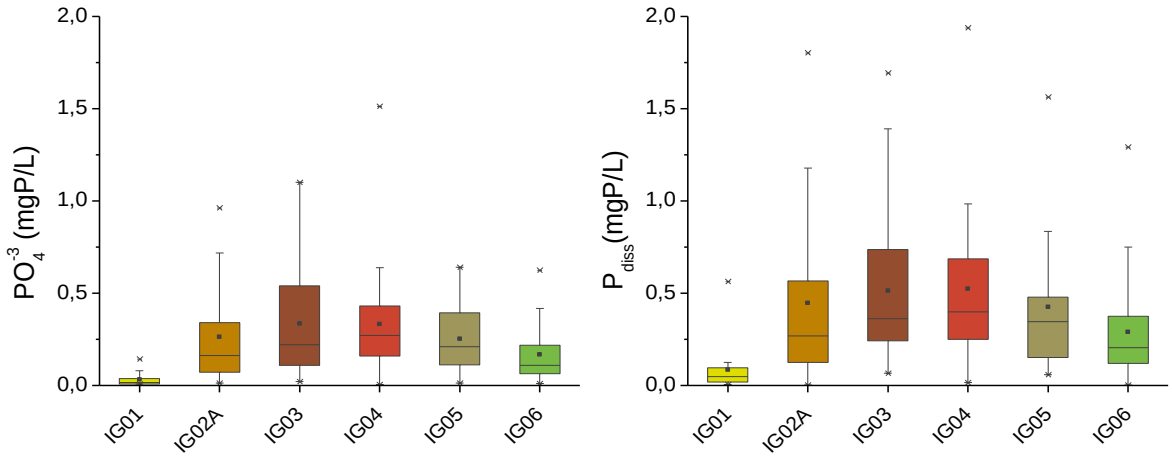


Figure 3: Variation of PO_4^{3-} (left) and Dissolved Phosphorous (right) for the monitoring sites. Samplings C26 to C48.

The concentrations of nitrogen (organic, ammonia, nitrite, and nitrate) showed similar spatial variation. Figure 4 presents the results for the 48 samplings along the Iguassu River. The median of organic nitrogen and ammonia from sites downstream of the urban development (IG02 to IG06) were significant higher than the median of water samples from the site with low anthropogenic impact (IG01) (Wilcox rank sum test, $p<0.05$). A different scale for site IG01 is also highlighted at Figure 4 for organic nitrogen and ammonia concentration.

The results of turbidity and conductivity are presented in Figure 5. These results also confirm the water quality degradation downstream site IG01. pH and temperature did not indicate difference between sites monitored, as illustrated in Figure 6.

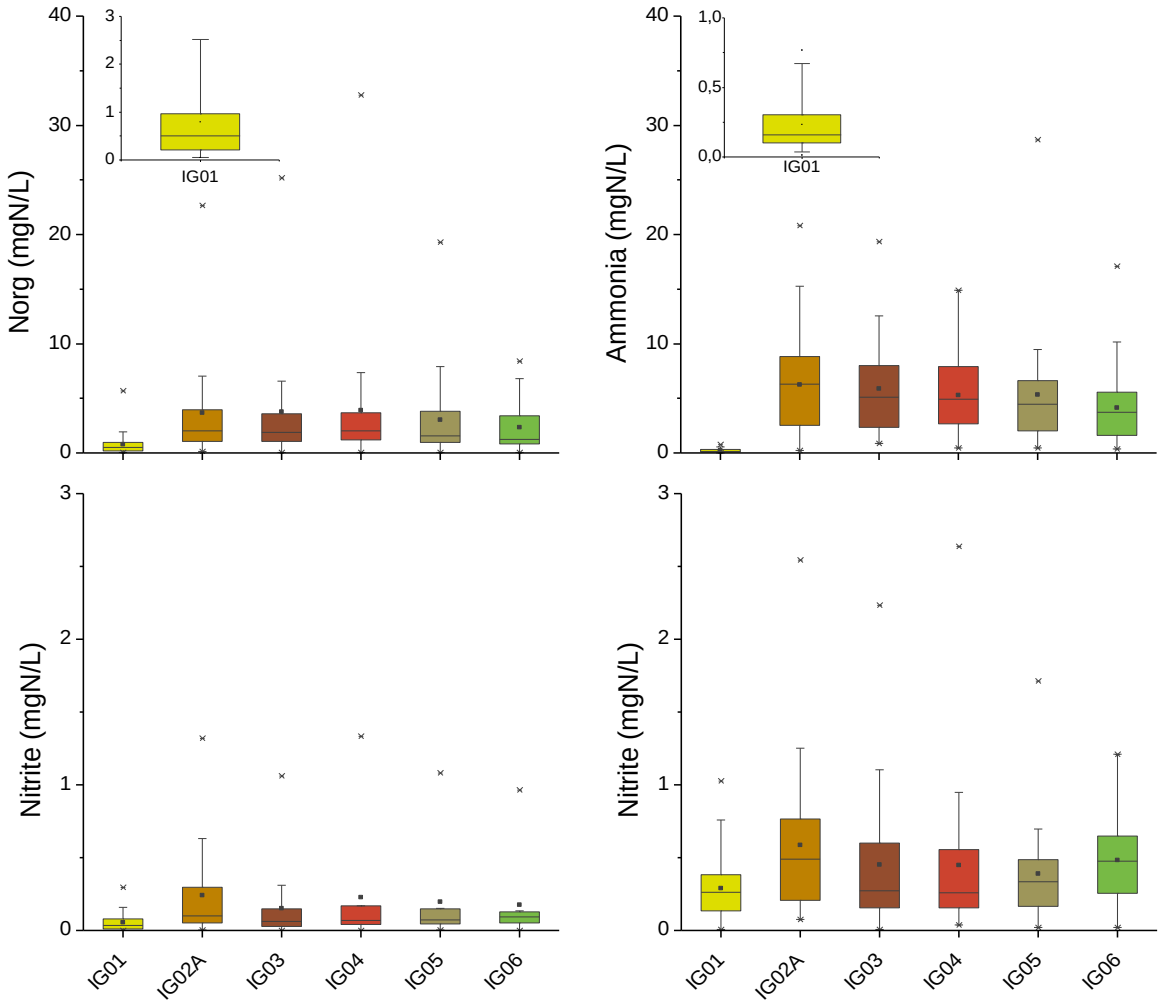


Figure 4: Variation of Organic Nitrogen (up, left), Ammonia (up, right), Nitrite (bottom, left), and Nitrate (bottom, right) for the monitoring sites. Samplings C01 to C48.

In addition, alkalinity and chlorophyll-*a* showed interesting spatial variation (Figure 7, Table 2). For alkalinity, site IG01 showed the lower value comparing to other sites monitored. The intermediate sites that represents the most polluted reach of Iguassu River, alkalinity was always higher than 75 mg/L. For chlorophyll-*a*, the results did not indicated opposite relationship between polluted and non-polluted sites, but its variation seems to be correlated with the increment of width size, and, consequently, lower velocity and/or turbulence. One hypothesis is that downstream the intermediate reach of Iguassu River, there are better conditions to an increase of biological activity. However, the overall concentrations of chlorophyll-*a* are small comparing to lentic systems, and, consequently, does not represent a parameter of concern.

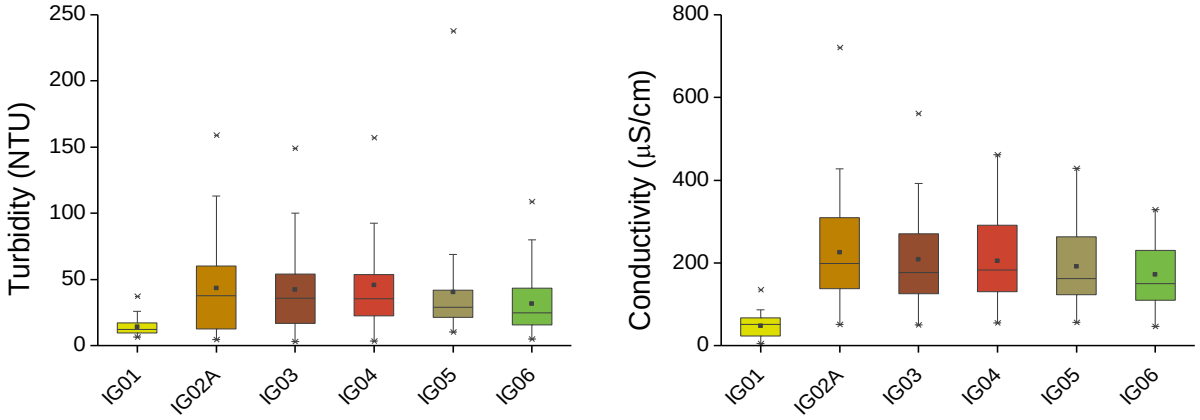


Figure 5: Variation of Turbidity (left) and Conductivity (right) for the monitoring sites. Samplings C01 to C48.

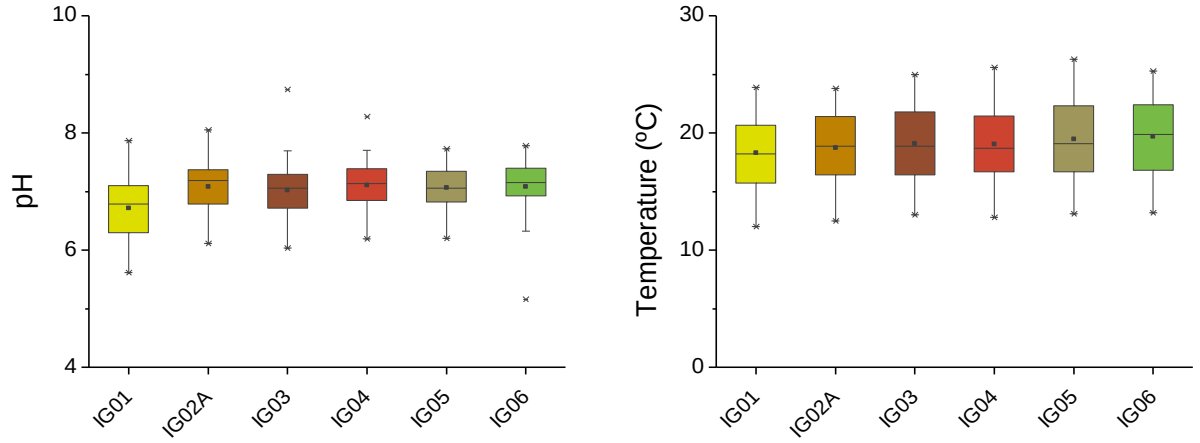


Figure 6: Variation of pH (left) and Temperature (right) for the monitoring sites. Samplings C01 to C48.

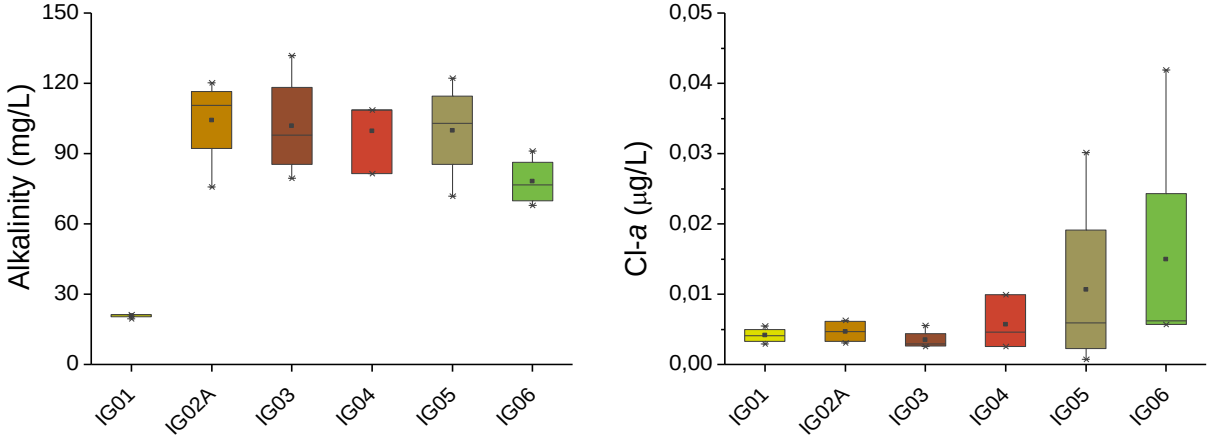


Figure 7: Variation of alkalinity (left) and chlorophyll-*a* (right) for the monitoring sites. Samplings C45 to C48.

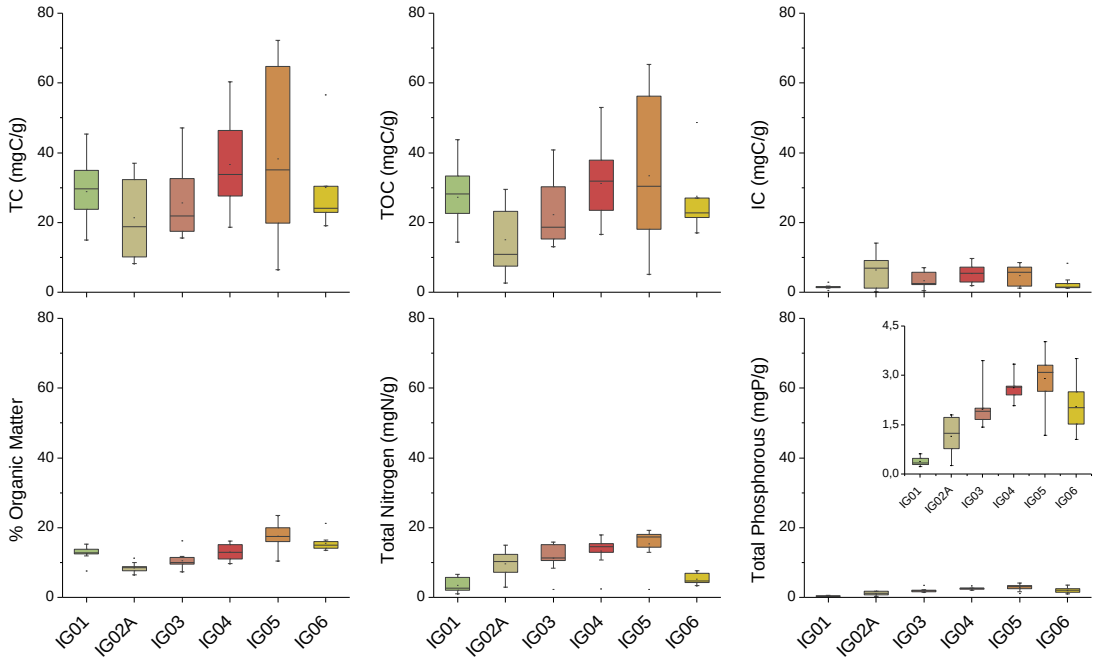


Figure 8: Box-plots of bottom sediment samples for TC (mgC/g), TOC (mg/g), IC (mg/g), OM (% dry mass/g), Total Nitrogen (mgN/g), and Total Phosphorous (mgP/g). (Sampling group: C39 to C48). Detail for different scale for Total Phosphorous.

The spatial variation of bottom sediment characteristics also indicates the answer of the system to the pollution sources matrix at Upper Iguassu Watershed. Figure 1 presents the box-plots of Total carbon (TC, mgC/g), Total organic carbon (TOC, mgC/g), Inorganic carbon (IC, mgC/g), Organic matter (% of dry mass/g), Total nitrogen (N_T, mgN/g), and Total phosphorous (P_T, mgP/g). According to the results, it can be observed that for N_T and P_T there is an increase of the dry mass for the intermediate sites, indicating a spatial relationship between anthropogenic occupation and bottom sediment characteristics. However, the mass of carbon and organic matter in the bottom sediment are not necessarily an indication of pollution. Site IG01, for example, showed similar results of TC, TOC, and %OM as observed in sites with anthropogenic impact (IG02, IG03, or IG04).

6.2.3 Total (TOC), Particulate (POC) and Dissolved Organic Carbon (DOC) Analyses

In general, only the dissolved fraction of organic carbon is included in a systematically monitoring plan, which can underestimate or result in incomplete information about the organic matter dynamics in the aquatic environment. Particularly in the case of Iguassu River, DOC and traditional parameters such as BOD or COD has been used for

water quality evaluation during the last years. Thus, the determination of TOC and POC proposed in this study to be part of a holistic understanding of the dynamics of organic matter, is an original strategy and complementarily parameters for the database analyzed. The analysis herein included, involves both a direct evaluation about the ranges of the different fractions of organic carbon and its variation through time and space, and the relationship between DOC, POC, and TOC with other parameters monitored. In addition, DOC values are used for EEMs normalization (inner-filtering effect correction) and for absorbance specific index evaluation.

Figure 9 and Table 3 presents the results for TOC, POC, and DOC at the 6 sites monitored at Iguassu River. Samplings C39 to C41 were performed during 2012 and C44 to C48 during 2013. A preliminary analysis of these results indicates two different patterns of TOC and POC between samplings C39, C40, and C41, and the second group of data. The first group of samplings (2012) was performed during low flow conditions, with evident pollution and color alteration at Iguassu River, which may have influenced the concentration of particulate compounds, and thus TOC analysis. TOC ranged from 3.6 ± 0.2 mgC/L at site IG01 (less anthropogenic impacts) to 34.8 ± 0.5 mgC/L at site IG04, while DOC ranged from 2.5 ± 0.2 mgC/L at site IG06 to 14.4 ± 0.04 mgC/L at site IG03 (Table 3). POC had an interesting variation, ranging from 0.1 ± 0.2 mgC/L at site IG01 to 27.4 ± 0.5 mgC/L at site IG04, with a median value of 2.0 ± 2.2 mgC/L and 7.4 ± 2.9 mgC/L, respectively for sites IG01 and IG04. Under the analytical methods used for the organic carbon analyses, TOC and POC data showed higher standard deviation than DOC. Complementarily, DOC showed less variation between samplings. Figure 10 presents the box-plots showing the variation of TOC, POC, and DOC for the sites monitored along the Iguassu River (samplings C39 to C48).

Table 3: Maximum, minimum, and median concentration for TOC, DOC, and POC for the sites monitored along the main river. (Samplings C39 to C48)

Parameter		IG01	IG02A	IG02B[(1)]	IG03	IG04	IG05	IG06
TOC (mgC/L)	*Maximum*	22.3 ± 1.9	24.3 ± 1.0	30.1 ± 4.8	27.4 ± 0.7	34.8 ± 0.5	34.0 ± 0.5	17.4 ± 0.4
	Minimum	3.6 ± 0.2	5.5 ± 0.1	8.7 ± 0.6	8.7 ± 1.2	8.21 ± 0.1	5.7 ± 0.3	3.8 ± 0.3
	Median	6.3 ± 1.9	10.9 ± 1.8	13.0 ± 4.0	10.8 ± 1.7	13.0 ± 2.6	8.5 ± 2.7	8.0 ± 1.9
DOC (mgC/L)	*Maximum*	8.5 ± 0.1	9.0 ± 0.1	9.8 ± 0.1	14.4 ± 0.04	8.0 ± 0.1	7.0 ± 0.1	6.0 ± 0.1
	Minimum	3.4 ± 0.5	3.4 ± 0.2	3.5 ± 0.2	4.8 ± 0.4	4.7 ± 0.8	2.6 ± 0.2	2.5 ± 0.2
	Median	4.6 ± 0.7	5.7 ± 0.6	6.9 ± 1.0	6.9 ± 1.2	5.7 ± 0.8	6.0 ± 1.2	5.0 ± 0.5
POC (mgC/L)	*Maximum*	13.9 ± 1.9	15.3 ± 1.0	20.3 ± 4.8	16.7 ± 0.7	27.4 ± 0.5	27.3 ± 1.5	11.9 ± 0.4
	Minimum	0.1 ± 0.2	1.7 ± 0.3	3.2 ± 1.8	1.3 ± 1.9	3.3 ± 0.9	0.2 ± 0.6	0.7 ± 0.2
	Median	2.0 ± 2.2	5.1 ± 2.0	7.0 ± 4.4	5.8 ± 2.2	7.4 ± 2.9	3.0 ± 3.3	2.9 ± 2.2

[(1)] Site IG02B is located in the opposite margin of site IG02A. For all sites n = 8, except for site IG04 (n=7).

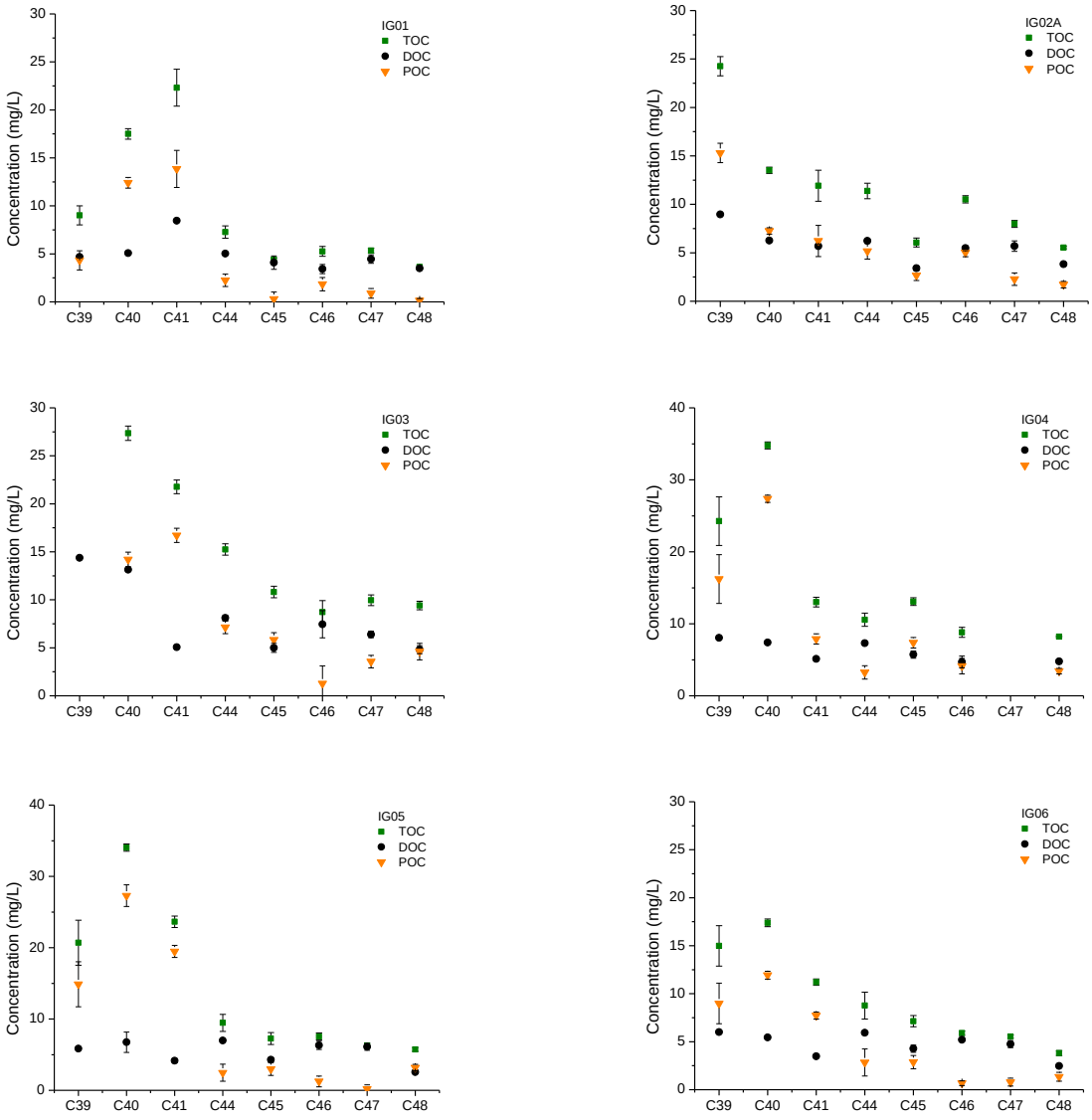


Figure 9: TOC, POC, and DOC concentration observed at Iguassu River during 8 samplings (C39 to C41 during 2012 and C44 to C48 during 2013). Monitoring points IG01 to IG06.

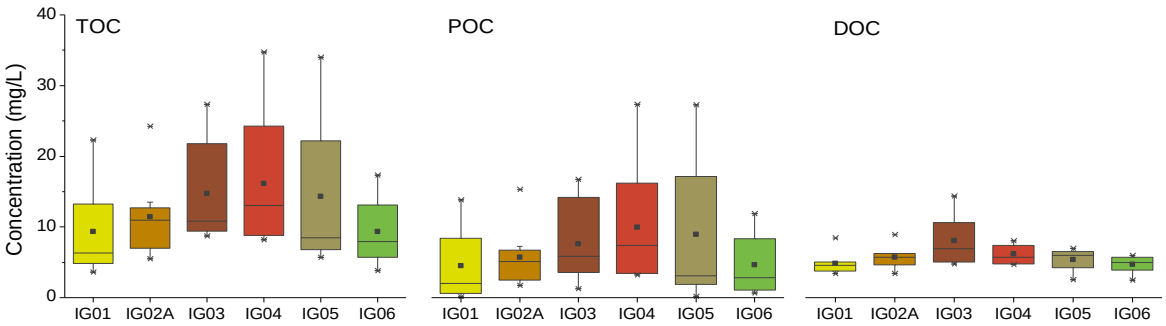


Figure 10: Box-plots showing the variation of TOC, POC, and DOC concentration observed at Iguassu River during 8 samplings (C39 to C41 during 2012 and C44 to C48 during 2013). Monitoring points IG01 (headwater) to IG06.

6.2.4 BOD, COD, and Organic Carbon Relationship

Figure 11 and Table 4 presents the box-plots for COD, BOD_5, TOC, POC, and DOC, at the 6 sites monitored at Iguassu River. According to the results, some interesting patterns can be observed. COD has the highest values and variance for all sites monitored. A minimum of 6.8 mgO_2/L was observed on site IG01, and a maximum of 106.4 mgO_2/L at site IG02B (opposite margin of site IG02A). BOD_5 ranged from a minimum of 1.0 mgO_2/L at site IG01, to a maximum of 56.0 mgO_2/L at site IG04.

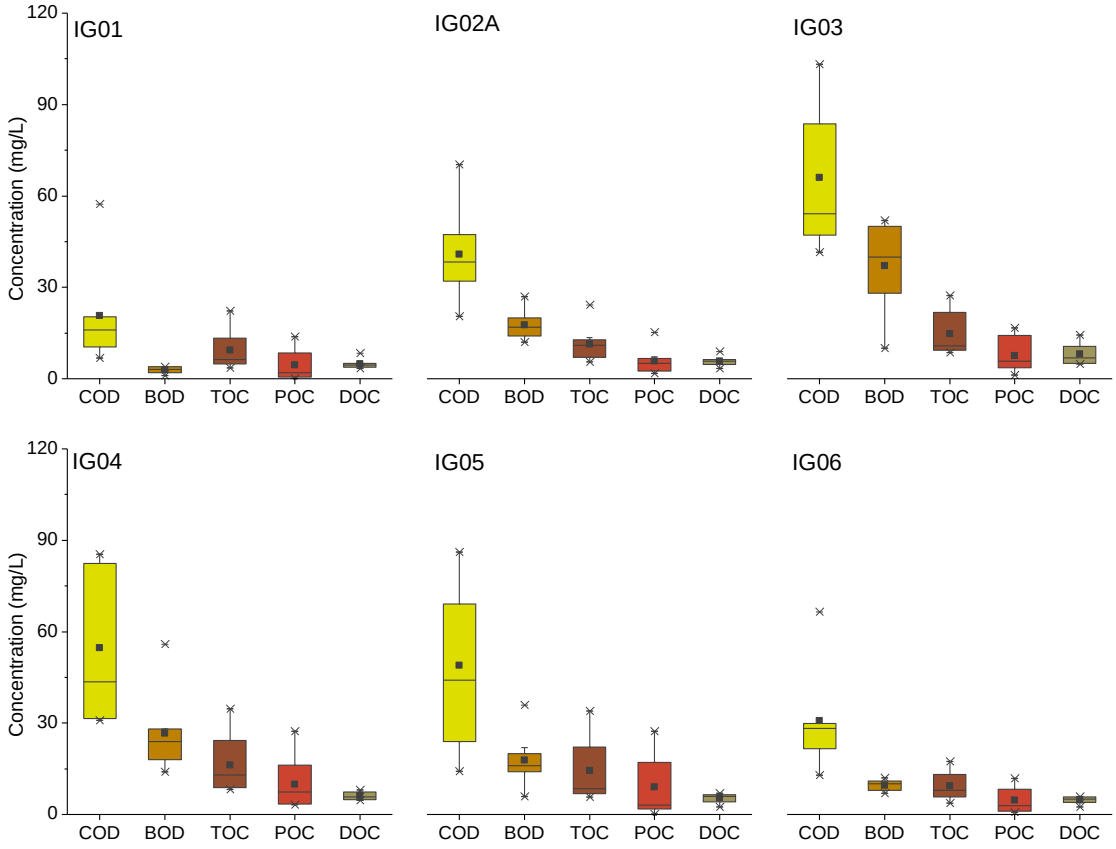


Figure 11: Box-plots showing the variation of COD (mgO_2/L), BOD_5 (mgO_2/L), TOC (mgC/L), POC (mgC/L), and DOC (mgC/L) concentration observed at Iguassu River during 8 samplings (C39 to C41 during 2012 and C44 to C48 during 2013). Monitoring sites IG01 to IG06.

Correlations between COD, TOC, and BOD_5 can indicate some interactions associated with the organic carbon in rivers. Figure 12 shows COD and TOC linear correlation with BOD_5 considering two periods: C39 to C41 (left) and C44 to C48 (right). The two periods showed good linear correlations between the parameters analyzed. Besides the linear correlation may be influenced by the reduced number of data of samplings C39 to

C41 (*r=0.8994, p<0.0001* – CODxBOD$_5$, n= 20; and *r=0.8016, p<0.0001* – TOCxBOD$_5$, n=20), these 3 samplings showed differences in the magnitude of TOC concentration (Figure 9) when comparing to samplings C44 to C48 (*r=0.51294, p<0.0001* – CODxBOD$_5$, n= 32; and *r=0.6763, p<0.0001* – TOCxBOD$_5$, n=32). In addition, samplings C39 to C41 showed color alteration, higher POC values, and low flow condition.

Table 4: Maximum, minimum, and median concentration for COD (mgO$_2$/L), BOD$_5$ (mgO$_2$/L), TOC (mgC/L), DOC (mgC/L), and POC (mgC/L) for the sites monitored along the main river (Samplings C39 to C48).

Parameter		IG01	IG02A	IG02B[1]	IG03	IG04	IG05	IG06
COD (mgO$_2$/L)	*Maximum*	57.4	70.4	106.4	103.2	85.5	86.2	66.5
	Minimum	6.8	20.5	28.9	35.5	22.1	14.2	12.9
	Median	15.9 ± 9.3	38.4 ± 9.1	51.5 ± 21.2	47.1 20.7	34.7 ± 21	38 ± 19.5	2.64 ± 9.6
BOD$_5$ (mgO$_2$/L)	*Maximum*	9.0	28.0	50.0	52.0	56.0	36.0	16.0
	Minimum	1.0	12.0	10.0	10.0	14.0	6.0	7.0
	Median	2.0 ± 1.0	19.0 ± 4.3	31.0 ± 9.3	36 ± 10.3	24.0 ± 7.6	16.0 ± 5.4	11.0 ± 2.2
TOC (mgC/L)	*Maximum*	22.3 ± 1.9	24.3 ± 1.0	30.1 ± 4.8	27.4 ± 0.7	34.8 ± 0.5	34.0 ± 0.5	17.4 ± 0.4
	Minimum	3.6 ± 0.2	5.5 ± 0.1	8.7 ± 0.6	8.7 ± 1.2	8.21 ± 0.1	5.7 ± 0.3	3.8 ± 0.3
	Median	6.3 ± 1.9	10.9 ± 1.8	13.0 ± 4.0	10.8 ± 1.7	13.0 ± 2.6	8.5 ± 2.7	8.0 ± 1.9
DOC (mgC/L)	*Maximum*	8.5 ± 0.1	9.0 ± 0.1	9.8 ± 0.1	14.4 ± 0.04	8.0 ± 0.1	7.0 ± 0.1	6.0 ± 0.1
	Minimum	3.4 ± 0.5	3.4 ± 0.2	3.5 ± 0.2	4.8 ± 0.4	4.7 ± 0.8	2.6 ± 0.2	2.5 ± 0.2
	Median	4.6 ± 0.7	5.7 ± 0.6	6.9 ± 1.0	6.9 ± 1.2	5.7 ± 0.8	6.0 ± 1.2	5.0 ± 0.5
POC (mgC/L)	*Maximum*	13.9 ± 1.9	15.3 ± 1.0	20.3 ± 4.8	16.7 ± 0.7	27.4 ± 0.5	27.3 ± 1.5	11.9 ± 0.4
	Minimum	0.1 ± 0.2	1.7 ± 0.3	3.2 ± 1.8	1.3 ± 1.9	3.3 ± 0.9	0.2 ± 0.6	0.7 ± 0.2
	Median	2.0 ± 2.2	5.1 ± 2.0	7.0 ± 4.4	5.8 ± 2.2	7.4 ± 2.9	3.0 ± 3.3	2.9 ± 2.2

[1] Site IG02B is located in the opposite margin of site IG02A. For all sites n = 8, except for site IG04 (n=7).

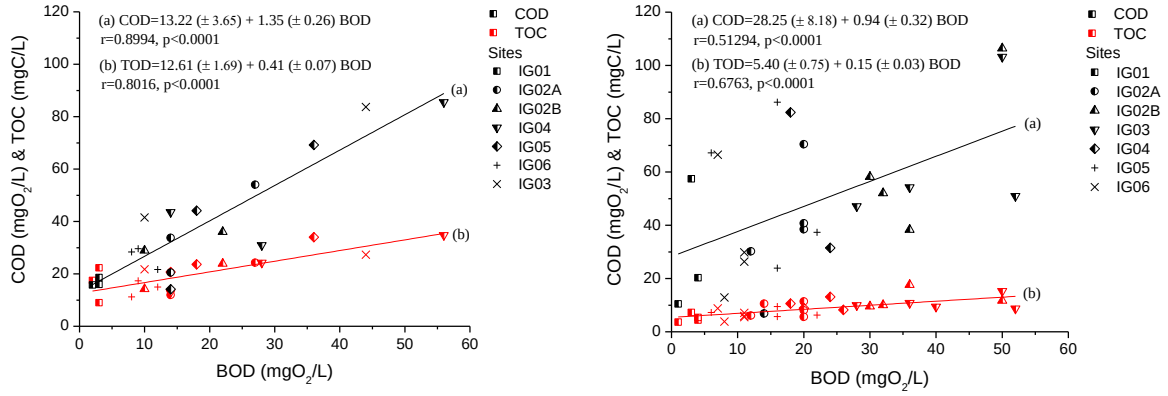


Figure 12: X-Y plots for COD and TOC x BOD for two sampling periods: C39-C41 (left) and C44-C48 (right). Solid lines represent the corresponding regression. r is the Pearson's linear coefficient and p is the significant level (C39 to C41, n=20 data; C44 to C48, n=32 data; site IG02 considered for left margin, IG02A, and right margin, IG02B).

The analysis of DOC also indicated an interesting interaction associate to the organic matter dynamics in rivers. Figure 13 shows the COD and DOC correlations with BOD, and the respective correlation between DOC and COD. The linear correlations with BOD considering the data from all monitoring sites (n=66) were *r=0.6217* (p<0.0001, COD), and *r= 0.5439* (p<0.0001, DOC). DOC and COD also presented a good linear correlation, *r=0.6779* (p<0.0001). While DOC ranged from 2.5 mgC/L to 14.4 mgC/L, values of COD above 100 mgO_2/L were observed at sites with an elevated number of inhabitants and population density, IG02 and IG03.

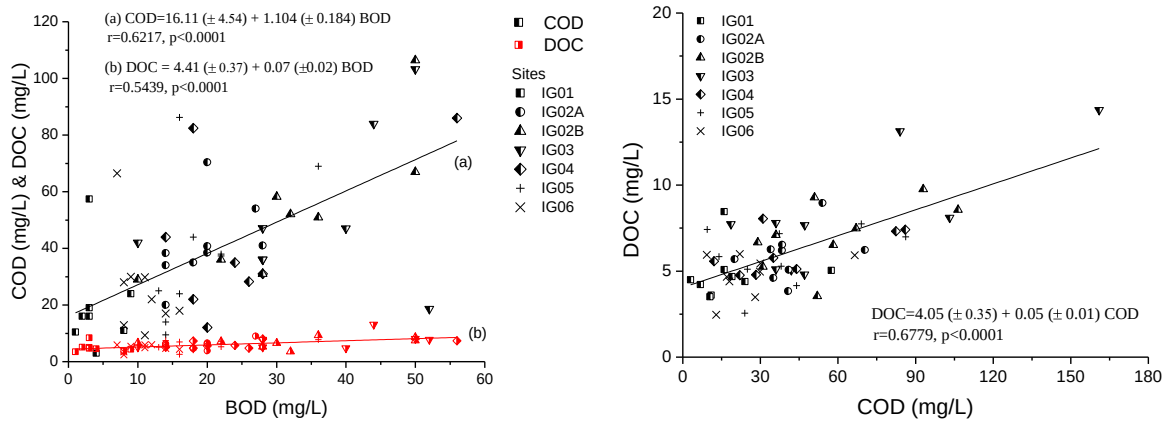


Figure 13: X-Y plots for COD and DOC x BOD (left) and DOC x COD (right). Solid lines represent the corresponding regression. r is the Pearson's linear coefficient and p is the significant level (C39 to C48, n=66 data; site IG02 considered for left margin, IG02A, and right margin, IG02B).

In essence, TOC, POC, DOC, BOD_5, and COD represent a different approach for the same organic matter that can be found in the sample. As described in details in Chapter 3, different organic compounds are suitable for determination by a set of analytical methods. However, not all the analytical methods can identify all the same compounds. One example is the BOD. While with BOD it is possible to evaluate the biodegradable organic matter, carbohydrates, and oxydisable minerals, this test cannot measure complex compounds such as humic substances and aliphatic and aromatic hydrocarbons (Thomas and Theraulaz, 2007). Since BOD is an indirect measure of the organic matter by the equivalent of oxygen consumed, the results will depend on the amount of biodegradable organic compounds and the adequate presence of microorganisms. Complementarily, COD and TOC data account for complex compounds, but while TOC measures only organic carbon compounds, COD can oxidize other substances. Thus, several factors can impact the interpretation and the strategy

to establish a well-defined rank to compare data in an environment with heterogeneous (natural and anthropogenic) sources of organic matter.

One first approach for an analysis strategy is to compare the ratio between the parameters. Table 5 presents a simplistic analysis of the ratio between BOD_5 and COD and TOC. For typical untreated domestic wastes BOD_5/COD varies from 0.4 to 0.8, and for BOD_5/TOC ratio varies from 1.0 to 1.6 (Metcalf and Eddy, 1991). The average ratio BOD_5/COD was equal to 0.14 at site IG01, and 0.38 at site IG06. For the other sites monitored (IG02A, IG02B, IG03, IG04, and IG05) the average ratio BOD_5/COD varied from 0.46 to 0.57. For BOD_5/TOC, the same patterns was observed, with low values at sites IG01 and IG06 (0.4 and 1.3), and values above 1.6 for the other sites. The intermediate sites (IG02A, IG02B, IG03, and IG04) are under the influence of important WWTP and drain the most urbanized area of the Upper Iguassu Watershed. Consequently, these sites are under strongly influence of treated and raw effluents, which contributes to the water quality deterioration.

Table 5: Average BOD_5/COD and BOD_5/TOC ratio for the sites monitored along the main river (Samplings C39 to C48).

Ratio	IG01	IG02A	IG02B[(1)]	IG03	IG04	IG05	IG06
BOD_5/COD	0.14	0.46	0.54	0.50	0.57	0.49	0.38
BOD_5/TOC	0.4	1.8	2.4	3.1	1.8	1.6	1.3

(1) Site IG02B is located in the opposite margin of site IG02A.

However, the interpretation of these ratios is not simple and the generalization of the relationship between BOD_5, COD, and TOC must be established under different conditions. Another strategy is to evaluate all the parameters and the respective ratios in terms of carbon equivalent or in molar concentration. The equivalent BOD_5 in mgC/L, rather than the conventional oxygen equivalent (mgO_2/L), may facilitate to relate values of BOD_5 and TOC and to evaluate different ranges of labile and refractory organic carbon. Table 6 presents a summary of results of COD, BOD_5, TOC, POC, and DOC in terms of carbon equivalent or the mass of carbon consumed (mgC/L) for the sites monitored along the Iguassu River. Figure 14 show a graphic representation of the variation in terms of carbon equivalent, comparing the respective scales for COD, BOD_5, TOC, POC, and DOC.

Table 6: Maximum, minimum, and median concentration for in terms of carbon equivalent for COD (mgC/L), BOD_5 (mgC/L), TOC (mgC/L), DOC (mgC/L), and POC (mg/L) for the sites monitored along the main river (Samplings C39 to C48).

Parameter		COD (mgC/L)	BOD_5 (mgC/L)	TOC (mgC/L)	DOC (mgC/L)	POC (mgC/L)
IG01	*Maximum*	21.5	1.5	22.3	13.9	8.5
	Minimum	2.6	0.4	3.6	0.1	3.4
	Median	6.0	1.1	6.3	2.0	4.6
IG02A	*Maximum*	26.4	10.1	24.3	15.3	9.0
	Minimum	7.7	4.5	5.5	1.7	3.4
	Median	14.4	6.4	10.9	5.1	5.7
IG02B	*Maximum*	39.9	18.8	30.1	20.3	9.8
	Minimum	10.9	3.8	8.7	3.2	3.5
	Median	19.3	11.6	13.0	7.0	6.9
IG03	*Maximum*	38.7	19.5	27.4	16.7	14.4
	Minimum	15.6	3.8	8.7	1.3	4.8
	Median	20.3	15.0	10.8	5.8	6.9
IG04	*Maximum*	32.1	21.0	34.8	27.4	8.0
	Minimum	11.6	5.3	8.2	3.2	4.7
	Median	16.3	9.0	13.0	7.4	5.7
IG05	*Maximum*	32.3	13.5	34.0	27.3	7.0
	Minimum	5.3	2.3	5.7	0.2	2.6
	Median	16.6	6.0	8.5	3.1	6.0
IG06	*Maximum*	24.9	4.5	17.4	11.9	6.0
	Minimum	4.8	2.6	3.8	0.7	2.5
	Median	10.6	3.8	8.0	2.9	5.0

(1) Site IG02B is located in the opposite margin of site IG02A. For all sites n = 8, except for site IG04 (n=7).

The analysis in terms of mass of carbon assimilated is useful to compare the ranges of values obtained throughout the space under investigation and to evaluate the proportion of labile and refractory organic carbon. If the composition is essentially labile, the organic carbon will be susceptible for biodegradation, and thus measured BOD_5 will have values closed to the observed TOC. If the composition is more refractory, as can be observed at site IG01 (Figure 14 and Table 6), the measured BOD_5 is significantly lower than TOC. One hypothesis for TOC values being higher than BOD_5 values in clean waters is that not all carbon compounds can be used as en energy source by microorganism when performing BOD_5 measurements (refractory organic carbon). Additionally, the effectiveness of the BOD_5 to measure the biodegradable organic matter depends on the amount of microorganism adapted to assimilate the organic compounds.

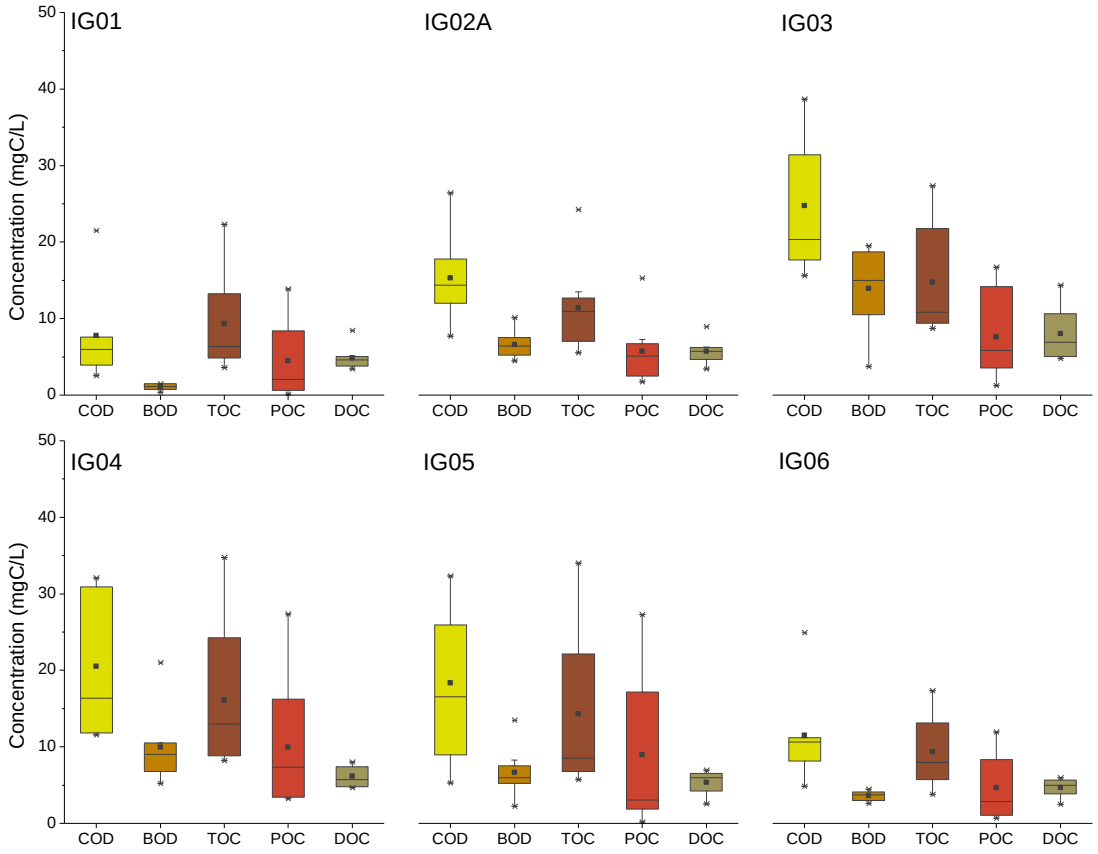


Figure 14: Box-plots showing the variation in terms of carbon equivalent of COD (mgC/L), BOD_5 (mgC/L), TOC (mgC/L), POC (mgC/L), and DOC (mgC/L) concentration observed at Iguassu River during 8 samplings (C39 to C41 during 2012 and C44 to C48 during 2013). Monitoring sites IG01 to IG06.

The same approach can be applied for TOC and COD data. In terms of mass of carbon assimilated, TOC and COD present similar results (Table 6). Since COD can oxidize not only organic carbon compounds, its results are generally higher than TOC in the most polluted sites (Figure 14). The water quality deterioration in the intermediate sites evaluated (IG02, IG03, IG04, and IG05) results from a complex and heterogeneous pollution matrix, with domestic and industrial wastewater, urban runoff, and natural sources (autochthonous and allochthonous pedogenic). Consequently, different compounds may be measured by COD analysis. In addition, COD measures the labile and the refractory fractions of organic matter. However, in terms of oxygen depletion, the refractory organic matter does not represent an immediate problem.

To overcome the challenge to interpret the different parameters limits focusing in water quality planning and management, one hypothesis or strategy is to estimate the proportion of labile and refractory organic carbon. The ratio between BOD_5 and TOC or COD, considering in molar concentration or in terms of mass of carbon assimilated, can be

used as a simple evaluation for this strategy. Table 7 presents the ratios BOD_5/TOC and BOD_5/COD for the sites monitored along the Iguassu River (samplings C39 to C48), and the respective percentage of refractory organic carbon.

Table 7: Average BOD_5/COD and BOD_5/TOC ratio for the sites monitored along the main river. Parameters in m.mol/L. Samplings C39 to C48.

Ratio	IG01	IG02A	IG02B[1]	IG03	IG04	IG05	IG06
BOD_5/COD	0.14	0.46	0.54	0.50	0.57	0.49	0.38
% of refractory OC	86%	54%	46%	50%	43%	51%	62%
BOD_5/TOC	0.16	0.58	0.46	0.39	0.60	0.43	0.48
% of refractory OC	84%	42%	54%	61%	40%	57%	52%

[1] Site IG02B is located in the opposite margin of site IG02A.

According to the results presented at Table 7, some interesting patterns can be highlighted. First, the less impacted site (IG01) showed the higher percentage of refractory organic carbon (86% and 84%), and thus, the lower ratios of BOD_5/COD and BOD_5/TOC. For the intermediate sites, the percentage of refractory organic carbon ranged from 40% to 61%. But, more important, the combined analysis of BOD and TOC in molar concentration or in terms of mass of carbon assimilated can be useful to indicate the percentage of the organic pollution that can really impact the oxygen depletion.

Comparing the ratios estimated considering BOD in terms of oxygen consumed, and in terms of mass of carbon assimilated (Table 5 and Table 7), two facts can be observed. For BOD_5/COD, since both parameters are an indirect measurement, the ratio will not alter due to the consideration about oxygen consumed or carbon assimilated. In the case of TOC, the second approach seems to be more representative to have a rapid interpretation. The results of Table 7 for BOD_5/TOC are similar to those obtained by the ratio between BOD_5 and COD, and retain a certain level of comparability when analyzing the percentage of labile and refractory organic carbon.

The weaknesses related to the interpretation of BOD_5/COD is that COD cannot measure all the complex organic carbon compounds, such as aromatic hydrocarbons (Thomas and Theraulaz, 2007), but can measure inorganic compounds (APHA, 1998). Even for raw effluents this relationship will depends on the effluent sources (Metcalf and Eddy, 1991). Additionally, COD is susceptible of interferences by oxidation time, reagent strength, and sample COD concentration (APHA, 1998).

Complementarily, the analysis of BOD_5/TOC is not trivial, besides it can suggest an indication about the magnitude of labile and refractory organic carbon. The problem, in this case, is that BOD is a subjective test and depends on several factors that may interfere in the final result (Comber et al., 1996). In addition to the oxygen consumed during biodegradation, the BOD test may also measure the oxygen used to oxidize inorganic compounds (sulfides and ferrous iron) and to oxidize reduced forms of nitrogen (nitrogenous demand), changing the magnitude of oxygen consumed (APHA, 1998).

Furthermore, not all the organic carbon consumed in a biological reaction will be used in respiration process (Davies, 2005). The biological metabolism can be (in a simplified manner) identified by two main processes: (i) respiration; and (ii) growth and division. These two processes represent the different pathways that the organic carbon can follow after being ingested by microorganisms. The first one is related to the respiration, when oxygen is used to convert the organic carbon in CO_2. This process is also named catabolism or energy metabolism, and represents the biological oxidation in which organic carbon is broken down to yield cellular energy. The energy is thus used for maintenance and growth, in the process named anabolism. After a series of biosynthetic reactions, part of the ingested organic carbon is used to form new biomass. Consequently, due to the basic principle of quantification, BOD_5 tests can only measure the carbon that follows the respiration pathway, when oxygen is consumed. In other words, BOD_5 can measure the organic carbon used to provide energy to growth, but not the organic carbon that is assimilated to form new biomass. In TOC analysis, all the organic carbon is quantified. Thus, a direct comparison of BOD_5 and TOC, even in terms of mass of carbon assimilated (Table 7), has a certain level of subjectivity.

In summary, the following guidelines can be indicated according to the values obtained for BOD_5 and TOC:

- If BOD_5 is expressed in terms of oxygen equivalent (mgO_2/L) and there is a high proportion of labile organic carbon, the expectation is that BOD_5 will be equal or higher than TOC. This result is generally expected for raw domestic effluents.
- If BOD_5 is expressed in terms of oxygen equivalent (mgO_2/L) and there is a low proportion of labile organic carbon (elevated refractory organic matter), the expectation is that BOD_5 will be equal or less than TOC. This is the case of unpolluted waters or with low anthropogenic impact.
- If BOD_5 is expressed in terms of carbon equivalent (mgC/L), the expectation is that BOD_5 will be equal or lesser than TOC. BOD_5 can be higher than TOC if other compounds (rather than carbon derived compounds) are being used by

microorganisms (ex. Sulfides and ferrous iron, nitrogen, oxydisable minerals), or with growth rate has significantly impact on the carbon assimilated. The presence of refractory organic carbon increases the difference between TOC and BOD_5 values.

Table 8: Summary of labile and refractory OC estimation relating BOD_5 and TOC analyses.

Parameter	TOC > BOD_5	TOC = BOD_5	TOC < BOD_5
BOD_5 (mgO_2/L)	↑ refractory OC	Refractory and labile OC	↑ labile OC
BOD_5 (mgC/L)	Refractory and labile OC	↑ labile OC	Other compounds(1)

(1) Sulfides and ferrous iron, nitrogen, oxydisable minerals.

The same analysis about the relationship between BOD_5 and TOC, and thus, the estimation of labile and refractory fractions of organic carbon, could be extending to DOC and POC. However, in this case, the BOD_5 test should be conducted for dissolved samples (same porosity size used for DOC analysis). If data of filtered BOD_5 is available, than the ratio with DOC and/or POC could indicate if the refractory organic carbon is predominant in the dissolved or particulate fraction. Another approach for the evaluation of the labile and refractory DOC and/or POC is the analysis of the biodegradation during a period of time, considering both fractions decay. Such analysis has been performed, with the main results being detailed at item 6.3.

6.2.5 UV-Visible and Fluorescence Spectroscopy

The analysis for absorbance and fluorescence spectroscopy is divided considering: (i) the peaks identification and the general information of each EEM; and (ii) the relationship woth the other parameters monitored. The first approach aims to mainly evaluate the source and the predominance of labile or refractory organic matter in the water column, in order to better evaluate the potential of oxygen depletion along the main river. The second approach has an investigative and exploratory character to identify correlation patterns between spectroscopic analyses and the traditional water quality parameters monitored.

6.2.1.4 Uv-vis and fluorescence spectroscopy main characteristics

The average values of $SUVA_{254}$ and A_{285}/DOC (Table 9) indicate a probable mixture of an autochthonous source of biological activity and an allochthonous source of dissolved organic matter. For example, when $SUVA_{254}$ values are low (lower than 4.4 L/mg.m), this may indicate that the dissolved organic matter may arise from anthropogenic allochthonous sources, such as domestic effluents, since DOC present in effluents has low absorption in the UV region, thus diminishing both the $SUVA_{254}$, and the A_{285}/DOC ratio (Rostan and Cellot, 1995; Westerhoff and Anning, 2000). Musikavong and Wattanachira (2007) also indicate that $SUVA_{254}$ values tend to increase (within this limit) in advanced levels of biological treatment of effluents, since biological activity tends to remove the fraction of the organic matter that is not sensitive to UV emission, i.e., the easily biodegradable organic compounds such as carbohydrates (Imai et al., 2002) and other aliphatic functional groups (Ma et al., 2001), diminishing DOC and maintaining absorbance.

Complementarily, the FR values (Table 9) indicate the presence of either autochthonous DOC (FR > 1.8), and DOC formed by compounds that do not present fluorescence emission in 450/500 nm with excitation of 370 nm wavelength, such as dissolved organic substances in domestic effluents (Westerhoff and Anning, 2000). Site IG01, the lower anthropogenic impacted site, the FR may indicate the predominance of allochthonous source of humic substances (FR ≤ 1.5).

Besides $SUVA_{254}$ and A_{285}/DOC are indicated to differentiate autochthonous and allochthonous organic matter (Rostan and Cellot, 1995; Westerhoff and Anning, 2000), it is important to highlight that its application for a river with a complex and heterogeneous pollution sources matrix does not provide a unique identification for the predominant

compound source. These indexes were primarily used to differentiate humic or fulvic acid compounds ($SUVA_{254}$ ≈ 4.4 L/mg.m, and A_{285}/DOC ≈ 20 L/g) from autochthonous and labile organic matter ($SUVA_{254}$ ≈ 1.2 L/mg.m, and A_{285}/DOC ≈ 10 L/g). Since in the river both compounds can be found in less or higher proportion, the result will be influenced either by the presence of complex compounds (higher indexes) and labile compounds. Thus, the results are interesting to have an indication about the compounds that can be found, but not necessarily it excludes the presence of other organic matter sources.

Table 9: Specific absorbance and fluorescence ratios for the sites monitored

Parameter	IG01	IG02A	IG02B	IG03	IG04	IG05	IG06
$SUVA_{254}$ (L/mg.m)	2.9 ± 0.4	2.7 ± 0.7	2.3 ± 0.7	2.4 ± 0.9	2.8 ± 0.7	2.9 ± 1.3	3.0 ± 1.2
A_{285}/COD (L/g.m)	21.1 ± 2.6	19.1 ± 4.4	16.3 ± 5.4	16.9 ± 5.3	19.8 ± 4.3	20.5 ± 6.1	21.5 ± 5.4
FR	1.4 ± 0.04	1.7 ± 0.1	1.8. ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	1.6 ± 0.1

FR: ratio between the intensities of fluorescence emitted at 450 and 500 nm wavelengths, with an excitation of 370 nm; $SUVA_{254}$ (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). Samplings C39 to C48.

A better interpretation of fluorescence variation as a function of the distinct organic compounds can be done with the analysis of the fluorescence peak intensity in an excitation-emission matrix (EEM). The variation of EEM between monitoring sites indicates a variation of peaks intensities according to the levels of organic pollution. Figure 15 presents a sequence of EEM for sites IG01 (headwater) through IG06 (downstream watershed) considering sampling performed in Nov/2012 (Sampling n. 42). In the EEM, the colour scale indicates the normalized intensity of fluorescence peaks (blue is the less intense and red represents the highest intensity). The results from EEM indicated an increase of fluorescence peaks intensity on site IG02, which can be related to the domestic effluents loads. Site IG02 is located after a sewage treatment plant (WWTP Atuba Sul) in the most urbanized area of the watershed. The presence of labile organic matter, peaks B (λ_{ex}= 230-275 nm/ λ_{em}= 310 nm), T_2 (λ_{ex}= 230 nm/ λ_{em}= 350 nm), and T_1 (λ_{ex}= 290 nm/ λ_{em}= 350 nm), were evident at sites IG02, IG03 and IG04. The low rates of anthropogenic occupation at site IG01 can be identified by the EEM analysis, with small contribution of labile organic matter (Peaks B, T_2, and T_1) and presence of humic compounds, peaks A (λ_{ex}= 230 nm/ λ_{em}= 400-500 nm), and C (λ_{ex}= 300-500 nm/ λ_{em}= 400-500 nm).

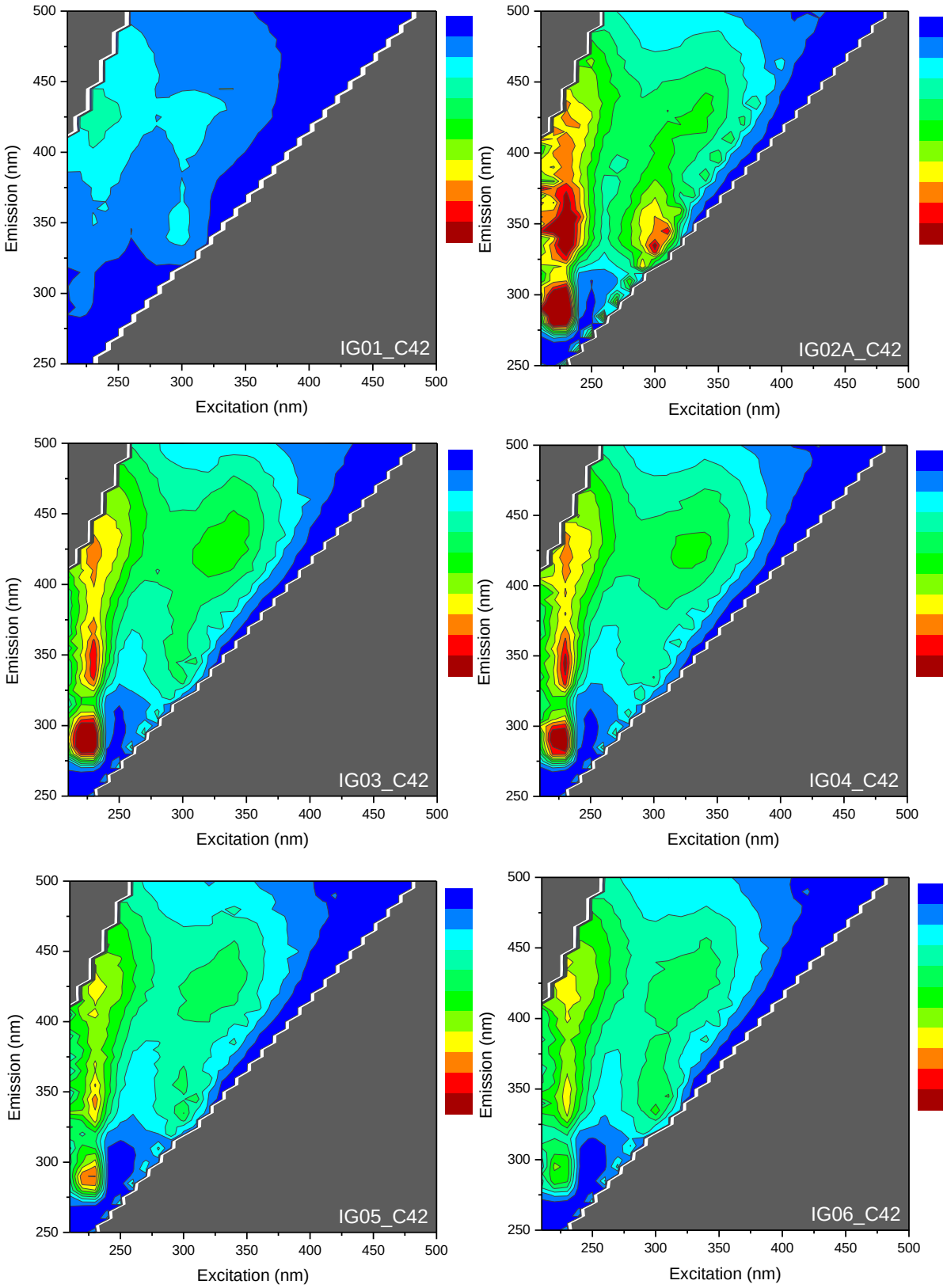


Figure 15: Example of fluorescence excitation-emission matrix (EEMS) for all sites monitored along main river (IG01 is located at headwater and IG06 is the most downstream site). Samples collected during field campaign n. 42 (Nov/2012). Fluorescence intensities are in Raman units. Color scale indicate the intensity of fluorescence peaks (blue is the less intense and red is the highest intensity).

The combination of commonly used analytical analysis with spectroscopic techniques has potential for the evaluation of organic matter dynamics in rivers. Through the results of fluorescence spectra and the identification of different regions with higher fluorescence intensity peaks (Hudson et al., 2007; Henderson et al., 2009; Goldman et al., 2012), it is possible to identify the presence labile organic matter (Westerhoff and Anning, 2000) and identify the location of the critical points due to the inputs of sewage along the main river (Figure 15). A visual analysis of these results show, for example, a non-affected part of the basin (site IG01), the evolution of the presence of sewage (Peaks B, T_2 and T_1) along the most urbanized points within the watershed (IG02 to IG04), and the recuperation of the water quality with the consumption of labile organic matter (sites IG05 and IG06). Due to its rapid determination, these methods can be useful for regulatory agencies for a primary identification of the most impacted points in an urban river.

6.2.1.5 Correlation between Uv-vis and fluorescence spectroscopy with other parameters

The relationship between spectroscopic fluorescence peaks intensities and BOD_5 also evidenced the presence of effluent derived organic matter at Iguassu River. Figure 16 presents the linear relationship between the intensity of fluorescence peaks B, T_2, T_1 (labile organic matter) and the concentration of BOD_5. The linear correlations considering the data from all monitoring sites (Samplings C39 to C48, n=66) were $r=0.7321$ ($p<0.0001$, Peak B), $r= 0.7560$ ($p<0.0001$, Peak T_2), and $r=0.6949$ ($p<0.0001$, Peak T_1). Complementarily to the evaluation of labile organic matter, the analysis of fluorescence peaks intensity related to the presence of humic compounds (Peaks A and C) also resulted in good correlation with BOD_5 (Figure 17). The linear correlations considering the data from all monitoring sites (Samplings C39 to C48, n=66), were $r=0.5523$ ($p<0.0001$, Peak A), and $r=0.6708$ ($p<0.0001$, Peak C).

Besides the tryptophan-like fluorescence peak (T_1) is commonly associated with the presence of labile organic matter (Carstea, 2012), the direct correlation with BOD may be not necessarily significant for all kind of samples (Baker, 2002; Cumberland and Baker, 2007). Generally, effluent samples have a strong correlation between T_1 and BOD_5 than unpolluted river samples. Hudson et al. (2007) found a good linear correlation between T_1 and BOD_5 for rivers ($r=0.612$, n=124 samples) and effluents ($r=0.714$, n=141 samples), while Baker (2002) did not found good correlations in an unpolluted river ($r=0.2$, n=91 samples). Since the organic matter dynamics depends on factors such as composition, compounds origin, physical and biological conditions for degradation, the estimated correlation between the

parameters analyzed will also depend on site-specific characteristics (Nataraja et al., 2006). In addition, for unpolluted waters, the weak correlations results from BOD_5 and tryptophan-like fluorescence reported by some studies may be affected due to inaccuracies of BOD_5 analysis for samples with low organic content (Comber et al., 1996; Henderson et al., 2009).

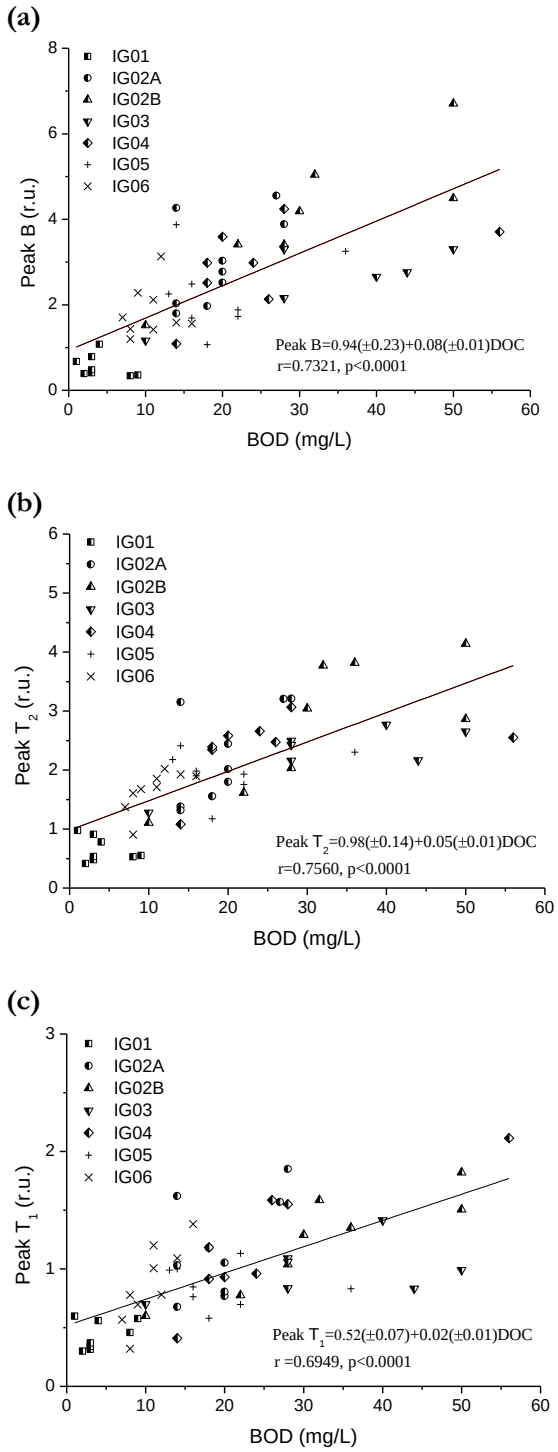


Figure 16: X-Y plots for (a) Peak B x BOD, (b) Peak T_2 x BOD, and (c) Peak T_1 x BOD. Solid lines represent the corresponding regression. r is the Pearson's linear coefficient and p is the significant level (n=66 data, all sampling sites. Site IG02 considered for left margin, IG02A, and right margin, IG02B).

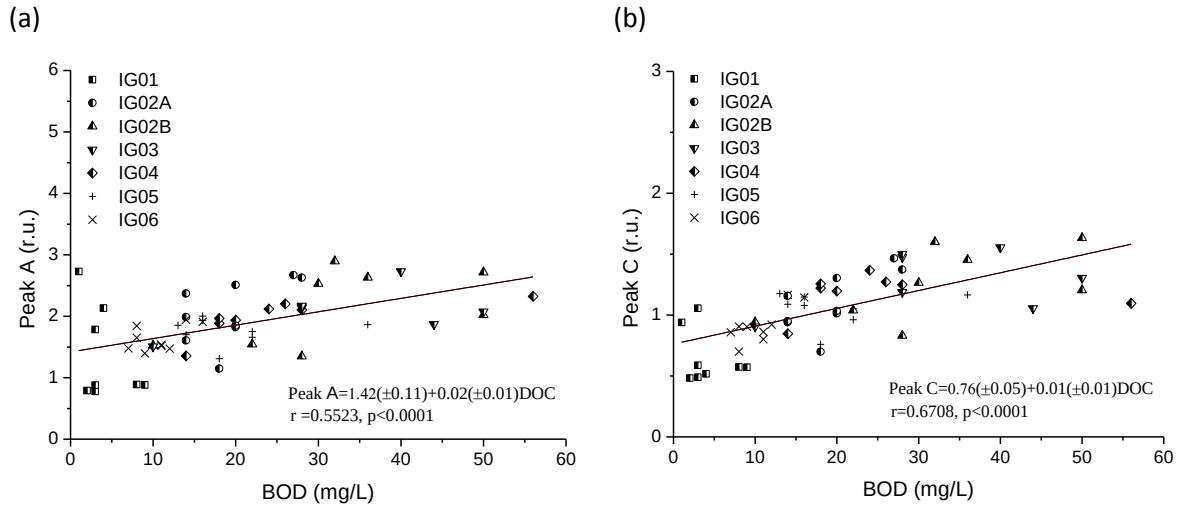


Figure 17: X-Y plots for (a) Peak A x BOD, (b) Peak C x BOD. Solid lines represent the corresponding regression. r is the Pearson's linear coefficient and p is the significant level (n=66 data, all sampling sites. (n=66 data, all sampling sites. Site IG02 considered for left margin, IG02A, and right margin, IG02B).

Considering the parameters evaluated through correlation analysis, it has been observed that BOD_5 represents a direct indication of the labile organic content, and thus results in good correlations for sewage or sewage impacted waters. For the COD, TOC, and DOC analyses, not only the labile organic matter is determined, but also the refractory fraction is measured. The different sources of organic pollution in rivers (sewage, industrial effluents, urban and agricultural runoff) may result in a complex mixture of compounds with distinct fluorescence and absorbance intensities. Consequently, both fluorescence and non-fluorescence compounds are quantified, which may affect the association of specific fluorescence peaks (Henderson et al., 2009).

In such a context, it is important to highlight that some studies found good correlations based on a data set with basic short temporal variation that would allow a more in depth reflection about potential parameters relationship. One example are the results presented by Hur et al. (2012), which relies on two samplings in a short time difference. The authors found good correlations between BOD, Total Nitrogen (TN), COD, and absorbance and PARAFAC (Parallel Factor Analysis Model) components. PARAFAC is a complementary analysis that is commonly used to separate and identify different peaks of the EEM data, and has been currently successfully applied in several on-going investigations related to water quality (Felipe-Sotelo et al., 2007; Stedmon and Bro, 2008; Hur and Cho, 2010; Cid et al., 2011).

However, to use the correlations data to associate fluorescence signals and use as a surrogate method for BOD_5 evaluation (on-line monitoring tool), as suggested by Hur et al.

(2012), more and deeply investigations are required. While the strong correlations found by the authors may indicate that fluorescence intensity peaks can be used as surrogates for BOD_5 analysis, it also may indicate that under the conditions of samplings performed, no variation was observed on flow regime, changes on sewage inputs, or seasonality factors. Additionally, Kokorite et al (2012) also discusses some factors which may affect concentrations of dissolved organic matter in surface waters, especially in large rivers basins. In their studies, discharge and correlated complex factors (such as long flow trajectories, different land-use types, soil and climate) can complicate interpretation of a river's dissolved organic matter content, confirming the results herein included.

As detailed in Chapter 3, a common conclusion from reported studies is that fluorescence and absorbance spectroscopy can be used to characterize natural waters or effluents (Westerhoff and Anning, 2000; Peuravuori et al., 2002; Pons *et al.*, 2004; Henderson et al., 2009; Goldman et al., 2012; Meng et al., 2013). For a highly polluted river such as the case study presented in this thesis, such procedures enable the water resources manager to better characterize and evaluate the organic matter content in a river. But the use of these techniques as substitutes for standard water quality parameters requires caution. First, because of the site-specific nature of the correlations with spectroscopic measurements, interpretation is limited since the relationships with traditional parameters may not be applicable at other sites. Second, since the organic pollution of an urban river is a result from a complex and heterogeneous mixture, spectroscopic analyses may indicate the presence of more than one type of organic matter source. Results reported from earlier research suggests that if a surrogate parameter is used to estimate the organic content in terms of BOD_5 or other water quality parameters, it is important that the results retain a degree of comparability with historical data (Comber et al., 1996).

6.2.6 Summary of Water Quality Assessment

Parameters such as BOD_5, ammonia, orthophosphate, and DO directly indicated the most polluted sites, while DOC did not show significantly variation according to the levels of urbanization and organic pollution in the river. The median values of specific absorbance $SUVA_{254}$ and A_{285}/DOC also did not varied significantly between the sites monitored. The respective results of absorbance indicated a mixture of anthropogenic allochthonous sources, i.e., domestic effluents, and compounds associated with humic and fulvic acids.

Considering the characterization of organic pollution in a river with different spatial characteristics, the analyses of fluorescence spectroscopy showed to be more representative than specific index of the absorbance spectrum. The fluorescence peaks B, T_2, and T_1, commonly associated with the presence of labile organic matter, had good positive correlation with BOD. In addition, the results of fluorescence EEMs also indicate the spatial change on the anthropogenic derived organic matter. Monitoring sites IG02, IG03, and IG04, located in the most urbanized region of the study, were more affected by the occurrence of tryptophan-like and tyrosine-like fluorescence.

The monitoring data confirms the existence of qualitative relationships between spectroscopic and parameters commonly-used in water quality parameters used in monitoring strategies. Considering the necessity of a rapid and direct analysis to the identification of organic pollution by regulatory agencies and management strategies, fluorescence spectroscopy can be applied to estimate the presence of labile organic matter. Due to its qualitative characteristic, fluorescence data can be used as a complementarily parameter in water quality planning and management strategies for basins with high inputs of organic pollution.

6.3 Organic Matter Biodegradation

In the aquatic environment, the organic matter dynamics involves an interconnected cycle of production and consumption. One important process of organic matter consumption is the biodegradation, in which microorganism assimilates and converts the organic compounds in energy, biomass and inorganic compounds (Davies, 2005). Thus, the motivation to conduct a biodegradation experiment is to understand the variation of the organic matter based on organic carbon quantification and characterization. The experimental procedure consisted in the quantification of DOC, TOC, and POC and the evaluation of the spectroscopic characteristics (absorbance and fluorescence) in an incubated reactor during a period of time. An open system (aerated flask with 2L of sample, named Experiment A) and a closed system (flask with 120 mL, named Experiment B) were evaluated.

Four sets of experiments were conducted during the last four samplings (C45 - Jul/13, C46 - Sep/13, C47 - Oct/13, and C48 - Dec/13). Three sites along the Iguassu River were considered for this exploratory analysis: sites IG01, IG02, and IG05. Site IG01 represents an area of low anthropogenic influence. Site IG02 has a direct influence of treated and non-treated effluent from high impacted tributaries of Iguassu River. Downstream in the watershed, site IG05 represents an area with relative anthropogenic influence, but also with dilution effects.

The first experiment was conducted during 40 days, in order to identify the time in which the organic carbon concentration would be stable. This is the basis of the BDOC essay, which considers as the biodegradable organic the difference between the initial values and the stable concentration reach after a period of time. For the other three experiments, the data were monitored for a period of 10 days, since during the first experiment the organic carbon concentration was stable after a small amount of days. The samples were collected daily for organic carbon analysis, and stored in carbon-free glass ampoules. After collection, samples were filtered (DOC analysis) and immediately acidified (for IC elimination).

Complementarily, UV-visible and fluorescence spectroscopic were analyzed for daily samples. These analyses are important to identify patterns of biodegradability according to the peaks in the excitation emission matrix (EEM). The expectation is that the decrease of specific peaks intensity would indicate the decomposition of labile fraction of organic carbon.

The results presented within this chapter are from experiments conducted during samplings C47 and C48 (October/13 and December/13, respectively). Supplementary data from other experiments are presented in Appendix 3.

6.3.1 Experiment Overview:

6.3.1.1 Batch Incubations

The evaluation of biodegradation process of anthropogenic derived organic matter was experimentally investigated for sampling sites IG01, IG02, and IG05. Carbon free amber glass bottles were used to collect three liters of sample in each monitoring site. An adapted method based on BDOC tests was conducted for organic matter degradation, based on procedures by Frias et al. (1995), Lim et al. (2008), Labanowski and Feuillade (2009) and Stutter et al. (2013). Batch incubations were performed in the dark, with controlled temperature at 20°C, for a period of 10 days, with regular intervals of sampling analysis. For each set of experiment, open and closed systems were tested to analyze its characteristics and results. In addition, a nutrient solution was added in the proportion of 0.5 mL/L of sample. The nutrient solution was prepared according to APHA (1998) for BOD analysis (phosphate buffer solution, magnesium sulfate solution, calcium chloride solution and ferric chloride solution).

For the open system (named Experiment A), 2L of sample were incubated in glass containers with an aeration and shaking apparatus. Ultrapure water with the same concentration of nutrients was used as a blank control. For the closed system (named Experiment B), amber glass bottles of 120 mL were used to incubate the samples. For Experiment B, ultrapure water with nutrients was used for dilution (APHA, 1998). A dilution of 50% was considered for all samples. A blank test with ultrapure water and the same concentration of nutrients was conducted for control.

In regular intervals of days, three subsamples of 10 mL from each bottle and/or container were collected with a glass syringe (pre-washed with acid). For DOC, fluorescence and absorbance analysis, samples were filtered in a pre-washed 0.45 μm PVDF syringe filter, and stored in a carbon free glass ampoule in the dark at 4°C. For total organic carbon (TOC) and DOC, 0.5% of the volume of H_2SO_4 P.A. was added for preservation (APHA, 1998). The measurements of DOC, TOC, fluorescence and absorbance were performed up according to the details described in the item 6.2.1. All glasses (collection bottles, ampoules, incubation bottles/containers) used during the experiment were acid washed and baked during 5 hours at 550°C.

6.3.1.2 Decay Models for Biodegradation Kinetics

The decomposition pattern of DOC, TOC, and POC were evaluated through exponential decay models. The kinetics rates were estimated according to the conceptualization of different DOC (or other parameter) pools of varying reactivity. Lønborg et al. (2009) and Koehler et al. (2012), considering long time dark incubation, used an exponential decay model with residual pool to represent the labile and refractory DOC (residual). In this model, the authors fitted a three parameter exponential model, according to the following equation:

$$C_t = C_L \cdot e^{-kt} + C_R \quad (1)$$

where C_t is the amount of DOC (or DOM, mg/L) remaining at time *t*, C_L is the bioavailable pool (labile DOC or BDOM, mg/L), *k* is the degradation rate (day^{-1}), *t* is time (days) and C_R is the residual pool (refractory DOC or DOM, mg/L).

Reuschenbach et al. (2003), Khan et al. (2005), and Tihomirova et al. (2012) used a first-order exponential decay model to represent the DOC biodegradation. This model considers that the rate of biodegradation is proportional to the concentration of the compound analyzed:

$$C_t = C_0 \cdot e^{-kt} \quad (2)$$

Where: C_t is the amount of DOC (mg/L) remaining at time *t*, C_0 is the initial DOC (mg/L), *k* is the degradation rate (day^{-1}), *t* is time (days). For the biodegradation experiments, two models of regression analysis were tested using the best-fit between DOC, TOC, or POC during the incubation time: (i) exponential decay model with residual pool (Equation 1, named Model 1); and (ii) first-order exponential decay model (Equation 2, named Model 2). The advantage of Model 1 is to consider the difference between labile and refractory pools, since refractory organic carbon can remain for long periods in the aquatic environment (Lønborg et al., 2009). The goodness of fitness was evaluated by the Nash-Sutcliffe efficiency (NSE), and the Mean Squared Error (MSE). The Nash-Sutcliffe efficiency is a normalized coefficient of the sum of squared of residuals between the measured and simulated series of the variable of interest. The equation to calculate the coefficient is (Nash and Sutcliffe, 1970):

$$E_{ns} = 1 - \frac{\sum_{i=1}^{N}\left(Y_i^{obs} - Y_i^{sim}\right)^2}{\sum_{i=1}^{N}\left(Y_i^{obs} - Y_i^{mean}\right)^2} \quad (3)$$

Where: E_{ns} is the Nash-Sutcliffe coefficient, Y_i^{sim} is the simulated data, Y_i^{obs} is the observed data, Y_i^{mean} is the averaged observed data, and *i=1,2,…,N*, where *N* is the total number of pairs simulated and observed data. NSE ranges between -∞ and 1.0 (1 inclusive), with NSE =1 being the optimal value. Complementarily, the MSE was used as an index to indicate the regression model goodness of fit, according to Equation 4:

$$MSE = \frac{\sum_{i=1}^{N}\left(Y_i^{obs} - Y_i^{sim}\right)^2}{N} \quad (4)$$

Where: MSE is the mean squared error coefficient, Y_i^{sim} is the simulated data, Y_i^{obs} is the observed data, *N* is the total number of pairs simulated and observed data. An MSE index of 0 indicates a perfect fit (Moriasi et al., 2007).

6.3.2 Temporal and Spatial Variation of Biodegradation Characteristics

The changes observed in organic matter indicates probable differences in its bioavailability for the anthropogenic impacted sites monitored. Table 10 presents a summary of the results of the biodegradable fractions of organic carbon from batch incubations.

Table 10: Summary of DOC, TOC, and POC concentration and the respective percentage and concentration of BDOC for each fraction. Experiment A is an open system with added nutrients, and Experiment B is a closed system with added nutrients. Site IG01 is located in a non-impacted area, and sites IG02 and IG05 are located in urbanized areas. Incubation time for both experiments was set on 10 days.

Sample dates	Sites	Dissolved			Total			Particulate		
		DOC (mg/L)	BDOC (mg/L)	BDOC (%)	TOC (mg/L)	BTOC[a] (mg/L)	BTOC[a] (%)	POC (mg/L)	BPOC[a] (mg/L)	BPOC[a] (%)
Oct/13	IG01 (Exp. A)	5.3	2.1	41%	6.1	2.5	42%	0.8	0.4	50%
	IG01 (Exp. B)	6.1	3.4	56%	6.4	2.6	41%	0.3	-	-
	IG02 (Exp. A)	8.1	4.2	51%	8.5	4.4	50%	0.3	0.2	75%
	IG02 (Exp. B)	5.8	2.4	42%	7.9	2.1	26%	-	-	-
	IG05 (Exp. A)	7.1	3.2	44%	10.4	6.0	57%	3.3	2.8	86%
	IG05 (Exp. B)	5.2	2.2	43%	6.8	2.7	39%	1.7	0.5	28%
Dec/13	IG01 (Exp. A)	4.1	0.8	21%	6.5	2.8	43%	2.4	1.9	80%
	IG01 (Exp. B)	5.0	1.1	22%	7.3	2.9	40%	2.3	1.8	78%
	IG02 (Exp. A)	6.6	1.9	29%	11.2	5.2	47%	4.7	3.3	71%
	IG02 (Exp. B)	7.9	1.6	20%	13.6	6.9	51%	5.7	5.3	94%
	IG05 (Exp. A)	6.7	1.8	27%	9.2	4.1	45%	2.4	2.3	94%
	IG05 (Exp. B)	7.3	2.4	33%	9.4	4.4	47%	2.1	2.0	95%

[a] For the purpose of this study, we consider the acronyms BTOC and BPOC in reference to biodegradable fractions of TOC and POC, respectively.

Comparing the average percentage of biodegradable fraction of initial DOC, sampling performed in October showed higher values, 46%, while during December the

average was about 25%. For TOC, differences between the percentage of biodegradable fraction was small between two sampling period (43% and 46%, respectively), while for POC, besides the small amount of data collected during the first sampling date, the average concentration and its percentage of biodegradable fraction was higher during the second sampling, 1.3 mg/L (60%) and 3.3 mg/L (85%), respectively.

The decay of DOC concentration during the incubation period indicated differences between collection time, organic matter composition, and degradation rates. Figure 18 presents the DOC concentration of experiments A and B, for sites IG01, IG02, and IG05 during the 10 days of the incubation period. According to the results, there was not a unique pattern of decay among the two sampling periods, what highlights the complexity and the challenging process to evaluate biodegradation in rivers. During the first sampling (October, 2013), the DOC decay and the higher values of BDOC indicated that there was more biodegradable organic matter present in the river comparing to the second sampling period.

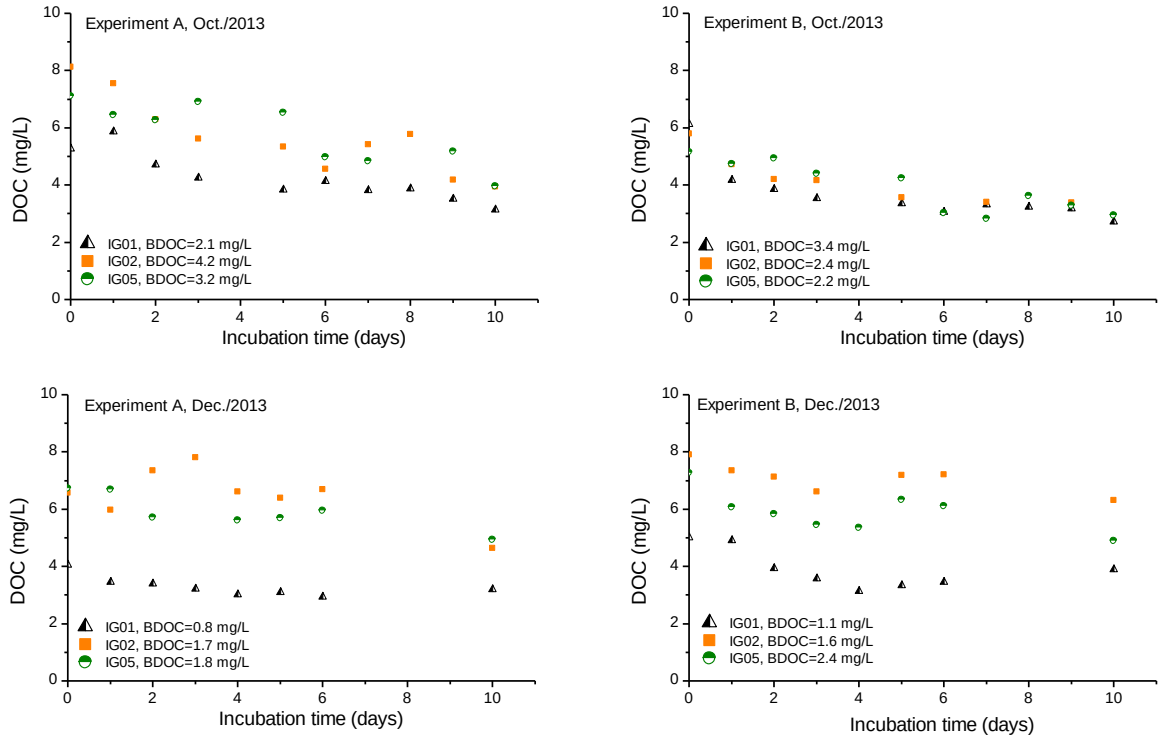


Figure 18: Example of decomposition curves showing the variation of DOC concentration for Experiment A (open system with added nutrients) and Experiment B (closed system with added nutrients). Batch incubations were performed for samples collected at Oct/2013 (top) and Dec/2013 (bottom), on sampling sites IG01, IG02, and IG05.

The characteristics of DOC decay comparing the open and closed systems were also different. Under a closed condition (Experiment B), DOC decay presented less variation over

consecutive days. Results from the open system showed a higher variability, in particular for sites IG02 and IG05. An increase of DOC concentration before a final decrease and consumption occurred with less frequency in Experiment B, and could be to small differences when shaking the sample and incubating in separate bottles. A formation of a biofilm within shaking and aeration apparatus, and consequently a release of the adsorbed compounds when collecting the sub-samples, could alter the results. Another hypothesis is the transformation of labile TOC into DOC by microbial assimilation and release. In Experiment A, shaking also allows the particulate material to remain in the water column, while in Experiment B settling occurred during the first hours of incubation.

Similarly DOC, TOC, and POC decay resulted in different decomposition patterns. Figure 19 presents an example of the decomposition curve and the normalized concentration for site IG02 (Experiment B). POC showed a significant consumption under the experiment conditions. The biodegradable fraction estimated for the period of experiment (10 days) was about 20% of DOC, 51% of TOC, and 94% of POC. The initial concentration for DOC, TOC, and POC was, respectively, 7.9 ± 0.1, 13.6 ± 0.6, and 5.7 ± 0.6. An average of 21% of the fluorescence intensities for peaks B, T_1 and T_2 reduced for site IG02. Commonly peaks B, T_1 and T_2 reflect the labile organic matter content, corroborating to the decrease of DOC concentration. Peaks A and C, often related to humic substances, showed less percentage of reduction, with 7 % and 17%, respectively. Specific absorbance at 254 and 285 nm increased during the period of the experiment.

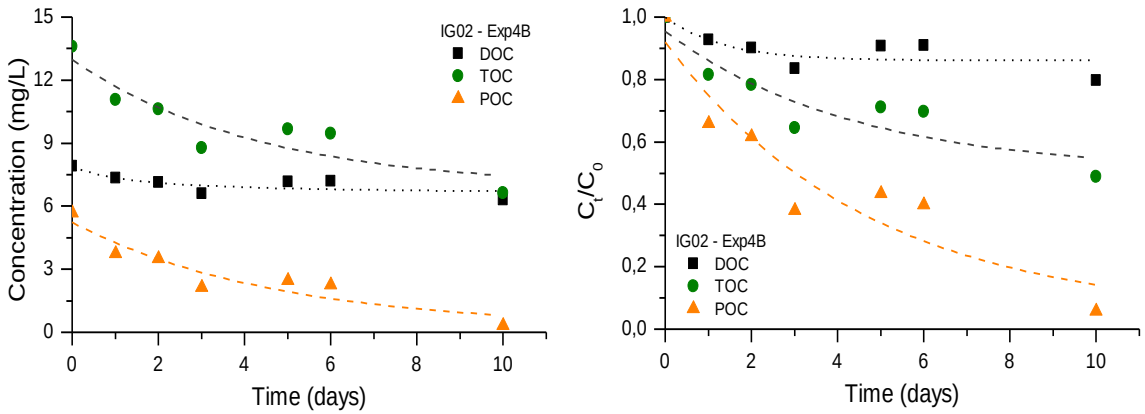


Figure 19: Example of decomposition curves showing the variation of DOC, TOC and POC concentration and the normalized values of the respective concentrations considering experiment B (closed system with added nutrients). Batch incubations were performed for samples collected during summer (05 Dec 2013) on sampling site IG02.

According to Mostofa et al. (2013a) biological degradation can mineralize up to 85% of DOC in natural waters. Studies have shown different values for BDOC, indicating that

intrinsic characteristics such as incubation period and initial DOC concentration interfere with decomposition rates. Khan et al. (2005) observed values from 37% to 44% considering an average initial DOC of 5.8 mg/L for surface waters in a batch reactor during 10 days of incubation. Wickland et al. (2012) conducting an experiment during 28 days at 15°C, found values of BDOC from 7% to 53% when analyzing samples from a river with initial DOC values of 2.7 mg/L to 17.3 mg/L. BDOC from effluents and leaf leachate showed higher values, respectively, 24% to 40% (Rhim et al., 2006) and 52% to 75% (Trulleyová and Rulík, 2004), since there is an elevated amount of labile organic matter. Lake waters, due to microbial and photodegradation processes and to the presence of humic and fulvic acids, have measured data of BDOC that are generally lower than other environments, from 12.4% (Søndergaard and Worm, 2000) to 29% (Lønborg et al., 2009). Unpolluted river and sea water showed intermediate values of 20% to 35% for an incubation time of 30 days (Servais et al., 1987).

In the Iguassu River, the average percentage of BDOC ranged from 20% to 56%, with initial DOC values from 4.1 mg/L to 8.1 mg/L (Table 10). In addition, our findings suggest that the quantification of both DOC and POC removal rates and biodegradable fractions are important to understand the transformation mechanisms and the resulting compounds. In general, the average observed values of the biodegradable fraction of particulate and total organic carbon were higher than BDOC, especially for samples collected during December 2013 (summer time). BPOC ranged from 50% to 95%, and BTOC ranged from 26% to 57% (Table 10). However, during the incubation period, absorption of both biodegradable and non-biodegradable DOC onto inorganic particle surfaces and biofilms surfaces can lead to an overestimation of BDOC values (Trulleyová and Rulík, 2004). The authors consider the absorption mechanism as the initial step in DOC removal from the solution, followed by hydrolysis and incorporation into biofilm. Microbial cells can then assimilate the biodegradable fraction of DOC.

6.3.3 Analyses of the Spectroscopic Characteristics from Biodegradation Experiment

Spectroscopic fluorescence peaks intensities and ratios also provided evidence of changes in the compositional quality of organic matter under experimental conditions. Figure 20 presents the linear relationship between the intensity of fluorescence peaks B, T_2, T_1, A, and C and the concentration of DOC for Experiment A and B (open and closed system)

during sampling performed on 05 Dec 13. Results indicated the degradation of labile (Peak B – tyrosine-like fluorescence, and Peaks T_2 ant T_1 – tryptophan-like fluorescence) and refractory (Peaks A and C – humic-like fluorescence) organic content during the period of incubation (10 days). The accentuated decrease of fluorescence intensity of Peaks B, T_2, and T_1 (Figure 20a and 20b) confirms the degradation of labile organic matter. The average percentage of fluorescence intensity reduction was 41%, 25%, and 29% for Peaks B (Figure 3a), T_2 (Figure 20b), and T_1 (Figure 20c), respectively. For peaks A (Figure 20d) and C (Figure 20e), the average percentage of fluorescence intensity reduction was less significant, with about 14% and 12%, considering an initial DOC range from 4.1 mg/L (IG01) to 7.9 mg/L (IG02).

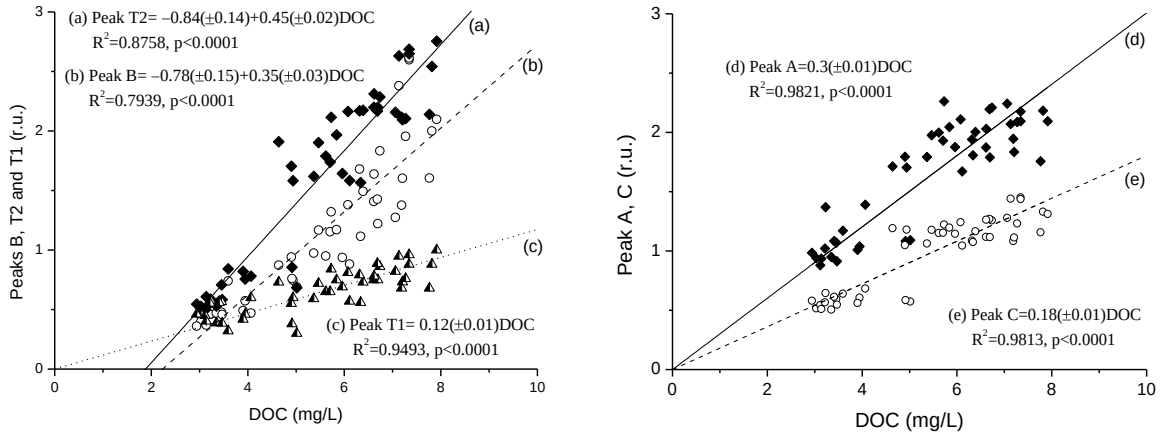


Figure 20: X-Y plots on the left being (a) Peak B x DOC, (b) Peak T2 x DOC and (c) Peak T1 x DOC, and plots on the right being (d) Peak A x DOC and (e) Peak C x DOC. Solid and dashed lines represent the corresponding regression. R^2 is the adjusted R square and p is the significant level. Batch incubations were performed for samples collected during summer (05 Dec 2013) at all sampling sites (n=46), considering open (Experiment A) and closed (Experiment B) systems. Incubation time: 10 days.

The decomposition of organic matter derived from humic compounds showed different intensities and consumption pattern between the sites monitored. Figure 21 presents the relationship between the intensity of fluorescence peaks A and C with DOC for Experiment A and B (open and closed system) during two sampling (Oct/2013 and Dec/2013). Peaks A and C, commonly related to humic derived compounds (Coble, 1996), were less intense and with a slow decrease at site IG01 compared to sites IG02 and IG05. One hypothesis is that at sites IG02 and IG05 there are more adapted microorganisms for biodegradation, since both sites receive effluents from sewage treatment plants. The experiments were performed without the addition of bacteria, since the objective was to analyze the biodegradation process with little interference or modifications. In addition, the

higher concentration of labile organic matter at sites IG02 and IG05 could facilitate the decomposition of complex compounds during the incubation period. Labanoviski et al. (2009) also supports the hypothesis that the abundance of easily biodegradable molecules may increase the degradation of more refractory organic matter in surface water by microorganisms. The decomposition of labile compounds contributes a primary source of energy. This energy is thus utilized in the degradation of aromatic structures which requires a great quantity of energy. Consequently, the microbial assimilation and respiration in anthropogenic impacted rivers is a rapid process, since DOM derived mainly from effluents has a labile composition. DOM observed in stream waters without or with low influence of effluents is typically recalcitrant to microbial degradation, showing a slow mineralization process (Mostofa et al., 2013b). Indeed, samples collected from sites downstream of sewage discharges (e.g. IG02) generally showed higher values of biodegradation rates.

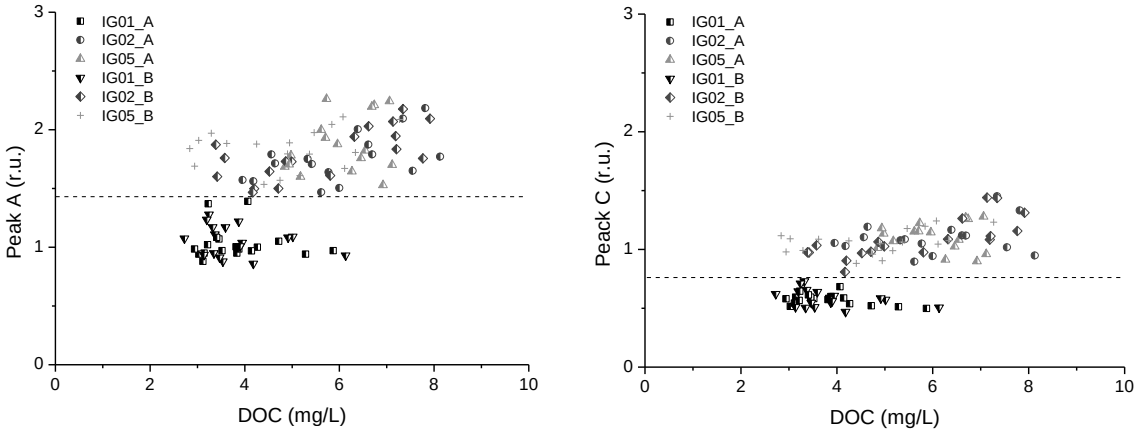


Figure 21: X-Y plots of Peak A x DOC (left) Peak C x DOC (right). Dashed lines separate data from site IG01 to IG02 and IG05. Batch incubations were performed for samples collected during Oct/2013 and Dec/2013, considering open (Experiment A) and closed (Experiment B) systems (n= 107). Incubation time: 10 days.

The variation of fluorescence EEMs between monitoring sites and incubation time indicates the pattern of biodegradation according to the level of organic pollution and the preferred compounds to be biologically degraded. Figure 22 presents a comparison of fluorescence EEMs for sites IG01, IG02, and IG05, considering Experiment A for sampling performed in Dec/2013. The results from EEMs indicated the decrease of fluorescence peaks intensity along the incubation period, which can be related to the consumption of specific organic compounds. The presence of labile organic matter (Peaks B, T_2 and T_1), was evident at sites IG02 and IG05. At IG01, the low rates of anthropogenic occupation reflects on the water quality, where the most expressive fluorescence intensity were located on the emission-excitation region often related to humic compound.

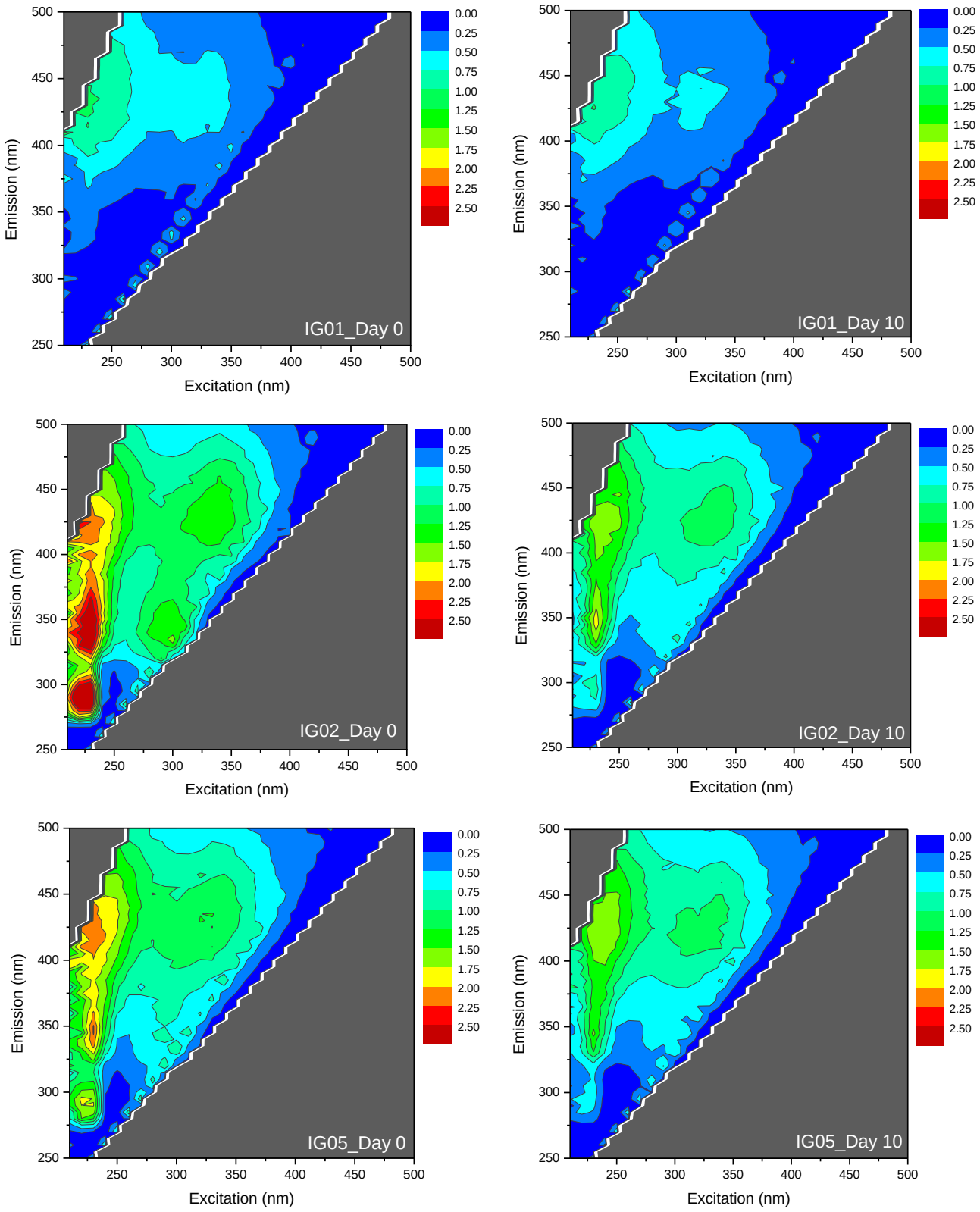


Figure 22: Excitation-emission matrices (EEMS) for sites IG01 (top), IG02 (middle), and IG05 (bottom). Batch incubations were performed for samples collected during Dec/2013, considering an open system (Experiment A). Incubation time: 10 days. Fluorescence intensities are in Raman units.

In particular, comparing EEMs at initial condition and the peaks intensities after 10 days (Figure 22), fluorescence peaks located at approximately 210/230 nm excitation and 300/310 nm emission showed a significant consumption for sites IG02 and IG05. This fluorophore, which has been attributed to a tyrosine-like fluorescence peak (Peak B - Coble, 1996) was the first group of compound consumed during the biodegradation experiment. The percentage of reduction from the initial values of Peak B were 13%, 73%, and 59% respectively for sites IG01, IG02 and IG05. Peaks T_2 and T_1 also showed significant reduction for the most polluted sites (39% and 48% for site IG02, and 31% and 30% for site IG05, respectively). For site IG01, peaks T_2 and T_1 where not visually significant, and had a small decrease, with 15% and 18%, respectively.

The degradation of complex compounds, such as humic and fulvic acids, occurred for all sites evaluated. Peak A, related to humic acid-like fluorescence, showed percentages of reduction of 7%, 27% and 24% for sites IG01, IG02 and IG05. The presence of labile organic matter may facilitate the decomposition of humic acids through a co-metabolism, i.e., utilization of energy released by the first step of degradation (Labanoviski et al., 2009). Another hypothesis for the consumption of more complex compounds at samples from sites IG02 and IG05 is the presence of more adapted microorganisms. Sites IG02 and IG05 are located downstream of urbanized areas, with inputs of raw and treated sewage. Thus, the presence of both labile organic matter and a high amount of microorganisms could increase the consumption of organic matter constituted by complex compounds. Peak C, also related to humic-like fluorescence (Coble, 1996), showed percentages of reduction of 13% and 20% for sites IG01 and IG02. At site IG05, the intensity of peak C was not relevant, with a reduction of 6% from the initial intensity.

The relationship between $SUVA_{254}$ and DOC is another evidence of the consumption of labile organic matter. Figure 23 presents the values of $SUVA_{254}$ for the biodegradation Experiments A and B. As DOC concentration decreases over time during the incubation period, values of $SUVA_{254}$ increased. Saadi et al. (2006) supports the hypothesis that during the incubation period there is a selective degradation of DOM with a lower UV absorption, which results in an increase of $SUVA_{254}$. Biological activity tends to remove the fraction that is not sensitive to UV emission, i.e., the most labile one that do not absorb in the UV-Vis region (Musikavong and Wattanachira, 2007). Since not all the organic matter is decomposed, the remaining organic compounds and small carboxylic acids as organic matter contribute to the organic carbon concentration (Thomas et al., 2007).

Under the conditions of Experiment A (open/aerated system), the specific absorptivity at the 254 nm wavelength decreased during the first days of incubation. Figure 24 presents the normalized specific absorptivity at 254 nm for Experiment A. The decrease of the specific absorptivity at 254 nm indicates that labile organic matter (protein-like or tryptophan-like) had been metabolized by microbial activity. Moreover, an increase of specific absorptivity for sites IG02 and IG05 at the end of incubation period (days 9 and 10) may indicate that nitrification occurred in samples collected during Oct/2013. Nitrification changes the absorptivity spectrum in the range of 200 to 275 nm wavelength according to the respective concentration (Roig et al., 2007). The more concentrate nitrate is in the water, the higher the value of specific absorption in the short wavelength. For Experiment A, the level of dissolved oxygen stabilized at approximately 7 mg/L. For the closed system, where the dissolved oxygen was constantly consumed, this change in the ultraviolet spectrum was not observed.

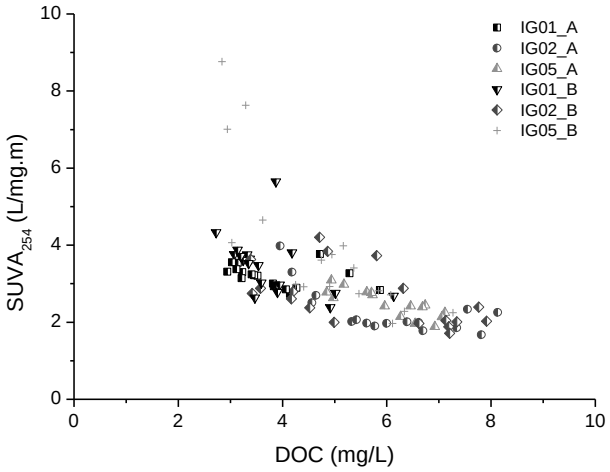


Figure 23: Relationship between $SUVA_{254}$ and DOC for Experiment A and B considering samples collected in Oct/2013 and Dec/2013, for sites IG01, IG02, and IG05 (n=107). Incubation time: 10 days.

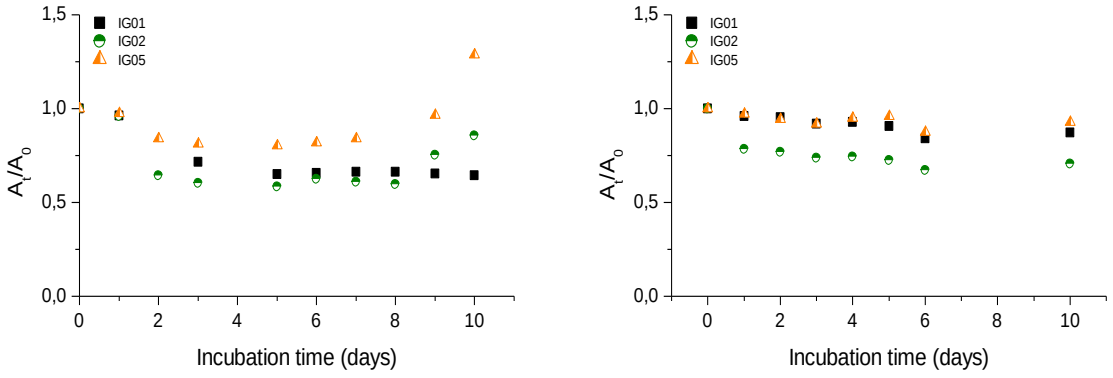


Figure 24: Variation of the normalized specific absorptivity at 254 nm wavelength for Experiment A (open system) considering samples collected on Oct/2013 (left) and Dec/2013 (right), for sites IG01, IG02, and IG05. Incubation time: 10 days.

Both increase and decrease of absorption observed in different studies (Trulleyová and Rulík, 2004; Saadi et al., 2006; Thomas et al., 2007; Mostofa et al., 2013b) occurs since biological decomposition changes the chromophoric dissolved organic matter (CDOM) properties. An increase of CDOM absorption in the longer wavelength region may be due to the presence of fulvic acids from upstream regions after photo induced or microbial degradation. Secondly, a decrease of CDOM absorption in the shorter wavelength region may result from the presence of autochthonous (protein-like or tryptophan-like) and agricultural CDOM, which are likely to undergo rapid microbial degradation (Mostofa et al., 2013b). In non-impacted waters, where fulvic acids predominate, studies demonstrate that biodegradation can modify the composition of fulvic acids, showing an increase in CDOM absorption (Mostofa et al., 2007; Musikavong and Wattanachira, 2007).

6.3.4 Organic Carbon Biodegradation Kinetics

The biodegradation kinetics estimated for DOC, TOC, and POC indicates probable differences in organic matter composition (labile and refractory pools) and, thus, the respective decomposition rates. Table 11 presents a summary of the results of decomposition rates, decay model parameters, and labile and refractory fractions of organic carbon from batch incubations considering an exponential decay model with labile and refractory pools (Model 1). Table 12 presents a summary of the results for biodegradation experiment considering a first-order exponential decay model (Model 2). A comparison between the refractory and labile fractions for experiments A and B is presented at Figure 25.

Table 11: Kinetic coefficients and parameters of kinetic model of BDOC, BTOC, and BPOC, considering labile and refractory pools of organic carbon (Model 1).

Site/ Experiment		Parameter	Initial Value (mg/L)	Biodegradable fraction [a]		Model: $C_t=C_L*\exp(-kt)+C_R$			Labile fraction[b] (%)	Refractory fraction[b] (%)	Fitness	
				(mg/L)	(%)	Labile - C_L (mg/L)	Refractory - C_R (mg/L)	k (day^{-1})			ENS	MSE
IG01	3A	DOC	5,29 ± 0,29	2,14	41%	2,99 ± 1,42	2,58 ± 1,55	0,13 ± 0,13	57%	49%	0,9951	0,0910
		TOC	6,06 ± 0,94	2,53	42%	4,21 ± 1,37	1,95 ± 1,44	0,10 ± 0,05	69%	32%	0,9991	0,0203
		POC	0,77 ± 1,23	0,38	50%	-						
	3B	DOC	6,13 ± 0,09	3,41	56%	2,91 ± 0,24	3,16 ± 0,10	0,83 ± 0,16	47%	52%	0,9974	0,0368
		TOC	6,42 ± 0,04	2,63	41%	2,51 ± 0,34	3,92 ± 0,13	1,02 ± 0,33	39%	61%	0,9963	0,0705
		POC	0,29 ± 0,13									
	4A	DOC	4,06 ± 0,24	0,85	21%	0,97 ± 0,13	3,08 ± 0,07	0,75 ± 0,25	24%	76%	0,9992	0,0089
		TOC	6,51 ± 0,85	2,80	43%	2,75 ± 0,21	3,81 ± 0,17	0,42 ± 0,08	42%	59%	0,9991	0,0205
		POC	2,44 ± 0,88	1,95	80%	1,99 ± 0,44	0,50 ± 0,45	0,25 ± 0,13	81%	21%	0,9822	0,0439
	4B	DOC	5,01 ± 0,10	1,11	22%	1,79 ± 0,43	3,43 ± 0,28	0,60 ± 0,35	36%	68%	0,9939	0,0960
		TOC	7,29 ± 0,08	2,90	40%	4,24 ± 1,61	3,12 ± 1,72	0,12 ± 0,08	58%	43%	0,9983	0,0606
		POC	2,27 ± 0,18	1,78	78%							
IG02	3A	DOC	8,13 ± 0,58	4,18	51%	3,85 ± 0,67	4,33 ± 0,64	0,29 ± 0,15	47%	53%	0,9927	0,2463
		TOC	8,45 ± 0,57	4,37	50%							
		POC	0,32 ± 1,15	0,20	75%							
	3B	DOC	5,81 ± 0,19	1,29	22%	2,37 ± 0,16	3,38 ± 0,12	0,48 ± 0,08	41%	58%	0,9993	0,0119
		TOC	7,94 ± 0,13	2,10	26%	3,16 ± 0,96	4,83 ± 0,47	1,12 ± 0,87	40%	61%	0,9865	0,4284
		POC	2,14 ± 0,32									
	4A	DOC	6,57 ± 0,10	1,28	22%							
		TOC	11,25 ± 0,02	5,23	47%	7,19 ± 10,95	3,34 ± 11,41	0,09 ± 0,20	64%	30%	0,9899	0,7815
		POC	4,68 ± 0,10	3,95	74%	3,25 ± 0,77	1,44 ± 0,39	0,85 ± 0,49	70%	31%	0,9487	0,3109
	4B	DOC	7,91 ± 0,09	1,60	20%	1,10 ± 0,44	6,81 ± 0,25	0,74 ± 0,71	14%	86%	0,9983	0,0879
		TOC	13,59 ± 0,55	6,94	51%	6,11 ± 1,94	6,87 ± 2,07	0,23 ± 0,18	45%	51%	0,9935	0,6701
		POC	5,67 ± 0,64	5,34	94%	5,00 ± 1,38	0,23 ± 1,49	0,22 ± 0,14	88%	4%	0,9744	0,2727
IG05	3A	DOC	7,12 ± 0,01	3,16	44%							
		TOC	10,37 ± 0,62	5,95	57%	8,37 ± 4,40	2,04 ± 4,68	0,11 ± 0,10	81%	20%		
		POC	3,25 ± 0,63	2,80	86%	3,14 ± 0,97	0,37 ± 0,97	0,28 ± 0,25	96%	11%	0,8918	0,4200
	3B	DOC	5,16 ± 0,12	2,22	43%	3,68 ± 3,03	1,57 ± 3,20	0,10 ± 0,14	71%	30%	0,9925	0,1211
		TOC	6,82 ± 0,16	2,68	39%	3,03 ± 0,53	3,79 ± 0,57	0,25 ± 0,13	44%	56%	0,9954	0,1176
		POC	1,65 ± 0,28	0,45	28%							
	4A	DOC	6,74 ± 0,11	1,80	27%	2,22 ± 1,88	4,46 ± 2,00	0,13 ± 0,19	33%	66%	0,9977	0,0796
		TOC	9,17 ± 0,24	4,09	45%							
		POC	2,43 ± 0,26	2,29	94%							
	4B	DOC	7,27 ± 0,20	2,37	33%	1,62 ± 0,57	5,64 ± 0,25	1,33 ± 1,30	22%	78%	0,9951	0,1723
		TOC	9,36 ± 0,11	4,36	47%							
		POC	2,10 ± 0,30	1,99	95%							

[a] Estimated by the difference between the initial and final observed data

[b] Estimated by the model parameters and the initial observed data

Table 12: Kinetic coefficients and parameters of kinetic model of BDOC, BTOC, and BPOC, considering first-order exponential decay (Model 2).

Site/ Experiment		Parameter	Initial Value (mg/L)	Biodegradable fraction [a] (mg/L)	(%)	Model: C_t=Co*exp(−kt) Co (mg/L)	k (day^{-1})	Fitness ENS	MSE
IG01	3A	DOC	5,29 ± 0,29	2,14	41%	5,45 ± 0,22	0,05 ± 0,01	0,9948	0,0962
		TOC	6,06 ± 0,94	2,53	42%	6,09 ± 0,11	0,06 ± 0,004	0,9990	0,0225
		POC	0,77 ± 1,23	0,38	50%				
	3B	DOC	6,13 ± 0,09	3,41	56%	4,98 ± 0,37	0,06 ± 0,02	0,9820	0,2568
		TOC	6,42 ± 0,04	2,63	41%	5,21 ± 0,42	0,04 ± 0,02	0,9811	0,3639
		POC	0,29 ± 0,13						
	4A	DOC	4,06 ± 0,24	0,85	21%	3,62 ± 0,17	0,02 ± 0,01	0,9947	0,0591
		TOC	6,51 ± 0,85	2,80	43%	5,94 ± 0,30	0,06 ± 0,01	0,9935	0,1522
		POC	2,44 ± 0,88	1,95	80%	2,40 ± 0,19	0,16 ± 0,03	0,9805	0,0483
	4B	DOC	5,01 ± 0,10	1,11	22%	4,51 ± 0,38	0,04 ± 0,02	0,9826	0,2742
		TOC	7,29 ± 0,08	2,90	40%	7,23 ± 0,20	0,05 ± 0,01	0,9981	0,0696
		POC	2,27 ± 0,18	1,78	78%	2,66 ± 0,38	0,07 ± 0,04	0,9474	0,2412
IG02	3A	DOC	8,13 ± 0,58	4,18	51%	7,64 ± 0,41	0,06 ± 0,01	0,9906	0,3201
		TOC	8,45 ± 0,57	4,37	50%	9,55 ± 0,59	0,07 ± 0,01	0,9873	0,6493
		POC	0,32 ± 1,15	0,20	75%				
	3B	DOC	5,81 ± 0,19	1,29	22%	5,21 ± 0,27	0,06 ± 0,01	0,9937	0,1146
		TOC	7,94 ± 0,13	2,10	26%				
		POC	2,14 ± 0,32						
	4A	DOC	6,57 ± 0,10	1,28	22%	7,16 ± 0,51	0,03 ± 0,02	0,9878	0,5240
		TOC	11,25 ± 0,02	5,23	47%	10,44 ± 0,66	0,05 ± 0,02	0,9898	0,7848
		POC	4,68 ± 0,10	3,95	74%	3,97 ± 0,75	0,21 ± 0,08	0,8923	0,6525
	4B	DOC	7,91 ± 0,09	1,60	20%	7,55 ± 0,23	0,02 ± 0,01	0,9981	0,0984
		TOC	13,59 ± 0,55	6,94	51%	12,39 ± 0,69	0,06 ± 0,01	0,9927	0,7591
		POC	5,67 ± 0,64	5,34	94%	5,18 ± 0,49	0,20 ± 0,04	0,9743	0,2735
IG05	3A	DOC	7,12 ± 0,01	3,16	44%	7,17 ± 0,36	0,05 ± 0,01	0,9928	0,2502
		TOC	10,37 ± 0,62	5,95	57%	10,31 ± 0,44	0,08 ± 0,01	0,9942	0,3365
		POC	3,25 ± 0,63	2,80	86%	3,42 ± 0,58	0,21 ± 0,07	0,8896	0,4286
	3B	DOC	5,16 ± 0,12	2,22	43%	5,20 ± 0,25	0,06 ± 0,01	0,9924	0,1230
		TOC	6,82 ± 0,16	2,68	39%	6,45 ± 0,29	0,05 ± 0,01	0,9935	0,1672
		POC	1,65 ± 0,28	0,45	28%				
	4A	DOC	6,74 ± 0,11	1,80	27%	6,59 ± 0,22	0,03 ± 0,01	0,9976	0,0833
		TOC	9,17 ± 0,24	4,09	45%	8,77 ± 0,57	0,04 ± 0,02	0,9898	0,6068
		POC	2,43 ± 0,26	2,29	94%				
	4B	DOC	7,27 ± 0,20	2,37	33%	6,53 ± 0,36	0,03 ± 0,01	0,9929	0,2516
		TOC	9,36 ± 0,11	4,36	47%	9,96 ± 0,86	0,04 ± 0,02	0,9818	1,3474
		POC	2,10 ± 0,30	1,99	95%				

[a] Estimated by the difference between the initial and final observed data

The observed and estimated data of the three sites monitored presented variation, indicating that both intrinsic properties of each monitoring site and sampling period affect the organic matter biodegradation. Site IG01, for an average initial concentration of 5.75 mg/L, and a BDOC estimated in 1.98 mg/L, had an estimated labile and refractory content of 52% and 50% during Oct/13, and 36% and 68% during Dec/13, respectively (Model 1). Site IG02 showed similar results, with an average estimate percentage of refractory DOC of 56% and 81% (Model 1), respectively for sampling performed during Oct/13 and Dec/13. In the case of IG02, the initial average DOC where 6.49 mg/L, with an estimated BDOC of 2.73 mg/L and 1.22 mg/L, respectively in Oct/13 and Dec/13. Complementarily, site IG05 had higher amount of labile DOC during Oct/13 (71%), with an estimated 72% of refractory DOC during Dec/13, for an initial DOC of 6.57 mg/L and estimated BDOC of 2.39 mg/L (Table 11). Thus, samples from Dec/13 had a common characteristic to have a higher proportion of refractory organic carbon (68%, 81%, and 72%, respectively for sites IG01, IG02, and IG05, based on the average estimated by Model 1). Complementarily, the average DOC kinetics rates were also higher during second sampling, indicating that the labile organic matter in the samples were rapid degraded (IG01: 0.48 d^{-1} and 0.60 d^{-1}; IG02: 0.38 d^{-1} and 0.75 d^{-1}; and IG05: 0.10 d^{-1} and 0.73 d^{-1}, respectively for the two samplings, Model 1).

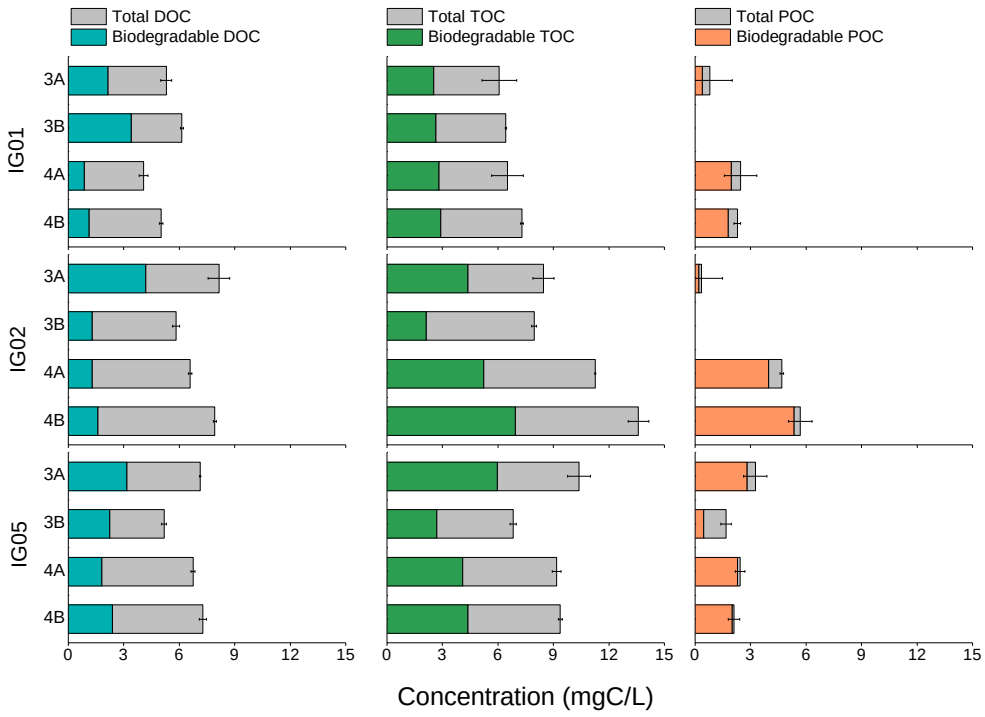


Figure 25: Variation of total and biodegradable concentration of DOC, TOC, and POC, for experiment performed in Oct/13 (3A and 3B, respectively open and closed system) and experiment performed in Dec/13 (4A and 4B, respectively open and closed system), sites IG01, IG02, and IG05.

Site IG01 is characterized by a less anthropogenic impact then other sites monitored. Consequently, the amount of effluent organic matter is not as high as observed at site IG02, and, with less proportion, at site IG05. However, under the conditions of the experiment, and considering the results of only two samplings, Model 1 indicated that the difference observed during the two samplings performed may have other factors influencing the amount of labile and refractory organic matter than only watershed main organic matter sources (e.g., effluent organic matter).

The specific absorptivity at 254 nm for Experiment A and sampling performed in Oct/13 (Figure 22) may indicate that nitrification occurred during the last days of incubation. In addition, nitrogen compounds were significantly higher during Oct/13. One condition to occur nitrification is the presence of dissolved oxygen, which was stable at approximately 7 mg/L for samples in Experiment A (open system). Thus, if nitrification occurred, it may have been a source of energy to the microorganism to convert inorganic carbon into organic compounds. Consequently, this process could affect the concentration of organic carbon in the sample, and the related decomposition kinetic rates estimated by the regression models.

Complementarily to the characteristics of each site, and the period of sampling, the reactor system, the organic carbon fraction and the type of regression model can result in different kinetics rates. Figure 26 and 27 presents the results for the estimation of the biodegradation kinetics rates for DOC, TOC, and POC, considering both samplings (Oct/13 and Dec/13), the two types of reactors analyzed (Open – Exp A, and Closed – Exp B), respectively for regression Model 1 and Model 2.

The characteristics of organic matter decay according to Model 1, comparing the open and closed systems, presented differences on the estimation of kinetics rates (Figures 26 and 27). For open (Experiment A) and closed (Experiment B) system, DOC decay presented less variation over consecutive days, and showed similar proportion of estimated labile and refractory DOC. In contrast, the decay of TOC presented distinct patterns for the systems analyzed. For site IG01, Experiment A resulted in 69% of labile content, while for Experiment B, the same sample resulted in 39% of labile TOC (Table 11, Figure 28). The respective kinetics rates for TOC were also distinct, 0.1 d^{-1} and 1.02 d^{-1}, respectively for open and closed system (Figure 28). Site IG02 also showed a higher TOC kinetic rate for labile content for closed system (0.23 d^{-1}) than for open system (0.09 d^{-1}). A similar difference was also observed at site IG05 for percentage of labile TOC (81% and 44%, for Experiment A and Experiment B, respectively) and kinetics rates (0.11 d^{-1} and 0.25 d^{-1}, respectively) but, in this case, the initial concentration of TOC may have influence on the results (10.37 mg/L for Experiment A, and 6.82 mg/L for Experiment B).

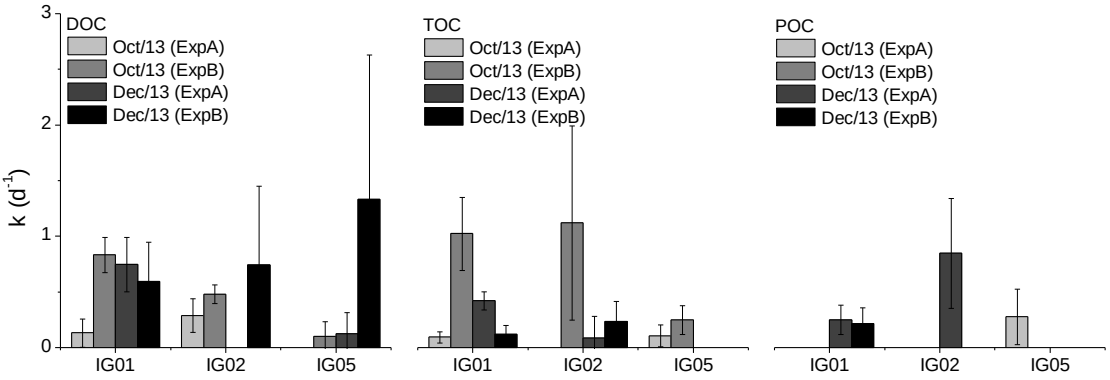


Figure 26: Variation on kinetics rates for DOC, TOC, and POC decay (Model 1) for sites IG01, IG02, and IG05, comparing Experiment A and B, for samplings performed at Oct/13 and Dec/13.

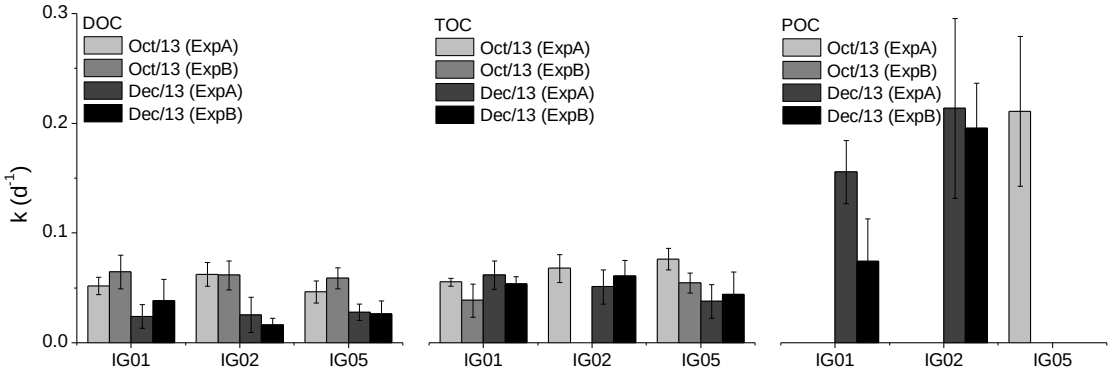


Figure 27: Variation on kinetics rates for DOC, TOC, and POC decay (Model 2) for sites IG01, IG02, and IG05, comparing Experiment A and B, for samplings performed at Oct/13 and Dec/13.

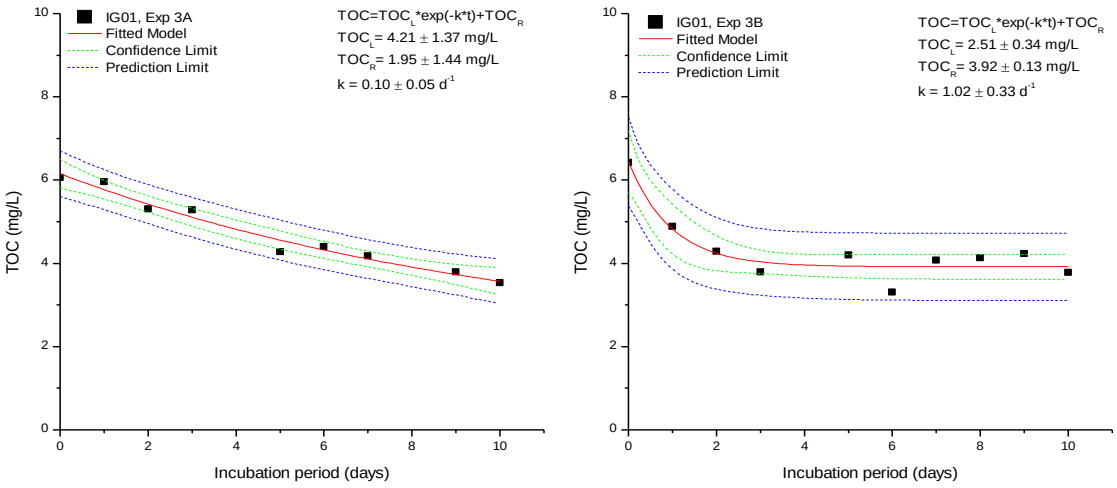


Figure 28: Example of decomposition curves showing the variation of TOC concentration for site IG01 (Model 1, Oct/2013), Experiment A (left) and Experiment B (right).

The decay of TOC, DOC, and POC during the incubation period indicated differences between degradation rates according to the organic carbon fractions. Figure 29 presents an example of decomposition patter for TOC, DOC, and POC for sites IG01 (Model 1, Dec/2013, Experiment A) and IG02 (Model 1, Dec/2013, Experiment B) during the 10 days incubation period. There was not a unique decay pattern among the fractions and sites considered. DOC concentration presented less variability than TOC and POC. Under the conditions of the experiment conducted for sampling performed in Dec/13, in average the decomposition of labile DOC reached its final stage during the fourth day of incubation, with a decay rate of 0.75 ± 0.25 d^{-1} and 0.74 ± 0.25 d^{-1}, respectively for sites IG01 and IG02. For site IG02, TOC and POC presented more variability, with an average kinetics rate of 0.23 ± 0.18 d^{-1} and 0.22 ± 0.12 d^{-1}, respectively for TOC and POC.

Generally, DOC observed data were more representative for model fitness than POC and TOC. When it was possible to fit the regression model, the kinetics rates estimated for POC decay also presented higher variability than DOC and TOC (Figures 26 and 27). In fact, POC and TOC evaluation are susceptible to errors and/or interference of other process that are not being controlled. One example is the sedimentation and ressuspention, which can underestimate or overestimate the amount of POC, and thus, TOC concentration. In addition, the results could be to small differences when shaking the sample and incubating in separate bottles. A formation of a biofilm in the shaking and aeration apparatus, and consequently a release of the adsorbed compounds when collecting the sub-samples, could alter the results. In Experiment A, shaking also allows the particulate material to remain in the water column, while in Experiment B settling occurred during the first hours of incubation.

The regression model considering labile and refractory pools of organic carbon was fitted considering 10 days of incubation time. Other studies indicated that this type of regression can better explain the organic carbon decay for a long period, such as more than hundred days (Lønborg et al., 2009) or years (Koehler et al., 2012). Besides the analysis of the coefficients used to test the goodness of fit (Table 3) show that Model 1 can explain the observed data, further investigations considering long period of incubation are relevant. The results presented here highlight an important result that the labile organic matter is fast degraded independent of pollution level.

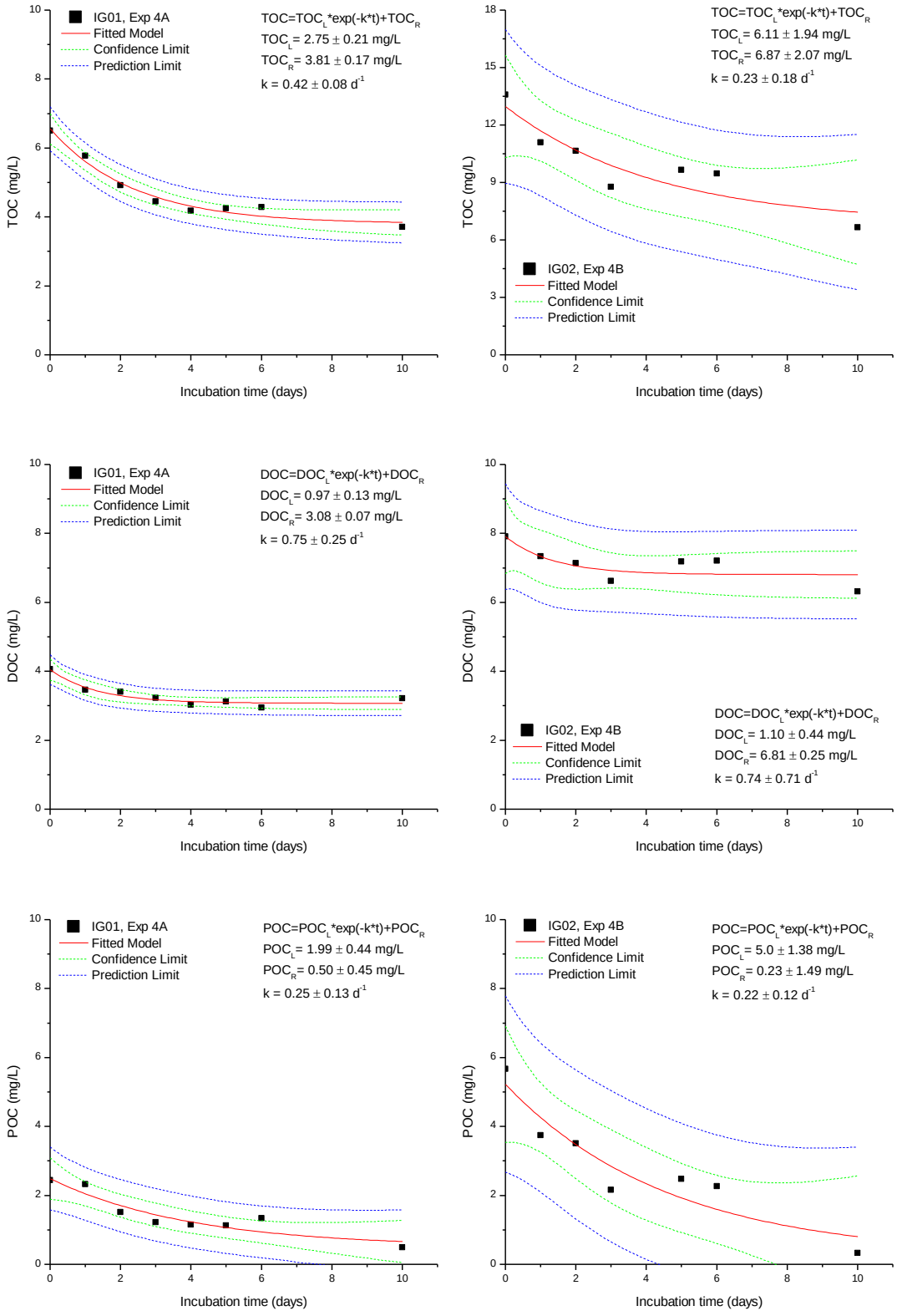


Figure 29: Example of decomposition curves showing the variation of TOC, DOC, and POC concentration for site IG01 (left) (Model 1, Dec/2013, experiment A, Open system with added nutrients) and site IG02 (Model 1, Dec/2013, experiment B, closed system with added nutrients).

The first-order exponential decay (Model 2) also explained the observed data (Table 12). The estimated kinetics rates obtained in this case were significantly lower (an average of 0.07 d^{-1}, with a maximum of 0.21 d^{-1}), with small or without difference between the two experiments (A and B) and samplings performed (Figure 29). Khan et al. (2005) also found DOC kinetic rates in the range between 0.05 and 0.08 d^{-1} for secondary effluent samples with an initial DOC concentration from 6.73 to 11.45 mg/L (28 days of incubation time). Koehler et al. (2012), considering a decay model with labile and refractory pools (Model 1, and long period of incubation), found even lower DOC kinetics rates for lake waters. According to their results, for clearwater lakes (DOC ranging from 4.6 to 15.84 mg/L) the average DOC kinetic rate was 0.0042 ± 0.0012 d^{-1}, and for brownwater lakes (DOC ranging from 14.63 to 24.82 mg/L), the average DOC kinetic rate was 0.0013 ± 0.0002 d^{-1}. The small values obtained by Koehler et al. (2012) are for lake water, with a refractory predominance, indicating, thus, the slow rate in which complex compounds are degraded in aquatic environments.

6.3.5 BOD and DOC Decomposition Kinetic Rates

The BOD decomposition kinetics can be described with a first-order exponential decay (Model 2). According to the model's assumption, the rate in which the organic matter is decomposed is proportional to the remaining organic matter in the sample. In other words, this model represents the decomposition of the bioavailable organic matter present in the sample.

The biodegradability of DOC and BOD has distinct characteristics. Figure 30 presents a comparison between DOC kinetics rates for monitoring site IG02, comparing Models 1 and 2, for samplings performed in Oct/13 and Dec/13. Figure 31 presents the BOD deoxygenation rate for site IG02, considering the same sampling period. Regression Model 2 was used for BOD kinetic rates evaluation. For the comparative analysis between DOC and BOD decomposition kinetics rates, only the results of Experiment B (closed system) are discussed. BOD was determined by respirometric method (Oxitop). Further information about BOD deoxygenation data and methods of evaluation can be found at Appendix 4.

According to the results, and under experiments conditions, BOD deoxygenation rate was equal to 0.14 d^{-1} for both samplings (Figure 31). Besides the same value obtained, an analysis of other samplings show a variation between 0.14 d^{-1} and 1.03 d^{-1} (Appendix 4). For DOC (Figure 32), the kinetic rates obtained from Model 1 were 0.74 ± 0.71 d^{-1} and 0.48 ± 0.05 d^{-1}, respectively for Oct/13 and Dec/13. The results from Model 2 were smaller, with 0.06 ± 0.01 d^{-1} and 0.02 ± 0.006 d^{-1}, respectively for Oct/13 and Dec/13. In terms concentration, BOD_5 was 20 mg/L for both samplings, while DOC for Oct/13 was 5.81 ± 0.19 mg/L, and in Dec/13 was 7.91 ± 0.09 mg/L.

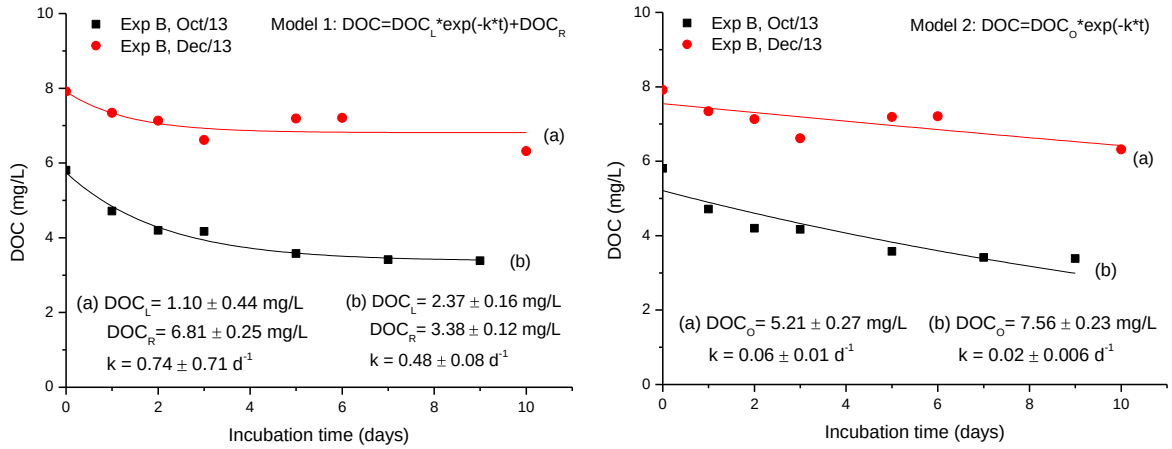


Figure 30: Comparison between DOC kinetics rate for site IG02, Experiment B, for samplings performed in Oct/13 and Dec/13, considering Model 1 (left) and Model 2 (right) for regression analysis. Incubation time: 10 days.

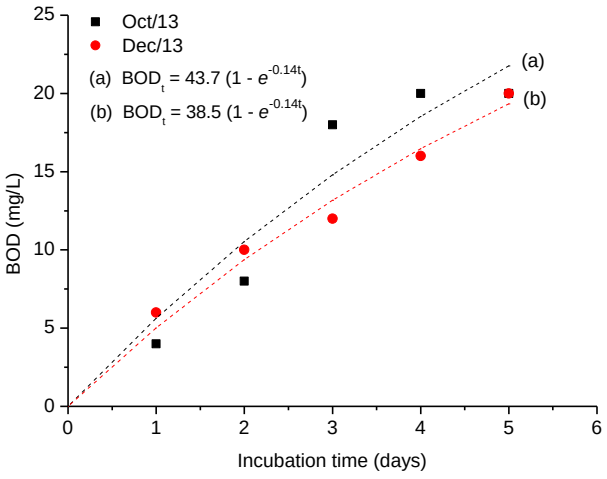


Figure 31: BOD kinetics rate for site IG02, for samplings performed in Oct/13 and Dec/13, considering Model 2 for regression analysis. Incubation time: 5 days.

The BOD deoxygenation rate depends on organic matter characteristics and sources (raw domestic effluents show higher values, while unpolluted waters have low values). Since it is a biological experiment, the properly determination of BOD kinetic rates may be affected by inhibitors, changes in the incubation temperature, presence of light (primarily production can increase oxygen levels), insufficient amount of initial microorganisms, rapid consumption of oxygen levels and anoxic condition. As BOD analysis, DOC biodegradability experiments are also susceptible of these interferences.

One difference between the experimental determination of DOC and BOD decomposition rates is the period necessary to complete de analysis. While 5 days are generally enough to the analysis of BOD, for biodegradability decomposition of DOC it is necessary more time to properly estimate the labile and the refractory pools. Thus, in the case of DOC (and other fractions, POC and TOC), and considering that the organic matter is not only formed by labile compounds, the hypothesis of a refractory pool can be a better assumption when analyzing the kinetics of organic carbon (Model 2).

6.3.6 Contribution of Biodegradability Analysis for Water Quality Modeling and Monitoring Strategies

Considering the water quality planning and management perspective, the evaluation of the biological decomposition in a polluted and urbanized river is essential to understand the fate and transformation mechanisms of organic matter under critical conditions. According to the results, DOC concentration ranged from 4.1 mg/L to 8.1 mg/L, while the average percentage of biodegradable DOC content in the samples ranged from 20% to 56%. Rather than only an exploratory analysis to evaluate biodegradability characteristics, these data indicates the presence of compounds potentially important for dissolved oxygen consumption in stream waters. In addition, differences between a non- impacted site (IG01) and sites located downstream of a highly urbanized region (IG02 and IG05), indicated that the compounds estimated have distinct properties and consequently, different degradation mechanisms.

The use of spectrophotometric techniques was suitable for a qualitative approach to identify and to evaluate the decay patterns of organic matter in stream water. $SUVA_{254}$ correlated inversely with DOC concentration, confirming the initial consumption of low weight compounds. The variation of the absorptivity spectrum during incubation period also indicated the beginning of nitrification under an aerated condition (Experiment A).

Fluorescence intensity peaks and ratios allowed estimating qualitative changes during biodegradation experiments. The variation of tryptophan and tyrosine like fluorescence peaks (peak T and B) confirms the rapid consumption of the labile fraction of organic matter in the first days of an experiment, with a large decay at sites IG02 (75%, 39%, and 48%, respectively) and IG05 (59%, 31%, and 30%, respectively). The results also showed a higher decrease of fluorescence peaks A and C intensities (humic compounds) at polluted sites (IG02 and IG05), corroborating to the hypothesis of a co-metabolism and energy exchange. As the labile organic matter is assimilated, the energy release may facilitate the degradation of complex molecules by biological activities (Volk et al., 1997; Labanoviski et al., 2009). Stutter et al. (2013) also highlighted that the consideration that the humic and fulvic substances dominate aquatic DOC and thus, have limited bioavailability in aquatic ecosystems, is one reason for the limited understanding of aquatic DOC reactivity. Organic matter is a heterogeneous mixture of compounds with temporal, spatial and reactivity variation. The presence of labile organic matter, and its proper estimation, is thus essential to understand the organic matter dynamics.

In an effluent-dominated river, BDOC rates are also influenced by the nature of the wastewater treatment plant process (Khan et al, 1998). Depending up on the level of organic pollution removal, or the utilization of a biological treatment process, the remaining organic content may have labile or recalcitrant characteristics. Though significant efforts have been applied to organic matter characterization over the years, further data are required to evaluate the biodegradable characteristics of effluents, and, consequently, its impact when released to surface waters.

Rivers have intrinsic physical, chemical, and biological components. Such characteristics will influence the transport and consumption mechanisms. Moreover, it is still not possible to isolate and identify all the different compounds (Frimmel, 1998; Leenheer and Croué, 2003; Fillela, 2009; Sharma et al., 2011), since organic matter is a heterogeneous mixture from allochthonous sources or originates as a product of river metabolism. The characterization at a molecular level, and thus the understanding about processes that produce organic matter such as biodegradation, condensation reactions, photolysis, is still a challenge (Leenheer and Croué, 2003).

As biodegradation, the photo induced process has implications for the fate of organic matter in aquatic ecosystems. Photodegradation in urbanized rivers is a current focus of investigation (Meng et al., 2013), but the association with the biodegradation process for polluted rivers is not yet well documented. On the one hand, there are difficulties in

evaluating BDOC in effluent-dominant rivers, and there are additional important aspects to consider for areas with low or without any anthropogenic sources of organic matter. The transformation mechanism of plant-derived BDOC is one example. Factors such as plant production rates, plant physical structure, C/N ratio, grazing pressure, disturbance and plant fiber content may affect the rate at which BDOC is leached from plants and decaying algae and became available in surface waters (Bachand and Horne, 2000; Lim et al., 2008). In fact, rivers are a "complex experimental laboratory", with still interesting research challenges for organic matter identification, quantification and modeling.

6.3.7 Summary of Biodegradation Experiment Main Results

The biodegradation experiment results were significant enough to state that the integrated evaluation of biodegradation tests (BDOC, BPOC, and BTOC), absorbance, and fluorescence analyses can provide information about the biodegradability characteristics of the anthropogenic derived organic matter in urbanized rivers. The experimental results suggest that protocols usually applied for the dissolved fraction of organic matter (BDOC) can be applied to evaluate biodegradable fractions of POC and TOC. Therefore, the interpretation of presented coupled data are important and relevant to understand the transformation mechanisms and the resulting compounds.

According to the results, the anthropogenic derived organic matter was susceptible to spatial changes. Monitoring site IG02, located in the most urbanized region of the study, and site IG05, located downstream, were more affected by the occurrence of tryptophan-like and tyrosine-like fluorescence. Both sites also presented high values of biodegradable DOC, POC, and TOC consumption during the experimental test procedures. The higher decrease of humic-like fluorescence peak intensity at polluted sites (IG02 and IG05), indicated that as the labile organic matter is assimilated, the energy released may facilitate the degradation of complex molecules by biological activity. Thus, biological process, especially for urbanized rivers with high inputs of domestic effluents, can be considered as an important degradation mechanism of organic matter dynamics in streams.

6.4 Organic Carbon Modeling

The third part of this chapter focus on the modeling approach of the proposed model: ROCS- Model. The analysis herein included of the model implementation comprises four main subgroups: (i) Comparative analyses between numerical and analytical solutions; (ii) Test the anoxic condition; (iii) Analysis of the automatic calibration routine; and (iv) Comparative analysis between BOD and organic carbon simulations for Iguassu River. With this strategy, the final objective of this chapter last section is to summarize the main improvements in the understanding of the water quality modeling considering the use of organic carbon to represent the organic matter content in a polluted river.

First, the input data and model parameters are summarized. For the simulations of the Iguassu River, a pollution sources matrix was used to allocate the point sources, tributaries, and other properties of flow and specific loads. Complementarily, Palmital River, a tributary of the Iguassu River, was used for two analyses.

The first subgroup of analysis consists in the comparison between numerical and analytical models. The same input data were simulated in the proposed model (ROCS-Model) and using Qual2e (Q2E) model (Brown and Barnwell, 1985). The case study adopted for this case was the Palmital River, a tributary of the Iguassu River. Hydraulic conditions and water quality variables (BOD, DO, nitrogen, and phosphorous) were evaluated in this analysis.

A second analysis was conducted to evaluate anoxic conditions. In polluted rivers with low flow conditions, previous studies had observed low concentrations of DO (Knapik, 2009), which may lead to differences when simulation the oxygen demand for BOD modeling. For this analysis, the Palmital River was used as the case study. To force the anoxic condition, a hypothetic scenario with low dissolved oxygen level was assumed.

The automatic calibration routine, based on PSO technique, was also tested to evaluate the algorithm implemented and the particularities related to the simulation of the proposed model, ROCS-Model. Following the same approach of the mentioned sensibility analysis, the Iguassu River was considered as a case study. Observed data from 6 sites monitored along the Iguassu River were used during the calibration procedure.

Finally, with the calibrated model for the Iguassu River, a comparative analysis with other aggregated variables is also presented. The objective of this investigation is to evaluate the overall aspects relating to the simulation of BOD and the new proposed model of organic carbon as the variable to represent the organic matter in a river. The main results of each subgroup of analyses are presented in the next following items.

6.4.1 Model Input Data

As described in Chapter 5, the model developed has different modules to estimate the non-point and point sources for BOD, nitrogen, phosphorous, and organic carbon. In summary, the non-point sources are a function of the drainage area, the specific flow, the percentages of land use and occupation, and the specific export rates for each variable simulated. Three categories of land use and occupation has been defined: urban, agricultural, and forest. Table 13 summarizes the export rates adopted for model simulation. For the ROCS-Model input data, it has been assumed that the non-point sources contribute only to the refractory organic carbon pool (allochthonous pedogenic). The loss of POC and DOC according to different land use and occupation was based on the review described in details in Chapter 3.

Table 13: Export rates for the variables considered in the simulation

Variable	Export rates (kg/km².year)			Reference
	Urban	Agriculture	Forest	
BOD	15[a]	5[a]	7[a]	Porto et al. (2007)
Total Nitrogen	10 – 2000[b]	50 - 5000[b]	130 - 1020[b]	Chapra (1997); Von Sperling, 2007
Organic Nitrogen	137.4	137.4	82.4	
Ammonia	308.8	308.8	185.0	
Nitrite	25.0	25.0	15.0	
Nitrate	28.0	28.0	17.0	
Total phosphorous	10-1000[b]	50-5000[b]	10-2000[b]	
Organic phosphorous	50.0	25.0	5.0	
Inorganic phosphorous	50.0	25.0	5.0	
RPOC	500	1000	1000	Mattson (2009)
RDOC	500	1000	1000	

[a] Value in concentration (mg/L). [b] Values indicated in the references. The respective fractions of nitrogen and phosphorous were estimated through a proportion of the monitored data.

The point sources are categorized domestic effluent, industrial effluent, and tributaries. The domestic effluent is estimated through the number of inhabitants in each watershed. The model considers the total number of inhabitants and the respective number of inhabitants that has the effluent collected and treated. The effluent flow was calculated using an average per capita flow of 200 L/inh.d, and considering that 80% returns to the

system (Von Sperling, 2007). A specific load per capita is used for each variable simulated (Table 14), according to the references indicated.

In the case of organic carbon modeling, some assumptions had to be taken to allocate the labile and refractory compounds. While treated domestic effluent had been considered as refractory source of organic carbon (RPOC and RDOC), raw domestic effluent was considered to contribute to the labile pool (LPOC and LDOC). The specific per capita loads suggested by Servais et al. (1999) where adapted in this case (Table 14).

The industrial effluent was considered in the pollution sources matrix according to the industry location, effluent flow and concentration of pollutants simulated (Porto et al., 2007; Knapik, 2009). The tributaries were considered as point sources. For each tributary simulated, the domestic sources were estimated through the methodology mentioned above, considering the specific per capita loads (Table 14) according to each watershed number of inhabitants. The non-point sources were also estimated through the drainage area, percentage of land use and occupation, and the export rates (Table 13). To evaluate the estimation of the input data, the monitoring sites along the Iguassu River were used to compare the range of the estimations. Figure 32 presents the estimated and observed concentration of POC, DOC, and TOC at the six monitoring sites. The estimated concentration was calculated through the flow and the respective loads for the drainage area upstream each monitoring site.

Table 14: Specific per capita loads for the variables simulated

Variable	Per capita loads (g/inh.d)		Reference
	Usual range	Value considered	
BOD	40.0-60.0	54.4	WHO, 1982
Total Nitrogen	6.0-12.0	[a]	Chapra et al., 1997; Von Sperling, 2007
Organic Nitrogen	2.5-5.0	2.5	
Ammonia	3.5-7.0	6.4	
Nitrite	≈ 0	0	
Nitrate	0-5.0	0.25	
Total phosphorous	0.7-2.5	[a]	
Organic phosphorous	0.2-1.0	0.3	
Inorganic phosphorous	0.5-1.5	0.7	
RPOC	[b]	20	Servais et al., 1999
RDOC	[b]	8	
LPOC	[b]	5	
LDOC	[b]	4	

[a] Not direct simulated. [b] Servais et al. (1999) evaluated raw and treated wastewater as detailed in Chapter 3.

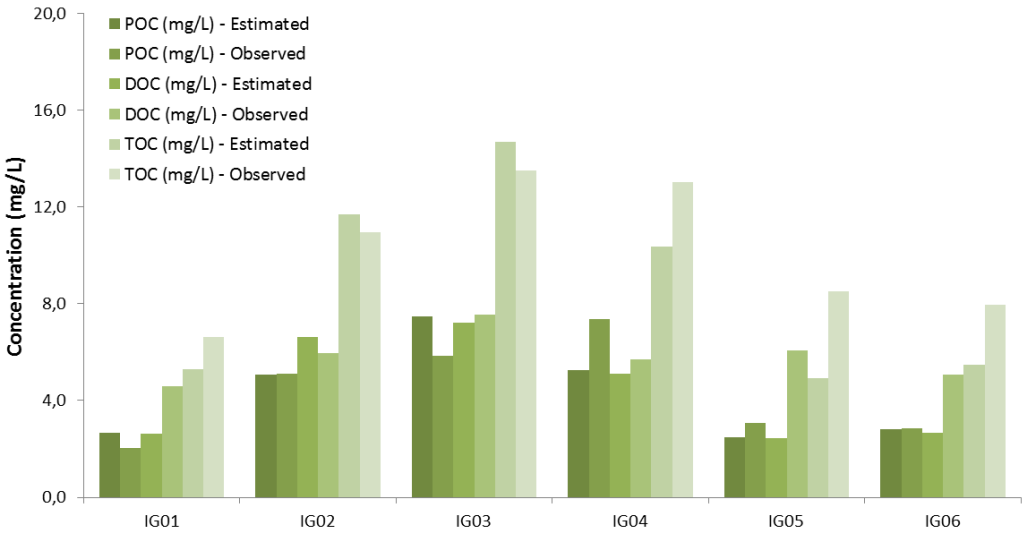


Figure 32: Estimated and observed data of DOC, POC, and TOC.

6.4.2 Analytical and Numerical Solution – Case study of Palmital River

Palmital River is a tributary located at Upper Iguassu Watershed. The length of the river is about 21 km, with a drainage area of 95 km². Two different land use and occupation are dominant in the basin: agriculture and urban occupation. There is a wastewater treatment plant located in the intermediate part of the watershed (WWTP Guaraituba), which can treat about 21% of the domestic effluents of the inhabitants within the basin (Total number of inhabitants: 219,264 according to Porto et al, 2007). Further details are described at Chapter 2.

Both hydraulic conditions and the mass balance for BOD, DO, nitrogen, and phosphorous were analyzed between the proposed model (analytical) and Qual2e (numeric). Figure 33 presents the results for flow, stream velocity, water level depth, and travel time.

According to the results (Figure 33), it can be observed that there is no difference between the two models, indicating that the flow simulation and the discretization and representation of the hydraulic properties are suitable for model implementation and for further comparison of mass balance modeling.

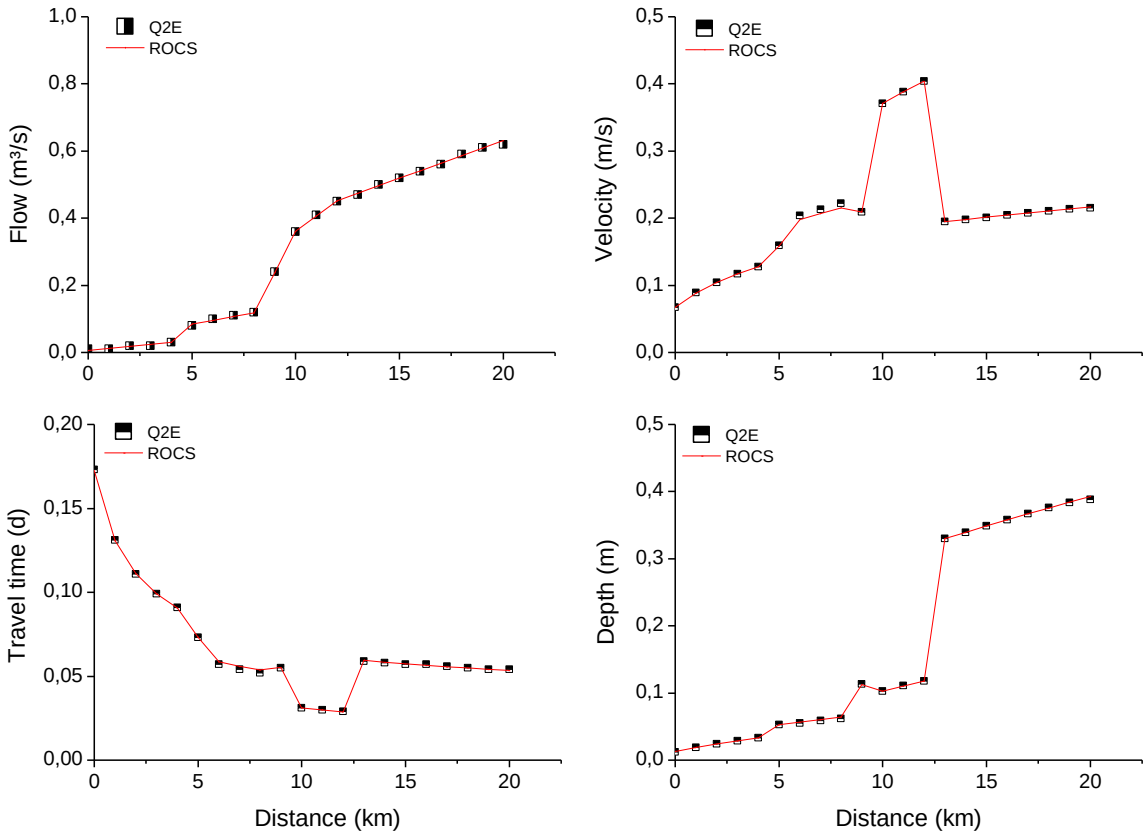


Figure 33: Comparison between numerical solution (Q2E) and the analytical solution (ROCS-Model) for Palmital River: Flow, Stream velocity, Depth, and Travel time.

The results for the nitrogen fractions (organic, ammonia, nitrite, and nitrate) and the organic and inorganic phosphorous are presented in Figure 34 and Figure 35, respectively. For some of the parameters (organic phosphorous, organic nitrogen, nitrite, and nitrate), it can be observed a small difference between the simulations. These differences could be either due to the analytical solution where dispersion effects are neglected, as some differences in the input data and the corresponding position of point and non-point sources. However, this difference is not significant when comparing the results for total nitrogen and total phosphorous (Figure 36).

BOD and DO were also evaluated in relation to both types of simulation. Figure 37 presents the simulation results for BOD and DO concentration for Palmital River. The processes considered for DO simulation include BOD deoxygenation, nitrification (nitrogenous oxygen demand), reaeration, and sediment oxygen demand. BOD results (Figure 37) did not presented significant differences between analytical and numerical solutions. Since the simulation of dissolved oxygen depends on the simulations of the other parameters, simulation errors for each parameter may have been propagated to the final mass balance.

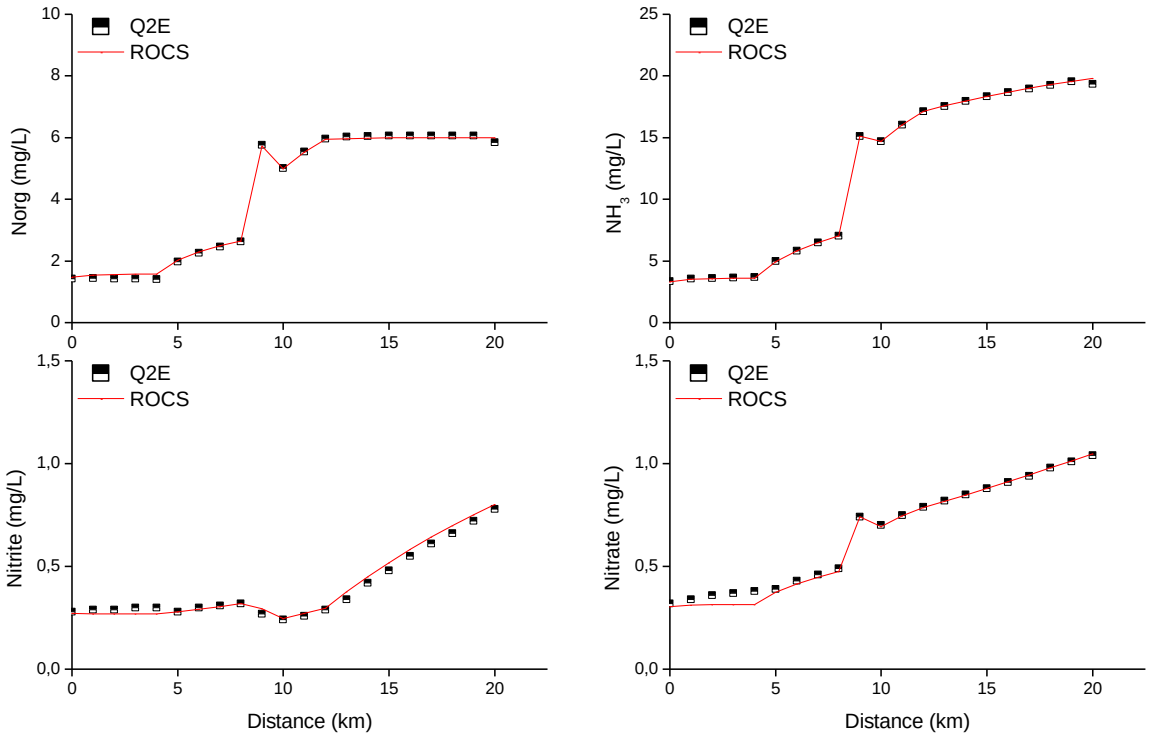


Figure 34: Comparison between numerical solution (Q2E) and the analytical solution (ROCS-Model): Organic nitrogen, Ammonia, Nitrite, and Nitrate of Palmital River.

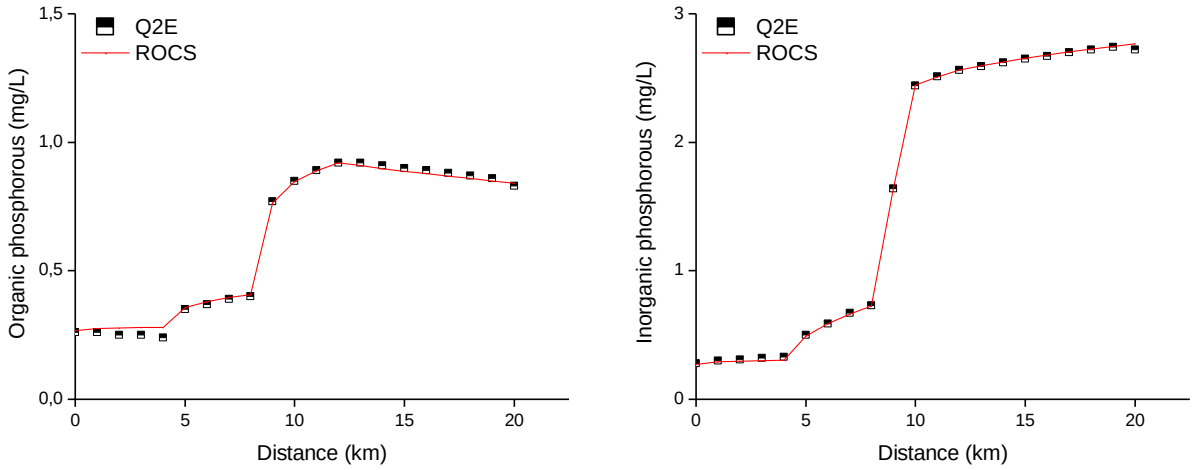


Figure 35: comparison between numerical solution (Q2E) and the analytical solution (ROCS-Model): Organic phosphorus and Dissolved inorganic phosphorous of Palmital River.

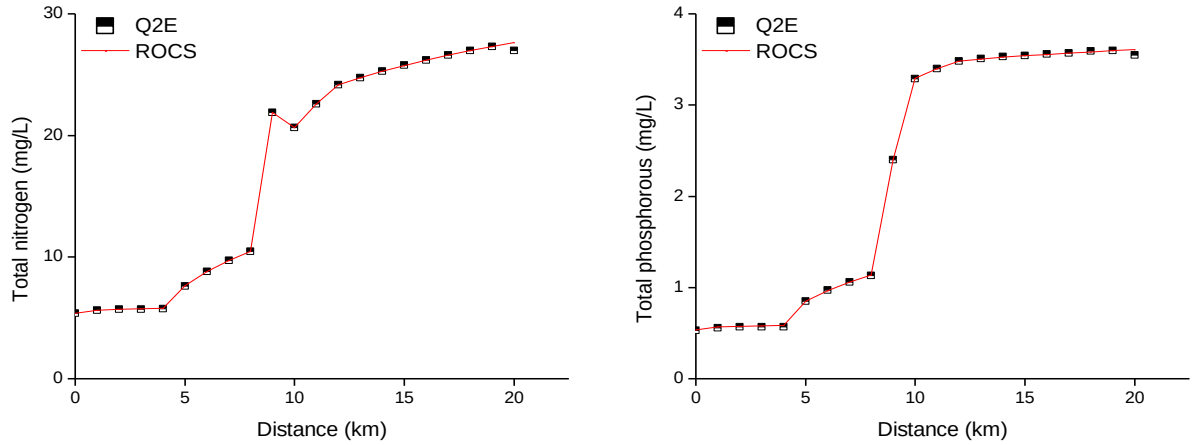


Figure 36: Comparison between numerical solution (Q2E) and the analytical solution (ROCS-Model): Total nitrogen, and Total phosphorous of Palmital River.

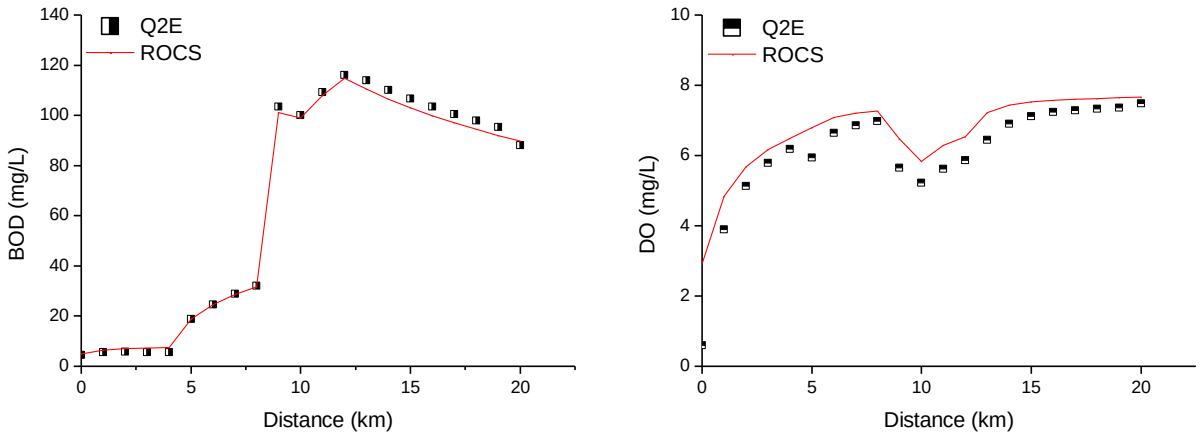


Figure 37: Comparison between numerical solution (Q2E) and the analytical solution (ROCS-Model), for BOD and Dissolved oxygen of Palmital River.

6.4.3 Anoxic Simulation – Case Study of Palmital River

Figure 37 presents the results for the comparison between BOD and DO concentration for Palmital River, considering a hypothetic scenario with low DO levels to simulate anoxic conditions. Curve 1 represents the simulation considering linear decay when the DO concentration is zero. Curve 2 represents the simulation without considering anoxic conditions for the BOD mass balance.

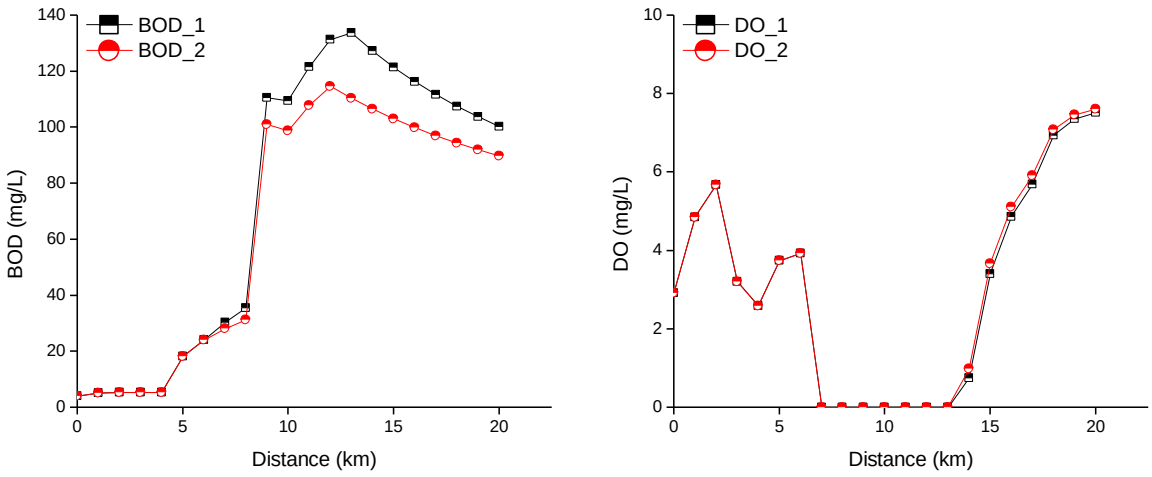


Figure 38: Comparison between BOD and Dissolved oxygen considering anoxic condition.

Comparing the results, it can be observed that there is a difference between the two methods for BOD simulation (Figure 37a). This difference is not usually accounted in models like Q2E, and, in some specific case for high polluted rivers, it may interfere in the simulated results for organic matter.

6.4.4 Automatic Calibration Algorithm Sensitivity Analysis – Case Study of Iguassu River

The model's calibration was performed separately considering: (i) flow calibration (based on an auxiliary coefficient applied in the specific flow at the diffuse sources module); (ii) organic carbon calibration (Chapra's model and ROCS-Model); (iii) organic and inorganic phosphorous based on the measured total phosphorous; and (iv) the remaining variables (BOD, nitrogen, and DO) were calibrated according the respective coefficients. The median of monitored data in the monitoring sites along the main river were used to estimate the quadratic difference between observed and simulated data as described in Chapter 5. Tests considering 10 up to 100 interactions were performed. The number of particles ranged from 100 to 600. The inertia weight and the acceleration coefficients were 0.4 and 2, respectively.

The first step on the model's calibration procedure is the adjustment of the observed and simulated flow. The flow was calibrated with the PSO technique, using an auxiliary coefficient in the incremental flow, as described in Chapter 5 (100 particles for a dimension of 6). The contribution of the tributaries was set as the long term average flow, according to

previously calibration performed by Knapik (2011). Figure 39 shows the calibrated flow for the Iguassu River. The box-plots indicate the observed data at the Iguassu River in the six sites monitored (IG01 being the headwater).

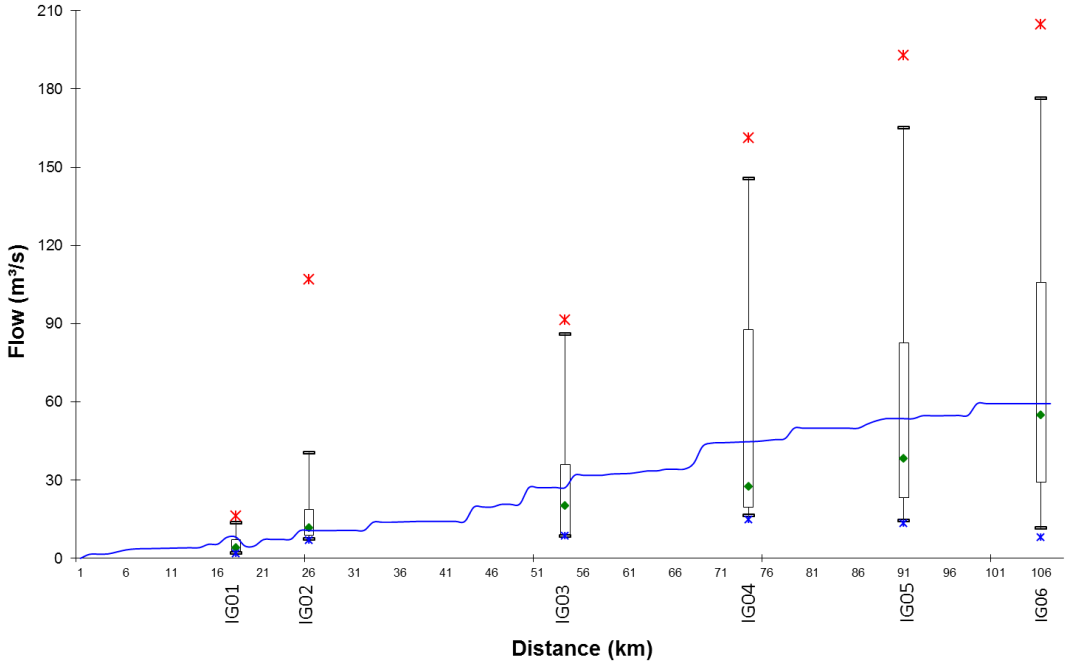


Figure 39: Calibrated flow for the Iguassu River and the box-plots of the six monitoring sites.

The final value of the coefficients, the decay pattern of the objective function, and the time of simulation varies according to the number of particles and the total number of interactions used in PSO algorithm. A test to evaluate the number of particles and total number of interactions was performed considering POC and DOC mass balance for 107 km of the Iguassu River. Six (6) control points along the main river were used to adjust the model coefficients with monitoring data (sites IG01 to IG06, as detailed previously in this chapter). The organic carbon mass balance was solved based on diffuse and point sources loads, considering first order decay and three coefficients (based on Chapra's Model, see Chapter 4 for further details).

The simulations were performed increasing the number of particles (100, 200, 300, and 600), considering 10 and 50 interactions. In this case, the dimension is 18 (6 control sites and 3 coefficients: k_p: POC dissolution rate, k_s: POC sedimentation rate, k_h: DOC hydrolysis rate). The weights for the objective function were $w_{POC} = 0.4$ and $w_{DOC} = 0.6$ for POC and DOC, respectively. The inertia weight and the acceleration coefficients were 0.4 and 2,

respectively. The lower and upper boundaries for k_p, k_s, and k_h were 0.001 and 2 d^{-1}, 0.01 and 2.4 d^{-1}, and 0.001 and 2 d^{-1}, respectively. The initial value of the objective function was 10.9007.

Table 15 presents the results of the objective function according to the number of particles and interactions performed. Figure 40 presents the decay pattern of the objective function for the simulations performed according to the number of particles. According to the results, it can be observed that when the number of particles is higher, the final value of the objective function is reached in a reduced number of interactions. While the condition of using n=600 can be performed with less interactions, for a reduced number of particle (n=300) the same range of minimum value of the objective function can be reached with an intermediate number of interactions. Thus, depending on the time processing, an intermediate number of particles and interactions could be choose.

Table 15: Variation of the object function according to the number of particles and interactions

Number of Interactions	Number of particles			
	100	**200**	**300**	**600**
10	2.2188	2.1462	1.9452	1.8883
50	1.8845	1.8965	1.9802	1.8869

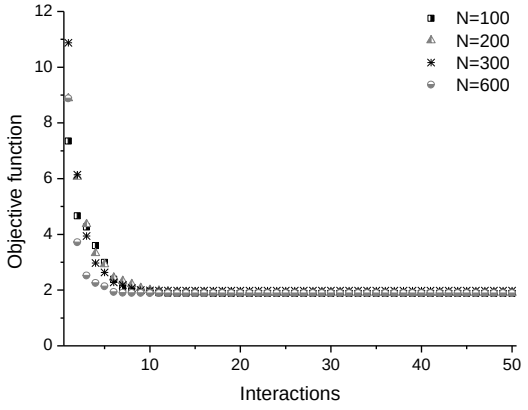


Figure 40: Variation of the object function according to the number of particles

The search mechanism of PSO algorithm, and other automatic calibration techniques, results in several combinations of coefficient values. Tables 16, 17, and 18 summarize the values of k_p, k_s, and k_h estimated by PSO algorithm, considering the initial conditions mentioned before. Figure 41 and 42 represent the calibrated curve and the

monitoring sites (box-plots), considering the average values of the coefficients (Tables 16, 17, and 18).

Table 16: Variation of k_p estimated for the 6 sites considering different number of particles and interactions.

Site	*N = 100*		*N = 200*		*N = 300*		*N = 600*		k_p ± sd
	i=10	*i=50*	*i=10*	*i=50*	*i=10*	*i=50*	*i=10*	*i=50*	
IG01	2	2	2	2	2	2	2	1.98	2 ± 0.01
IG02	0.001	0.111	0.442	0.001	0.313	0.001	0.095	0.001	0.12 ± 0.17
IG03	0.001	0.001	0.237	0.185	0.123	0.001	0.001	0.001	0.07 ± 0.11
IG04	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001 ± 0.00
IG05	0.059	0.834	0.142	0.580	1.44	0.572	1.75	0.580	0.75 ± 0.59
IG06	0.001	0.001	0.001	0.232	0.101	0.235	0.001	0.219	0.10 ± 0.11

Table 17: Variation of k_s estimated for the 6 sites considering different number of particles and interactions.

Site	*N = 100*		*N = 200*		*N = 300*		*N = 600*		k_s ± sd
	i=10	*i=50*	*i=10*	*i=50*	*i=10*	*i=50*	*i=10*	*i=50*	
IG01	0.01	2.4	0.90	2.4	0.01	1.2	1.03	0.01	1 ± 0.99
IG02	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.40 ± 0.00
IG03	0.01	0.01	0.01	0.01	0.01	0.01	0.19	0.22	0.06 ± 0.09
IG04	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.010 ± 0.00
IG05	1.96	1.15	1.68	1.29	0.65	1.42	0.01	1.28	1.18 ± 0.61
IG06	0.01	0.24	0.01	0.01	0.01	0.01	0.38	0.03	0.09 ± 0.14

Table 18: Variation of k_h estimated for the 6 sites considering different number of particles and interactions.

Site	*N = 100*		*N = 200*		*N = 300*		*N = 600*		k_h ± *sd*
	i=10	*i=50*	*i=10*	*i=50*	*i=10*	*i=50*	*i=10*	*i=50*	
IG01	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001 ± 0.00
IG02	2	2	2	2	2	2	2	2	2.00 ± 0.00
IG03	0.001	0.001	0.303	0.123	0.001	0.001	0.001	0.001	0.05 ± 0.11
IG04	0.001	0.001	0.001	0.283	0.384	0.244	0.272	0.241	0.178 ± 0.15
IG05	0.001	0.373	0.001	0.001	0.586	0.001	0.786	0.001	0.22 ± 0.32
IG06	0.314	0.454	0.001	0.579	0.533	0.581	0.451	0.577	0.44 ± 0.20

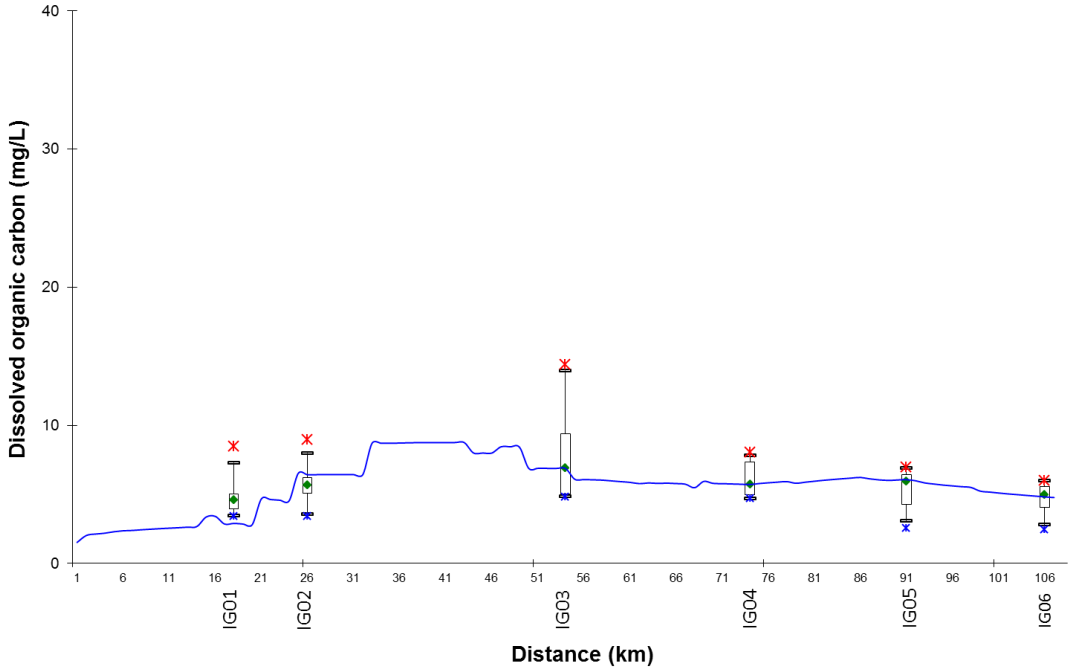


Figure 41: Calibrated curve for DOC concentration, considering the average coefficients estimated by PSO algorithm.

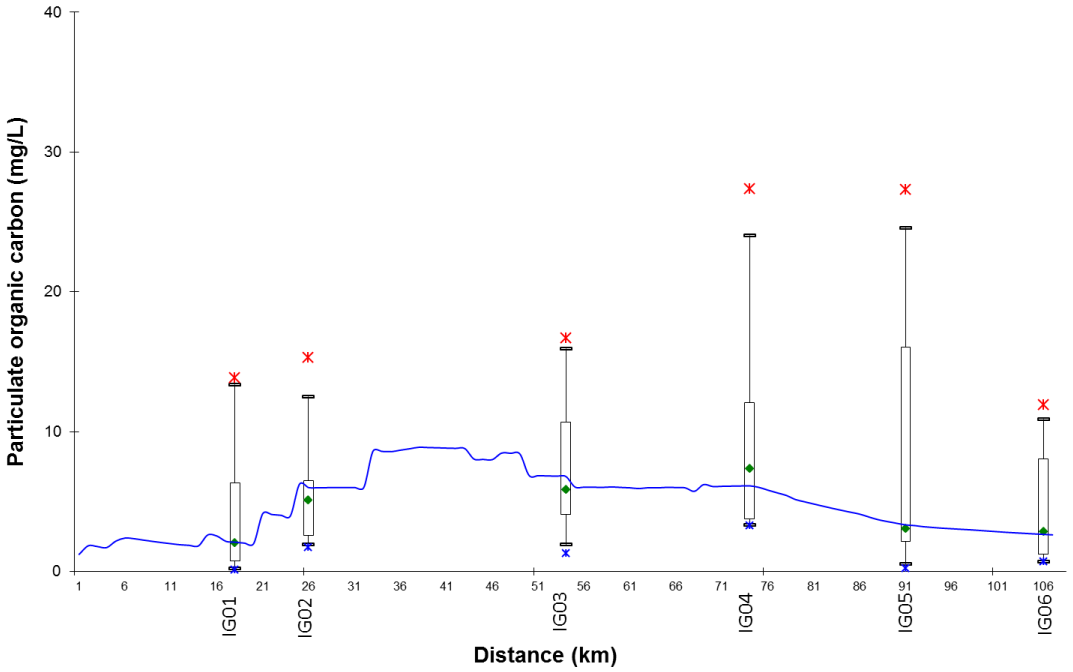


Figure 42: Calibrated curve for POC concentration, considering the average coefficients estimated by PSO algorithm.

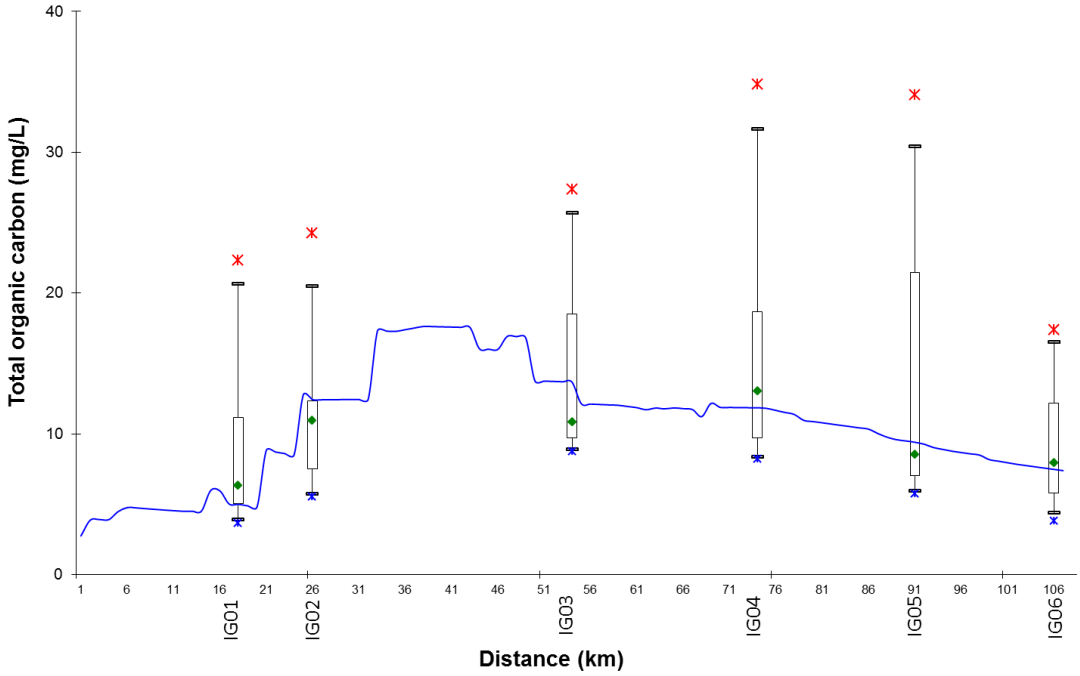


Figure 43: Calibrated curve for TOC concentration, considering the sum of POC and DOC calibrated data.

According to Figure 41, the simulated DOC at site IG01 were lower than the median observed data, while POC show good adjustment in the same river reach. One hypothesis is that the input DOC data may be underestimated upstream site IG01. An important contribution upstream is the Iraí Reservoir, used for water supply. For the simulation, it was assumed a concentration of 5 mg/L of DOC, and 2.5 mg/L of POC. Besides the difference of the calibrate data and the observed DOC concentration at IG01, TOC calibration curve (Figure 43) showed good results.

For the calibration of the model considering labile and refractory pools of DOC and POC (ROCS-Model), 13 coefficients have been adjusted. As described at Chapter 5, the observed POC concentration was used to evaluated the sum of LPOC and RPOC (considered as simulated POC), and the observed DOC concentration was used to evaluate the sum of LDOC and RDOC (considered as simulated DOC). Both simulated and observed TOC was considered as the sum of POC and DOC. The number of particles was set on 250, considering 50 interactions for a 78 dimension optimization problem. The lower and upper boundaries for the coefficients were set on 0.001 and 1, respectively. Figures 44, 45, and 46 presents the results for DOC, POC, and TOC according to ROCS-Model simulation.

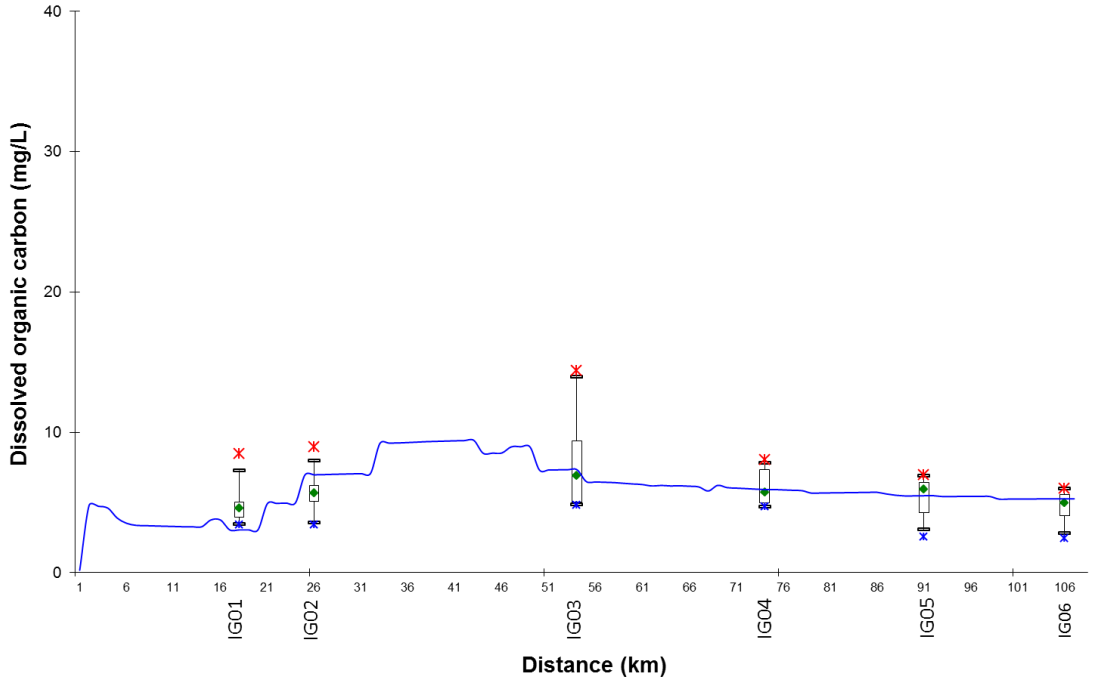


Figure 44: Calibrated curve for DOC concentration, considering the coefficients estimated by PSO algorithm for ROCS-Model simulation.

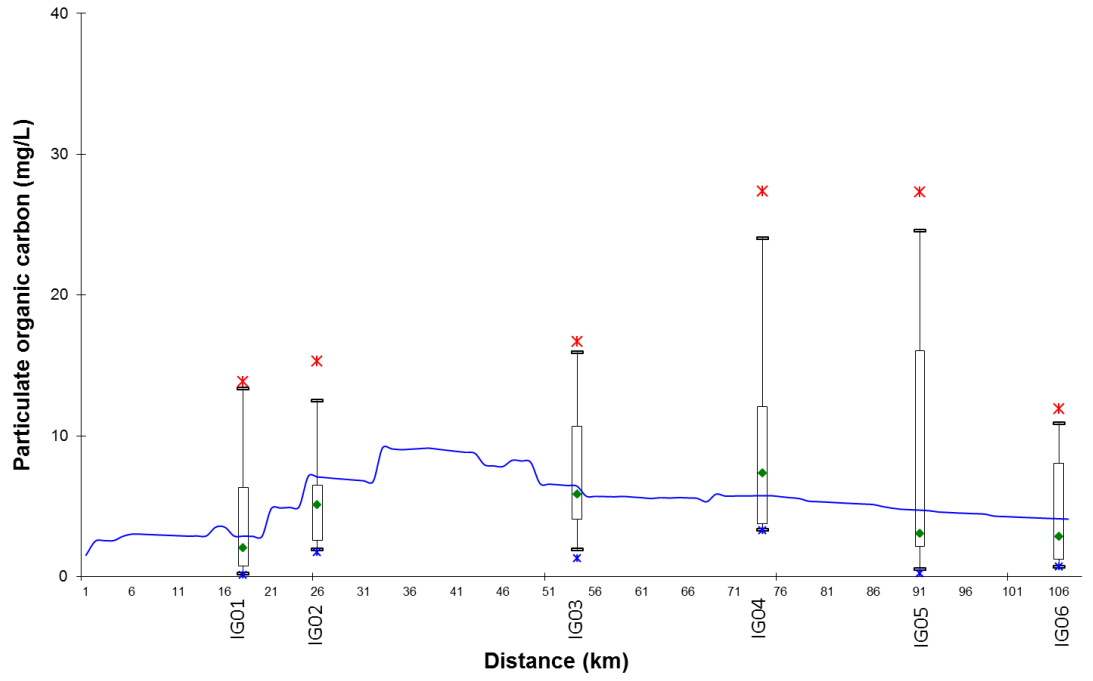


Figure 45: Calibrated curve for POC concentration, considering the coefficients estimated by PSO algorithm for ROCS-Model simulation.

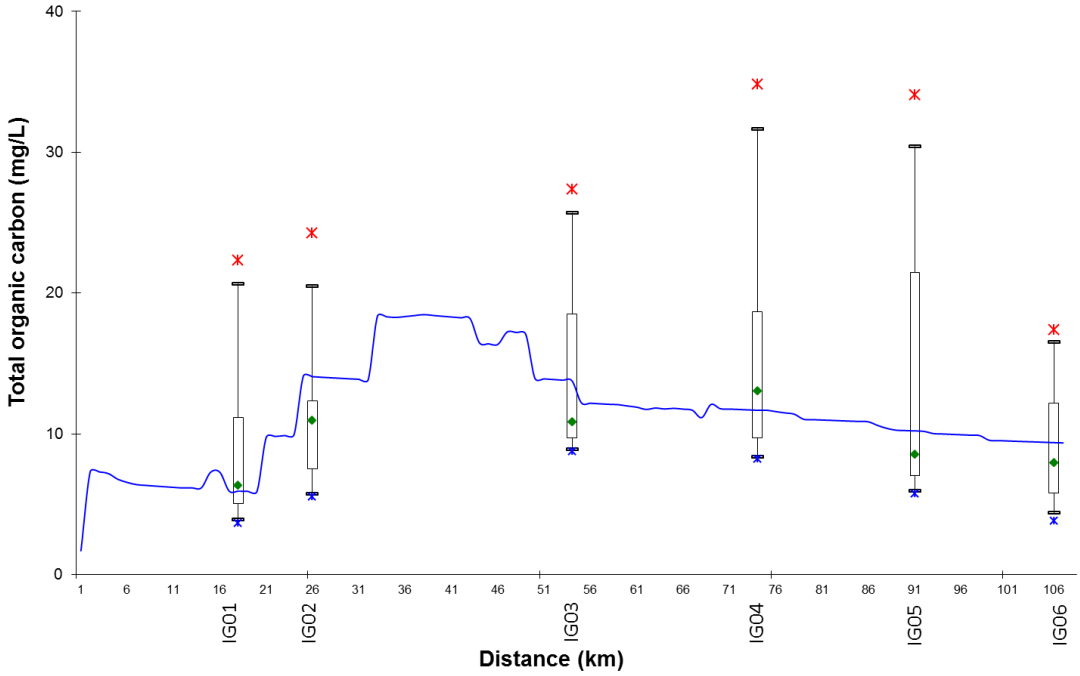


Figure 46: Calibrated curve for TOC concentration, considering the coefficients estimated by PSO algorithm for ROCS-Model simulation.

Peculiarly, the PSO algorithm converges to a common set of coefficients. Table 19 summarizes the results for the coefficients in the organic carbon simulation based on 50 interactions and 250 particles. Other tests with different conditions (number of particles and interactions) also resulted in the same range or repetition of the same value. The initial value of the objective function to be minimized by the optimization mechanism decays in the first interactions and reaches a constant value (Figure 47). The restrictions applied in this case or the dependence of the objective function on two terms (POC as the sum of LPOC and RPOC, and DOC as the LDOC and RDOC) may be affecting the evolution of the search to an optimum group of data. Besides that, the model can be considered as calibrated for DOC, POC, and TOC.

Table 19: Summary of coefficients estimated for the 6 sites considering n=250 particles

Coefficient	*IG01*	*IG02*	*IG03*	*IG04*	*IG05*	*IG06*
K_{L1}	0.1	0.1	0.1	0.01	0.1	0.1
K_{L2}	0.0873	0.0001	0.0001	0.0076	0.0001	0.0001
K_{L3}	0.01	0.1	0.1	0.1	0.1	0.1
K_{L4}	0.1	0.07	0.1	0.1	0.1	0.1
K_{L5}	0.1	0.1	0.1	0.01	0.1	0.1
K_{L6}	0.001	0.1	0.1	0.079	0.1	0.1
K_{L7}	0.1	0.1	0.0797	0.1	0.1	0.1
K_{L8}	0.1	0.1	0.1	0.1	0.1	0.1
K_{R1}	0.1	0.1	0.1	0.1	0.038	0.1
K_{R2}	0.0880	0.1	0.1	0.0001	0.0881	0.0001
K_{R3}	0.1	0.1	0.1	0.1	0.0001	0.1
K_{R4}	0.0001	0.1	0.1	0.1	0.1	0.1
K_{R5}	0.001	0.089	0.003	0.023	0.039	0.1

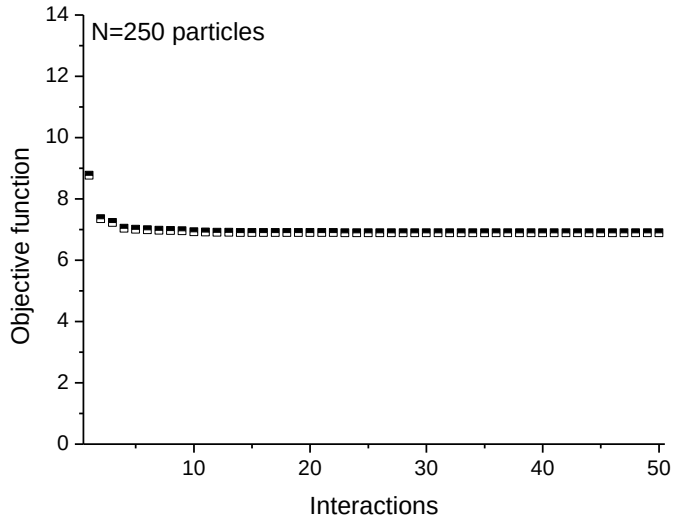


Figure 47: Variation of the object function considering 50 interactions and 250 particles

For the calibration of BOD, DO, Organic nitrogen, ammonia, nitrite, and nitrate, 8 coefficients were adjusted through PSO algorithm (dimension = 48). The inertia weight and the acceleration coefficients were 0.4 and 2, respectively. The total number of interactions was set on 100, for a population of 500 particles. The lower and upper boundaries for the coefficients were, respectively: K_1 and K_3: 0.02 – 3.4 d^{-1}; K_2: 3 – 100 d^{-1}; K_4: 0.05 – 2 gm^{-2} d^{-1}; K_{oa}: 0.02 – 0.4 d^{-1}; K_{os}: 0.001 – 0.1 d^{-1}; K_{ai}: 0.01 – 1 d^{-1}; and K_{in}: 0.2 – 2 d^{-1}. For this set of simulations, and considering the number of restrictions, the time of model execution

increased significantly. Figures 48 to 55 presents the calibrated curve for BOD, DO, nitrogen fractions (organic nitrogen, ammonia, nitrite, nitrate, and total), and total phosphorous.

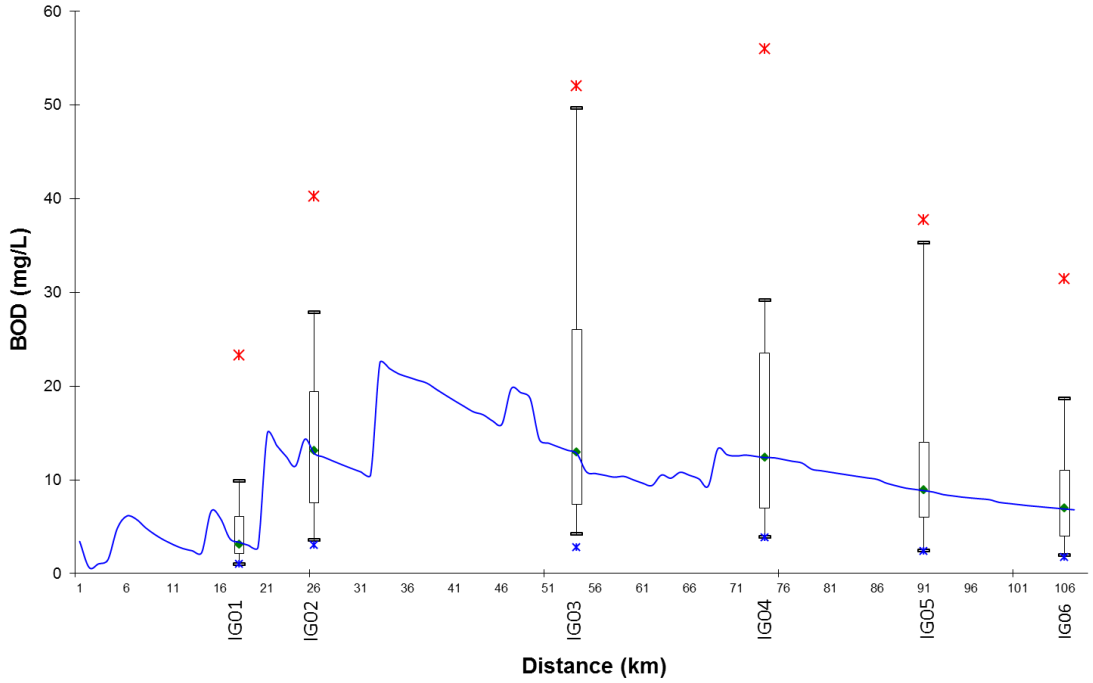


Figure 48: Calibrated curve for BOD concentration, considering the coefficients estimated by PSO algorithm for ROCS-Model simulation.

According to the results, it is possible to observe that while for some variables the model fitted perfectly the simulated and observed data in the six control sites (e. g., BOD, Figure 48), for other variables the results were not satisfactory for all the control points considering the observed median value as the optimum data (e.g., Ammonia, Figure 51). In addition, considering the simulation of DO (Figure 49), the calibration resulted in a good fitness for the control sites. However, the model faults to simulate the data between two consecutive sites (e.g., IG03 and IG04). Other tests performed also showed the same pattern of simulation. Thus, additional monitored data could be relevant to overcome this weakness.

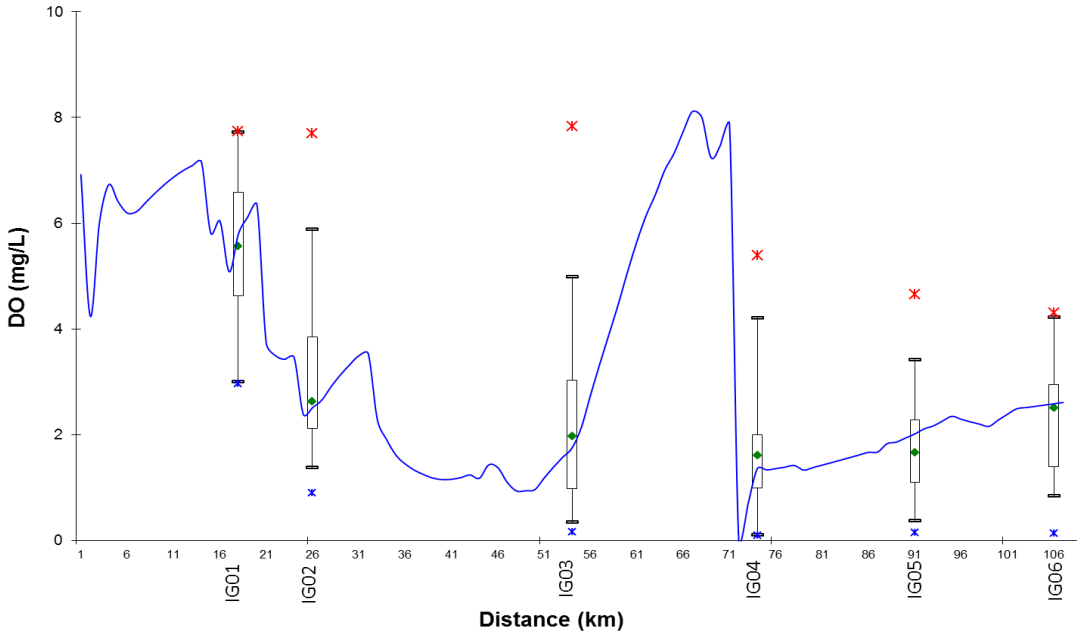


Figure 49: Calibrated curve for DO concentration, considering the coefficients estimated by PSO algorithm for ROCS-Model simulation.

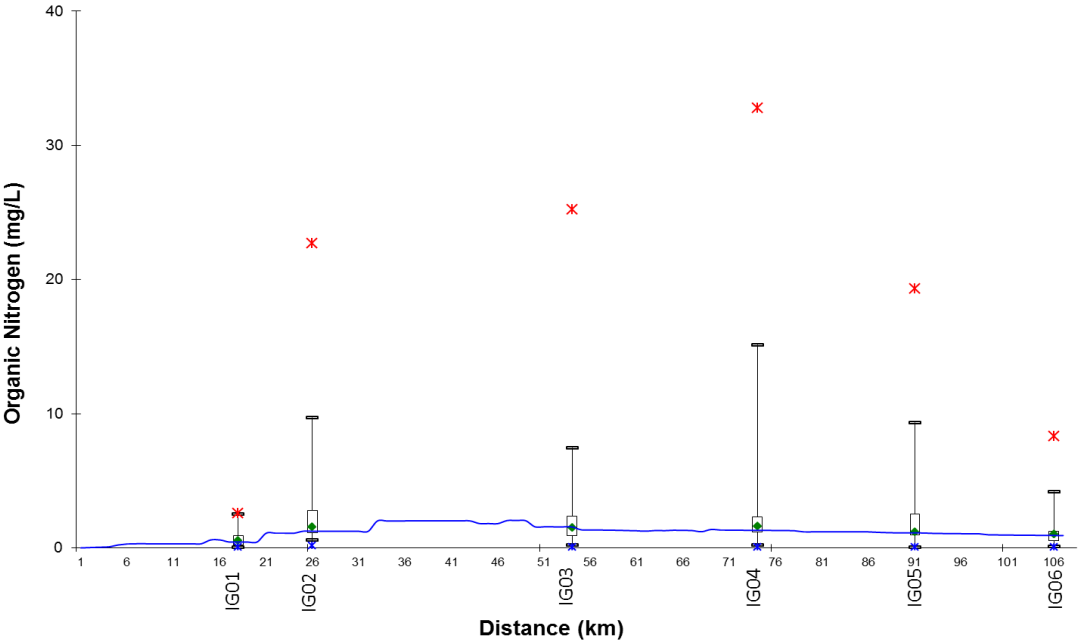


Figure 50: Calibrated curve for Organic Nitrogen concentration, considering the coefficients estimated by PSO algorithm for ROCS-Model simulation.

For the calibration of nitrogen, the two opposite fractions of model's process formulation (organic nitrogen and nitrate), showed the best model adjustment in the control sites (Figures 50 and 53). Ammonia and nitrite concentration did not show a good adjustment for all control points (Figure 51 and 52). Consequently, the total nitrogen, which represents the sum of the four calibrated nitrogen fractions, did not present a good adjustment. Further analysis considering changing the PSO algorithm parameters (number of particles, interactions, weight and inertia parameters) and/or the restrictions and search limits, could improve the model calibration for all variables simulated.

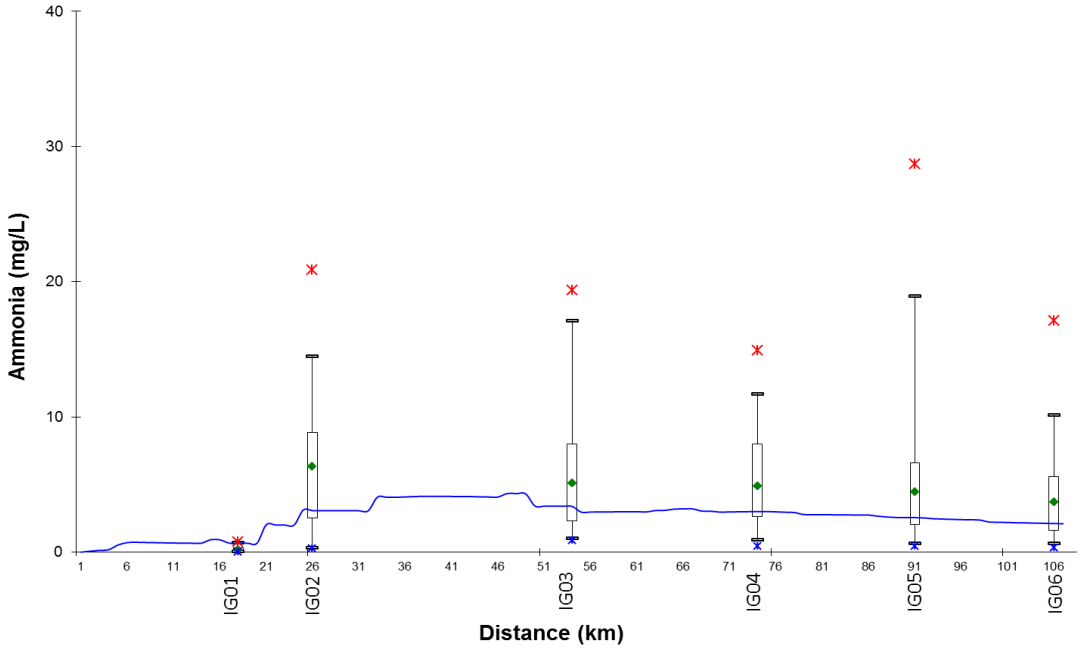


Figure 51: Calibrated curve for Ammonia concentration, considering the coefficients estimated by PSO algorithm for ROCS-Model simulation.

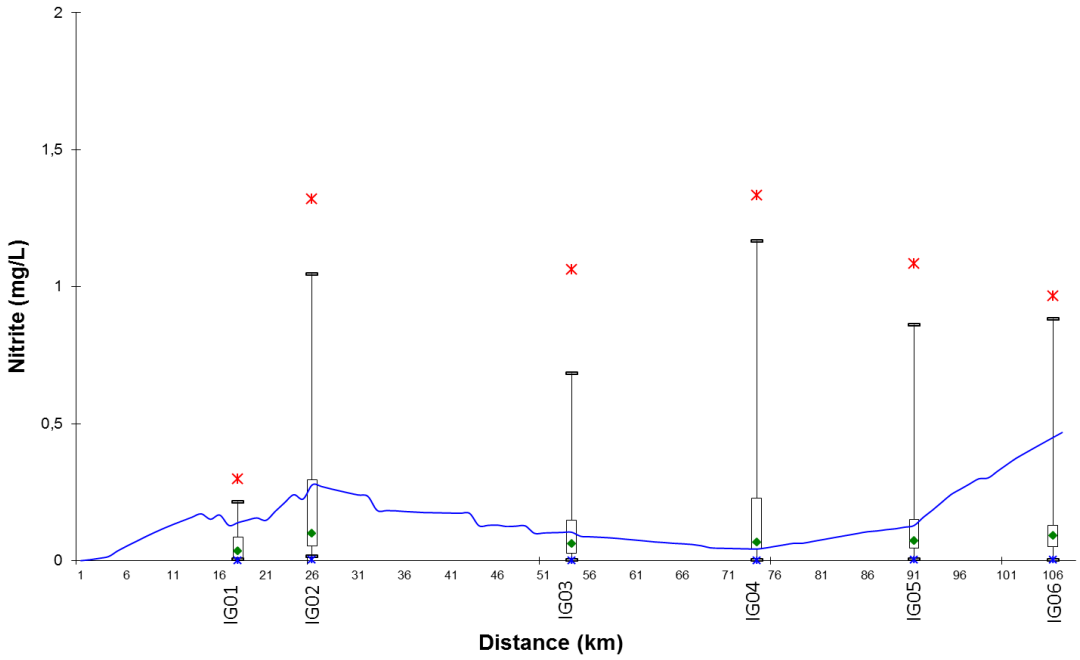


Figure 52: Calibrated curve for Nitrite concentration, considering the coefficients estimated by PSO algorithm for ROCS-Model simulation.

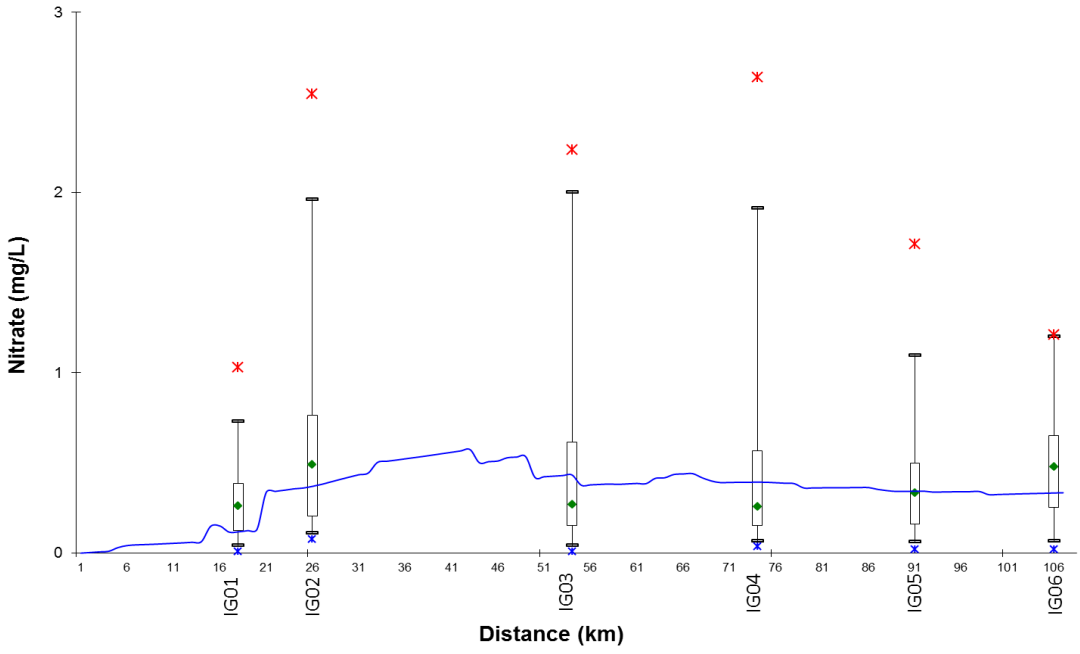


Figure 53: Calibrated curve for Nitrate concentration, considering the coefficients estimated by PSO algorithm for ROCS-Model simulation.

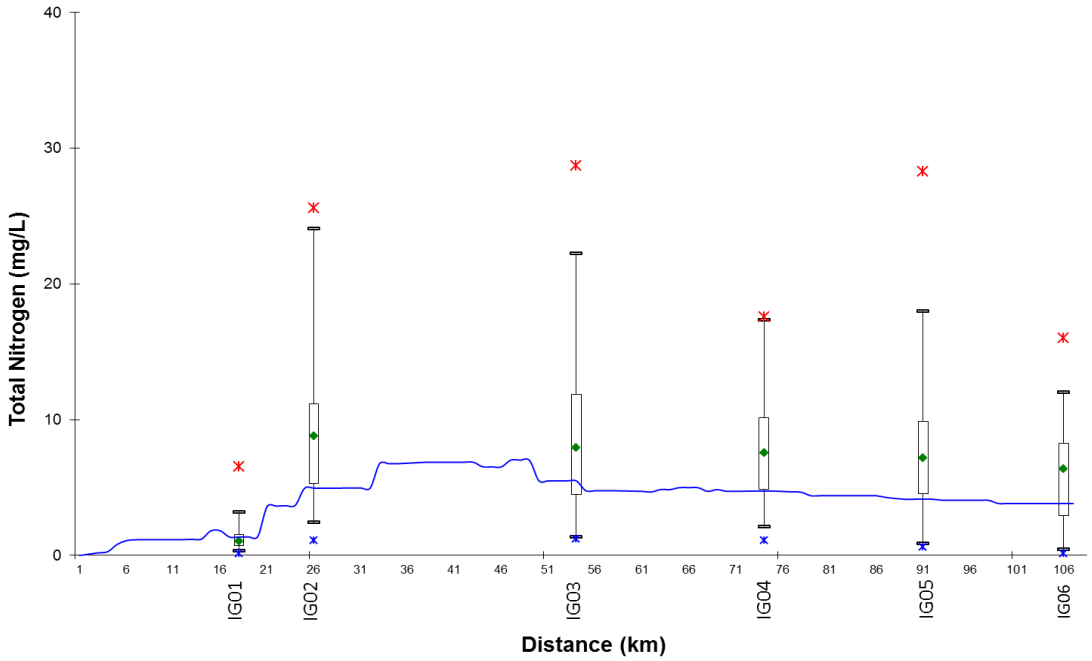


Figure 54: Calibrated curve for Total Nitrogen concentration, considering the coefficients estimated by PSO algorithm for ROCS-Model simulation.

Figure 55 presents the calibration for total phosphorous. The lower and upper boundaries for the coefficients were, respectively, K_{ps} from 0.001 to 1.4 d^{-1}, and K_{pd} from 0.01 to 1.4 d^{-1}. As the same case of organic carbon, an assumption had to be made to adjust the coefficients of organic and inorganic phosphorous model through the observed total phosphorous. This procedure may be affecting the search mechanism by increasing the model execution time and, consequently, in the final calibrated curve. Regardless, the model showed a good fitness to the observed data in five of the six control points. The higher concentration of simulated total phosphorous at site IG01 may also be a result for the input data from the Iraí Reservoir, located upstream in the Watershed.

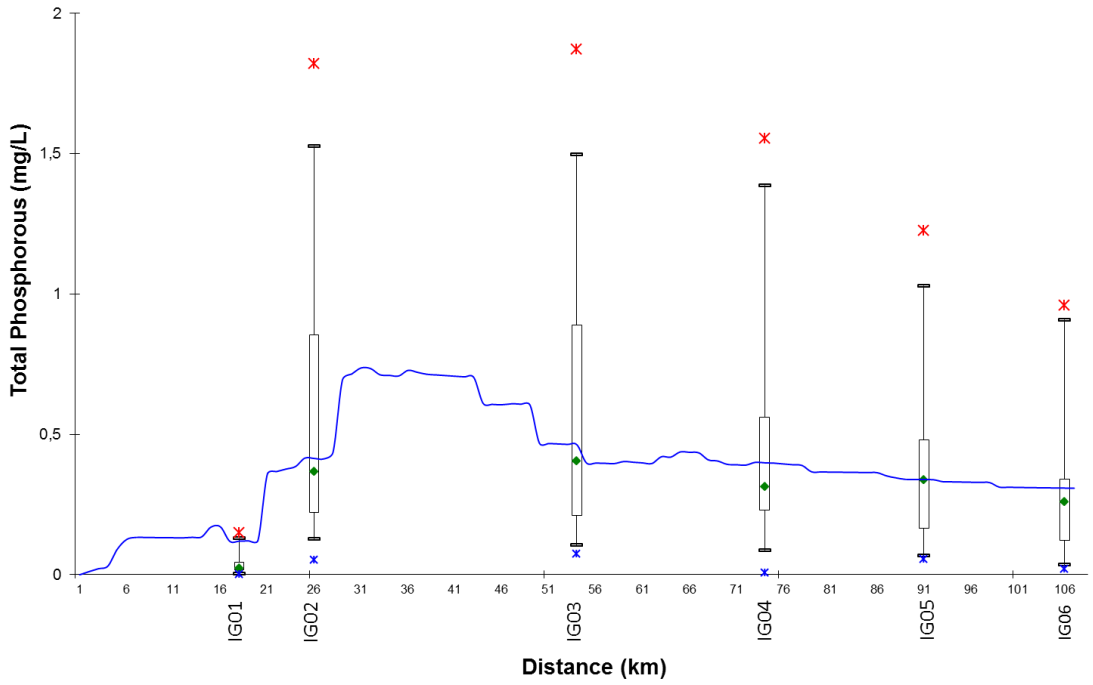


Figure 55: Calibrated curve for Total Phosphorous concentration, considering the coefficients estimated by PSO algorithm for ROCS-Model simulation.

A summary of the calibrated coefficients are presented in Table 20. Several combinations of coefficients found by the PSO search algorithm can result in good model fitness. In some cases, the values obtained may not be a real meaning when comparing to measured data. For example, the deoxygenation rate (K_1) estimated for site IG01, 3.31 d^{-1}, is significantly higher than the usual range of values indicated for low polluted sites (≈ 0.1 d^{-1}). One issue related to the PSO algorithm implemented in the model is that the initial value of the coefficients is randomly generated. Thus, in every simulation performed, the best values obtained previously are not considered, which may increase the time efforts for model calibration. Another improvement could be to increase the lower and upper boundaries for the coefficients search limits. The limits considered were based on previous studies or other model's default values (such as Qual2e model). In addition, for the case that variables with distinct values ranges (e.g., BOD compared to Nitrite), other objective functions may be tested to avoid weights during the function minimization. Thus, further analyses to investigate the appropriate range of values could improve the calibration results.

Table 20: Summary of coefficients estimated for the 6 sites considering n=500 particles and 100 interactions.

Coeficients	*IG01*	*IG02*	*IG03*	*IG04*	*IG05*	*IG06*
K_1	3.31	3.4	1.5	0.02	0.72	0.02
K_3	3.4	2.21	0.02	1.47	0.04	0.76
K_2	21.96	15.45	9.14	3	3	4.9
K_4	2	0.4	1.28	1.99	1.36	0.1
K_{oa}	0.21	0.39	0.052	0.4	0.02	0.33
K_{os}	0.1	0.001	0.001	0.1	0.004	0.06
K_{ai}	0.997	1.0	0.08	0.01	0.12	0.57
K_{in}	0.72	1.92	2	2	0.2	0.2
K_{ps}	0.34	1.37	0.34	0.88	1.02	0.9
K_{pd}	1.18	0.84	1.22	1.25	0.13	1.13

6.4.5 Organic Carbon and BOD Modeling for Iguassu River: Comparative Analysis

An interesting consideration about the proposed modeling approach is the segmentation between labile and refractory organic carbon. While the simulation of TOC gives an idea about the overall organic carbon throughout the main river, the distinct fractions allow a deeper interpretation about the amount of organic matter that can be easily degraded, and, consequently, direct impact of the overall water quality conditions. Figure 56 presents the concentration of TOC and the respective labile and refractory fractions for dissolved and particulate organic carbon (LPOC, RPOC, LDOC, RDOC).

According to the results, the labile fraction, LPOC and LDOC, represents the most part of the TOC under the conditions of the simulation for the Iguassu River. RTOC showed a constant concentration of 2.6 mgC/L (in average), while LTOC ranged from 3.2 to 16.2 mgC/L (Figure 56). In average, the labile fraction represented 75% of the TOC, while the refractory fraction 25%. Figure 57 shows the variation of the respective percentages of labile total organic carbon (LTOC) and refractory total organic carbon (RTOC). It can be observed that downstream the most impacted area of the Watershed (from site IG02 to IG04), LTOC predominates. Upstream site IG02, the proportion of refractory organic carbon was higher, with RTOC about 39%, while LTOC was 61%, in average. In addition, the RTOC showed a tendency to increase progressively throughout the watershed.

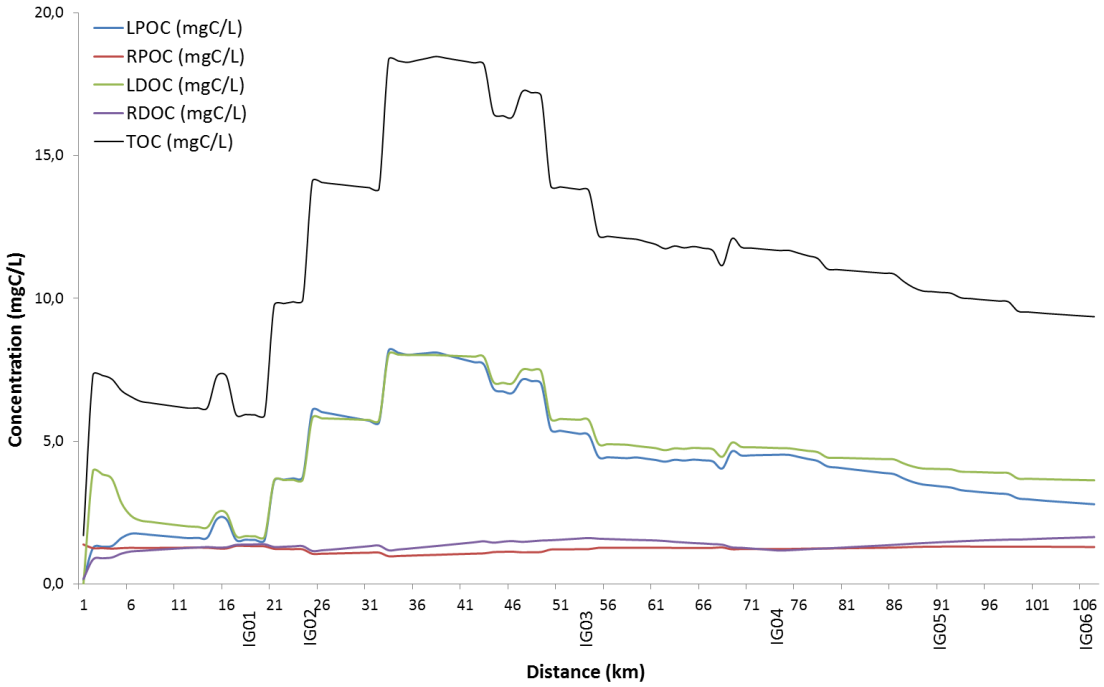


Figure 56: Variation of TOC and the labile and refractory fractions of dissolved and particulate organic carbon.

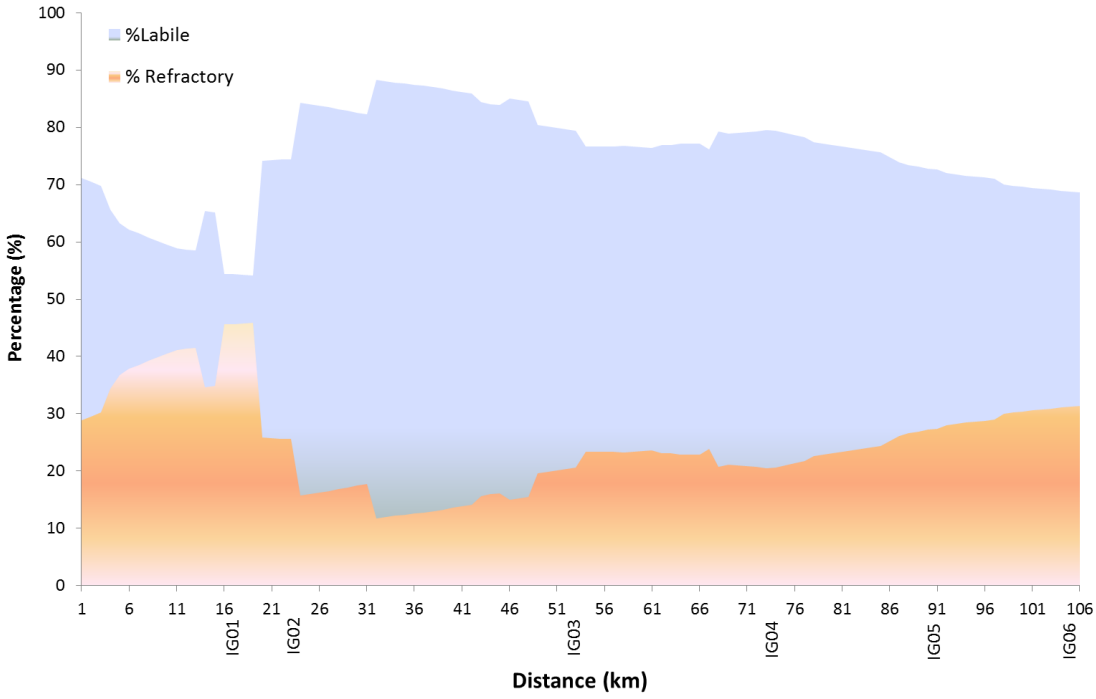


Figure 57: Percentage from TOC of labile and refractory organic carbon for the Iguassu River

Complementarily, the combined interpretation of the organic carbon labile fraction and the BOD data can be relevant to identify the most polluted areas. Figure 58 shows the simulated curves for BOD, TOC, and the respective labile (LTOC), and refractory (RTOC) fractions. According to the results, it can be observed that BOD and LTOC have a similar configuration throughout the watershed. The most polluted reaches are located in the intermediate sites (IG02 to IG04), where important urban and polluted tributaries are located (Atuba, Belém, and Barigui Rivers).

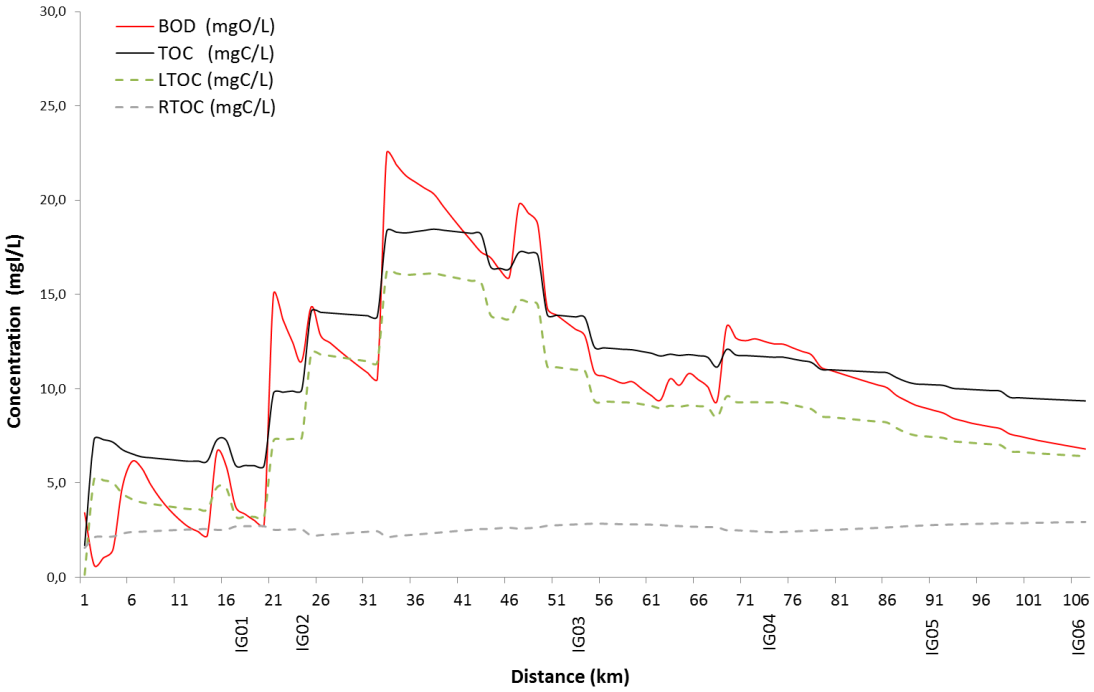


Figure 58: Concentration for simulated data of BOD, TOC, and the labile and refractory fractions of TOC (LTOC and RTOC)

In addition, while BOD presents a fast decay, TOC show a slowly decrease. The reason for this different pattern is that the proposed modeling approach for the organic carbon simulation considers not only the consumption through mineralization, but also the conversion between the distinct fractions simulated. For example, part of the particulate organic carbon can lead to dissolution and increase the dissolved pool. In addition, the estimated rates for the process considered were lower for organic carbon than the estimated coefficients for BOD modeling (Tables 19 and 20, respectively).

A complementarily interpretation of BOD and TOC simulated curves can be done through the molar concentration. In the same unit, it is possible to evaluate and analyze the

ranges of BOD and TOC data. Figure 59 shows the variation of BOD, TOC, LTOC, and RTOC considering the respective molar concentration. Under this consideration, the units of BOD can be easier interpreted as the biodegradable part of the TOC in the river. The expectation is that the LTOC and BOD have a similar concentration. In this case, BOD presented a good calibration with the observed that, while the calibration of LTOC was performed through a simplification considering the sum of labile and refractory fractions. Thus, the higher values of LTOC may indicate that or the simulated data of labile DOC and POC are overestimated, or the decay rates are lower than the expected. This aspect shows how relevant is to look for the combination of both biodegradable fractions with organic carbon fractions. Definitely, in polluted rivers, the chemical reactions are truly influenced by all different conditions, as a consequence of the complex nature of mixing distinct aspects of the pollution source matrix. These results remind the necessity of additional decay experiments to better clarify the results.

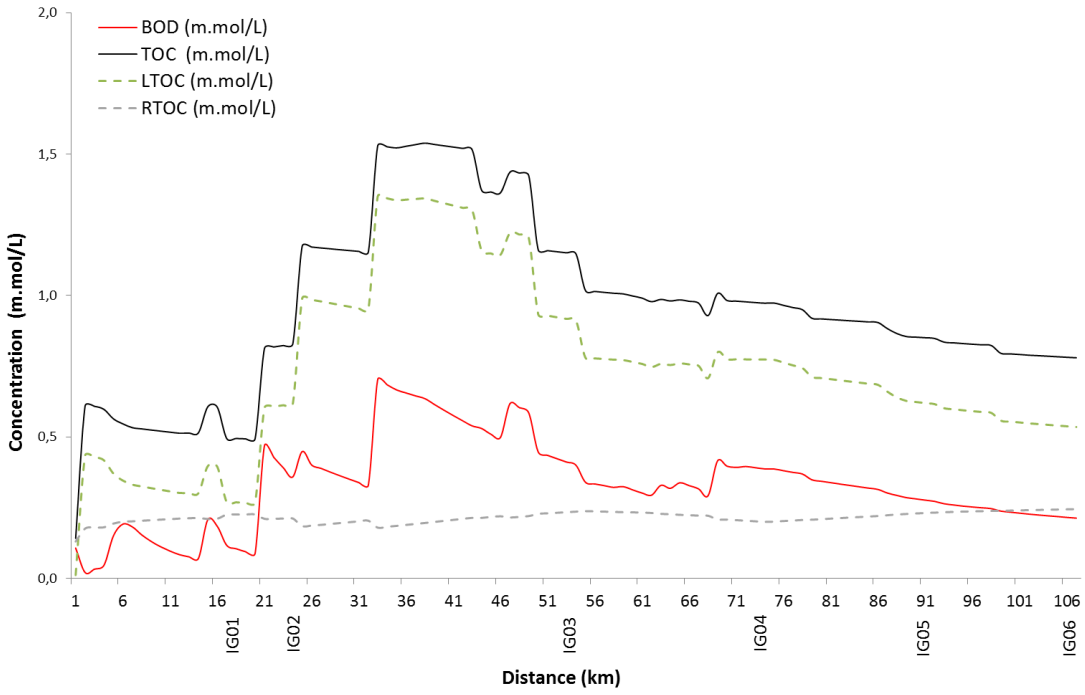


Figure 59: Molar concentration for simulated data of BOD, TOC, and the labile and refractory fractions of TOC (LTOC and RTOC)

Finally, the labile and refractory organic matter estimated through the specific fluorescence intensity peaks also confirms the simulated patterns indicated in the previous

results. Figure 60 presents the variation of fluorescence intensity peaks B, T_2, and T_1, representing the labile organic matter for the six sites monitored along the Iguassu River. Figure 61 presents the variation of fluorescence intensity peaks C and A, representing the refractory organic matter.

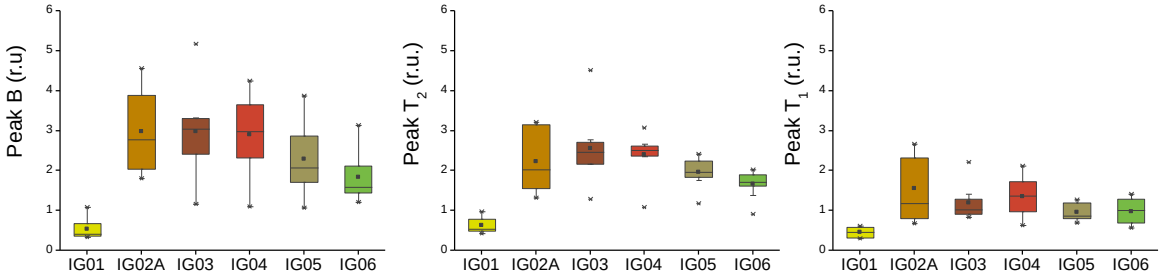


Figure 60: Variation of fluorescence intensity peaks B, T_2, and T_1, relating the labile organic matter in the six sites monitored. Data from samplings C39 to C48.

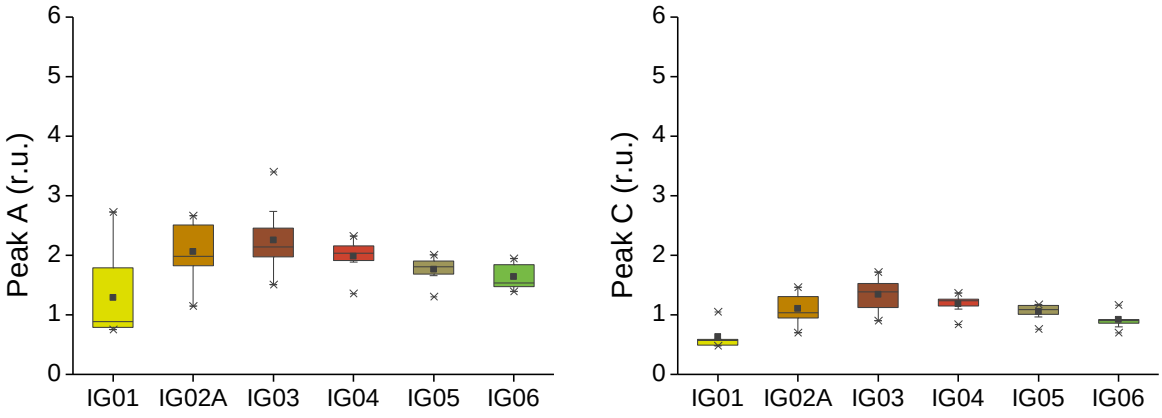


Figure 61: Variation of fluorescence intensity peaks A and C, relating the refractory organic matter in the six sites monitored. Data from samplings C39 to C48.

The results clearly indicate a changing point between site IG01 and IG02 corresponding the beginning of the influence of the urbanization and the increase sources of domestic wastewater. In addition, downstream site IG04, the presence of labile organic matter starts to decrease, as can be observed in the simulated data (Figure 59). The refractory organic matter shows small variation when comparing the Peaks A and C, and an approximately constant simulated concentration along the Iguassu River. These results indicate that even though the labile organic matter predominates and are a direct consequence of the urbanization and organic pollution derived from domestic wastewater, there is still a percentage of refractory organic matter in the water column.

6.4.6 Advantages and Disadvantages of the Proposed Model

Based on the proposed model concepts and structure developed, and under the conditions of the simulations performed, some advantages and disadvantages should be highlighted. The proposed model, ROCS-Model, is applicable to rivers, considering one-dimensional advection transport and steady-state condition. ROCS-Model advances in the simulation of different fractions of organic carbon, considering labile and refractory compounds for dissolved and particulate forms. In addition, the model's structure was idealized to be a database of watershed main data, coefficients, discretization, non-point and point sources estimation, and calibration. Important advantages and disadvantages include the input data, model equation solution, calibration algorithm, and main structure.

In terms of measured data to calibrate the model, it has being estimated the percentages of labile and refractory organic carbon. The consideration about the labile and refractory organic carbon provides a gain in terms of quantification of the potential compounds that have a direct impact in oxygen depletion and water quality deterioration. Complementarily, the measurement of DOC, POC, and TOC have less subjectivity and are not time-consuming as BOD test, being another positive issue relating to the reliability of the input data or the observed data used for model calibration.

Relating to the proposed model concepts and process simulations, the solution method is one issue that could be improved. Currently, the simulation is done by analytical solution. While the comparison between a numerical model and the analytical solutions were similar, this method of solution has some limitations when considering a large group of variables. To apply the analytical solution some hypothesis and simplification had to be assumed. In addition, the restrictions for the model coefficients to operate PSO algorithm may increase the runtime.

The use of an optimization technique to calibrate the model can facilitate the search for the model's coefficients. For the proposed configuration of the model, PSO was used as calibration tool, considering six control sites along the main river.

Considering the structure developed, one advantage of the proposed model is the possibility to use the interface as a data base for the general data of the basin, and the monitoring data. The first one is important to generate all the loads within the model structure, which can allow the user to identify possible errors, missing data, or look for new strategies for watershed management. The second feature is important to have a set of observed data to calibrate the model and, thus, evaluate its performance and modify inputs,

coefficients or parameters. The integration of both types of data in the same structure makes the proposed model a more independent interface to be used in different field of studies. Additionally, the input data and the results are in a format that can be easily understood, with a graphic interface implemented in Excel spreadsheets.

6.4.7 Summary of Water Quality Modeling

The third part of this chapter focused in the evaluation of the ROCS – Model (River Organic Carbon Simulation Model). The tests comparing numerical and analytical solutions were satisfactory, confirming that the model discretization and execution is reliable. The modules for point and non-point sources estimation, and the other model features, showed good results and easy implementation.

The use of PSO algorithm facilitates the calibration procedure. Considering the multiple coefficients, the respective restrictions, and the assumptions to compare the available measured data with the simulated variables, the results were consistently and robust. However, further analysis are necessary to evaluate the effect of different objective functions, coefficients upper and lower boundaries, minimum number of interactions, particles, and control sites.

The modeling results confirm the spatial variation of the organic pollution throughout the Upper Iguassu Watershed. As demonstrated by the water quality assessment, the intermediate sites, IG02 to IG04, represents the most impacted reaches. Downstream site IG05 the river starts to assimilate the organic pollution and recovering the quality of water. The comparison in molar concentration gives an idea about the proportion of labile and refractory organic carbon and the relationship with BOD modeling.

In addition, the proposed modeling approach advanced in the representation of organic carbon through labile and refractory characteristics, in both particulate and dissolved fraction. This forms are intend to represent the slow (refractory) and fast (labile) organic carbon decomposition. Consequently, more than just use biodegradable fraction of the organic pollution, identified through BOD modeling, the hypothesis presented in this thesis focus on a better representation of the organic matter in an urban and polluted river.

6.5 Summary

This chapter summarizes the main monitoring results and the proposed model structure and simulations performed. The data base, composed by a set of water quality parameters, is fundamental to evaluate the organic matter dynamics for the particular case of a high polluted watershed, with distinct and conflicting pollution problems. Complementarily, the water quality model, based on a more detailed organic matter consideration, is a key issue to consolidate this thesis main contribution.

The monitoring strategy had a clearly focus on a better characterization of the organic matter in a river with a complex pollution sources matrix. The determination of organic carbon fractions, spectroscopic analysis and biodegradability experiment are the differential for this approach. In an integrated approach, these analyses can provide information about the presence of labile and refractory compounds, and, consequently, the biodegradability characteristics of the anthropogenic derived organic matter in urbanized rivers. Due to the mixture of different compounds and process that are occurring in the river, its analysis is not trivial. The strategy relies on the identification of possible patterns between the parameters monitored, and the main process that may interfere on the organic matter dynamics.

The concepts and processes considered in the ROCS-Model implementation resulted in interesting considerations about the labile and refractory organic carbon content in the Iguassu River. The model advanced in the simulation of DOC, POC, and TOC, but also in the discretization of labile and refractory compounds. The simulation also confirmed the spatial configuration of organic pollution and the most impacted sites.

Finally, one issue related to the challenges in water quality planning and management considering the proposed model is how to analyze the results of organic carbon modeling in a legal basis of only BOD levels. It still needs to be carefully evaluated, since a direct and linear correlation between these two groups of data is not well defined. Furthermore, even though in a mathematical concept, BOD and organic carbon can represent the same "big variable", i. e., organic matter, their chemical history of analysis, and consequently, its interpretation in water quality planning and management is still a research challenge.

Chapter 7

Final Remarks – Conclusions, Thesis Main Contribution and Issues of Further Work and Research

"These are a few of the research challenges; there are undoubtedly more. Dissolved organic matter research is rapidly advancing with many exciting and useful applications. As the need for water resources and recycling continues to increase, so will the need for understanding the nature and properties of dissolved organic matter."

Leenheer, Jerry. A. & Croué, Jean-Philippe. 2003. Characterizing dissolved aquatic organic matter. Environmental Science and Technology, v. 37 (1), p. 18-26.

7.1 Thesis Main Contribution and Conclusions

The water quality deterioration in rivers is a multivariate problem and a direct consequence of the fast urban development. Anthropogenic activities can impact the physical, chemical, and biological properties in aquatic ecosystems. The properly evaluation of these impacts, and the spatial and temporal consequences throughout a watershed, implies in alternatives and distinct monitoring strategies based on quantitative and qualitative

approaches. Up until now however, management restoration efforts focus only on a set of quantitative variables that typically includes biological and chemical oxygen demand, nutrients, and sediments.

The understanding of the pollution problem in urban rivers depends also on the transport and decomposition mechanisms. Water quality models are an important instrument to analyze in an integrated manner the effects of organic pollution. The challenge relies on the identification of an appropriate variable or group of variables that better represents the physical, chemical, and biological process related to the organic matter dynamics in rivers.

Thus, this thesis focused in understanding of how the organic carbon can be used to represent the process related to the organic matter that may occur in an urbanized and polluted river in a context of defining a better water quality strategy for planning and management purposes.

Considering the monitoring perspective, this thesis advanced quantitatively in the determination of DOC, POC, and TOC, and qualitatively by applying spectroscopic techniques to evaluate the organic matter in polluted rivers. The results herein discussed indicated that the determination of BOD, as is commonly monitored, is not enough for the understanding of the organic matter dynamics in aquatic ecosystems.

Besides the information from BOD tests are relevant to the evaluation of the impact in terms of oxygen consumed for biodegradation, other aspects relating to the presence of labile and refractory compounds cannot be identified. In addition, the subjectivity of BOD tests may affect the proper investigation and its use in water quality planning and management.

However, the procedures for DOC, POC, and TOC are not trivial. Differences may be encountered due collection practices, storage, filtration, preservation, and equipment operation. For the properly quantification, it is necessary to conduct the analysis with criteria and following standards and protocols. Moreover, it is important to highlight that the determination of the organic matter is not feasible by a single laboratory procedure. The organic matter is, in essence, a complex mixture of compounds originated by distinct sources, and with specific properties. Thus, the integrated use of different techniques enables the understanding of specific fractions and the evaluation of sources and decay mechanisms. In such a context, this thesis contributed to the application and interpretation of these procedures focusing in a better water quality monitoring strategy for urban and polluted watersheds.

This thesis also advanced in the investigation of organic carbon biodegradation pathways. The experimental procedure herein designed gave important insights about the biological assimilation of DOC, POC, and TOC considering different levels of anthropogenic impacts. The evaluation of the biodegradation kinetics and the evolution of fluorescence EEM throughout the experiment showed relevant considerations about the labile and refractory percentages and decomposition properties.

In addition, the reason for a more comprehensive approach, compared with other water quality models developed to date, is that the process relating organic matter in the aquatic ecosystem cannot be represented only by the biodegradable fraction. Besides BOD-DO mass balance has a well-known history of water quality modelling applications, it represents indirectly only one characteristic of the organic matter. Consequently, a new modelling approach that considers the representation of labile and refractory organic matter, both for dissolved and particulate fractions, complements this thesis main contribution.

In summary, this thesis advanced in four interconnected branches: the understanding of organic matter dynamics in urban watersheds; the quantitative and qualitative measurement of organic matter in polluted rivers; the development of a simulation model for organic carbon; and the evaluation of the proposed monitoring and modelling approaches in water quality planning and management. The four main contribution of this thesis are represented in Figure 1.

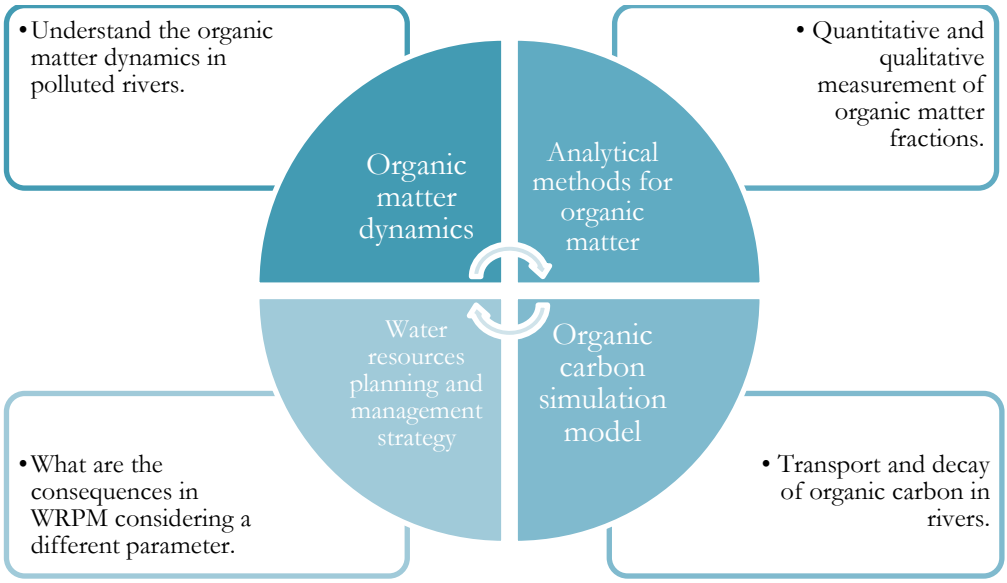


Figure 1: Overview of thesis main contribution

More specifically, this thesis focused on answer the following questions:

"What is the relative importance of allochthonous organic carbon in aquatic ecosystem"?

In the aquatic environment, the organic matter plays an important role in the production and consumption cycles. Through photosynthesis, autotrophs organisms produce organic matter and oxygen, converting inorganic nutrients and CO_2 using solar energy. On the other hand, the available organic matter is assimilated and converted into energy and biomass through a set of metabolic reactions. The organic matter also influences the structure of aquatic communities, the transport of adsorbed pollutants, micronutrient availability, and biological productivity in waters.

Three major sources contributes to the organic matter in aquatic systems: (i) allochthonous, i.e., from outside of the system such as atmospheric deposition or soil material transported by runoff; (ii) autochthonous or surface water-derived of algal or phytoplankton origin; and (iii) allochthonous synthetic organic substances of anthropogenic sources, i.e., effluent organic matter. The distinct sources drives not only the amount of organic matter released or produced in the water column, but also the process and transformation mechanism.

Thus, this question motivated the monitoring strategy and the evaluation of distinct analytical procedures for the characterization and differentiation of organic matter compounds. The results indicated that in human dominated watersheds, labile organic matter predominates and directs contributes for the water quality deterioration. The problem relies on the properly removal and treatment of the organic matter.

Finally, in terms of water resources planning and management strategies for polluted rivers that have to attend formal regulations (i.e. 9.433/97 Brazilian Water Resources Law), this question also highlights an important aspect not considered in traditional water quality monitoring plans. The problem is that not all the organic matter is derived from anthropogenic pollution. Thus, to overcome potential conflicts in understanding the real nature of pollution, it is necessary to evaluate the main characteristics of organic matter. The techniques herein applied and discussed seem to be an interesting alternative.

"What is the meaning of organic carbon as a monitoring parameter in the water quality characterization"?

Due to the organic matter complex composition, mainly formed by biochemically well-defined compounds (polysaccharides, proteins, peptides, and lipids) and humic and fulvic substances (Zumstein and Buffle, 1989), its characterization based on analysis of individual compounds and their properties is still not possible (Leenheer and Croué, 2003). Thus, distinct approaches can be used to characterize the presence of different group of compounds. Examples are BOD, COD, TOC, and spectroscopic techniques. Some of these methods are more suitable for drinking water treatment analysis due to its low detection limits, while for effluent organic matter evaluation other methods can be more easily applied in a routinely way. These methods can provide a better understanding in both quantitative and qualitative terms of the organic content, their origin, and how it will be degraded in the aquatic ecosystem.

Considering these group of parameters, the organic carbon measurement is considered as the most comprehensive measurement widely used to quantify the presence of organic matter in aquatic systems (Leenheer and Croué, 2003). The advantages of TOC or DOC (filtered sample) measurement is the direct quantification of the organic carbon in the sample. BOD, an indirect measurement of the oxygen consumed due to the organic matter biodegradation, has important interferences, resulting in a subjective interpretation.

In addition, while with BOD it is possible to evaluate the biodegradable organic matter, carbohydrates and oxydisable minerals, this test cannot measure complex compounds such as humic substances and aliphatic and aromatic hydrocarbons. COD and TOC analysis account for complex compounds, but while TOC measures only organic carbon compounds, COD can oxidize other substances.

However, one issue when analyzing TOC or DOC concentration in an isolated manner for river water samples, is that the difference of scales resulting from natural or anthropogenic sources are often not as high as observed in other parameters. For example, at the Iguassu River, TOC, DOC, BOD, and COD concentration in a non-impacted site (IG01) was, respectively, 6.3 mgC/L, 4.6 mgC/L, 3.0 mgO_2/L, and 15.0 mgO_2/L. For a human impacted site, IG02, the median observed for the same parameter change to 10.9 mgC/L, 5.7 mgC/L, 17.0 mgO_2/L, and 38.4 mgO_2/L, respectively.

While this could bring complexity to the data interpretation, the measurement of TOC and/or DOC allows more in-depth interpretations. One example if the identification of probable properties related to labile and refractory compounds. This information can be

achieved through the integrated analysis of TOC with BOD, COD and fluorescence EEM. The ratio between TOC and BOD, for example, gives an idea about the labile content of the sample, and, consequently, its immediate impact in terms of water quality deterioration. At the Upper Iguassu Watershed, it has been observed that upstream site IG01, the refractory organic carbon predominates, while downstream site IG02 the labile content reflects the high inputs of wastewater. DOC is also important for Uv-vis and fluorescence spectroscopy. DOC is fundamental for spectrum normalization, and, consequently, to the evaluation of absorbance and emission properties of organic compounds.

Finally, TOC, POC and/or DOC have a fast and easy measurement. Comparing to other parameters, the data interpretation is less susceptible to subjectivities and interferences. These properties are important, especially in human dominated watersheds, where a monitoring approach focusing in a distinct set of parameters is fundamental and would bring relevant information to the decisions makers.

"What is the proportion of organic carbon considering the anthropogenic allochthonous organic matter"?

The organic matter is a complex mixture of organic compounds, and occurs either by natural allochthonous (soil transport and atmospheric deposition) and autochthonous (primary production) sources, as allochthonous synthetic organic substances of anthropogenic sources (i.e., effluent organic matter).

The occurrence of DOC, POC, and TOC is significantly variable and depend on the type of aquatic system and sources. For example, referenced data of measured TOC ranged from less than 1mgC/L in underground or seawaters, to 2-10 mgC/L in lake or river waters, up to 10 gC/L in marshes and fens, while DOC may represent 0.1 mgC/L in groundwater to 50 mgC/L in swamps (Leenheer and Croué, 2003; Visco et al., 2005).

In urbanized watersheds, wastewater effluents contribute to a high proportion anthropogenic allochthonous organic matter. Raw wastewater commonly presents TOC concentration between 80 mgC/L to 290 mgC/L (Metcalf and Eddy, 1991), which may represent a specific per capita TOC load of 26.4 and 28.3 gC/inh.d (Servais et al., 2009). DOC varies from 72 mgC/L in raw wastewater, to values ranging from 5 to 35 mgC/L depending on the wastewater treatment, resulting in a specific DOC per capita load of 6.5 to 8.5 gC/inh.d (Servais et al., 1999; Katsoyiannis and Samara, 2007; Sharma et al., 2011).

The measured data of TOC and the complementarily interpretation of EEM fluorescence intensity peaks at the Iguassu River clearly indicates that the low rates of wastewater collection and treatment represents and important impact in the water quality in more than 50% of the river extension. Sites IG02 to IG04 drains important watersheds, with approximately 2.8 million of inhabitants. The impact in the water quality can be observed through the measured data. The less impacted site (IG01) showed the higher percentage of refractory organic carbon (86% and 84%), and thus, the lower ratios of BOD_5/COD and BOD_5/TOC (0.14 and 0.16, respectively) when comparing molar concentration. For the intermediate sites, the percentage of refractory organic carbon ranged from 40% to 61%.

In summary, anthropogenic sources of organic matter represent a great contribution for increasing of organic matter levels in surface waters, especially in developing countries. The fast urban development and the low rates of wastewater collection and treatment are currently issues of concern. The integrated interpretation of the analytical procedures herein discussed showed that these parameters can contribute to the monitoring and evaluation of spatial and temporal changes in the quality of water in polluted and urbanized rivers.

"How to characterize and differentiate between autochthonous and allochthonous organic matter in human dominated basins"?

The currently available techniques for organic matter characterization are important instruments for water quality planning and management. The basic principle is the identification of common properties that may be used to differentiate the organic matter sources. The integrated use of DOC, UV-visible absorbance, and fluorescence spectroscopy have being successfully applied in aquatic environments for this purpose. The challenge, in the case of Upper Iguassu Watershed, is the complex and heterogeneous organic matter sources and the constant changes and mixtures throughout the rivers. Anthropogenic and natural sources share the space and the evolution of water quality deterioration and recovery throughout the Upper Iguassu Watershed.

The strategy to achieve this question focused on the monitoring of consecutive sites along the Iguassu River, considering distinct properties for the drainage area. For example, site IG01, the most upstream site, drains an area with low anthropogenic occupation. Sites IG02 to IG04 receive continuously an important amount of organic pollution. Sites IG05 and IG06, located downstream in the watershed with progressive less anthropogenic activities, represents the results of compounds dilution and assimilation effects.

Three analytical techniques were used for the primary investigation: DOC, specific UV-visible absorbance at 254 and 285 nm, and fluorescence EEM. Complementarily, two commonly parameters used in organic pollution monitoring and control was used to analyze correlations: BOD_5 and COD. Differences in the biodegradation pathway also supported the characterization and differentiation between human derived organic matter from natural sources.

The results of fluorescence intensity peaks B, T_2, and T_1, commonly related to the properties of the labile organic matter clearly indicate the presence of anthropogenic allochthonous organic matter in the intermediate sites (IG02 to IG04). These peaks showed good correlation with BOD_5. According to the fluorescence peaks intensity, there is a changing point between site IG01 and IG02, corresponding to the beginning of the influence of the urbanization and the increase sources of domestic wastewater. Monitoring sites IG02, IG03, and IG04, located in the most urbanized region of the study, were more affected by the occurrence of tryptophan-like and tyrosine-like fluorescence. Downstream site IG04, where the watershed has less urban development and anthropogenic activities, the presence of labile organic matter starts to decrease and the refractory organic matter becomes proportionally relevant. Refractory compounds are derived either by the labile organic matter decay, as the inputs of allochthonous pedogenic compounds.

Fluorescence spectroscopy showed to be more representative than other techniques for the organic matter differentiation. Interestingly, the results of the fluorescence EEM indicate that even though the labile organic matter predominates and are a direct consequence of the urbanization and organic pollution derived from domestic wastewater, there is still a percentage of refractory organic matter in the water column. Other tests, such as the specific absorbance at 254 and 285 nm, did not provide enough information about the differentiation of labile and refractory organic matter at the Iguassu River. In addition, DOC did not show significantly variation between the sites monitored. However, the determination of DOC is necessary for the fluorescence EEM inner filtering effects normalization.

Finally, the biodegradation experiment results were also relevant to the evaluation of the differences between biodegradable organic matter in impacted and non-impacted sites. Sites IG02 and IG05 presented high values of biodegradable DOC, POC, and TOC consumption during the experimental test. The higher decrease of humic-like fluorescence peak intensity at the polluted sites, compared to site IG01, may indicate that as the labile organic matter is assimilated, the energy released may facilitate the degradation of complex molecules by biological activity.

"What is the difference between the dynamics of organic matter in rivers and other aquatic environments"?

The differences on the transformation mechanism of organic matter when comparing lakes and rivers occurs not only due to physical and geomorphological characteristics, but also as a function of organic matter characteristics. Consequently, both monitoring and modelling approaches implies in distinct strategies.

In general, low human impacted lakes are autotrophic systems (i.e., produces carbon through photosynthesis), while streams are heterotrophic systems (i.e., allochthonous source of organic matter such as terrestrial plants and runoff) (Thurman, 1985). In an urbanized river, as is the Iguassu River at Curitiba and metropolitan region, allochthonous synthetic organic substances of anthropogenic sources represents the most part of the organic matter in the water column. Consequently, biodegradation and other related process may be influenced by the presence of labile compounds.

Considering geomorphological characteristics, the residence time and flow regime has a distinct interference in the biodegradation and sedimentation in streams comparing to lakes. For example, biodegradation may be affected by changes in microorganism adaptation. In rivers, microorganisms are susceptible to physical interferences, i.e., resuspension of settled material during turbulent events, as the presence of inhibitors due to the presence of specific effluent and/or contaminant. Sedimentation also depends on water depth, stream water velocity, and other geomorphological properties. Such conditions promote less interference in sedimentation process in lakes and reservoirs than it affects the respective process in rivers (Thurman, 1985).

Another important aspect is the light penetration. In lakes, in general, photoinduced process are responsible for 21-36% of DOC decrease during one photoperiod, while in rivers the mineralization through photodegradation is less than 2% under the same conditions (Mostofa et al., 2013b).

To represent the transport mechanism and transformation process for the Iguassu River, a one-dimensional advection transport and steady-state model was proposed. ROCS-Model, River Organic Carbon Simulation Model, advanced in the simulation of different fractions of organic carbon, considering labile and refractory compounds for dissolved and particulate forms. The simulated results were consistent with the observed data, and confirm predominance of labile compounds in anthropogenic impacted areas (downstream site IG02, in average 78% of LTOC).

"The current database is sufficient to model the processes involved in organic matter dynamics consistently"?

The modelling approach proposed in this thesis consists in the compartmentalization of the organic carbon primarily in dissolved and particulate fractions, and complementarily in labile and dissolved compounds. This strategy aimed to represent physically and chemically the specific characteristics of the organic matter and to separate the fractions susceptible to a fast and slow decomposition.

In terms of organic carbon monitoring, when it is monitored, only DOC is measured. In addition, the distinct modelling approach hypothesized in this thesis, implies in other input data, such as carbon export rates and specific per capita loads.

For the model implementation and simulation, this thesis advanced in the measurement of other fractions rather than only DOC, and in the evaluation of previous researches suggestions about the carbon export rates as a function of different land use and occupation, and the specific per capita loads for raw and treated wastewater.

For the organic carbon measurement, an adaptation of the standard protocols was proposed for POC and TOC measurement. The results indicated that TOC ranged from 3.6 ± 0.2 mgC/L at site IG01 (less anthropogenic impacts) to 34.8 ± 0.5 mgC/L at site IG04 (high organic pollution inputs), while DOC ranged from 2.5 ± 0.2 mgC/L at site IG06 to 14.4 ± 0.04 mgC/L at site IG03. POC had an interesting variation, ranging from 0.1 ± 0.2 mgC/L at site IG01 to 27.4 ± 0.5 mgC/L at site IG04, with a median value of 2.0 ± 2.2 mgC/L and 7.4 ± 2.9 mgC/L, respectively for sites IG01 and IG04.

The non-point sources were categorized in urban, agricultural, and forest areas. For the ROCS-Model input data, it has been assumed that the non-point sources contribute only to the refractory organic carbon pool (allochthonous pedogenic). In addition, raw and treated domestic wastewater has being estimated to simulate the point sources. The specific per capita loads adopted ranged from 4 to 20 g/inh.d, according to Servais et al. (1999).

For the calibration procedure, some assumptions and simplifications had to be made to adjust the observed and simulated data. In the case of ROCS- Model, that considers four state variables (LPOC, RPOC, LDOC, and RDOC), there was not measured data to directly calculate the objective function to be minimized by the PSO algorithm. Thus, it had been assumed that the observed DOC and POC were equivalent to the sum of LDOC and RDOC, and LPOC and RPOC, respectively. Besides this strategy could mask the search for specific coefficients, the model calibrated the data for the most monitored sites along the Iguassu

River. In addition, the results of fluorescence spectroscopy, through the EEM specific peak intensity, were important to confirm the areas which refractory or labile organic matter predominates.

In summary, to consider the proposed modelling approach, ROCS- Model, there is a necessity of, at least quantitatively, the measurement of DOC, POC, and TOC. Fluorescence EEM data complement qualitatively the differentiation between labile and refractory organic matter, and thus helped in the modelling results interpretation. The biodegradation experiments were also important for the identification of decay patterns and the coefficients ranges. Further studies may incorporate the experimental approach herein proposed to evaluate the organic carbon specific per capita load for different WWTP efficiencies and treatment characteristics.

"Is organic carbon a better variable to model and to represent the organic matter dynamics in a river"?

One of the key questions of this thesis was the evaluation if the organic carbon would be feasible enough to be used in water quality planning and management as a monitoring parameter and a state variable in a mathematical modelling. To achieve this question, the strategy focused on the quantitative and qualitative monitoring, and on the development of a mathematical model to simulate the mechanisms of transport and decay of organic carbon in rivers.

As discussed in previous chapters, and in the aforementioned introductory questions, the organic carbon has important characteristics that justify its inclusion in monitoring and modelling activities. The measurement of TOC, POC, and DOC are less susceptible to interferences rather than other commonly used parameters in water quality monitoring and modelling. BOD, for example, represents an indirect measurement of the oxygen consumed for organic matter biodegradation. The problem concerning with BOD test is mostly related to the subjectivity of the analytical method, once the amount of the oxygen consumed will depend on several factors that are difficult to be rigorously controlled and compared.

The challenge is that not all the organic carbon measured in the water column represents a pollution problem. While the simulation of BOD is restrict to the biodegradable fraction of the organic matter, a model based on TOC simulation gives an idea about the overall organic carbon throughout the main river. Moreover, the distinct fractions allow a

deeper interpretation about the amount of organic matter that can be easily degraded, and, consequently, direct impact of the overall water quality conditions.

The results of the organic carbon modeling indicated that before urbanization (site IG01), the proportion of refractory organic carbon was higher, with RTOC about 39%, while LTOC was 61%, in average. Downstream site IG02, the proportion changed to 22% of RTOC and 78% of LTOC. These results are in accordance with other studies, that indicates a percentage from 40 to 80% of humic substances (refractory organic matter) in non-polluted aquatic environments (Zumstein and Buffle, 1989; Benner, 2003; Filella, 2009).

Clearly, the proposed modelling approach advances in the organic matter modelling in rivers, specifically in the labile and refractory organic carbon evaluation. The process based on labile and refractory organic carbon provides a gain in terms of quantification of the potential compounds that have a direct impact in oxygen depletion and water quality deterioration. Besides in Brazil there is still no legal basis for organic carbon monitoring and control, the monitoring and modelling strategies herein presented are an important contribution for water resources planning and management.

7.2 Organic Matter Dynamics in Polluted Rivers: Further Experiments

This thesis advanced in the measurement of TOC and POC, as the specific biodegradation rates through a set of experimental determinations. Complementarily, some improvements to the experimental evaluation of organic matter dynamics can be highlighted and be considered for future studies.

As discussed at chapter 6, BOD_5 and organic carbon analysis (TOC, POC, and DOC) are associated. In the case study presented, TOC, COD, and BOD ratios were analyzed to evaluate probable percentages of labile and refractory organic carbon. For example, when comparing BOD_5 in molar concentration or in terms of mass of carbon assimilated, the comparison with TOC values could provide interesting information. In this case, if TOC and BOD_5 showed similar results, it may indicate that the organic carbon measured by TOC has labile properties. If TOC is higher than BOD_5, then the composition may be more refractory. And, if BOD_5 exceeds TOC, then it could be an indication that BOD_5 measured other compounds, such as nitrogen, ferric iron, sulfide, or the growth rate of microorganism are significant.

Thus, to extend this analysis, filtered BOD_5 data could be useful. If BOD_5 test is conducted with samples filtered with the same porosity size of DOC analysis, than the associated ratios could also indicate the percentages of labile and refractory DOC. The same approach could be interesting for the particulate fraction of organic carbon.

Another possibility to advance in the organic matter dynamics in polluted rivers is to conduct experiments to evaluate the effects of photodegradation. It is well documented that photoinduced process contributes to the most part of the total organic matter assimilation and degradation in lakes (Thurman, 1985; Mostofa et al., 2013a). However, besides the influence of turbidity, which decreases light penetration, and the constant turbulence and mixture due to the hydrodynamics of a river, little is known about the effective contribution of photodegradation to the overall organic matter decomposition in rivers, and, in this case, in polluted rivers.

7.3 Absorbance and Fluorescence Spectroscopy: what came next

According to the literature review detailed in Chapter 3, absorbance and fluorescence spectroscopy have been successfully applied in the last decades in different fields related to water resources. The main principle is the identification of specific compounds that emit or absorb energy in different wavelength. Depending on the source or the composition of the organic compounds, the results of these analyses are direct and easy to be evaluated. However, when there are different sources of organic matter (natural allochthonous, autochthonous, anthropogenic allochthonous), or the control volume is not well define or susceptible to high variability on time and space, the results are often only an indication of the probable composition.

Thus, with the data collected and the experience obtained with the strategy defined in this thesis, further work ay incorporate the application of PARAFAC modelling (Felipe-Sotelo et al., 2007; Stedmon and Bro, 2008). PARAFAC – Parallel Factor Analysis, can be used to evaluate the predominance of different peaks intensities according to watershed scale and anthropogenic occupation. Considering decomposition experiments, this analysis could also be used to evaluate and identify groups of compounds that are more likely to be decomposed according to the catchment characteristics and pollution sources.

7.4 Effluent Organic Matter Characterization

There is also a lack of data regarding to effluent organic matter quantification and characterization. For example, the quantification and evaluation of DOC, POC, ant TOC specific loads and the respective labile and refractory percentages for raw sewage and after different treatments would bring additional data and important information for modelling and management purposes. Servais et al. (1999) conducted a detailed study with the aim of determining the main effluent characteristics according to different types of treatment and removal efficiencies. In their research, the authors evaluated the variation of DOC, POC, TOC, and its respective biodegradable fractions, as the corresponding specific loads for 5 wastewater treatment plants (WWTP).

Future studies might also incorporate the evaluation suggested by Goldman et al. (2012), who applied fluorescence EEM and statistical techniques for predicting percent wastewater in an urban stream. The authors investigated the amount of wastewater in the river as function of the relative abundance of specific fluorescence excitation/emission pairs. In common, these studies focused directly on the effluent organic matter characterization and quantification. Similar approach could provide an overview about the removal rates and specific per capita loads of organic carbon for the WWTP located at Upper Iguassu Watershed and other watersheds.

7.5 Organic Carbon Export Rates

While the results of ROCS-Model simulation indicated that for a polluted river the main contribution of organic pollution is not from non-point sources, the properly identification and quantification of allochthonous organic matter is still relevant for modelling purposes. Thus, the organic carbon export rates are another issue that could be considered for further studies.

The export rates vary according to the soil type, vegetal coverage, land use and occupation. Besides there are good references about loss of organic carbon under different land use and occupation (See Chapter 3 for a list of references), it is still a challenge to adapt and use this data for modelling purposes. Most part of the studies was conducted in temperate watersheds, with forested areas, or distinct land and crop practices. In addition, the

rates of organic carbon loss were estimated through river water samples, which may have effects of transport, mixture and decomposition (Hope et al., 1994).

One approach to quantify the export rates of DOC, POC, and TOC from different land use and occupation could be through the evaluation of loss of organic carbon in a controlled site. Lysimeters installed in distinct watersheds can be used for this purpose (McTiernan et al., 2001; Sandford et al., 2013). In this case, artificial rainfall with increasing intensity could also be part of the evaluation.

Another approach could be through a continuous monitoring system, by collecting several samples in the river during a rainfall event. This system has being successfully applied in an urban basin to evaluate water quality variation during different rainfall intensities (Braga et al., 2013).

7.6 Non-impacted Watersheds: What happens?

It is also interesting to highlight that future monitoring strategies in non-impacted watersheds including the particulate and dissolved fractions of organic carbon (POC, DOC, and TOC), and the combination of fluorescence and absorbance spectrophotometry, would bring an important contribution to the understanding of organic matter dynamics in different conditions. The expectation is that in non-impacted watersheds, this investigation would provide distinct data due to the characteristics of organic carbon export (allochthonous pedogenic humic compounds), and, consequently, on the degradation rates in the water column.

Additionally, it would also be interesting the evaluation of how the refractory organic carbon is distributed along a non-polluted river, and the ratio between DOC and POC. This analysis could lead to a better understanding of the organic matter dynamics in a "natural" condition, with less interference of other compounds. Consequently, the integrated analyses with other conventional parameters used in water quality monitoring strategies would also bring new information about acceptable levels of DOC, POC, and TOC.

7.7 The Water Quality Planning and Management Perspective

One question that relies on this strategy is "Is the organic carbon the right parameter for water resources planning and management?" First, there are some advantages about the organic carbon analysis. The determination of DOC, POC, and TOC has less interference comparing to parameters such as BOD. For effluents evaluation and control, COD and BOD have a successfully historical application. However, for surface waters quality monitoring, and thus, the use of COD and BOD in planning and management, has well documented fragilities (Comber et al., 1996).

Due to the incubation time, the necessity to rigorously control of the microorganisms condition and adaptation, the presence of inhibitors or other compounds, the results of BOD tests have uncertainties (20% of acceptable error, according to APHA, 1998) and logistical difficulties (5 days as a minimum for incubation time). COD is also a critical parameter for surface water quality monitoring, since it is difficult to evaluate the characteristics of the organic matter that is being oxidized.

When the protocols are strictly followed, DOC, POC, and TOC seem to be an interesting alternative. More than just measure the biodegradable fraction, as is the result of BOD tests, the determination of organic carbon fractions allows a deeper interpretation about the labile and refractory content. Moreover, the integrated analysis with UV-vis and fluorescence spectroscopy qualitatively provides insights about the main sources of organic matter in the aquatic system.

As emphasized before, the Brazilian water resources regulation, CONAMA 357/11 and 9.433/97 Law, do not consider the organic carbon as a parameter for surface water monitoring and control. Besides both regulations advanced in several aspects about the on-going water resources planning and management deficiencies in the Brazilian territory, there is still a necessity to consider distinct strategies to efficiently apply the management instruments outlined. One of these strategies is the search for a more representative parameter, or a group of parameter, for water quality monitoring. Thus, the results discussed in this thesis can be an important contribution for a changing in this strategy.

Chapter 8

References

"If I have seen farther, it is by standing on the shoulders of giants."

Isaac Newton, in a letter to Robert Hooke, Cambridge, February 5, 1675.

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Appendix 1

Monitoring Sites

This additional appendix presents some illustrations of the monitoring sites during the samplings performed during 2012 and 2013.

Figure 1: Sampling site IG01 (left) and sampling site IG02 (right) – Iguassu River.

Figure 2: Sampling site IG02B (left) and sampling site IG03 – Iguassu River.

Figure 3: Sampling site IG05 (left) and sampling site IG6 – Iguassu River.

Figure 4: Equipment used for samples preservation and collection.

Figure 5: Courageous people during the samplings activities.

Appendix 2

Analytical Methods for DOC, POC, TOC, and Summary of Methods of Other Parameters

This additional appendix describes the analytical methods considered for dissolved organic carbon (DOC), particulate organic carbon (POC), and total organic carbon (TOC). It is also presented the calibration curves, solution used, detection limits and other supplementary information. Complementarily, it is presented a summary of the other parameters used for the water quality monitoring.

1. Organic carbon measurement

The concentration of Dissolved (DOC) and Total Organic Carbon (TOC) were measured using a TOC-VCPH analyzer (Shimadzu). The inorganic carbon was eliminated before the analysis, with a 10 min sparging time using Nitrogen as the carrier gas. For each sample, 100 μL was injected at least 6 times (a direct injection in the combustion tube was adapted using a gas tight micro syringe). For DOC concentrations, samples were filtered

with a pre-washed 0.45 µm PVDF syringe filter. The particulate organic carbon (POC) was calculated by the difference between TOC and DOC.

The equipment was calibrated using potassium hydrogen phthalate, 0.5% of the volume of H_2SO_4 P.A. for IC elimination (APHA, 1998), and 10 minute of sparging time. The limit of detection (LOD) and the limit of quantification (LOQ) were estimated though the standard deviation and the slope of the calibration curve:

$$LOD = 3 \cdot \frac{S_a}{b}$$

$$LOQ = 10 \cdot \frac{S_a}{b}$$

Where: *LOD* is the limit of detection; *LOQ* is the limit of quantification; *Sa* is the standard deviation of the measured data; and *b* is the slope of the calibration curve. The LOD ranged from 0.016 mgC/L to 0.107 mgC/L, and the LOQ ranged from 0.053 mgC/L to 0.357 mgC/L. Figures 1, 2, and 3 presents the calibrated curves for three sets of measurement.

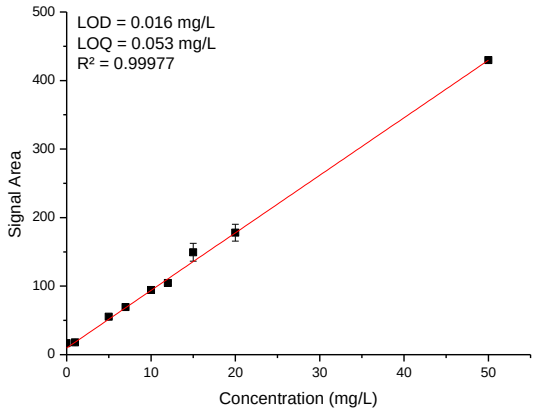

Figure 1: Calibration curve for organic carbon, considering manual direct injection (syringe), potassium hydrogen phthalate, 0.5% of H_2SO_4 and 10 minute sparging time.

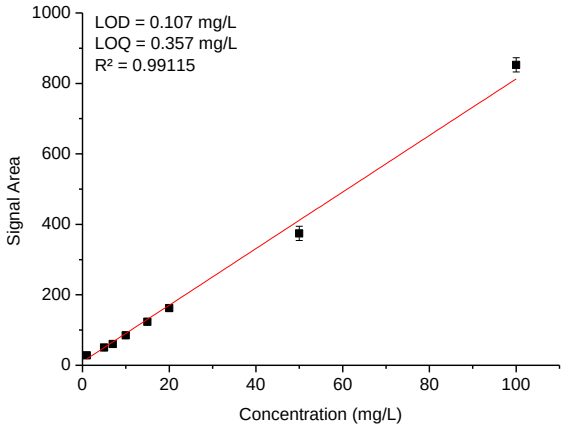

Figure 2: Calibration curve for organic carbon, considering manual direct injection (syringe), potassium hydrogen phthalate, 0.5% of H_2SO_4 and 10 minute sparging time.

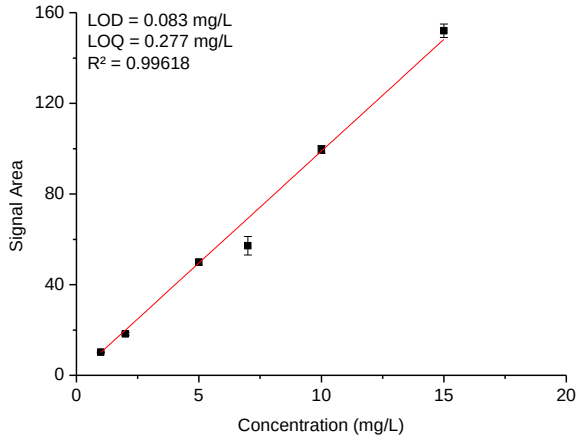

Figure 3: Calibration curve for organic carbon, considering manual direct injection (syringe), potassium hydrogen phthalate, 0.5% of H_2SO_4 and 10 minute sparging time.

2. Summary of water quality parameters and chemical analysis.

The physical-chemical parameters measured were: dissolved oxygen, water temperature, conductivity, pH, turbidity, and alkalinity. The parameters determined in laboratory were: BOD_5, COD, organic and ammonia nitrogen, nitrite and nitrate, total phosphorus, orthophosphate, and solids. The following tables present a summary of the parameters and the respective analytical methods, and the detection range.

Table 1: Summary of the analytical method and detection limit of the monitored parameters

Parameter	Analytical Method	Detection Range	Reference
BOD_5	Respirometric method	0 – 4.000 mg/L	WTW user manual.
COD	Closed reflux, titrimetric method	420 nm: 0 – 90 mgO_2/L 600 nm: 100 – 900 mgO_2/L	5220 D (APHA, 1998)
Total Nitrogen	Phenate method	> 2.9 mg/L	4500 – N C (APHA, 1998)
Ammonia	Phenate method	10 – 2000 μ g/L	4500 – NH_3 F (APHA, 1998)
Nitrite	Colorimetric method	5 – 1000 μ gNO_2^-/L	4500 – NO_2^- B (APHA, 1998)
Nitrate	Colorimetric method	10 – 1000 μ gNO_3^-/L	4500 - NO_3^- E (APHA, 1998)
Total phosphorous	Sulphuric acid – nitric acid digestion followed by stannous chloride colorimetric method	5 – 1500 μ g/L	4500-P E (APHA, 1998)
Orthophosphate	Ascorbic acid method	5 – 1500 μ gPO_4^{-3}/L	4500-P E (APHA, 1998)
Alkalinity	Titrimetric method	In the range of 10 – 500 mg/L, it is accepted a standard deviation of 1 mg/L of $CaCO_3$	2320 A (APHA, 1998)
Chlorophyll-a	Spectrophotometric	5.0 μ g/L	10200 H (APHA, 1998)
Total Solids	Dried at 103 – 105 ºC	< 200 mg	2540 B (APHA, 1998)
Fixed and Volatile Solids	Ignition method at 550 ºC	< 200 mg d	2540 E (APHA, 1998)

Table 2: Summary of equipment technical details for the in situ monitored parameters

Parameter	Sensor	Details
Dissolved Oxygen	LDO101 – Hach	- Range: 0.1 to 20.0 mg/L (1 to 200% of saturation); - Accuracy: ± 0.1 mg/L for 0 to 8 mg/L and ± 0.2 mg/L for greater than 8 mg/L. - Operating Temperature: 0 to 50ºC (32 to 122 F) - Minimum Sample Depth: 25 mm (0.984 in.)
pH	pHC101 - Hach	- Slope: -59mV/pH (90 to 110% at 25ºC (77 F) per Nernstian theoretical value) - Temperature Accuracy: ± 0.3 ºC (± 0.54 ºC) - Operating Temperature: 0 to 50ºC (32 to 122 F) - Minimum Sample Depth: 20 mm (0.79 in.)
Conductivity	CDC401 - Hach	- Range: 0.01 ìS/cm to 200.0 mS/cm; - TDS (total dissolved solids range): 0 to 50,000 mg/L as NaCl; - Operating Temperature: -10 to 110ºC (14 to 230 F); - Minimum Sample Depth: 45 mm (1.77 in.)
Temperature	Hach	- Range: -10.0 to 110.0; Resolution: 0.01 ºC - Accuracy: ± 0.3 ºC (± 0.54 F)
Turbidity	2100Q - Hach	- Range: 0 to 1000 NTU; Accuracy: ± 2% of reading plus stray light; Operating Temperature: 0 to 50ºC (32 to 122 F)

Appendix 3

Biodegradation Experiment: Supplementary Data

This additional appendix presents supplementary data and analysis of the biodegradation experiment.

1. Experimental design

The evaluation of biodegradation process of anthropogenic derived organic matter was experimentally investigated for sampling sites IG01, IG02, and IG05. Carbon free amber glass bottles were used to collect three liters of sample in each monitoring site. An adapted method based on BDOC tests was conducted for organic matter degradation, based on procedures by Frias et al. (1995), Lim et al. (2008), Labanowski and Feuillade (2009) and Stutter et al. (2013). Batch incubations were performed in the dark, with controlled temperature at 20°C, for a period of 10 days, with regular intervals of sampling analysis. For each set of experiment, open and closed systems were tested to analyze its characteristics and results. In addition, a nutrient solution was added in the proportion of 0.5 mL/L of sample. The nutrient solution was prepared according to APHA (1998) for

BOD analysis (phosphate buffer solution, magnesium sulfate solution, calcium chloride solution and ferric chloride solution).

For the open system (named Experiment A), 2L of sample were incubated in glass containers with an aeration and shaking apparatus. Ultrapure water with the same concentration of nutrients was used as blank control. For the closed system (named Experiment B), amber glass bottles of 120 mL were used to incubate the samples. For Experiment B, ultrapure water with nutrients was used for dilution (APHA, 1998). A dilution of 50% was considered for all samples. A blank test with ultrapure water and the same concentration of nutrients was conducted for control.

In regular intervals of days, three subsamples of 10 mL from each bottle and/or container were collected with a glass syringe (pre-washed with acid). For DOC, fluorescence and absorbance analysis, samples were filtered in a pre-washed 0.45 μm PVDF syringe filter, and stored in a carbon free glass ampoule in the dark at 4°C. For total organic carbon (TOC) and DOC, 0.5% of the volume of H_2SO_4 P.A. was added for preservation (APHA, 1998). All glasses (collection bottles, ampoules, incubation bottles/containers) used during the experiment were acid washed and baked during 5 hours at 550°C. A schematic representation of the experiments and the adaptation in the TOC analyzer is presented in Figures 1, 2, and 3.

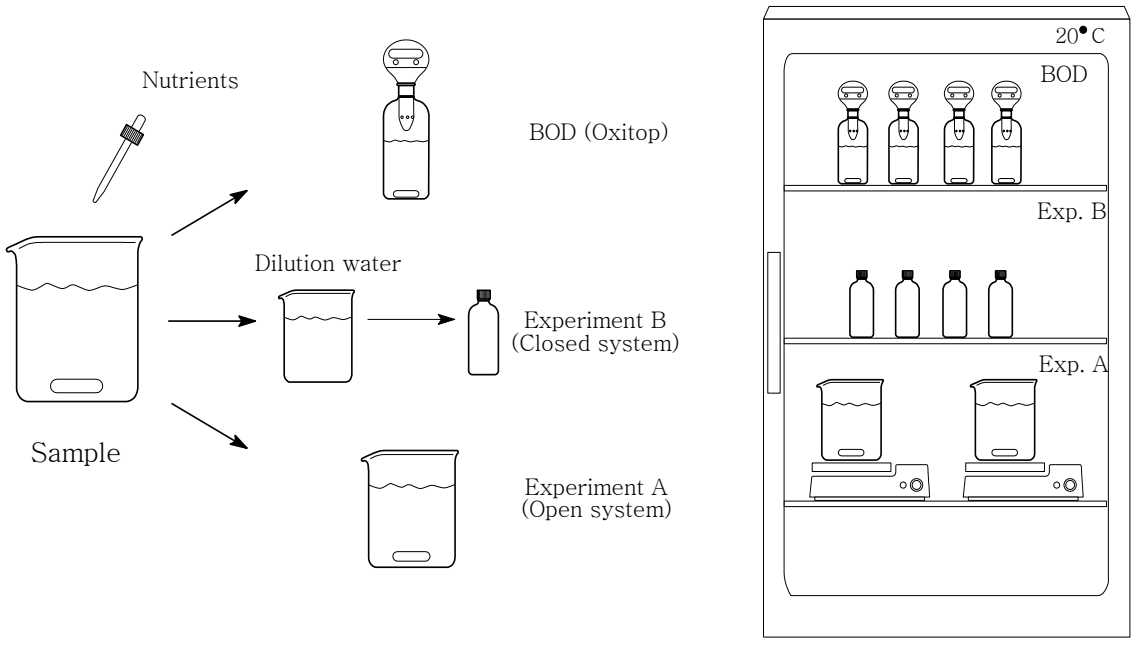

Figure 1: Schematic representation of the biodegradation experiment (TOC and DOC analysis) and the determination of BOD kinetics rates (Oxitop). Experiment A and B were incubated during 10 days. BOD samples were incubated during 5 days.

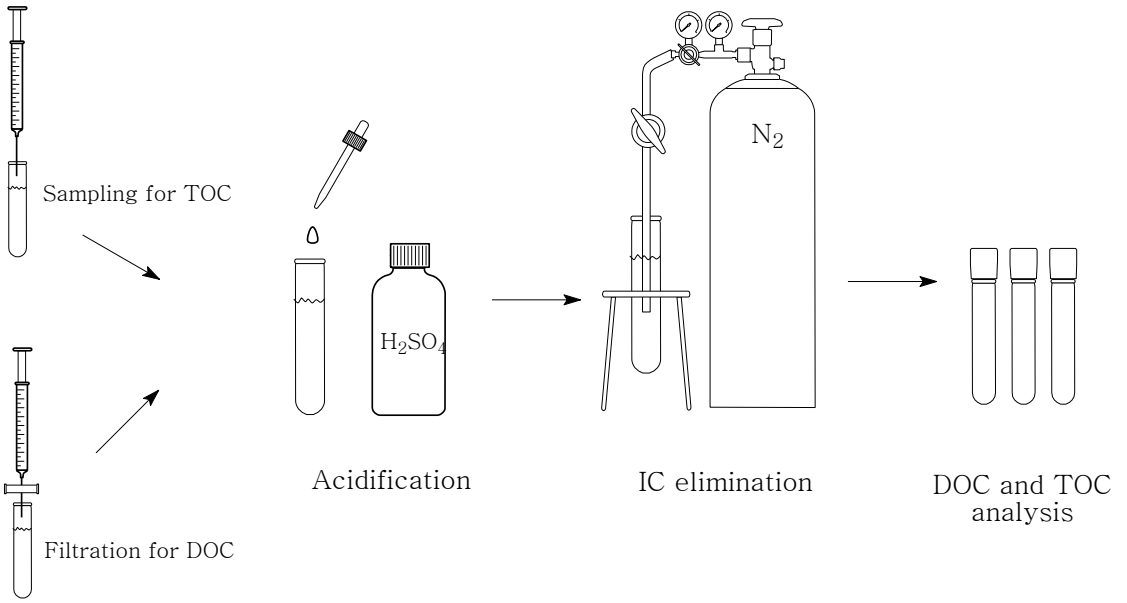

Figure 2: Schematic representation of sampling, filtration (for DOC samples), acidification, IC elimination (10 min. sparging with N_2).

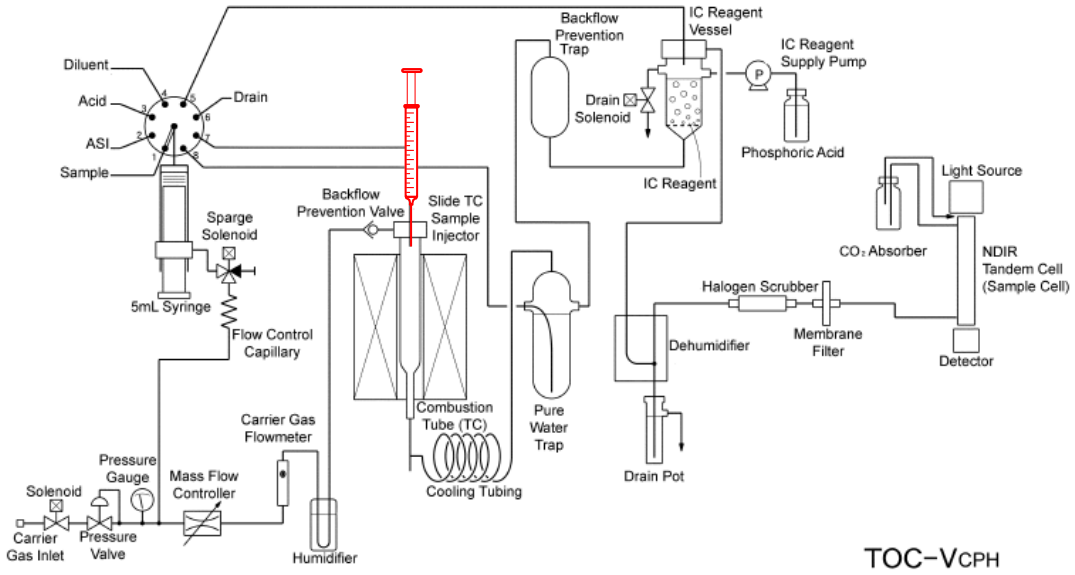

Figure 3: Technical scheme of standard TOC equipment (TOC-VCPH, Shimadzu) and the adapted direct injection of liquid samples in the combustion tube (red syringe). The direction injection was adapted with a Hamilton gas tight syringe. Source: Adapted from Shimadzu TOC-VPH user manual.

Figures 4 and 5 present some illustrations of the experiment. TOC was measured through an adapted direct injection of liquid samples in the combustion tube of the equipment (Figure 4). Samples were collected through a glass syringe, and storage in free carbon glass ampoules (Figure 5).

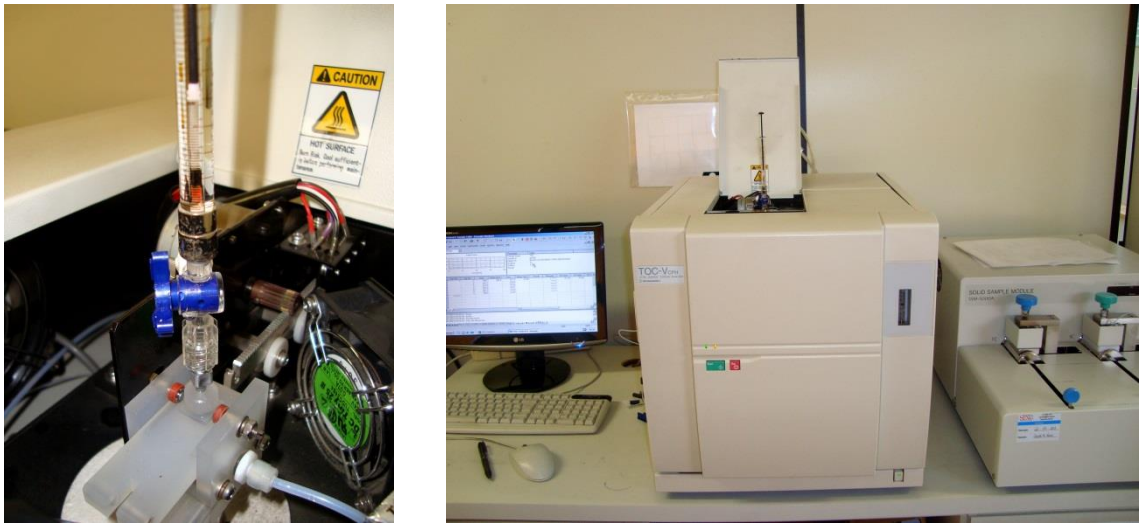

Figure 4: The adapted direct injection of liquid samples in the combustion tube (left) and the TOC equipment (TOC-VCOH, Shimadzu).

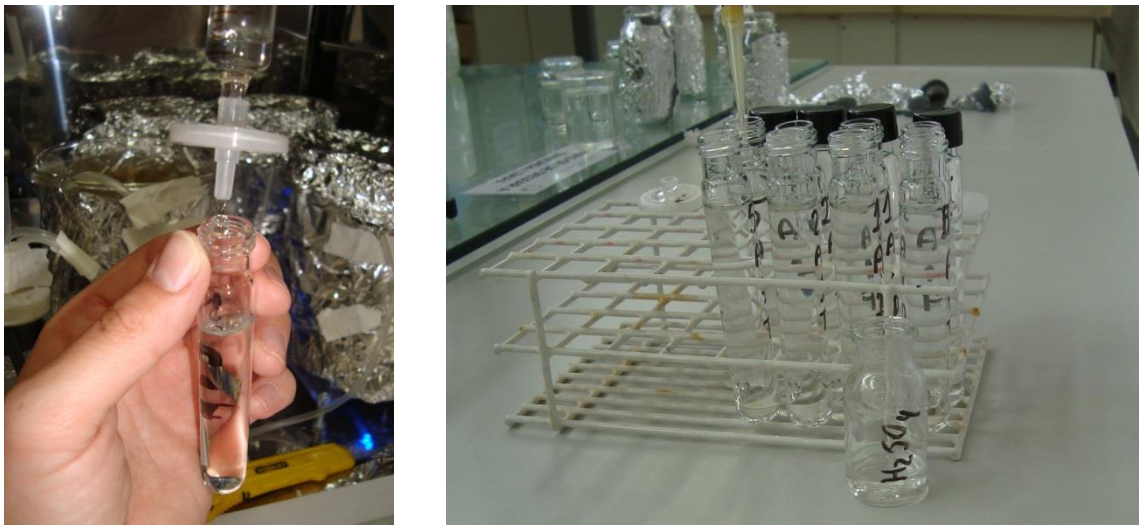

Figure 5: Detail of sample collection and filtration (left) and the acidification for sample preservation (right).

Figure 6 and 7 present illustrations of Experiment A. In the first two sets of experiment (July/13 and Set/13), it had been observed that suspended material was retained in the aeration apparatus was (Figure 6), which could lead to an underestimation of POC and TOC. During the other samplings, the aeration was not used. Figure 7 shows the complementarily measurement of DO and pH in the samples.

Figure 6: Experiment A (open system) inside the incubator (left) and the detail of suspended material retained in the aeration system during the second experiment (right).

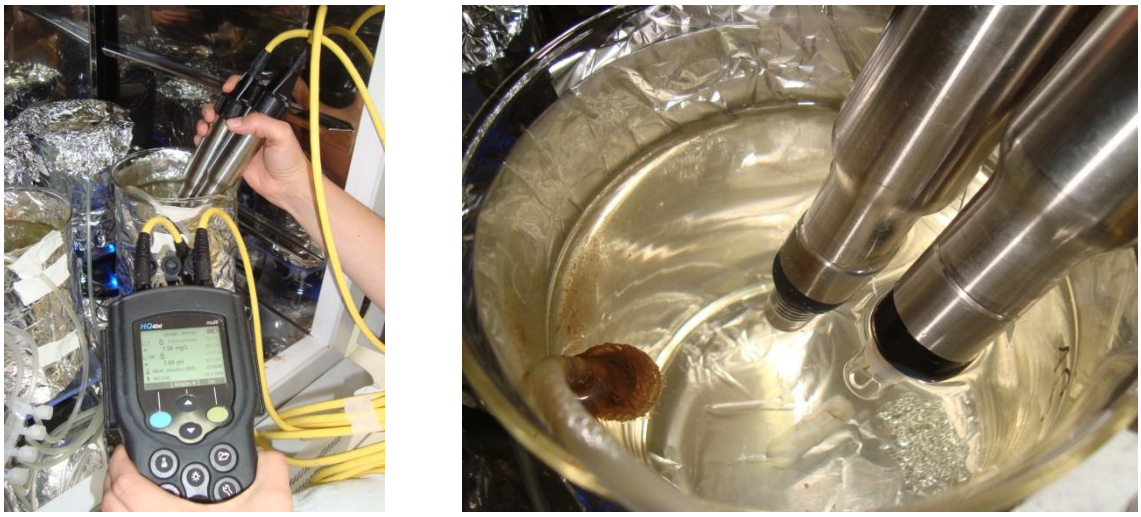

Figure 7: Measurement of DO and pH (left) and detail of the sensors used for DO and pH measurement (right).

2. Additional measured data

Complementarily, DO and pH were measured during the incubation period. The following Figures presents the results for experiments performed in Oct/13 and Dec/13.

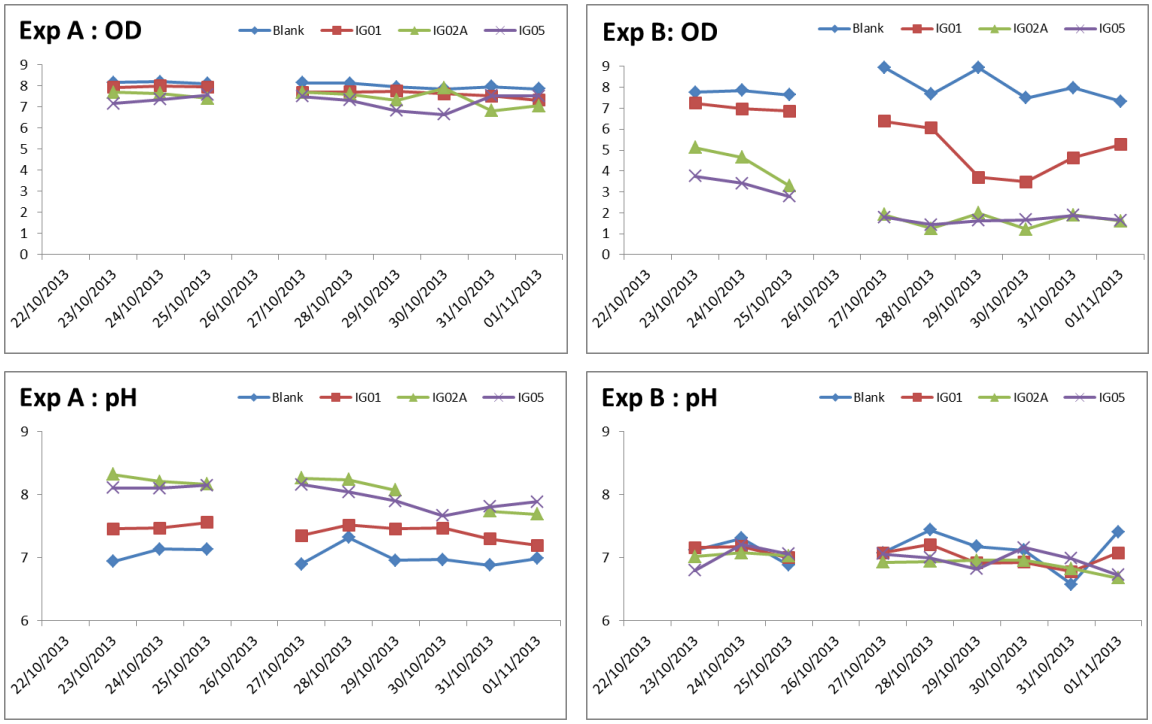

Figure 8: Additional measured data for experiment A and B, Oct/13.

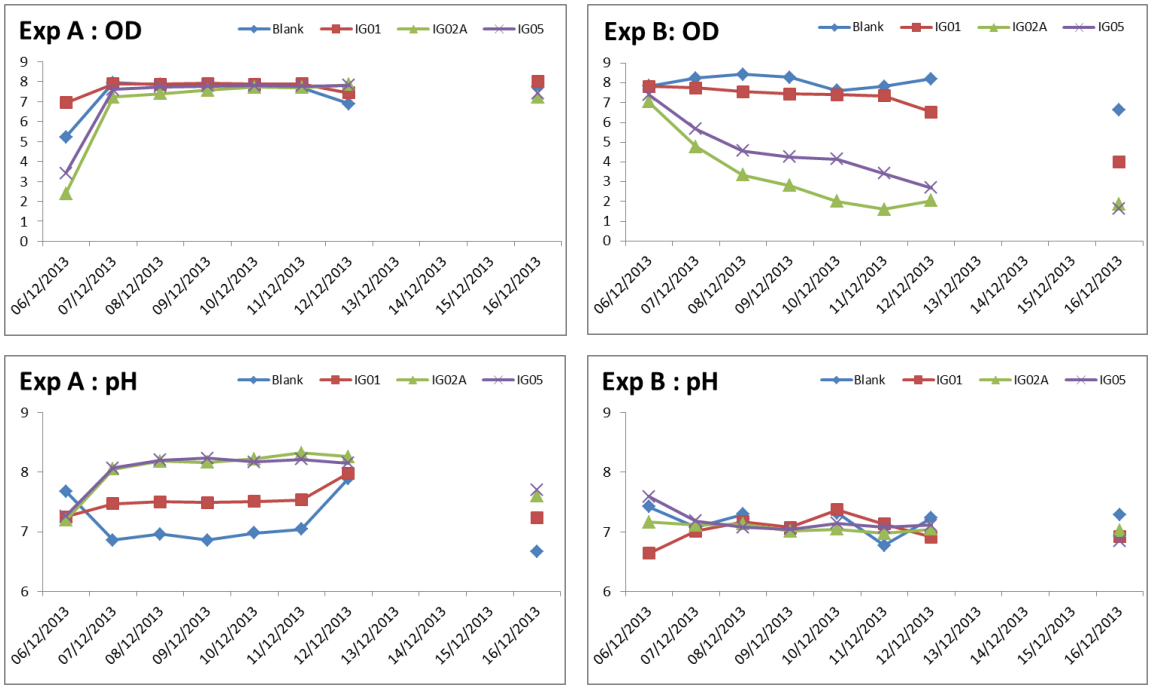

Figure 9: Additional measured data for experiment A and B, Dec/13.

3. Experimental difficulties: identification of data errors

It is also important to report some of the difficulties and/or errors detected during the biodegradation experiment. A first observation is about the filtration process. It was detected during the first and second experiments some contamination with organic compounds during the experimental procedure (DOC measured was higher than TOC for the same sample, Figure 10). Some of the hypotheses lead to the investigation of the filter system, membrane used or some of the material used to collect the sample. Tests were realized in an attempt to identify which are the probable causes, but with no conclusive findings. To overcome this problem of contamination during the last two experiments (Oct/13 and Dec/13), syringe filters were used, and the results indicated no contamination for the samples analyzed.

Another adaptation of the experiment between the first and third set of analysis were the amount of nutrient solution and the nitrification inhibitor added to the samples. The nutrient solution was added according to the protocols for BOD essays. The adaptation was in order to keep the same proportion of nutrient add per volume of raw sample used, even for the diluted experiment (Experiment B, closed system). In relation to the nitrification inhibitor, only on the second set of experiment it was added to the sample, since during the first experiment it was observed that the absorbance signal after 5 days of experiment had being changed, indicating that a nitrification process was occurring in the sample (open system, Experiment A). However, due to the composition of the inhibitor, problems were observed during the organic carbon analysis and on the absorbance and fluorescence signals.

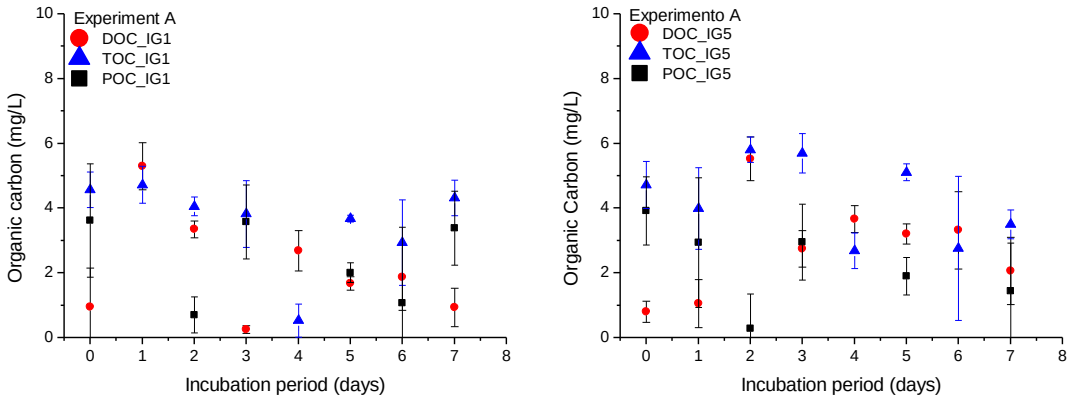

Figure 10: Decay of organic carbon for Experiment B during sampling performed at Sep/13.

Another example of contamination can be observed in Figures 9 and 10. Considering experiment B, a probable contamination could be due to the lids used. It has being observed that the contamination did not propagate for the samplings for the other incubated bottles.

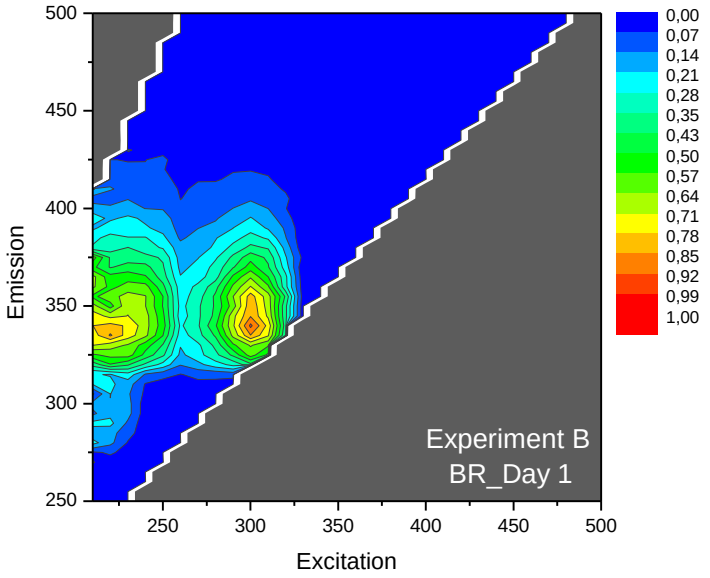

Figure 11: Example of contamination observed during experiment performed at Oct/13 (C47), for closed system (Experiment B), in the blank control.

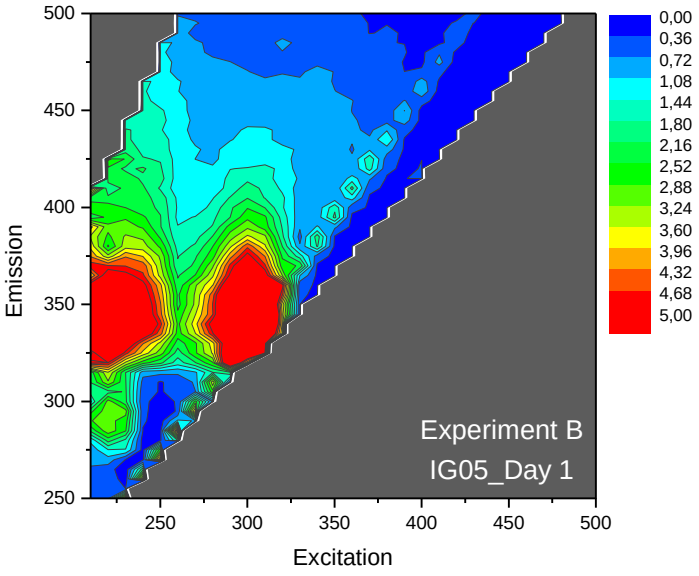

Figure 12: Example of contamination observed during experiment performed at Sep/13 (C46), for closed system (Experiment B), monitoring site IG05.

Complementarily, Figure 11 and 12 shows and example of contamination identification considering fluorescence intensity of peak T1 and the excitation-emission matrix.

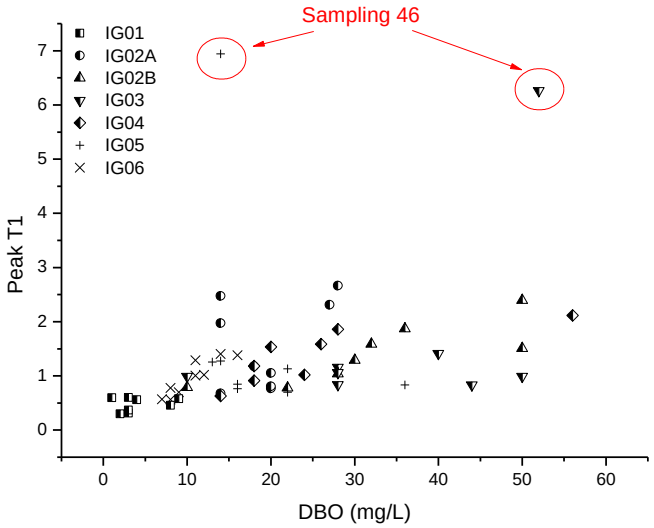

Figure 13: Example of contamination observed during sampling C46 (Set/13), for monitoring sites IG05 and IG03.

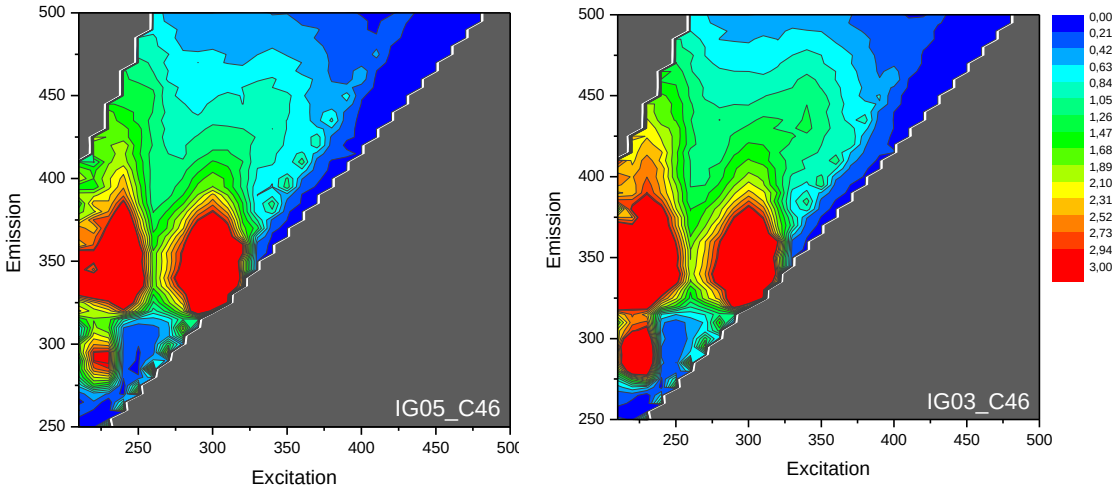

Figure 14: Example of contamination observed during sampling C46 (Set/13), for monitoring sites IG05 and IG03

4. Additional data

The following Figures presents the results for normalized value of DOC and TOC, and the EEM during the biodegradability experiment A (open system) and B (closed system) realized with samples collected in October/2013.

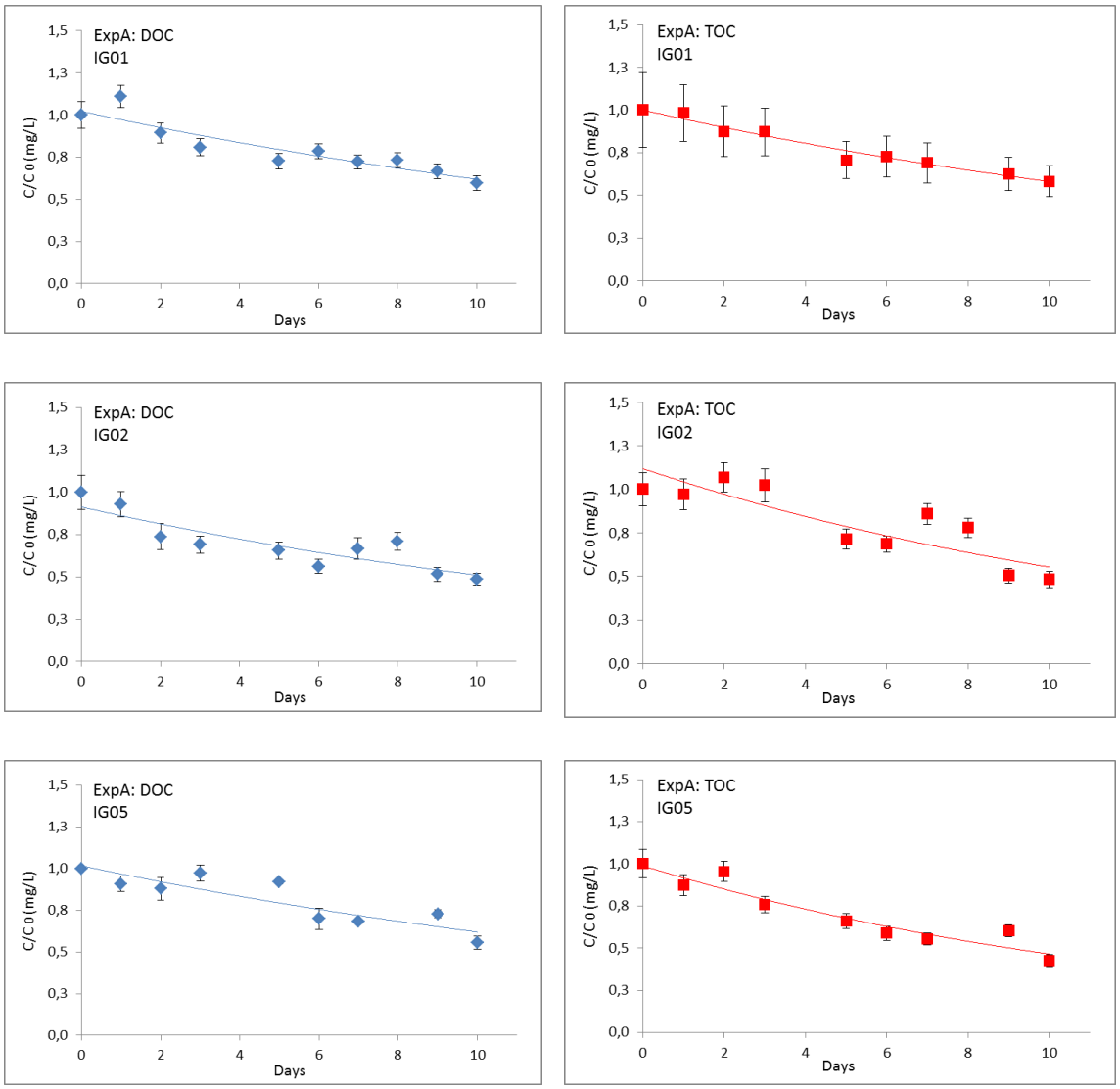

Figure 15: Normalized data of DOC and TOC for Biodegradation experiment A (Oct/13)

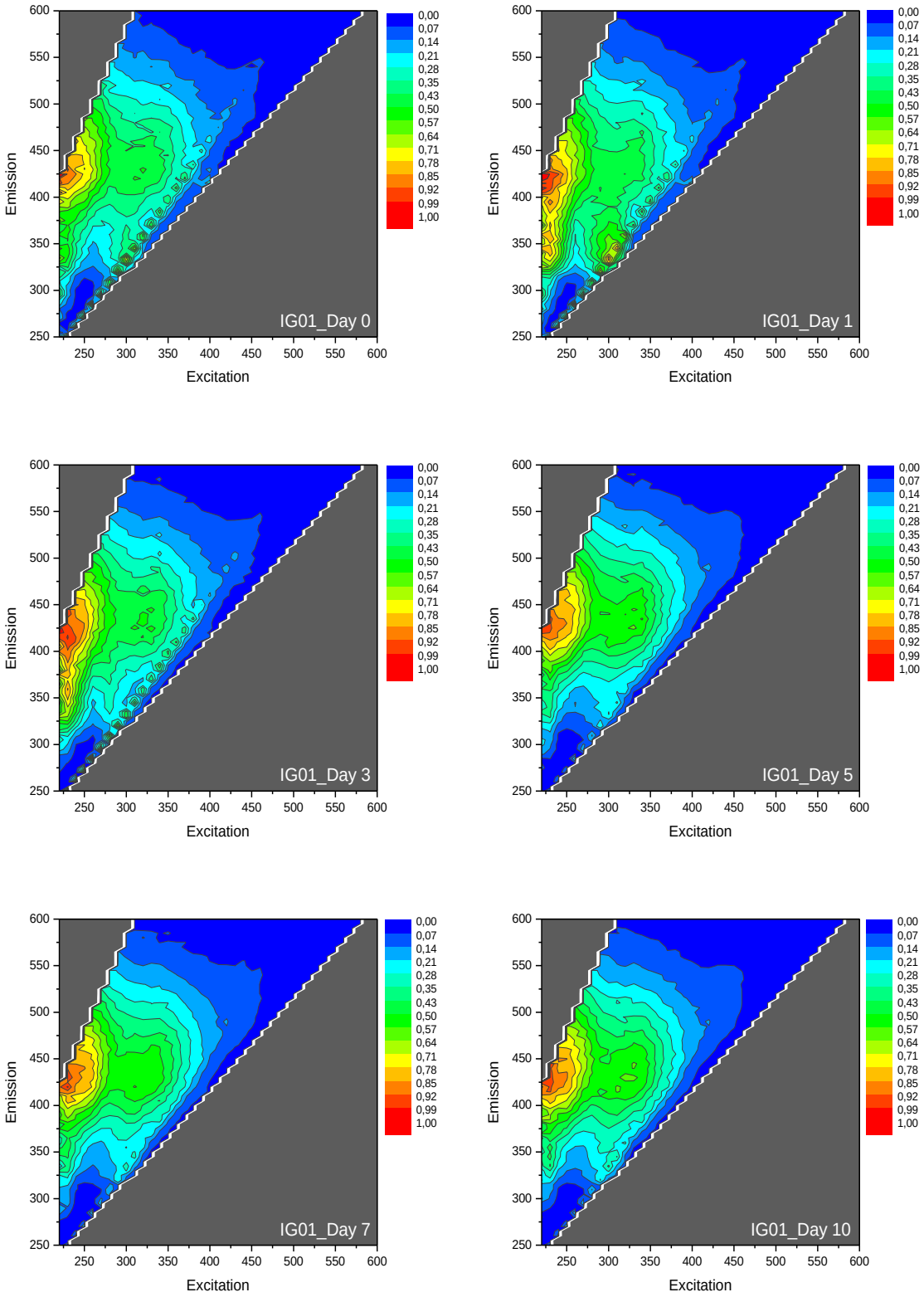

Figure 16: Excitation-emission matrix evolution, exp. A, sample IG01 (Oct/13)

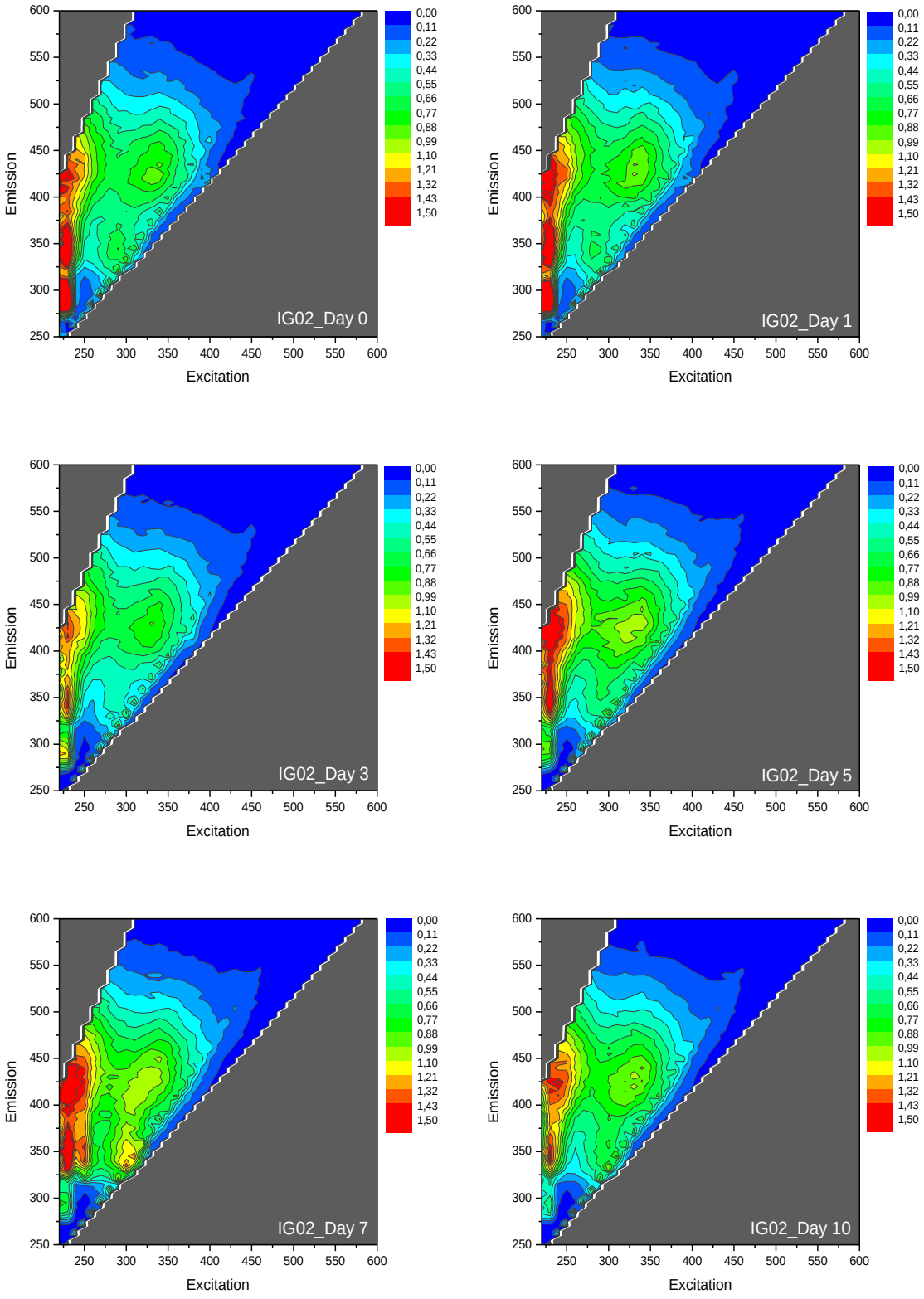

Figure 17: Excitation-emission matrix evolution, exp. A, sample IG02 (Oct/13)

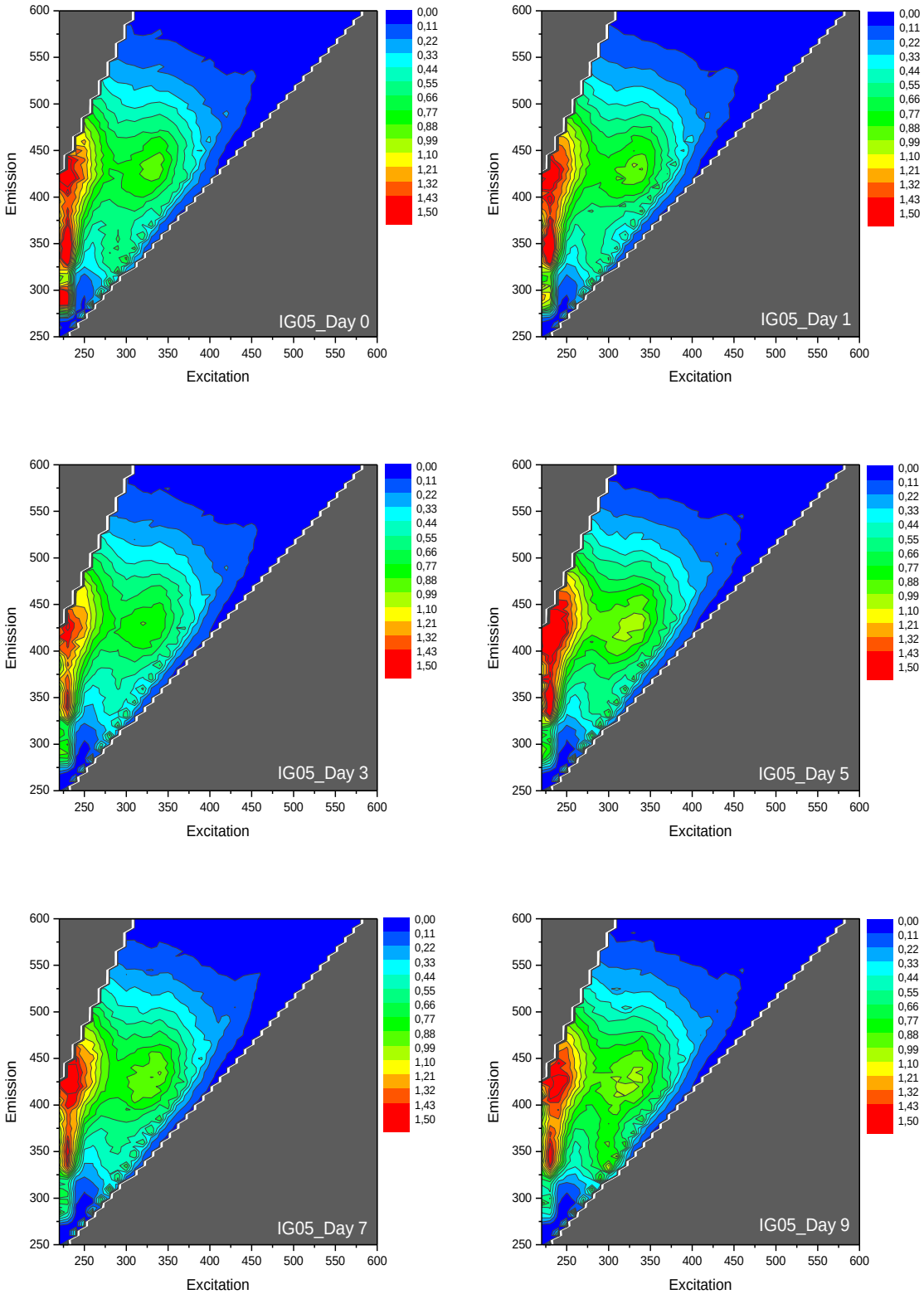

Figure 18: Excitation-emission matrix evolution, exp. A, sample IG05 (Oct/13)

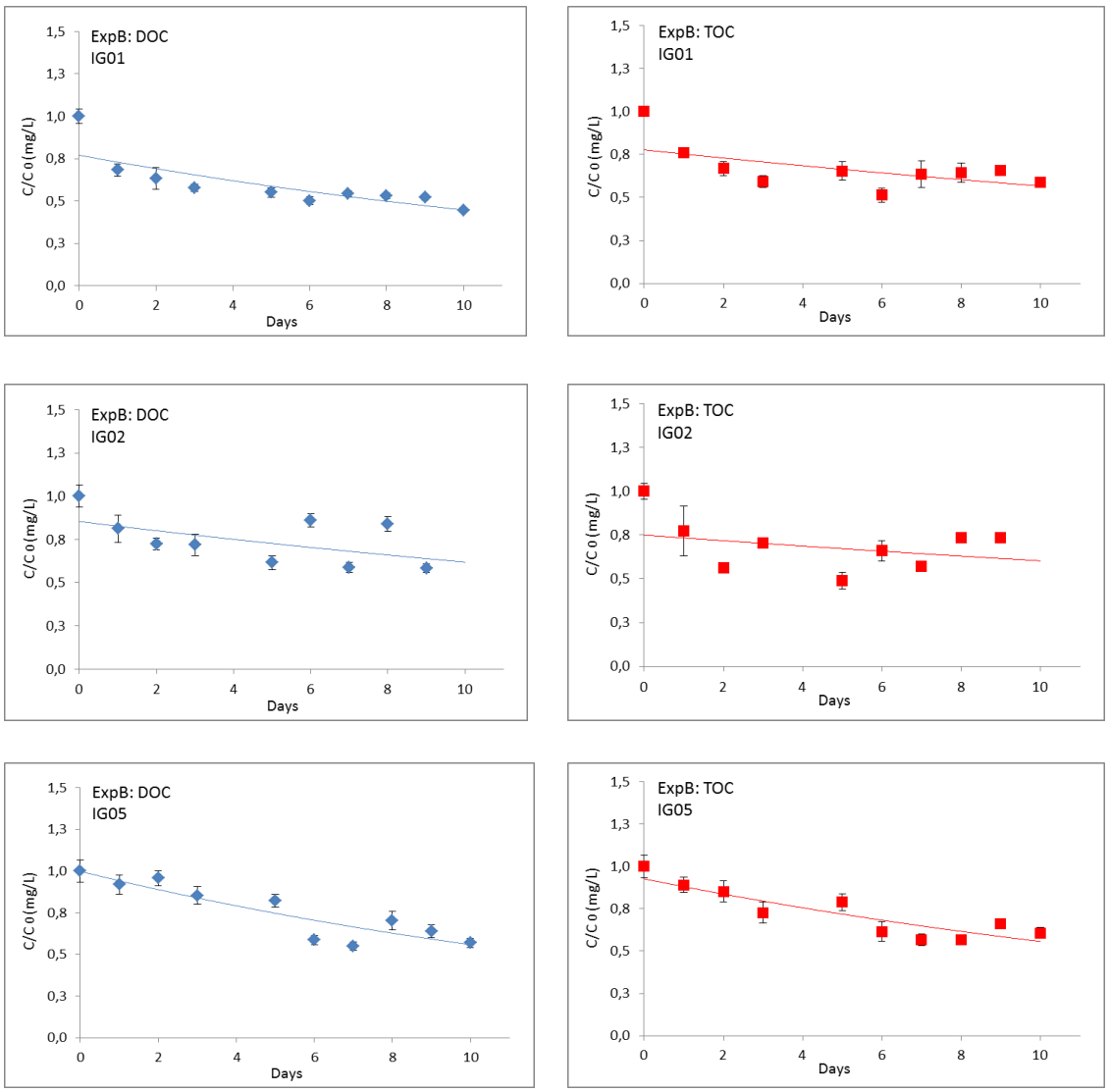

Figure 19: Normalized data of DOC and TOC for Biodegradation experiment B, Oct/13.

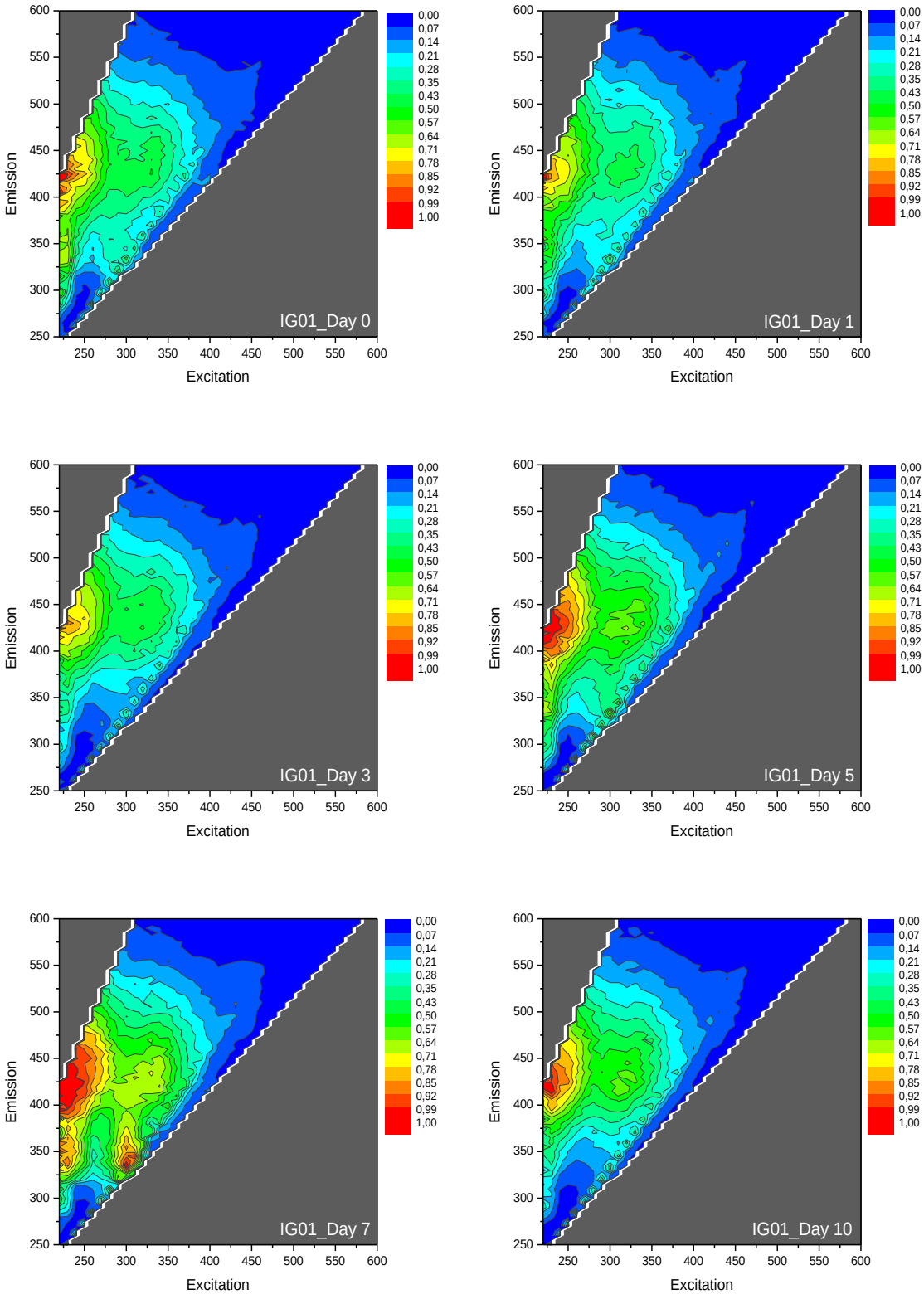

Figure 20: Excitation-emission matrix evolution, exp. B, sample IG01 (Oct/13)

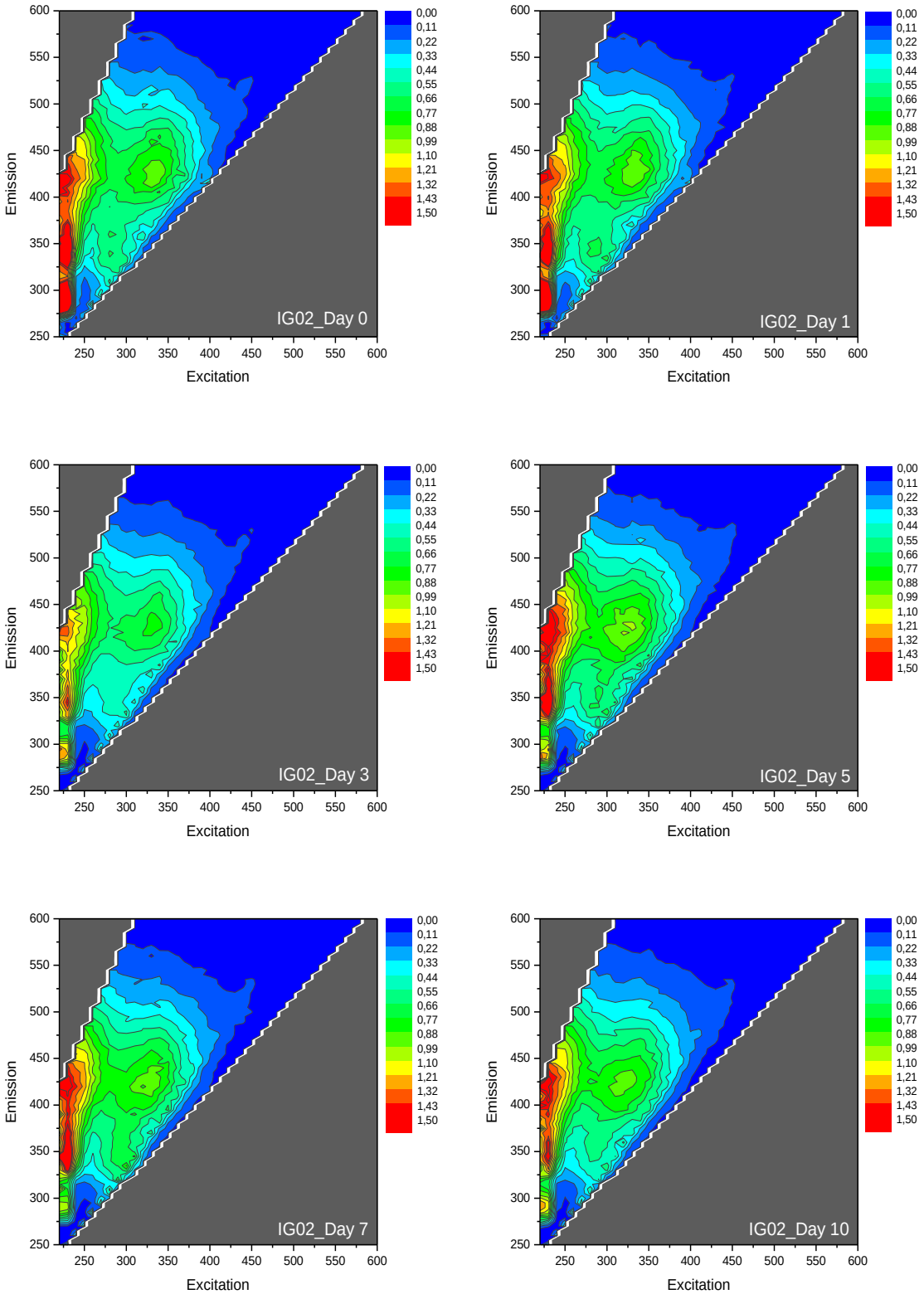

Figure 21: Excitation-emission matrix evolution, exp. B, sample IG02 (Oct/13)

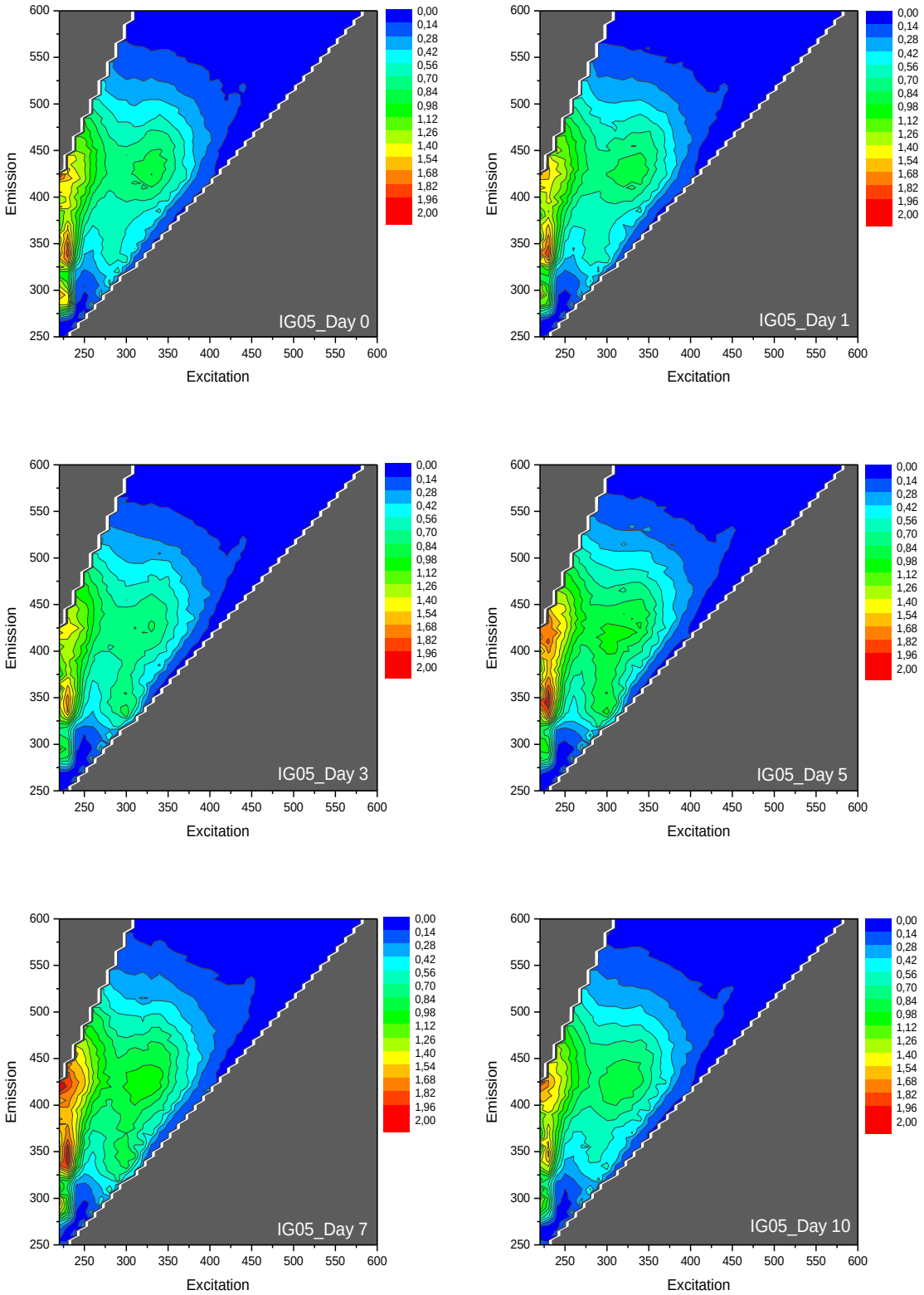

Figure 22: Excitation-emission matrix evolution, exp. B, sample IG05 (Oct/13)

Appendix 4

BOD deoxygenation rates (k_1)

This additional appendix summarizes the main results of Biochemical Oxygen Demand deoxygenation rates. These rates where estimated for samplings performed during 2012 and 2013 in 6 monitoring sites (Iguassu River).

BOD deoxygenation rates *(k_1):* Experimental determination

The mass balance for the amount of oxydisable organic matter in the sample can be described by a first-order kinetics equation:

$$\frac{dL}{dt} = -k_1 L \quad (1)$$

According to equation (1), it can be observed that the rate in which the organic matter is decomposed is proportional to the remaining organic matter in the sample. The integration of equation (1) considering L_0 as the initial remaining BOD (equivalent to the total amount of oxygen consumed) results in the following equation:

$$L = L_0 e^{-k_1 t} \quad (2)$$

The oxygen consumed during the decomposition process can be defined as the difference between the initial BOD and the amount of oxydisable organic matter remaining in the sample at time t:

$$y = L_0 - L \tag{3}$$

The substitution of (3) in (2) results:

$$y = L_0(1 - e^{-k_1 t}) \tag{4}$$

Where: y: Amount of oxygen consumed at time t (BOD) [mgO_2/L]; L: Amount of oxydisable organic matter remaining in the sample [mgO_2/L]; $L_0 = BOD_u$: Initial concentration of oxydisable organic matter or the total amount of oxygen consumed in the reaction (ultimate BOD) [mgO_2/L]; k_1: Reaction constant [d^{-1}]; t : Time elapsed since the start of the assay [d];

There are several methods for the simultaneous determination of k_1 and L_0, such as: (i) linear regression method (minimum squared method); (ii) Thomas method; (iii) Fugimoto method; and (iv) non-linear regression method. Basically, the experimental data of BOD for consecutive days are used to estimate the rate in which the organic matter is decomposed under controlled temperature conditions. The deoxygenation rate (k_1) depends on organic matter characteristics and sources (raw domestic effluents show higher values, while unpolluted waters have low values of k_1). Since it is a biological experiment, the properly determination of k_1 may be affected by inhibitors, changes in the incubation temperature, presence of light (primarily production can increase oxygen levels), insufficient amount of initial microorganisms, rapid consumption of oxygen levels and anoxic condition (most common in polluted waters when conducting Winkler method for BOD determination).

For the objectives of the present study, samples were collected at six monitoring sites along 107 km of the Iguassu River from Aug/2012 to Dec/2013 (10 samplings, being 4 during Spring, 2 during Summer, 1 during Fall, and 3 during Winter). One site (IG01) was located upstream of the urbanization area, three sites were located in the most impacted area and downstream from wastewater treatment plants (IG02, IG03 and IG04), and two control sites were selected downstream of the urbanization area (IG05 and IG06). Data from site IG02 were collected in two margins (IG02A and IG02B). Biological

Oxygen Demand (BOD_5) was determined by respirometric method (Oxitop), according to manufacture guidelines. The estimation of k_1 (BOD deoxygenation rate) and *BODu* (ultimate BOD) were carried out in electronic spreadsheet, considering non-linear regression analysis through Solver function (GRG method).

The goodness of fit was calculated by the Nash-Sutcliffe coefficient (E_{NS}) and the Mean squared error (MSE). The Nash-Sutcliffe efficiency was used for evaluation the performance of different algorithms. This coefficient is a normalized form of the sum of squared of residuals between the measured and simulated series of the variable of interest. The equation to calculate the coefficient is (Nash and Sutcliffe, 1970):

$$E_{ns} = 1 - \frac{\sum_{i=1}^{N}(O_i - P_i)^2}{\sum_{i=1}^{N}(O_i - \bar{O})^2} \quad (6)$$

where E_{ns} is the Nash-Sutcliffe coefficient, P is the model simulated value, O is the observed data, $\bar{O}$ is the mean for the entire time period of the evaluation, and $i=1,2,\ldots,N$, where N is the total number of pairs simulated and observed data.

Figure 1 presents the estimated and observed BOD data for site IG02 (left) considering four different samplings (C40, C42, C43, and C45), and a comparison between all sites monitored during sampling C42 (right). According to Figure 1, it can be observed that different values of deoxygenation rates can be estimated for the same sampling site. Factors such as flow, variation on effluents discharge, temperature, presence of other compounds, presence of inhibitors, can affect the determination of the BOD biodegradation rates.

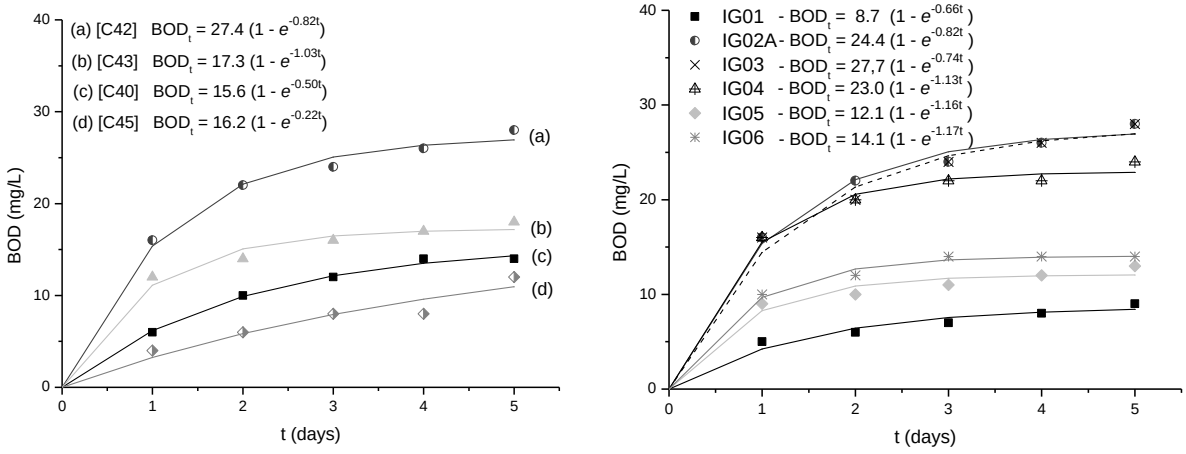

Figure 1: Example of estimated and observed BOD data and the respective decomposition rates (*k1*) for monitoring site IG02A (left), samplings n. 42 (a), 43 (b), 40 (c), and 45 (d), and all sites in sampling n. 42 (right). Solid lines represent the calculated curve based on *k1* and *BODu*.

Table 1 presents a summary of all data estimated during 10 samplings in 2012 and 2013. Figures 2 and 3 show the variation of k_1 according to the samplings performed and to the monitoring sites.

Table 1: Numerical results of k_1 coefficient and BOD_u (ultimate BOD) by non-linear estimation for 10 samplings performed at Iguassu River, in 6 monitoring sites during 2012 and 2013.

Sampling	Site	k1	BODu	ENS	MSE	Sampling	Site	k1	BODu	ENS	MSE
C39	IG01					C44	IG01				
C39	IG02A	0,26	38,3	0,984	0,80	C44	IG02A	0,22	31,7	0,947	1,89
C39	IG02B	0,47	57,3	0,929	11,26	C44	IG02B	0,16	91,4	0,978	4,46
C39	IG03	1,12	44,1	0,937	2,22	C44	IG03	0,34	60,1	0,997	0,39
C39	IG04	0,20	45,0	0,993	0,42	C44	IG04	0,38	20,9	0,984	0,27
C39	IG05	0,26	19,7	0,992	0,11	C44	IG05	0,18	27,7	0,992	0,14
C39	IG06	0,09	33,5	0,982	0,21	C44	IG06	0,11	17,2	0,985	0,05
C40	IG01					C45	IG01				
C40	IG02A	0,50	15,6	0,991	0,08	C45	IG02A	0,22	16,4	0,878	0,86
C40	IG02B	0,39	25,6	0,998	0,05	C45	IG02B				
C40	IG03	0,53	46,3	0,991	0,64	C45	IG03				
C40	IG04	0,29	74,8	0,996	0,68	C45	IG04				
C40	IG05	0,43	39,7	0,990	0,59	C45	IG05	0,42	7,3	0,919	0,21
C40	IG06	0,50	9,5	0,978	0,07	C45	IG06				
C41	IG01					C46	IG01				
C41	IG02A					C46	IG02A				
C41	IG02B	0,25	13,9	0,932	0,50	C46	IG02B	0,15	68,6	0,990	0,92
C41	IG03	0,36	12,3	0,977	0,13	C46	IG03				
C41	IG04					C46	IG04	0,21	29,1	0,928	1,56
C41	IG05	0,08	53,1	0,992	0,18	C46	IG05	0,15	27,3	0,950	0,84
C41	IG06	0,19	12,7	0,958	0,18	C46	IG06				
C42	IG01	0,66	8,7	0,860	0,28	C47	IG01				
C42	IG02A	0,82	27,4	0,967	0,56	C47	IG02A	0,14	43,7	0,890	4,92
C42	IG02B	0,93	48,2	0,955	2,09	C47	IG02B	0,23	45,8	0,983	1,14
C42	IG03	0,74	27,7	0,938	1,15	C47	IG03				
C42	IG04	1,13	23,0	0,937	0,46	C47	IG04				
C42	IG05	1,16	12,1	0,729	0,54	C47	IG05				
C42	IG06	1,17	14,1	0,943	0,15	C47	IG06	0,08	34,0	0,957	0,44
C43	IG01					C48	IG01				
C43	IG02A	1,03	17,3	0,876	0,57	C48	IG02A	0,14	38,5	0,971	0,68
C43	IG02B	1,07	27,2	0,974	0,31	C48	IG02B	0,19	50,1	0,989	0,65
C43	IG03	1,20	27,5	0,932	0,61	C48	IG03				
C43	IG04	1,19	17,7	0,896	0,39	C48	IG04	0,14	50,1	0,921	3,24
C43	IG05	1,11	21,2	0,922	0,48	C48	IG05				
C43	IG06					C48	IG06				

BODu: is the total amount of oxygen consumed in the reaction (or ultimate BOD)
MSE: mean squared error
ENS: Nash-Sutcliffe efficiency

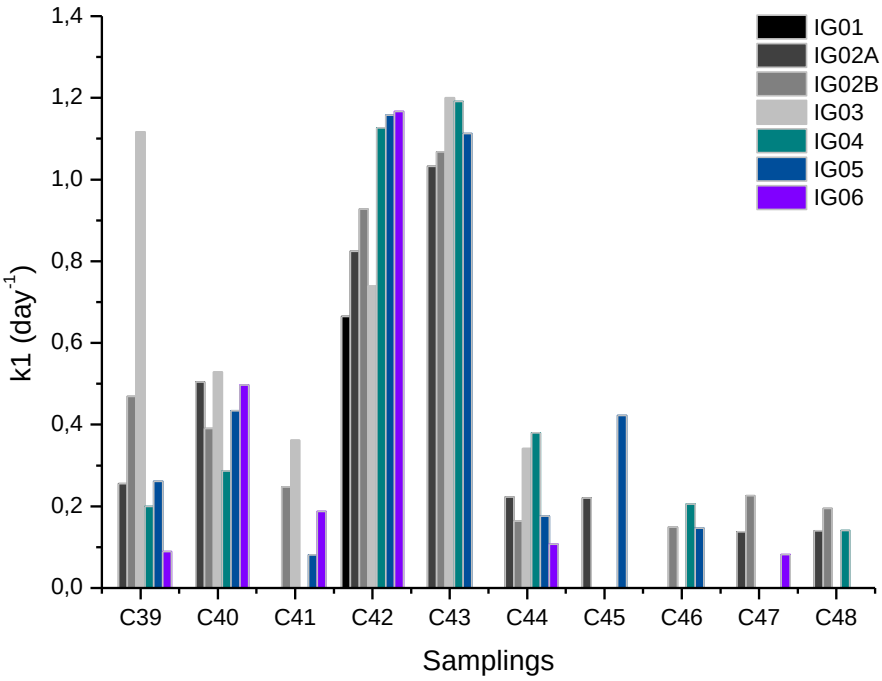

Figure 2: Variation of the deoxygenation rates *(k_1)* during samplings performed in 2012, C39 to C43, and 2013, C44 to C48 (site IG02 considered for left margin, IG02A, and right margin, IG02B).

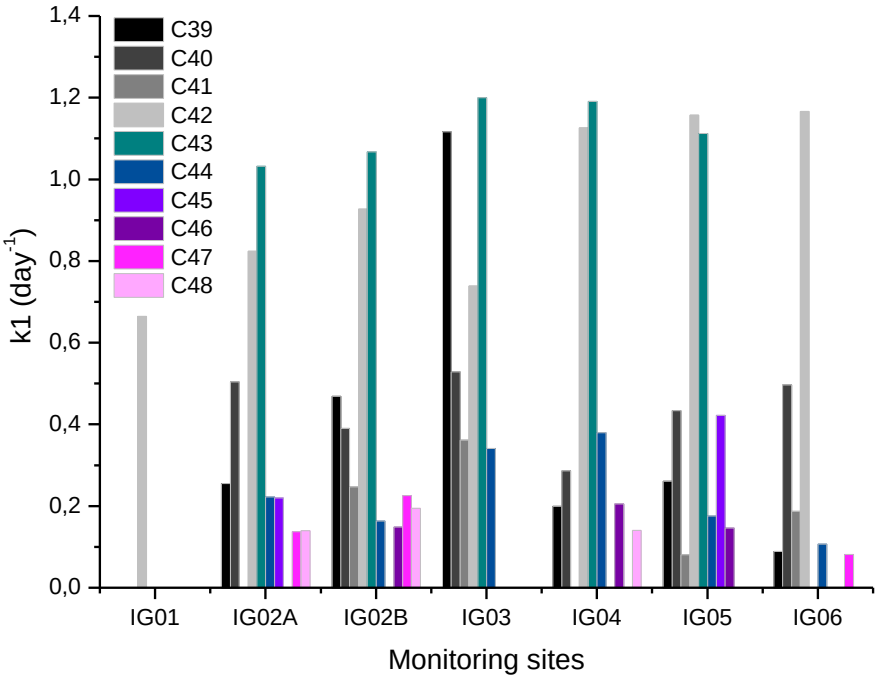

Figure 3: Variation of the deoxygenation rates (k_1) for the monitoring sites at Iguassu River, IG01 (headwater) to IG06 (site IG02 considered for left margin, IG02A, and right margin, IG02B).