

Evaluating Dissolved Organic Matter Loads in Tropical Alpine Rivers Using FDOM Measurements

by

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MANUSCRIPT-BASED THESIS PRESENTED TO ÉCOLE DE
TECHNOLOGIE SUPÉRIEURE IN PARTIAL FULFILLMENT FOR A
MASTER'S DEGREE WITH THESIS IN ENVIRONMENTAL
ENGINEERING
M.A.Sc.

MONTREAL, OCTOBER 21, 2019

ÉCOLE DE TECHNOLOGIE SUPÉRIEURE
UNIVERSITÉ DU QUÉBEC



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ACKNOWLEDGMENTS

I would like to start by thanking, Michel Baraër. First, for accepting to be my project supervisor and trusting me during these years. It was a pleasure to work with you. I am grateful for your advice, your patience and for sharing your knowledge with me. Thank you for the support during the fieldwork in Peru, one of the most enriching trips I have ever made and without a doubt an experience I will always talk about. Thank you for your support, not only academic, for always having a smile, good humor and basically for being the amazing human being you are. Lastly but not least, thank you for all the coffee and all the pens. I feel so lucky to have counted on you, you are the best!

I would also like to thank, Jeff McKenzie, Bryan Mark, and Lauren Sommers, for the support and the good moments we had during the fieldwork expedition in Peru. You are an incredible team. It was truly my pleasure to work with you guys.

To the DRAME, for the support and company during this journey. Special thanks to Anna and Mariana not only for the academic support and help but also for your friendship. Thank you, girls, for the laughs, jokes and good moments.

This one goes to, Alex. Thank you for your love, care, patience, support, advice and constant encouragement. This adventure has been way much better with you by my side. I am very lucky to have you in my life. I love you!

Lastly, I would like to thank especially my grandparents, Rafael Avella, and Gladys Macias, without whom none of this would have been possible. Thank you for raising me right and for not giving up on me. Thank you for encouraging me to be better, for showing your support without hesitation in my decision to come to Canada. Thanks for your patience, love, and wisdom. I wouldn't be where I am today without you guys and I couldn't be more thankful for all that you both have done for me. I love you to the moon and back.

Évaluation de la contamination organique dans les rivières alpines tropicales en utilisant des mesures FDOM

Katherine RODRIGUEZ AVELLA

RÉSUMÉ

Le Pérou possède environ 70% des glaciers tropicaux du monde. En raison principalement du changement climatique, ils ont perdu 40% de leur superficie depuis les années 1970. Ce recul a eu pour effet de réduire l'apport d'eau de fonte dans les cours d'eau, surtout en saison sèche, ce qui entraîne une augmentation de la concentration de contaminants. La matière organique dissoute (DOM) est un indicateur de la qualité de l'eau car elle est influencée à la fois par les activités naturelles et par les activités anthropiques. Les développements technologiques récents permettent de quantifier et / ou de qualifier la matière organique dissoute fluorescente (FDOM), une fraction de la DOM, en utilisant soit des capteurs dans des fluoromètres portables et soit des mesures spectroscopiques de fluorescence en laboratoire couplée à une analyse par facteur parallèle (PARAFAC). L'objectif de cette recherche était d'évaluer l'évolution des mesures de FDOM dans deux bassins versants glaciaires tropicaux du Pérou : le Shullcas et le Upper Santa. En utilisant un fluoromètre portable et un multimètre portable, 38 mesures sur site de FDOM, de pH et de turbidité ont été effectuées. En même temps, 38 échantillons ont été prélevés pour être analysés par spectroscopie de fluorescence. Les performances des résultats FDOM ont été comparées aux mesures de carbone organique dissous (COD) utilisant l'oxydation catalytique à haute température comme méthode de référence. Nos résultats montrent que les lectures de fluorimètres portables sont limitées à la partie de la matière organique dissoute fluorescente qui correspond à des substances de type humique en raison de sa bande passante étroite. La spectroscopie de fluorescence couplée à PARAFAC était la seule méthode capable à la fois de différencier les sources potentielles de DOM et d'estimer les concentrations relatives de différents types de substances organiques. La capacité de cette méthode à produire des estimés fiables de concentration de FDOM dans le contexte d'une étude couvrant un bassin versant entier reste tout de même à démontrer. Cette recherche nous a permis de mettre en évidence le fort impact des villes sur la qualité de l'eau pour les deux bassins d'étude.

Mots clés : Matière organique dissoute, Carbone organique dissous, Matière organique dissoute fluorescente, Analyse des facteurs parallèles, Spectroscopie de fluorescence, Qualité de l'eau.

Evaluating Dissolved Organic Matter Loads in Tropical Alpine Rivers Using FDOM Measurements

Katherine RODRIGUEZ AVELLA

ABSTRACT

Peru is home to about 70% of the world's tropical glaciers. However, due to climate change mainly, they have lost 40% of their surface area since the 1970s. This retreat has been decreasing the meltwater contribution to the streams especially in the dry season, which increases the concentration of contaminants. Dissolved organic matter (DOM) is an indicator of water quality as it is influenced by natural as well as by anthropogenic activities. Quantifying and characterizing DOM is challenging because of its complexity. Recent technological development allows us to quantify and/or qualify fluorescent dissolved organic matter (FDOM), the fraction of DOM that has fluorescence properties, by employing portable fluorometers or benchtop measurements of fluorescence spectroscopy coupled with parallel factor analysis (PARAFAC). The objective of this research was to evaluate the evolution of FDOM measurements throughout two glacierized tropical alpine watersheds in Peru: the Shullcas and the Upper-Santa. Using a portable fluorometer equipped with a CDOM/FDOM sensor and a portable multimeter, 38 on-site measurements of FDOM, pH and turbidity were completed. Simultaneously 38 samples were taken to be analyzed with fluorescence spectroscopy followed by parallel factor analysis. FDOM results performances were compared with Dissolved Organic Carbon (DOC) measurements using high-temperature catalytic oxidation used as a reference method. Our results show that the portable fluorometer readings are limited to the portion of fluorescent dissolved organic matter that corresponds to humic-like substances due to the narrow bandwidth of the sensor used. The fluorescence spectroscopy coupled with PARAFAC was the only method able to both differentiate between potential sources of DOM and estimate relative concentrations of different organic substances types. The ability of the method to provide accurate estimations of the total FDOM load at the watershed scale remains, however, to be proven. Among others, this study shows cities have a strong impact on the water quality of studied catchments, mainly due to the absence of proper sewage systems.

Keywords: Dissolved Organic Matter, Dissolved Organic Carbon, Fluorescent Dissolved Organic Matter, Parallel Factor Analysis, Fluorescence Spectroscopy, Water Quality.

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LIST OF ABBREVIATIONS

DOM	Dissolved Organic Matter
CDOM	Chromophoric Dissolved Organic Matter
FDOM	Fluorescent dissolved organic matter
DOC	Dissolved organic carbon
POC	Particulate Organic Matter
PPB	Parts Per billion
EEM	Excitation and Emission Matrix
R.U.	Raman Units
nm	nanometers
NTU	Nephelometric Turbidity Units

INTRODUCTION

Peru is home to about 70% of the world's tropical glaciers. Due to climate change, these glaciers have diminished by approximately 35% since 1975 (Veetil, 2018). This glacier retreat affects glacially fed streams as there is a decrease in meltwater contribution (Cannone et al., 2008; Meier et al., 2007) especially during the dry season (Baraer et al., 2012). Consequently, this will lead to a significant reduction of the rivers discharge in the coming years triggering a reduction in water availability and thus, leading to an increase in the concentration of contaminants affecting water quality (H. Slemmons et al., 2013). In addition to this, there is lack of proper sewage treatment in most of the cities and most of the waste is poured into the streams (Guittard et al., 2017), increasing the risk for people that use the water for consumption, irrigation, agriculture, industrial use, and power generation.

Dissolved organic matter is one of the main indicators of water quality and has been an active area of research due to its complexity and non-conservative behavior (Alling et al., 2010). It is a mixture of organic compounds such as humic acids and proteins (Thurman, 1985; Zhao et al., 2015). DOM influences nutrients and pollutants transport (Chen et al., 2019; Old et al., 2019), it is a major reactant and a product of biogeochemical processes in which it serves as energy and controls levels of dissolved oxygen, nitrogen, phosphorus, sulfur, numerous trace metals, and acidity, and since it has an influence on the interaction of nutrients and microbial activity it is considered a good indicator of water quality (Kaplan and Newbold, 2000; Leenheer and Croué, 2003). Aquatic systems obtain DOM inputs from allochthonous sources (sources external to the system) and from autochthonous sources (produced within the system) (DeVilbiss et al., 2016; Kieber et al., 2006; Zhou et al., 2019). DOM is strongly affected by human activities such as treated and/or untreated wastewater inputs in an urbanized area (Tang et al., 2019).

Carbon is an element that represents the reactive fraction of the organic matter. It represents roughly 65% of the total DOM mass (Bolan et al., 2011). Consequently, dissolved organic

matter can be estimated by quantifying the carbon it contains. Therefore, it is commonly found expressing DOM as DOC (Mostofa et al., 2013b; Qualls et al., 2013).

Dissolved organic matter can exhibit unique optical properties i.e., absorbance and fluorescence, that can be used for analytical purposes (Coble, 2007). A fraction of DOM is known as chromophoric dissolved organic matter (CDOM), it is able to absorb visible and UV radiation. A fraction of the CDOM is able to fluoresce after absorbing radiation, this fraction is what is known as fluorescent dissolved organic matter (FDOM) (Mostofa et al., 2013b). The fraction that corresponds to fluorescent dissolved organic matter is chromophoric, however, not all chromophoric dissolved organic matter is fluorescent (Coble, 2007).

The characterization of DOM is challenging as it represents a mixture of compounds (Coble et al., 2014). However, typically there are two fluorescence categories observed in natural waters (Coble, 1996; Lakowicz, 2006; Maie et al., 2007; Mopper and Schultz, 1993) : the protein-like fluorescence which includes the 3 fluorescent aromatic amino-acids: phenylalanine, tyrosine and tryptophan (Coble, 1996; Lakowicz, 2006; Maie et al., 2007; Mopper and Schultz, 1993) and the humic-like fluorescence that corresponds to signals of humic substances that arise from the breakdown of vegetal sources (Coble, 2007, 1996; Kalle, 1949; Mopper and Schultz, 1993; Stedmon et al., 2003).

For decades the DOM has been measured through several different techniques: the spectrophotometric method, wet oxidation, dry combustion and molecular weight fractions (Bolan et al., 1996; Chatterjee et al., 2009). However, the technique that exhibited better results and showed to cover a larger part of DOC is the high-temperature catalytic oxidation method introduced by Sugimura and Suzuki, (1988).

FDOM comprises only around 1% of the total DOM pool (Cory et al., 2011). Still, it has the advantage of allowing the use of its optical properties for analytical purposes. A reason for this is that fluorescence occurs at specific excitation and emission wavelengths, depending on FDOM composition i.e., humic-like and protein-like, and the intensity of the signal is

proportional to the concentration (Cory et al., 2011; Fellman et al., 2010). Being able to monitor fluorescence is attractive as it is a fast technique and there is no specific sample preparation required prior to the analysis (Henderson et al., 2009). It is highly sensitive and is easy to develop at relatively low costs (Lee, Osburn et al. 2018), making fluorescence measurements attractive approaches to monitor water quality (Tang et al., 2019). The strong fluorescence signal of DOM that has been observed in sewage has shown to be an interesting surrogate to existing water quality parameters commonly employed in the monitoring of river pollution (Henderson et al., 2009). This has driven the increased usage of fluorometry to monitor key macro-nutrients and DOM (Blaen et al., 2016).

There are two popular techniques that can be considered for analyzing FDOM in natural waters. One of them is the fluorescence spectroscopy that results in a 3D contour plot of fluorescence intensities for an array of excitation and emission wavelengths known as excitation/emission matrix (EEM). The use of this bench-top technique has grown in popularity and, to date, it has been widely used to identify sources and compositions of fluorescent DOM in natural watersheds (Zhang and Liang, 2019).

In addition to this, a growing number of studies have applied the parallel factor analysis (Harshman, 1970) (PARAFAC) for the interpretation of EEMs (Baghoth et al., 2011, 2011; Coble, 1996; Murphy et al., 2013, 2011; Stedmon et al., 2003; Stedmon and Markager, 2005; Yamashita et al., 2008). The PARAFAC, decomposes the EEMs into individual fluorescent components (Weiwei et al., 2019) and it has been successfully used to identify allochthonous and autochthonous fluorescent DOM components (e.g. DeVilbiss et al., 2016; Henderson et al., 2009) as it measures the abundance and the type of fluorescing compounds found in DOM.

The on-site optical sensing for fluorometry is another technique that has grown in popularity due to technical advances and cost reduction (Blaen et al., 2016). This technique does not require sample manipulation, transportation nor conservation, which enables accurate measurements considering the instability of organic components (Hansen et al., 2016). Data

treatment should be applied to results due to the effect that turbidity and temperature have in light scattering (Downing et al., 2012; Saraceno et al., 2017; Watras et al., 2011).

The study of DOM at a watershed scale is important as rivers are important contributors to the global organic matter distribution (Ward et al., 2017). It is, therefore, important to consider the land use close to rivers as it influences the quantity and quality of DOM that is exported to natural waters (Stedmon et al., 2003). However, regardless of the number of studies that apply fluorescence-based techniques to monitor DOM (Bierozza et al., 2010; Henderson et al., 2009; Mopper and Schultz, 1993), there is no study that compares the performances of the techniques and its variations at a watershed scale.

In this study, the overall objective was to evaluate the dissolved organic matter loads of two glacierized watersheds in the Peruvian Andes. To achieve this, we compared the performance of the two fluorometric methods, to assess to what extent those methods can provide information on the DOM dynamics in natural waters. We compared the measurements obtained using an on-site optical fluorometer equipped with a CDOM/FDOM sensor, FDOM bench-top fluorescence spectroscopy and high-temperature combustion DOC analysis for 38 sampling points strategically chosen over the two selected glacierized catchments. These watersheds were strategically chosen because they both present high spatial variability in DOM sources and concentration, flowing from pristine proglacial lakes to points of obvious anthropogenic influences.

CHAPTER 1

LITERATURE REVIEW

This chapter gives a brief overview of how glacier retreat is affecting water availability and quality in Peru. It then details what dissolved organic matter (DOM) is, how it is measured and why it is fundamental when speaking about water quality. This is followed by the presentation of fluorescent dissolved organic matter (FDOM) and how it is considered a proxy of DOM. Finally, it presents the two fluorescence-based analytical techniques used in this study: the on-site fluorometry and the fluorescence spectroscopy.



Figure 1-1 Views of the glacierized cordillera Blanca in the Huascarán national park located in the Ancash region in Peru, 2017

1.1 Glacier retreat affecting water availability and quality in Peru.

Glaciers are an important source of freshwater input into many rivers worldwide due to the melt-water contribution (Kaser and Osmaston, 2006). They represent an important source of water for humans, agricultural activities, industrial use, and hydroelectric power generation. Unfortunately, glaciers in many regions are in a strong imbalance due to global warming and are destined to further substantial ice loss (Rabatel et al., 2013).

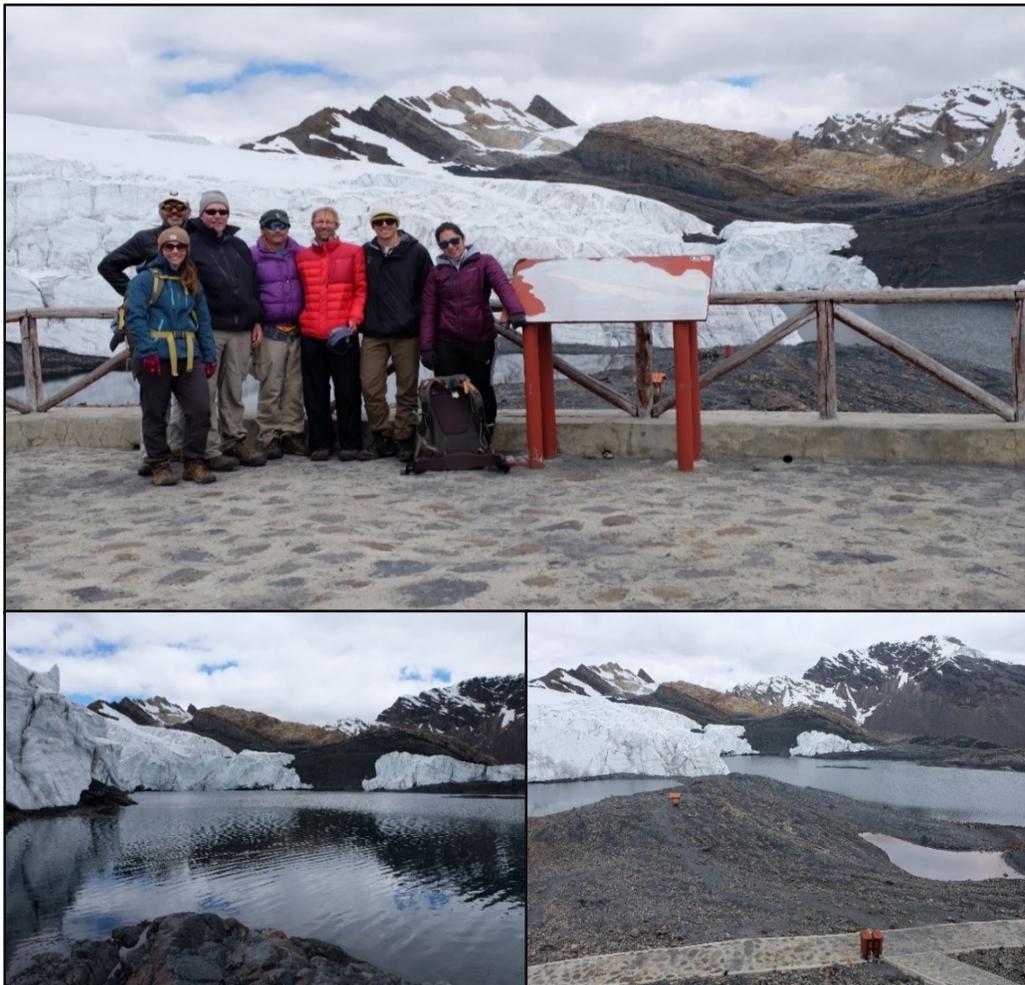


Figure 1-2 Views of the pastoruri glacier, located in the southern part of the Cordillera Blanca. Located in the Ancash region. Year, 2017

Peru, a country in western South America, is home to about 70% of the world's tropical glaciers. The major glacier concentration in Peru is found in the Cordillera Blanca (Kaser and Osmaston, 2006). Figure 1-1 provides views of the glaciers located in the Cordillera Blanca, in the national park of Huascarán. Figure 1-2, presents the views of the Pastoruri glacier, located in the southern part of the Cordillera Blanca, which represents the first tourist destination in Peru to be closed due to its climatic conditions (Wang and Zhou, 2019).

The Cordillera Blanca has an average altitude of 3700 m.a.s.l, which is a determinant factor on its climatic conditions. (Patricio Aceituno, 1996). One of the main climatic features is that the region is characterized by strong seasonality in precipitation: rainfalls remain extremely rare during most of the year but are abundant during the austral summer (November to March) when intense storms transport significant rain (Garreaud, 2009).



Figure 1-3 Observed discharge in the Shullcas River flowing through the city of Huancayo during the dry season June-July 2017



Figure 1-4 Evidence of Fisheries, agriculture and pastoralism in various locations of the Shullcas Watershed. Pictures taken during the dry season June-July 2017

A large percentage of the western part of the Cordillera Blanca drains toward the Pacific Ocean feeding the Santa River (Mark and Seltzer, 2003), one of Peru's most important rivers (Carey et al., 2014a). Unfortunately, Peru has been ranked as one of the most vulnerable countries in the world to climate change (UNEP, 2013). Due to global warming, glaciers have diminished by approximately 35% since 1975 (Veettil, 2018). This situation affects glacially-fed streams as the Santa River and the Shullcas River in the Andes and it has a negative influence in water quality since the decrease in meltwater contribution as a response of glacier retreat, especially during the dry season (Baraer et al., 2012), leads to a significant reduction of the river's discharge. Figure 1-3, shows the observed discharge in the Shullcas River, flowing through the mid-size city, Huancayo, during the field-work expedition of this study.

As a result of water supply shortages there will be difficulties to have access to water, affecting tremendously the population. It is projected that the life, livelihood and maintenance of rural

populations in these regions will be threatened (Mark et al., 2010). It will also have an effect in the economic activities (Carey et al., 2014a) such as: pastoralism, fish farming, and small-scale agriculture, Figure 1-4 presents evidence of this activities. It is also alarming since, as a result of a reduction in water availability, it will lead to an increase in the concentration of contaminants affecting water quality, especially considering that most of the cities along the watershed that receive melt-water contribution do not count with a proper sewage treatment plant, and therefore, most of these cities pour wastewater directly into the rivers (Guittard et al., 2017). Figure 1-5 presents evidence of wastewater inputs into the streams. This situation and the decrease in meltwater contribution are detrimental to the health of people in the area



Figure 1-5 Evidence of the contamination in the Shullcas river. Pictures taken in the city of Huancayo. Year, 2017.



Figure 1-6 Rio Santa Head waters in the Huascarán national park. Pictures taken during the dry season June-July 2017

This region not only presents suitable characteristics to study the evolution and the dynamics of water quality as both, the Santa and the Shullcas watersheds, flow from pristine headwaters of glacial origin, as seen in Figure 1-6, through natural environments and gradually increasing population density with spots of obvious anthropogenic influences, as seen in Figure 1-4. Besides this, the glacier mass loss at the headwaters of both watersheds is raising concerns about the future of water quality in the region (Guittard et al., 2017). This should be considered and studies regarding the water quality of glacially fed streams should be carried out.

1.2 Dissolved organic matter in aquatic environments

The Organic Matter (OM) in water is composed of two major fractions: dissolved and non-dissolved (Mostofa et al., 2013b). The term dissolved organic matter (DOM) represents the largest pool of organic matter in aquatic environments (Alvarez-Cobelas et al., 2012) and refers

to the fraction of total organic matter which can pass through a filter size of 0.45 micrometers, the fraction that remains in the filter is Particulate Organic Matter (POM).

DOM plays a vital role in aquatic environments, it controls levels of dissolved oxygen, nitrogen, phosphorus, sulfur, numerous trace metals, and acidity (Leenheer and Croué, 2003), it is a potential source that provides energy and carbon that contribute to the stream ecosystem (Kaplan and Newbold, 2000; Thomas, 1997), and since it influences the interaction of nutrients and microbial activity it is considered a good indicator of water quality.

However, due to its complexity and non-conservative behavior (Alling et al., 2010) further research is needed to better understand its dynamics. The composition of dissolved organic matter is mainly organic acids (80%) such as fulvic and humic acid, the remainder consists of organic compounds like free amino acids and proteins among others (Thurman, 1985).

Dissolved organic matter and its components can be derived from two different sources: Allochthonous, sources external to the system, this includes rainwater (Kieber et al., 2006), windborne material, or surface flow (Thomas, 1997) and autochthonous, sources within the system that consist mainly of non-humic substances, such as phytoplankton (Zhang et al., 2009) and aquatic vegetation. Consequently, DOM composition and concentration are significantly variable.

The complexity and non-conservative behavior of DOM responds to the fact that DOM has biogeochemical roles in microbial, chemical and geochemical processes in natural waters (Mostofa et al., 2013b). DOM has microbial functions as it is decomposed by microorganisms (Tranvik, 1998; Wu et al., 2018). This also plays an important role in cycling nutrients (Christie-Oleza et al., 2017) as they are produced by degradation of DOM and elements like (N) nitrogen, (P) phosphorus and (C) carbon, are assimilated and re-synthesize, improving water quality, and or absorbing heavy metals (Liu et al., 2016; Refaey et al., 2014; Zhu et al., 2014).

Dissolved Organic Matter also presents unique optical properties i.e., absorbance and fluorescence. A fraction of DOM is termed chromophoric or colored dissolved organic matter (CDOM) considering its availability to absorb UV radiation. A fraction of the mentioned CDOM emits fluorescence after absorbing radiation, this is what is termed as the Fluorescent Dissolved Organic Matter (FDOM) (Mostofa et al., 2013b). In other words, the fraction that corresponds to Fluorescent dissolved organic matter is chromophoric or colored. however, not all chromophoric dissolved organic matter is fluorescent (Coble, 2007).

1.2.1 DOM measurement

Carbon is the element that represents the reactive fraction of the organic matter, with about 67% (Bolan et al., 2011). Therefore, the Dissolved Organic Matter is commonly determined as DOC (Mostofa et al., 2013b; Qualls et al., 2013). This is considered a reliable measurement method considering the complexity of the molecules found in the dissolved organic load (Kaplan and Cory, 2016; Thurman, 1985). Different methods have been used to quantify DOC. They can be divided into three groups: spectrophotometric method, wet oxidation and dry combustion (Bolan et al., 1996; Chatterjee et al., 2009).

However, Sugimura and Suzuki, (1988) introduced the Hight-Temperature Catalytic Oxidation method, stating the possibility that the other methods presented inconsistencies and values obtained represent only a part of the whole DOM. The method consisted in the injection of sample in and oxidation column which is carried out in a platinum catalyst at a temperature of 680 °C under an oxygen atmosphere, then the non-volatile fraction of dissolved organic carbon is oxidized to CO₂, the concentration CO₂ obtained is measured with a non-dispersive IR detector. The reaction time per sample of the whole process is about 4 minutes.

1.3 Fluorescent dissolved organic matter

As discussed previously, dissolved organic matter is a mixture of complex an heterogeneous organic compounds (Zhao et al., 2015). A fraction of these organic compounds exhibits optical

behavior that can be used for analytical purposes (Coble, 2007; Mostofa et al., 2013b). This optically active fraction is called Chromophoric Dissolved Organic Matter (CDOM). It contains molecules that absorb light and in presence of high concentrations of CDOM, waters exhibit a yellow color, so, it is also found as colored dissolved organic matter. After the absorption of light, part of the CDOM will emit light exhibiting fluorescence properties and is referred to as the Fluorescent Dissolved Organic Matter (FDOM) (Stedmon and Nelson, 2015).

1.3.1 Fluorescence process

In order to understand the importance of FDOM it is recommended firstly to understand how the fluorescence process works. The first person to describe fluorescence was the Irish physicist Sir George Gabriel Stokes in the year 1852. The fluorescence process can be summarized in three important steps (Lakowicz, 2006; Reynolds, 2014): Step 1. Excitation of the fluorophore (chemical compound that can re-emit light) through the absorption of light at certain wavelength. Step 2. Loss of energy by vibrational relaxation. Step 3. Return of the fluorophore to its ground state and emission of light (photons) at a longer wavelength. As it emits a photon at lower energy, the wavelength is longer than the excitation light, this difference can be distinguished analytically. Different excitation and emission wavelengths can be used to target different components within a sample.

1.3.2 Fluorescent compounds of interest in DOM

Advances in the application of fluorescence spectroscopy to analyze Dissolved Organic Matter (Bridgeman et al., 2011; Coble, 1996; Fellman et al., 2010; Wasswa et al., 2019) have provided a way to identify a number of compounds or group of fluorophores of different origin. Typically, there are two fluorescence signals observed in seawater and natural waters: protein-like (Coble, 1996; Lakowicz, 2006; Maie et al., 2007; Mopper and Schultz, 1993) and humic-like (Coble, 2007, 1996; Kalle, 1963, 1949; Mopper and Schultz, 1993). These signals are restricted to emission wavelengths from 300-600 nm and excitation wavelengths of 240-500

nm. Therefore, when processing data it is important to remove parts where there is more noise than signal i.e., $E_m > 600$ and $E_x < 250$ (Murphy et al., 2013).

The protein-like fluorescence receives its name as it is alike to three aromatic and fluorescent amino-acids that build up proteins: tryptophan, tyrosine, and phenylalanine (Lakowicz, 2006). Phenylalanine has a weak fluorescent signal and therefore, it is considered of little importance to the DOM fluorescence (Aiken, 2014).

The fluorophore that is associated with tyrosine is a simple phenol that fluoresces strongly at approximately an excitation of 275 nm and emission of 303 nm (Lackowicz, 2006). However, the fluorescence properties of tyrosine are importantly quenched when found as proteins, being only about 10 to 50% as intense if compared to free tyrosine (Wolfbeis, 1985).

On the other hand, tryptophan, whose fluorophore contains an indole group, also fluoresces strongly at excitation of 287 nm and an emission of 348 nm (Aiken, 2014). These fluorophores are of special interest as there is evidence in previous studies that the protein-like fluorescence intensity increases as anthropogenic wastewater inputs increase (Baker, 2001; Baker et al., 2004) and as farm waste inputs increase (Baker, 2002).

The humic-like fluorescence is the main signal in most dissolved organic matter and is due to the occurrence of humic substances that arise from the remineralization of organic matter and from the breakdown of vegetal sources (Stedmon et al., 2003) happening in soils, on land and in the water column (Coble et al., 2014). It is associated to chemical compounds that result from abiotic humification processes that produce humic and fulvic acids.

1.3.3 Factors affecting FDOM

Fluorescence readings and measurements can be affected by certain factors, some are of chemical indole, and some others are due to environmental conditions. Among the factors affecting FDOM readings we have Temperature, Turbidity and pH.

1.3.3.1 Temperature

Temperature is considered one of the most important parameters to watch out when doing fluorescence measurements, especially when using on-site equipment as it is impossible to maintain a constant temperature. Fluorescence quenching is described as the process that reduces the fluorescence intensity of a fluorophore (Aiken, 2014), it responds to the fact that when an excited molecule is returning to its ground state it is temperature-sensitive (Lakowicz, 2006) as a rise in temperature increases the probability that electrons fall to its ground state by a radiation-less process (Baker, 2005). Several studies have aimed to find the most appropriate method of temperature compensation (de Oliveira et al., 2018; Downing et al., 2012; Khamis et al., 2015; Wasswa and Mladenov, 2018; Watras et al., 2011), however, it is stated the importance of identifying the most suitable one based on the needs, as the behavior for freshwater is different as for sea water.

It is also important to consider that due to global warming, aquatic processes that are temperature dependent will have an impact on microbial processes as well as biogeochemical processes. However, there is no study conducted to examine the effect on photochemical, microbial and metal-complexation.

1.3.3.2 Turbidity

As temperature, turbidity is an important parameter to consider when measuring fluorescent dissolved organic matter on site due to light attenuation. Suspended particles may absorb and scatter the light (Downing et al., 2012), thus, FDOM measurements decrease as turbidity increase. Still, while temperature correction is straightforward with the employment of a compensation equation, the correction for turbidity is not that simple considering that turbidity can be affected by the size of particles and mineralogy (Lee et al., 2015) therefore, turbidity corrections may also be site-specific (Saraceno et al., 2017). On the other hand, Downing et al., (2012) states that turbidity corrections vary among different equipment manufacturers. Consequently, more studies should be carried out to evaluate the effect of particles and their

properties, however the studies published to date (Downing et al., 2012; Khamis et al., 2015; Saraceno et al., 2017) represent an improvement in the understanding and discovery of the best approach to turbidity corrections.

1.3.3.3 pH

The effect of pH on fluorescent dissolved organic matter has been studied and significant effects have been found (Romera-Castillo et al., 2014). The fluorescence of humic-like substances is dependent on pH, the intensities of Fulvic Acids and Humic Acids increase with a rise in pH (Ghosh and Schnitzer, 1980; Timko et al., 2015). However, natural waters generally have a pH that is usually between 4 to 10, therefore, it is possible and reliable to measure fluorescence without pH adjustment (Aiken, 2014). It was also found that pH affects the solubility of humic-like substances, at pH 2 the solubility is 0% and at pH 7.5 the solubility is 97% (J Wu et al., 2002). Consequently, it is important to consider that in acid waters measurements considering humic substances may not be accurate.

1.3.4 Analytical techniques

Fluorescent dissolved organic matter monitoring is appealing as it is a rapid, reagent-less and requires no sample preparation prior to analysis (Henderson et al., 2009), it is highly sensitive and easy to process at relative low costs (Lee, Osburn et al. 2018). Therefore, fluorescence measurements are effective and attractive approaches to monitor water quality (Tang et al., 2019). Researches have been focused on two analytical techniques, the on-site fluorometry and the fluorescence Spectroscopy coupled with a parallel factor analysis.

1.3.4.1 On site fluorometers

On-Site optical sensing using fluorescence is a technique that has grown in popularity due to technical advances and cost reduction (Blaen et al., 2016), these measurements do not require sample manipulation, transportation nor conservation, clear benefits considering the

characteristic instability of labile organic components (Hansen et al., 2016). Being an on-site technique, it is fast enough to serve in researches of small-scale variation on DOM composition

These sensors measure the intensity of fluorophores, the molecules that absorb UV light and re-emit it with less energy at longer wavelengths. To achieve this, these types of sensors use an LED (Light-emitting-diode) as light source. The wavelengths of these sensors vary as upon manufacturers or variabilities within the model line (Lee et al., 2015). Figure 1-7 corresponds to the deployed On-site fluorometer used in this study, a C3 Turner Designs fluorometer equipped with a CDOM/FDOM sensor that uses an excitation wavelength of $325\pm 60\text{nm}$ and an emission wavelength of $470\pm 30\text{nm}$.



Figure 1-7 C3 Turner Designs fluorometer equipped with a CDOM/FDOM sensor used in this study.

The application of these sensors has allowed scientists to quantify DOM in high-frequency sampling as concentrations experience high variations. Allowing measurements considering depth, temperature or turbidity. However, it is necessary, as discussed previously, to apply

turbidity and temperature corrections when large variations occur. (Downing et al., 2012; Saraceno et al., 2017).

1.3.4.2 Fluorescence spectroscopy

Fluorescence spectroscopy is one of the analytical techniques available to generate Excitation and Emission matrices (EEM), a 3D scan that results in a contour plot of excitation wavelength vs. emission wavelength vs. fluorescence intensity. The fluorescence spectroscopy equipments use a xenon lamp as light source and counts with monochromators to allow the selection of excitation and emission wavelengths, these monochromators are motorized to allow automatic scanning of wavelength (Lakowicz, 1999).

In the last decade, the number of studies characterizing DOM by applying this analytical technique has grown (Bridgeman et al., 2011; Coble, 1996; Fellman et al., 2010; Liu et al., 2016, 2016; Murphy et al., 2013; Parlanti et al., 2000; Wasswa et al., 2019) as it measures the abundance and the type of fluorescing compounds found in DOM, as mentioned previously, the two fluorescent signals: biogeochemically labile (protein-like) and relatively recalcitrant (humic-like). Even though fluorescent DOM comprise a relatively small fraction of the bulk DOM, around 1% of the total DOM pool (Cory et al., 2011), changes in the fluorescence of DOM can be used to assume changes in the whole DOM pool (Coble, 2007; Stedmon et al., 2003).

In addition, fluorescence spectroscopy is a fast analytical technique that needs a small volume of sample, it is very well suited for DOM studies that are limited in sample volume and in DOM concentration (Fellman et al., 2010).

Since the application of fluorescence spectroscopy to the study of DOM in natural waters, a growing number of studies apply the parallel factor analysis (PARAFAC)(Bro, 1997) for EEMs interpretation (Baghoth et al., 2011, 2011; Coble, 1996; Murphy et al., 2013, 2011;

Stedmon et al., 2003; Stedmon and Markager, 2005; Yamashita et al., 2008) which (Harshman, 1970) decomposes the EEMs into its individual fluorescent components (Weiwei et al., 2019).

1.3.4.2.2 Parallel Factor Analysis (PARAFAC)

The parallel factor analysis was originally used in psychometrics (Harshman, 1970) i.e., theory and techniques of psychological measurement. However, it has gained popularity as it is applicable to chemometrics (Bro, 1997) i.e., use of mathematical and statistical methods to have optimal measurements and obtain relevant chemical information. It is part of multi-way methods that in the last decades have been used in a number of studies (Baghoth et al., 2011; Carstea et al., 2014; Murphy et al., 2011; Stedmon and Bro, 2008; Stedmon and Markager, 2005, 2005; Yamashita et al., 2008, 2008; Zhao et al., 2015) as a tool to analyse EEMs and decompose them into individual fluorescent components (Weiwei et al., 2019).

It is applicable to data that is set in three or more order array (e.g., excitation wavelength vs. emission wavelength vs. fluorescence intensity). There are 5 overall steps to obtain a PARAFAC model (Murphy et al., 2013):

- (1) **Data import and assemble.** The first step is to transfer the data from the analytical equipment to the chosen software that supports the PARAFAC analysis. MATLAB is an efficient software that handles data arrays and the PARAFAC algorithms are available through third-party toolboxes. Data is then assembling into three-way data structures.
- (2) **Preprocessing.** The preprocessing step has the objective of: correcting any systematic biases in the data, remove noisy signals unrelated to fluorescence and normalize the data set considering large intensity difference between samples.
- (3) **Exploratory phase.** One of the main goals in this step is to identify and remove poor quality data and/or outliers.
- (4) **Model validation.** In order to the PARAFAC to validate in an accurate way the right number of components should be fitted. If fewer components are used in the model than there really are, the model may approximate the combined signal of chemical

distinct components. When more components are specified, two or more PARAFAC components are used to represent a single moiety.

(5) Results interpretation. PARAFAC components in validated models are interpreted to represent fluorophores or groups of fluorophores with very similar spectra.

As Murphy et al., (2013) states, there are also three important assumptions to consider for successfully decomposing a 3-array data using the PARAFAC:

- Variability, there can't be two chemical components with identical spectra or with perfectly covarying fluorescence intensities.
- Trilinearity, the emission spectra must be invariant across the excitation wavelengths and excitation spectra must be invariant across the emission wavelengths. Lastly and very important the fluorescence signal increases linearly with concentration.
- Additivity, the total signal within a sample is due to the linear superposition of a fixed number of components.

The PARAFAC, is a second order calibration algorithm (Chen et al., 2014; Li et al., 2011) which allows the analysis of second-order data i.e., when the result is one matrix per sample (Gómez and Callao, 2008), it is therefore known to have the 'second order advantage' (Booksh and Kowalski, 1994). This advantage results very important when applying the PARAFAC to analytical chemistry especially when there are spectral interferences. The algorithm is mathematically able to separate any possible spectrally overlapping data from the EEMs into fluorescence independent chemical components (Stedmon et al., 2003), the overlapping fluorescence data is decomposed into score and loading vectors which are estimates of the spectra and the concentration of the chemical analytes. With this information the PARAFAC model can perform a mathematical chromatography on the data, allowing the identification and the consequent quantification of independent analytes. (Bro, 1997).

The combination of EEM fluorescence with the statistical Parallel Factor Analysis has been proved to be a promising tool to quantify and qualify DOM. This approach gives a rise to

potentially use it in drinking water treatment plants, wastewater treatment plants, evaluation of ballast water exchange in ships (Stedmon and Bro, 2008), among other applications.

CHAPTER 2

COMPARING THE PERFORMANCE OF ON-DISPERSIVE INFRARED DETECTION, FLUORESCENCE SPECTROSCOPY AND ON-SITE FLUOROMETRY PERFORMANCES IN ASSESSING DOM DYNAMICS AT THE WATERSHED SCALE

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This paper was submitted on August 2019 for publication
in *Water Research*

2.1 Abstract

Dissolved organic matter (DOM) is recognized as an indicator of water quality as its concentration is influenced by anthropogenic activities. Recent technological advances allow for the rapid quantification and/or characterization of fluorescent dissolved organic matter (FDOM), the fraction of DOM that fluoresces. Portable FDOM sensors and in-laboratory fluorescence excitation and emission spectroscopy coupled with parallel factor analysis (EEM-PARAFAC) show a high applicability potential. Despite their rising popularity, there is still a need to assess the extent to which those techniques can assess DOM dynamics at the watershed scale. We compare the performance of *in-situ* measurements of FDOM with laboratory measurements of fluorescence spectroscopy in the context of two glacierized watersheds in Peru. Glacierized watersheds represent unique testing environments with contrasting DOM conditions, flowing from pristine, vegetation-free headwaters to or through locations with obvious anthropogenic influences. We used an *in-situ* fluorometer and a portable multimeter

to do 38 measurements of FDOM, pH, and turbidity throughout the two catchments. Additionally, samples were analyzed with fluorescence spectroscopy. Results were compared to Dissolved Organic Carbon (DOC) measurements using standard high-temperature catalytic oxidation methods. Our results show that the three techniques were able to identify high DOM concentration spots, confirming that FDOM measurements comprise a proxy for the state of a river in terms of dissolved organic matter. Measuring DOC with the reference method gives an overview of the state of the water body but no details on its composition. The portable fluorometer's results were limited to quantifying the portion of fluorescent dissolved organic matter that corresponds to humic-like substances due to its narrow bandwidth. The fluorescence spectroscopy coupled with PARAFAC was the only method able to both differentiate between potential sources of DOM and provide an estimate of relative concentrations of different organic components. The accuracy of relative concentration estimates remains to be fully estimated at the watershed scale.

2.2 Introduction

Dissolved organic matter (DOM), is a complex and heterogeneous mixture of organic compounds like humic acids, proteins, and carbohydrates (Zhao et al., 2015), that is widely distributed in natural aquatic ecosystems (e.g Zhou et al., 2015). DOM is a major reactant and product of biogeochemical processes as it provides an energy source for biota and controls concentrations of dissolved oxygen, nitrogen, phosphorus, sulfur, numerous trace metals, and acidity (Leenheer and Croué, 2003). DOM is involved in the solubility and transport of both inorganic and organic molecules and colloids (Conte and Kucerik, 2016) and it influences nutrients and pollutants transport at the watershed scale (Chen et al., 2019; Derrien et al., 2019; Old et al., 2019).

Aquatic systems receive DOM inputs from allochthonous sources, such as riverine discharge and autochthonous sources, such as *in situ* production (DeVilbiss et al., 2016; Zhou et al., 2019). Allochthonous DOM is strongly linked to human activities such as treated and/or untreated wastewater inputs in urbanized areas (Tang et al., 2019). As such, DOM can be used

to trace the source, species and migration of contaminants (Peng et al., 2018), making it an excellent water quality indicator. In recent decades climate change, eutrophication and human activities have contributed to an increase in the inputs of terrestrial DOM to aquatic ecosystems (Massicotte et al., 2017), making it important to understand DOM dynamics at the watershed scale. However, the characterization of aquatic organic matter remains challenging as it is the result of a mixture of organic compounds from different sources and processes, which require different analytical approaches (Coble et al., 2014).

There are several proxies which can be used to characterize DOM. As carbon represents around 65% of the elements in dissolved organic matter (Bolan et al., 2011), it is common to express DOM as dissolved organic carbon, DOC (Qualls et al., 2013). DOC is considered a reliable proxy for DOM, considering the molecular complexity found in the dissolved organic load (Thurman, 1985). An alternate method to characterize DOM is by measuring FDOM, the fluorescent dissolved organic matter. FDOM is the fraction of chromophoric DOM (CDOM), that fluoresces as it releases absorbed energy at a different wavelength than the absorbed one. A distinction can be made between two fluorescent signals: protein-like fluorescence and humic-like fluorescence (Coble, 1996). The protein-like fluorescence includes the three aromatic amino-acids that are fluorescent: phenylalanine, tyrosine and tryptophan. (Lakowicz, 2006). Humic-like fluorescence corresponds to signals of humic substances that arise from the breakdown of vegetal sources (Stedmon et al., 2003).

Different techniques exist for measuring DOC. These include spectrophotometry, wet oxidation, dry combustion and molecular weight fractions (Bolan et al., 2011). The high-temperature catalytic oxidation method (Sugimura and Suzuki, 1988) is one of the most widely used and accepted, improved by Suzuki et al. (1992). Even though DOC analysis is widely used, it can be expensive (Chatterjee et al., 2009) and methods that employ chemical oxidation are limited by incomplete oxidation of protein-like molecules, which affects their accuracy. Another disadvantage of those methods is that sample contamination can occur by using reagents (Bisutti et al., 2004).

Measuring FDOM accounts for only a limited fraction of DOM (approximately 1%;(Cory et al., 2011), but has the advantage of being non-destructive and requires little time and preparation. Fluorescence occurs at specific excitation and emission wavelengths, depending on FDOM composition, and the intensity of the signal is proportional to concentration (Cory et al., 2011; Fellman et al., 2010). Fluorescence monitoring is attractive as it is a rapid, reagent-less technique that requires no sample preparation prior to analysis (Henderson et al., 2009). In addition, it is highly sensitive at relatively low costs (Lee et al., 2018). The strong fluorescence signal of sewage-derived DOM has led to fluorescence being considered as an alternative to existing water quality parameters commonly employed in the monitoring of river pollution (Tang et al., 2019).

In-situ optical sensing using fluorescence is one of the FDOM measurement techniques that has increased usage due to technical advances and cost reduction (Blaen et al., 2016). Photosensors also allow continuous quantification of DOM changes through time. *In situ* measurements do not require sample manipulation, transportation or conservation, clear advantages given the inherent instability of labile organic components (Hansen et al., 2016). However, temperature and turbidity corrections are potentially required (Downing et al., 2012; Saraceno et al., 2017; Watras et al., 2011).

Fluorescence spectroscopy, another emerging technique, generates three-dimensional excitation and emission matrices (EEMs) from the spectra emitted in response to the selected excitation wavelength. This bench-top technique has been widely used to identify sources and compositions of fluorescent DOM in natural watersheds (Zhang and Liang, 2019). A growing number of studies apply the parallel factor analysis (PARAFAC) for EEM interpretation (Baghoth et al., 2011; Coble, 1996; Murphy et al., 2013, 2011; Stedmon et al., 2003; Yamashita and Jaffé, 2008). PARAFAC decomposes the EEMs into individual fluorescent components (Weiwei et al., 2019) and has been successfully used to identify allochthonous and autochthonous fluorescent DOM components in a diverse range of natural environments (Henderson et al., 2009).

Despite the recent rise in applications of fluorescence-based techniques to monitor DOM, few studies compare their performance. Among those, (Lee et al., 2015) compared UV-VIS and FDOM sensors for on-site monitoring and (Wasswa et al., 2019) compared the performance of portable sensors and benchtop fluorometers in control samples and samples from different stages of a water reuse facility. However, there is still a need to further assess the limits of measuring FDOM to characterize DOM dynamics at the watershed scale.

In the present study, measurements from on-site optical FDOM sensor, benchtop fluorescence spectroscopy and high-temperature combustion DOC analysis are compared both visually and by calculating correlation coefficients for 38 sampling points spread over two mesoscale glacierized catchments in the Peruvian Andes. The watersheds were selected because they have high spatial variability in DOM sources and concentration and they flow from pristine mineral headwaters to points of obvious anthropogenic influences. The overall objective of this study is to evaluate the performance of the two fluorometric methods in a watershed context and to assess to what extent those methods are representative of what is measured using a traditional DOC technique.

2.3 Materials and Methods

2.3.1 Study sites

The Shullcas and Santa Rivers both drain glacierized watersheds situated in the Peruvian Andes (Figure 2-1). The region is characterized by a strong seasonality in precipitation with almost no rain during the dry season and abundant rainfall during the austral summer (Garreaud, 2009). Dry season conditions are favorable for synoptic sampling for mesoscale watersheds because glacier melt contribution to base flow is predominant over precipitation runoff (Mark et al., 2005).

Both rivers flow from glacial origin headwaters, through naturally vegetated lands and progressively greater presence of human activities, to urbanised areas with contamination by untreated wastewater.

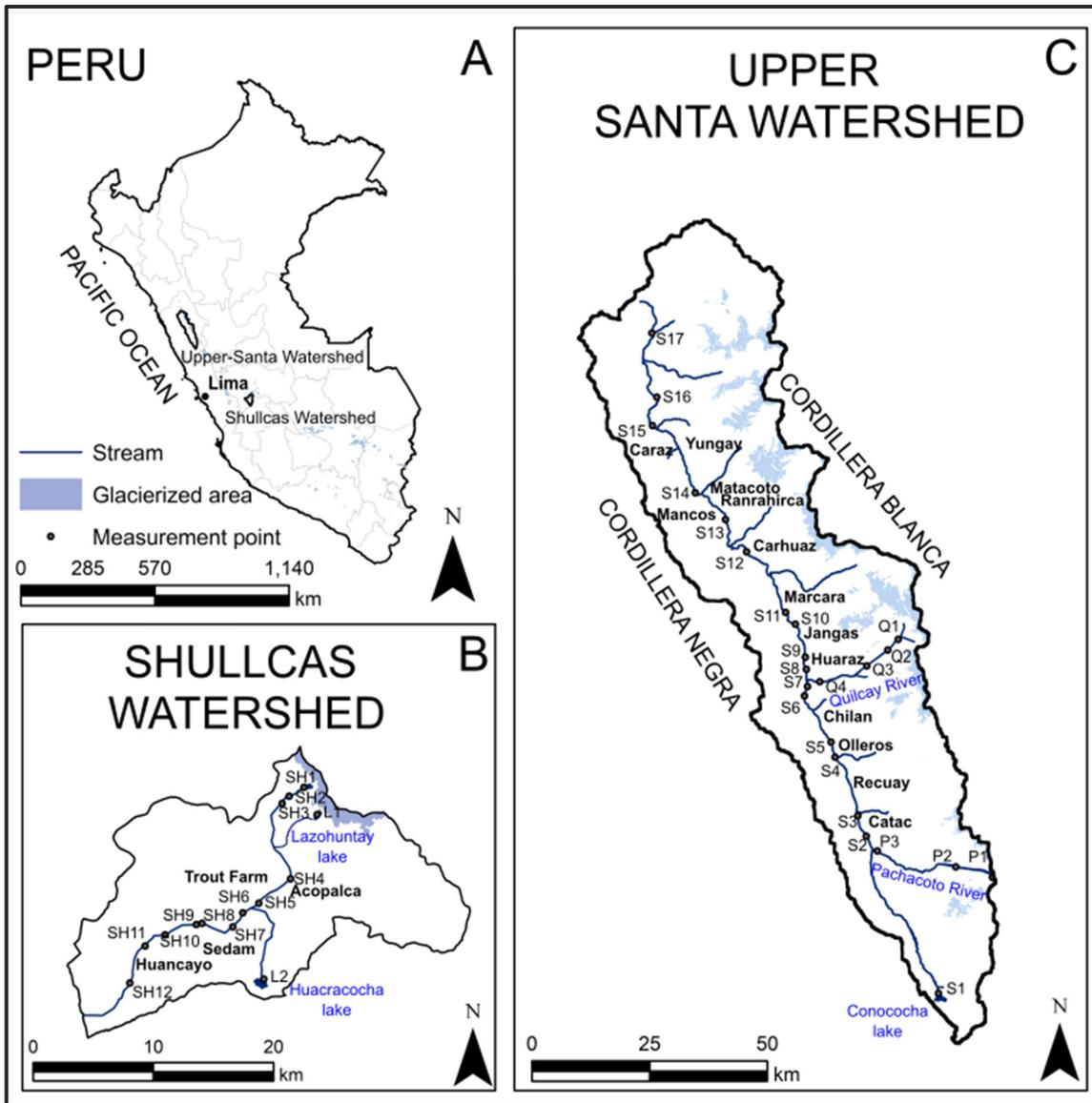


Figure 2-1 A. Peru map indicating location of study watersheds. B. Upper-Santa watershed. C. Shullcas watershed. Glacierized areas are colored in grey, sampling points are located using black dots. Samples and main cities names are written in black while water bodies names are in blue.

As glaciers in the Peruvian Andes retreat, the decrease in meltwater is predicted to lead to a significant decline in river discharge in the coming decades, particularly during the dry season (Baraer et al., 2012). and this is raising concerns about water quality (Guittard et al., 2017). Overall, these factors motivate the selection of these study sites to evaluate and compare DOC and FDOM measurements.

2.3.1.1 Shullcas Watershed

The Shullcas Watershed is located upstream of the City of Huancayo (Figure 2-1B). It flows from the Chuspicocha and Lasohuntay proglacial lakes, located on the western side of the glacierized Huaytapallana Mountain with a peak elevation of 5557 m.a.s.l. The Huaytapallana glaciers currently cover 22 km² but have lost 56% of their total surface area between 1984 and 2011 (López-Moreno et al., 2014). The Shullcas River flows through several villages, agricultural areas and trout farms before reaching Huancayo. The population density increases progressively until the river reaches Huancayo (pop. 470,000).

2.3.1.2 Upper Santa Watershed

The Santa River drains the western slopes of the Cordillera Blanca, the mountain chain with the highest density of tropical glaciers in the world (Figure 2-1C). The river flows from Conococha Lake at the southern end of the watershed into the Pacific Ocean (Baraer et al., 2015).

During the dry season, meltwater represents more than 30% of the total runoff at the upper Santa River watershed (Baraer et al., 2012; Mark et al., 2005). The River receives water from glacierized tributaries such as the Quilcay (17% glacierized in 1997) and Pachacotto (8% glacierized in 1997) (Mark and Seltzer, 2003) and provides water for stakeholder and industrial agriculture, mining, domestic water and hydropower production (Gurgiser et al., 2016). The glacierized headwaters of the tributaries are above 5000 m.a.s.l. and are not impacted by significant human settlements. None of the cities along the upper Santa River is equipped with

a proper sewage treatment plant and most discharge wastewater directly into the Santa River (Guittard et al., 2017), making the lower portion of the Upper Santa an anthropogenically impacted river.

2.3.2 Method

2.3.2.1 On-site measurements

At each sampling site, we measured FDOM using a C3-Turner Designs Submersible Fluorometer. The FDOM sensor has a wavelength of 325nm +/- 60nm for excitation and 470nm +/- 30nm for emission. The C3 was calibrated using standard solution prior to the sampling campaign. To ensure accuracy a solid secondary standard was used in the field to check any loss in sensitivity or stability on a daily basis. The fluorometer was in the water at each site for a minimum of 5 minutes, with a 30 second sampling interval. Turbidity, temperature and pH were measured with a portable multiparameter probe.

The Santa watershed *in-situ* measurements and sampling were performed from June 21 to June 27, 2017 in 17 points (Figure 2-1c) and the Shullcas watershed between June 29 and July 2, 2017 in 14 points (Figure 2-1b). Field observations, including sampling points surroundings, land use and visual watershed characteristics were recorded during the whole sampling campaign.

2.3.2.2 Sample collection

Water samples were collected at each sample site and filtered using a 0.45um filter. Samples were stored in sealed 60 ml HDPE amber bottles and were stored at 4°C until laboratory analysis.

2.3.2.3 Data processing for *in-situ* measurements

Readings from the C3 fluorometer were first preprocessed. Highest, lowest, first and last readings per site were considered as potential outliers and erased from records. Remaining values were averaged to obtain a unique value of uncorrected FDOM. Previous studies have shown that temperature (Wasswa and Mladenov, 2018; Watras et al., 2011) and turbidity (Downing et al., 2012; Saraceno et al., 2017) can both affect optical measurements due to thermal quenching and light scattering. Therefore, uncorrected FDOM values were corrected for temperature following (Watras et al., 2011). Considering that the attenuation of FDOM signal is of 10% to 20% of the maximum for turbidity values under 50 NTU (Saraceno et al., 2017), and that the average turbidity measured at all sites was 24.66 NTU with a standard deviation of 23.95 NTU, it has been assumed that no correction for turbidity was required in the context of the present study.

2.3.2.4 DOC laboratory measurements

Filtered water samples were analyzed for DOC concentration using an Apollo 9000 TOC analyzer (high-temperature catalytic oxidation method). Each sample was first acidified and sparged to eliminate non organic dissolved carbon. It was then transferred into a quartz cell and heated at 700 °C so that all the organic carbon was transformed to CO₂ that was quantifying infrared absorbance. The mean of the three readings is reported for every sample. Prior to analysis, a calibration curve was obtained using potassium hydrogen phthalate.

2.3.2.5 Fluorescence spectroscopy and EEM matrices generation

EEMs were produced for each sample using a Cary Eclipse fluorescence spectrophotometer. Slits were set to 5 nm for both excitation and emission. In order to obtain EEMs the excitation spectra wavelength was set from 290 nm to 455 nm and the emission spectra wavelength from 250 to 455 nm with a scanning speed of 1200 nm/s. Eight samples selected randomly from the Shullcas River and 15 from Upper-Santa River were tested twice in order to verify the

replicability of the method. As pure water has a clear scatter peak (i.e. the Raman peak) that could alter the fluorescence response (Cross et al., 1937), emission spectrum of blanks were performed every day the equipment was used. This Raman Test was performed on nanopure water using an excitation wavelength of 275 nm and an emission spectrum of 285-450 nm with 1 nm interval. After the Raman signal was subtracted from samples EEMs, it was then normalized and expressed in Raman units (R.U., nm^{-1}).

EEMs were corrected for possible systematic biases due to variations and/or imperfections in the optical components that could end up distorting the data and the validation of the PARAFAC that is carried out later. This was achieved by multiplying each EEM by a correction matrix specific of the instrument. The Raman peak was also removed using the drEEM Matlab toolbox (Murphy et al., 2013).

2.3.2.6 PARAFAC modeling

Two parallel factor analyses were applied to the corrected EEMs to decompose the spectra into its different components. This multi-way statistical data analysis is used to describe data with more than two dimensions. This analysis is known to have a ‘second order advantage’ (Booksh and Kowalski, 1994), meaning that the algorithm is mathematically able to separate any possible spectrally overlapping data from the EEMs into fluorescence independent chemical components (Stedmon et al., 2003). The application of the PARAFAC approach includes spectral correction, calibration, removal of scatter, identification and removal of outliers, and model validation (Stedmon and Bro, 2008). The PARAFAC method was used following the procedure recommended by (Murphy et al., 2013) using MATLAB R2017B. Model validation was performed by conducting a split analysis that consists of generating different models from subsample groups. The model for the entire dataset was validated, including when the loadings from the different subgroups were identical to the one from the entire dataset (Murphy et al., 2013). The components identified through PARAFAC were classified as Humic-like or protein-like substances based on components with similar spectra identified in previous

studies. Component relative concentration was estimated by considering its maximum peak intensity called *Fmax* and expressed in Raman units (R.U., nm⁻¹).

2.4 Results

2.4.1 The Shullcas Watershed

Figure 2-2 presents a synthesis of analytical results for the Shullcas watershed. DOC, FDOM (*in-situ*) and PARAFAC results are presented on a unique graph to facilitate comparison. Results for each analytical technique are individually discussed, followed by the performance comparison.

2.4.1.1 DOC measurements

DOC measurements vary from 380 ppb at the headwaters to ~4500 ppb at Huancayo city. DOC concentrations increase gradually as we move away from the pro-glacial lakes to SH7, a sampling point situated in the middle of the catchment where it reaches 1100 ppb. Between SH7 to SH10, DOC declines slightly reaching 780 ppb at the entrance of the urban area. DOC then shows an exponential-like increase from SH10 to SH12 where it reaches 4500 ppb.

The increase in DOC throughout the River's journey corresponds to qualitative observations of land cover and land use. In absence of human activities and with very limited vegetation cover, proglacial lake areas accumulate glacier melt water and do not show obvious signs of intense biological activities. As the river flows through the watershed, the vegetation coverage, pastoral and agricultural activities gradually increase. Downstream of point SH5, the River crosses the Acopalca village and a trout farm where it shows an increase in DOC. Effect of mixing with major tributaries is observed twice in the upper watershed. Firstly, DOC shows no increase after the confluence with the stream that flows from the Lazohuntay proglacial lake where the lowest value of the entire watershed has been measured (220 ppb). Several km downstream, the DOC of the Shullcas River shows an increase immediately after the

confluence with the stream that flows out of the Huacracocha lake, in which the highest DOC value of the watershed was measured (5750 ppb). This value responds to herding and fishing activities, some shepherd's settlements, and the organic-rich soil surrounding the lake.

The slight decreasing trend in DOC observed between SH7 and SH10 is due to the SH7-SH8 drop mostly. Several hypotheses are proposed for this: absence of major settlements, possible groundwater discharge, dilution by numerous small confluents. The slight increase in DOC observed between SH8 and SH9 points can be explained by the water use of the major trout farm. Points SH11 and SH12 are situated in the urbanised area of the watershed. Numerous points of wastewater input can be observed within that area and different indications of water pollution were noticed at sampling: turbidity, color, smell. This was especially the case at point SH12 where DOC reached 4500 ppb.

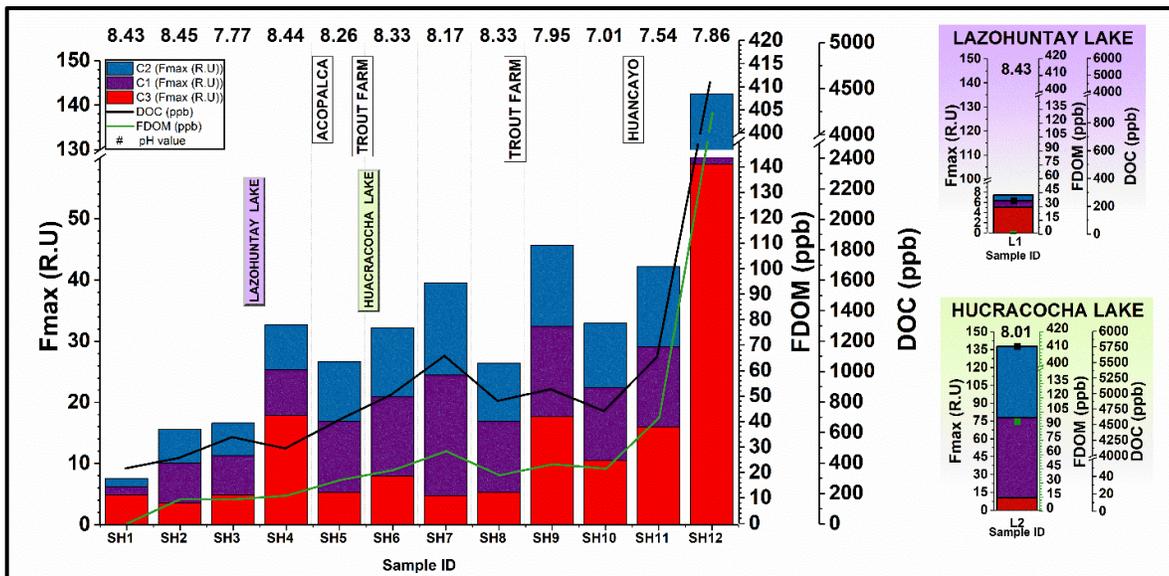


Figure 2-2 Analytical results for the Shullcas watershed. DOC is shown as a black line or points. FDOM is shown in green. PARAFAC components Fmax appear as bar graphs where purple corresponds to C1, blue to C2 and red to C3. Names in the boxes indicate cities and tributaries. Site locations are in Figure 2-1b

2.4.1.2 FDOM *in-situ* measurements

With an R^2 of 0.98 and a p-value of 8.20×10^{-9} . The FDOM profile of the Shullcas River reproduces closely the DOC profile. (Figure 2-2). FDOM readings vary from 0 ppb to 405 ppb. Like DOC, FDOM measurements fit well to qualitative land use observations. Few discrepancies exist between DOC and FDOM. The Huacracocho lake on-site FDOM readings yield a concentration of 90 ppb that is far from the highest concentration for the watershed (405 ppb at SH12). Noticeably, the Lazohuntay Lake (L1) FDOM on-site measurement was 0 ppb, which suggests there is no presence of any fluorescence component or that the value is under the detection limit of the equipment.

2.4.1.3 PARAFAC EEMS

Three fluorescent components were identified and validated through the PARAFAC-EEMS analysis for the Shullcas Basin. Figure 2-3a-c shows the contour plots of each of the different components. Component one (C1) is made of two peaks with maxima excitation at 255 and 360 nm with an emission range from 485 to 530 nm. Component two (C2) has a fingerprint comparable to C1 with emission values slightly below. It shows two peaks with a maxima excitation of 260 nm and 310 nm and an emission peak maximum of 435. Component 3 (C3) shows a single peak of excitation at 285 nm and a single peak of emission at 330 nm.

For each component identified with the PARAFAC-EEMS analysis, Table 1 provides excitation and emission wavelengths of the main peaks, wavelengths of comparable components found in the literature and their possible source.

According to the PARAFAC-EEMS results, the Shullcas River's FDOM is comprised of two main Humic-like substance groups, C1 and C2, probably of terrestrial origin and one substance group, C3, identified as tryptophan, an amino acid often associated with the presence of microbial activity like what is found in wastewater.

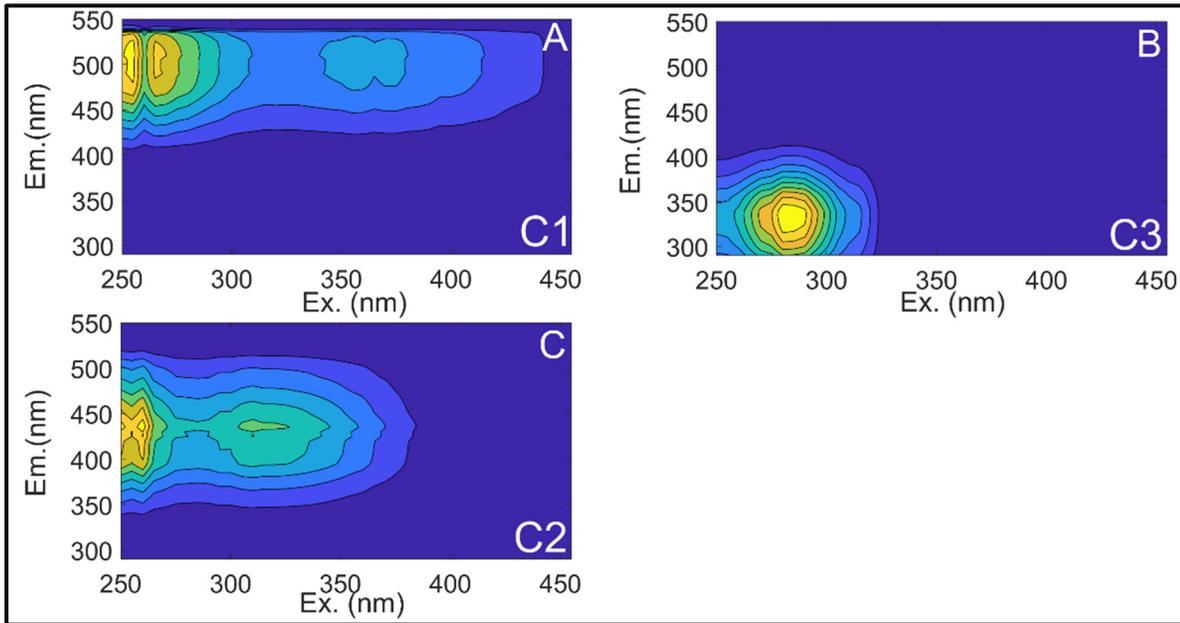


Figure 2-3 Three different components identified by the PARAFAC model for the Shullcas basin.

Fmax intensities that are proportional to the component concentration are reported in Figure 2-2. The data describes a general agreement between PARAFAC-EEM and DOC ($R^2=0.96$, p-value 1.05×10^{-8} .) and between PARAFAC-EEM and FDOM ($R^2=0.94$, p-value 0.04). The DOM evolution pattern described by DOC and FDOM measurements can also be observed in the sum of the Fmax measured for the three components.

Table 2-1 Components identified in this study for the Shullcas Basin with the corresponding wavelength position of its maximal fluorescence. Tentative description is based on components identified in previous studies.

Component	λ (nm) (Ex/Em)	λ (nm) of possible analogue (Ex/Em)	Description	Reference
C1	255(360)/485-530	237-260/400-500 250(385)/504 260(360)/480	Humic Fulvic acid terrestrial or autochthonous Terrestrial Humic Substances	Bridgeman et al (2011) Stedmon and Markager (2005) Baghoth et al (2011)
C2	260(310)/435	260/380-460 237-260/400-500 250(340)/440	Humic-like Humic Terrestrial humic substances	Coble (1996) Bridgeman et al (2011) Baghoth (2011)
C3	285/330	275/340 275/340 280/344	Tryptophan-like, protein-like Tryptophan Tryptophan-like, autochthonous	Coble (1996) Bridgeman et al (2011) Stedmon and Markager (2005)

Two sampling points make exception to the general agreement within the three tested methods: SH4 and SH9. The PARAFAC-EEMS method shows SH4 has the second highest total fluorescence in the SH1-SH7 transect while the DOC measurements rank 4th and FDOM 5th. In a comparable way, the PARAFAC-EEMS method situates the fluorescence level at SH9 proportionally higher than the two other methods. Interestingly, in both cases the high Fmax values are associated with particularly high C3 Fmax. SH4 is situated just after the confluence with the stream that drains the Lazohuntay Lake that shows relatively high C3 Fmax too. The trout farm, whose impact can be assessed by the two nearby points SH8 and SH9, appears to be increasing the load of protein like C3 in the main stream.

PARAFAC-EEMS provide additional information to the DOC and FDOM measurements. This is the case at SH12, within the Huancayo city, where we observe high values for all the three components, suggesting diverse sources of dissolved organic matter at that point. At the confluence with the stream flowing from Huacracochoa lake, the PARAFAC-EEMS shows a humic origin in the increase of FDOM at SH6 and SH7, the same type of component that shows being the origin of the high fluorescence in the Lake.

2.4.2 The Upper-Santa watershed

A synthesis of analytical results for the Upper-Santa watershed are presented in Figure 2-4.

2.4.2.1 DOC measurements

The highest recorded concentration was measured at S1 (Conococha Lake, 940 ppb), headwaters of the Santa River. The lake is shallow and is a refuge for migratory birds, regularly visited by livestock, and collects wastewater from nearby human settlements. Downstream from Conococha, the Santa River presents a fluctuating DOC with a net negative trend as it flows through the upper watershed. Peaks are observed downstream of Huaraz city (S8), near Jangas (S11) and upstream of Caraz city.

The most pronounced drop in DOC is observed between S1 and S2, downstream of Conococha Lake. The Santa River collects water from the west side of the Cordillera Blanca where glacier meltwater is a large component of the dry season discharge. The Pachacoto River, a glacierized tributary, flows from a proglacial lake with the lowest DOC value (62 ppb; P1) measured in the Upper Santa watershed, and joins the Santa River between S1 and S2. As the Pachacoto River flows to the Santa River it passes through pasture lands and small settlements and its DOC concentration increases to 600 ppb at P2 and 476 ppb at P3.

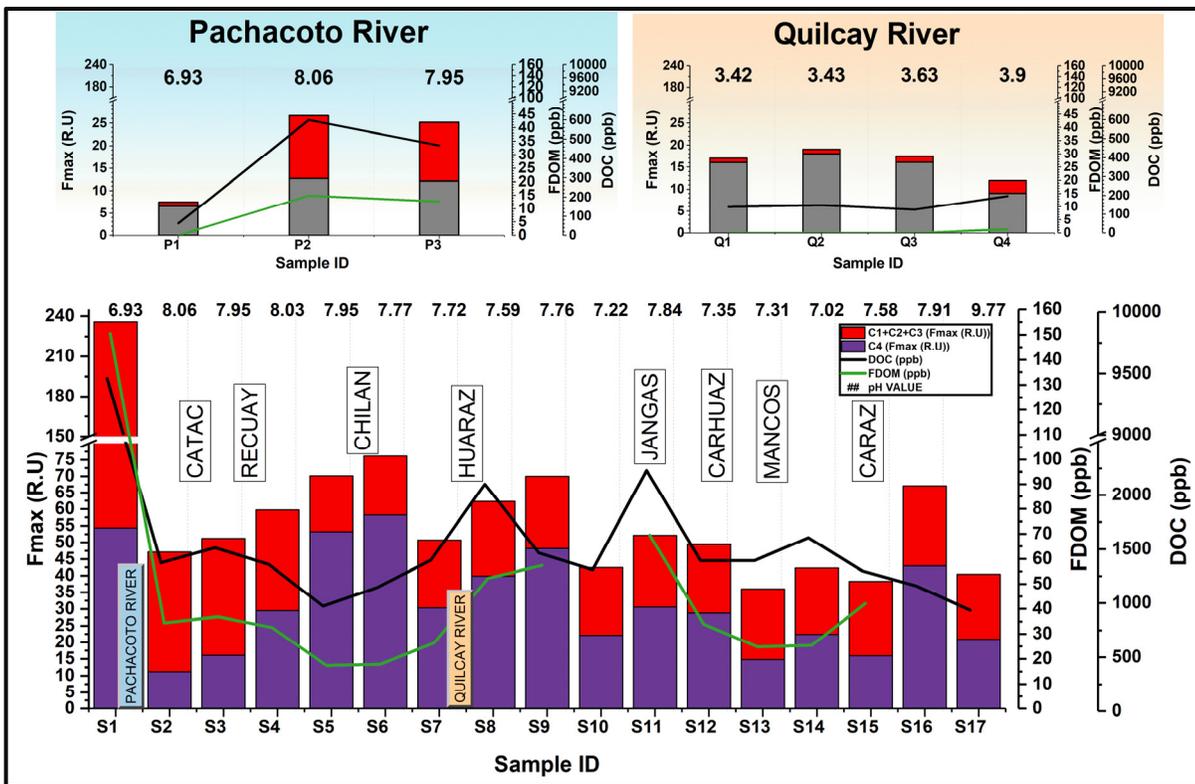


Figure 2-4 Analytical results for Upper-Santa watershed. DOC is shown as a black line and points. FDOM is shown in green. PARAFAC components Fmax appear as bar graphs where purple corresponds to C4 and red represents the sum of C1, C2 and C3 Fmax

From S2 to S5, DOC is relatively stable. Point S5 is just downstream of the confluence with the Rio Negro and is the DOC minimum for the river (980 ppb). DOC increases between S5 and S8 (2150 ppb), the latter being below Huaraz city, just after the confluence with the Quilcay River (Q1 to Q4). Similar to Pachacoto, the Quilcay tributary originates from proglacial lakes. DOC concentrations at Quilcay vary between 150 and 200 ppb, with the highest value being recorded furthest downstream at Q4, just above Huaraz.

Below Huaraz, DOC in the Santa River decreases slightly as the population density decreases (S9 and S10) and several tributaries join the main river. DOC increases again at S11 in Jangas to almost 2200 ppb, which corresponds to field observations, which describe the site as trash laden and with a bad odour. From Jangas, DOC gradually decreases and reaches a minimum at S17 at the hydropower facility river intake. Declining DOC in this portion of the river occurs simultaneously with large tributary inflows and limited human activities as the river incises and valley slopes become steeper. With the exception of the DOC peak at S11, DOC variations match field observations of urbanization and watershed dynamics.

2.4.2.2 FDOM *in-situ* measurements

The FDOM profile of the Santa River closely mimics that of DOC between S1 and S9. The highest FDOM value was measured in the Conococha Lake (155 ppb). FDOM then drops to 40 ppb as the Santa River by presence of tributary The FDOM spatial variability along the Pachacoto River has a similar pattern as the DOC with the difference that the P1 FDOM is zero ppb, suggesting the proglacial lake is free of organic matter. Along the Santa River, FDOM peaks just after Huaraz and at the confluence with the Quilcay River (S8) to a value of 57 ppb, similar in pattern to DOC. The Quilcay River has 0 ppb for points Q1 to Q3 suggesting, similar to the Shullcas watershed, that proglacial lakes do not have significant biological activities, confirming the DOC measurement. The last sampling point of the Quilcay River (Q4) shows a slight increase in FDOM to 2 ppb.

Immediately after S8 in the Santa River, FDOM decreases slightly along with DOC, to 52 ppb. Unfortunately, the measurement results are not available for S10, S11, S16, and S17 due to an equipment failure. Between S12 and S15, the FDOM curve does not mimic the DOC, particularly between S14 and S15 where there are opposite trends. Those disagreements between the two methods are reflected within the R^2 value of 0.89 and a p-value of 1.21×10^{-3} that is almost 0.1 below than the one calculated at Shullcas.

2.4.2.3 PARAFAC-EMMS

After subtracting the Raman signal and normalizing the data, four fluorescent components were identified for the upper Santa watershed with the PARAFAC-EEMS analysis. Figure 2-4a-d shows the contour plots of each of the different components. Component one (C1) has a F_{max} excitation wavelength of 255 nm with an emission ranging from 440 to 490 nm (Figure 2-4a). Components two (C2), three (C3) and four (C4) exhibit peak excitation wavelengths at 295, 265-280 and below 250 nm respectively while their peak emission wavelengths are 335-360, 320-350 and 350 nm (Figures 2-4b, 2-4c and 2-4d respectively).

As in the Shullcas study, the spectral characteristics of the four components were then compared to those of components described in the literature for excitation and emission wavelength position of the peaks (Table 2).

In summary the PARAFAC-EEM results from the Upper Santa basin contain one component tentatively corresponding to Humic-like substances (C1) and three components whose fingerprint tentatively correspond to protein-like (C2, C3, C4). The evolution of the components along the rivers are shown as bars in Figure 2-4.

Comparing total PARAFAC-EEMS fluorescence to DOC and FDOM results shows a mixture of matching and diverging trends. The R^2 for PARAFAC-EEMS and DOC is 0.91 (p-value $.6.5 \times 10^{-4}$) and for PARAFAC-EEMS and FDOM is 0.82 (p-value = 0.1), both values being

below what was obtained at Schulcas. Visually, we observe that from S1 to S3, the three methods have similar trends of DOM. In the same area, samples taken in the Pachacoto River have a good agreement between the three methods. Starting at S4, we observe differences in the concentration profiles between the three detection methods. The most noticeable differences between the PARAFAC-EEMS, the DOC and the FDOM profiles in the Santa River occur between S4 and S9 where PARAFAC-EEMS has a gradual increase of fluorescence, reaching a peak just after Chilan that marks the beginning of the Huaraz urban area. A drop in DOM is then observed at S7, the most upstream sample taken in the Huaraz region. This method situates the peak in DOM associated to the city of Huaraz fingerprint at S9, four kilometers downstream of S8 where the DOC method measured its maximum value for the Huaraz urban area.

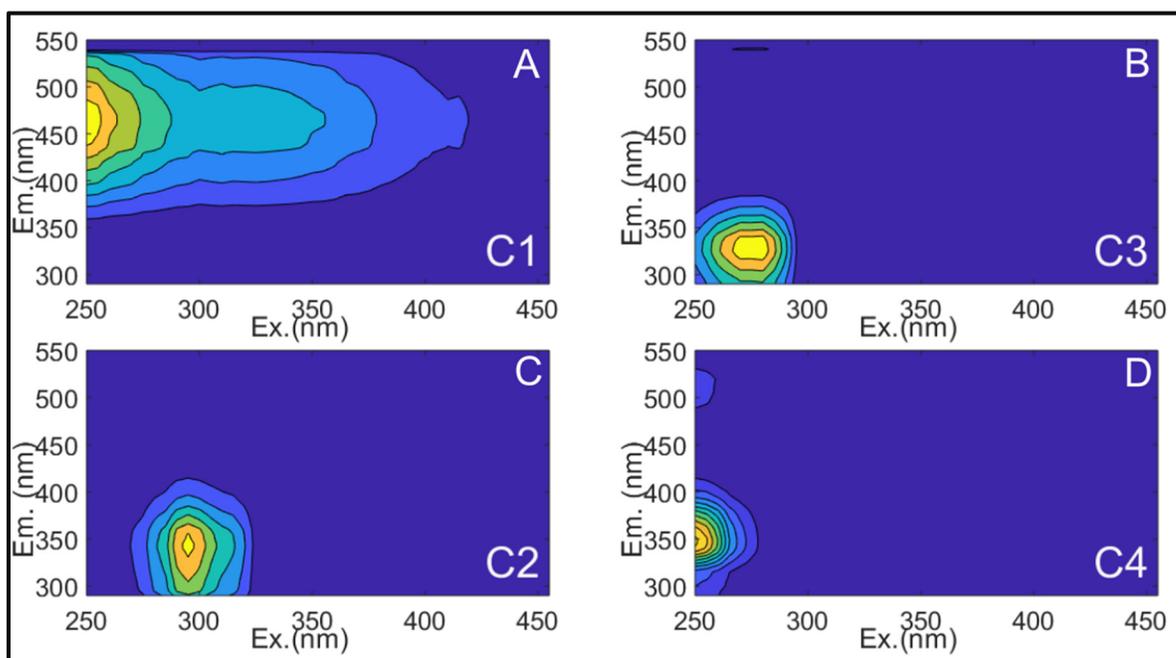


Figure 2-5 Four different components identified by the PARAFAC model for the Upper-Santa basin.

Unlike the DOC method, the PARAFAC-EEMS method does not identify Jangas (S11) as a particularly high DOM hotspot. Finally, the PARAFAC-EEMS method suggests a particularly low DOM concentration at S15, just upstream of the Caraz city, followed by a peak just

downstream of the city (S16). The increase in DOM downstream of Caraz could be related to the lack of sewage treatment by the city.

The PARAFAC-EES split between humic-like and protein-like components provide additional insights regarding DOM dynamics in the Upper Santa watershed (Figure 2-4). Except for S14 and S15, results show a good agreement between FDOM and the C1 PARAFAC component, including the two sampled tributaries. The non-detectable FDOM in proglacial lakes, where significant concentrations of DOC were measured, suggests that fluorescence is mainly of protein-like origin. The very low concentration in humic-like substances, even in Q3 that flows through large alpine meadows, can be explained by the low solubility of humic acids at pH lower than 5 (Wu et al., 2002). The significant amount of protein-like DOM in the pristine proglacial lakes of the Quilcay River can find an explanation in the pH values too. Waters of the Quilcay upper watershed have been described by Fortner et al. (2011) as very acidic and containing above the average metal concentrations arising from sulfide oxidation weathering, a process likely accelerated by bacterial mats. The protein-like high Fmax signal would therefore confirm those intense microbial activities suggested.

Table 2-2 Components identified in this study for the Upper Santa Basin with the corresponding wavelength position of its fluorescence maxima. Tentative description is based on components identified in previous studies

Component	λ (nm) (Ex/Em)	λ (nm) of possible analogue (Ex/Em)	Description	Reference
C1	<255/440-490	260/380-460 237-260/400-500 255(350)/460 <255/448 250(340)/440 <260/458	Humic -like Humic -like Terrestrial humic-like Terrestrial humic-like Terrestrial humic-like Terrestrial humic-like	Coble (1996) Bridgeman et al (2011) Zhao et al (2016) Stedmon and Markager (2005) Baghoth (2011) Yamashita et al (2008)
C2	295/335-360	225(290)/360 <250(290)/360 285/362	Autochthonous Tryptophan-like Amino acids or protein bounds Non humic-like	Zhao et al (2016) Baghoth (2011) Yamashita et al (2008)
C3	265(280)/320-350	275/340 275/340 280/344	Tryptophan-like, protein-like Tryptophan Tryptophan-like, autochthonous	Coble (1996) Bridgeman et al (2011) Stedmon and Markager (2005)
C4	<250/350	275/340 275/340 280/344	Protein-like, Tryptophan, Tyrosine Autochthonous Tryptophan-like Amino acids free or protein bounds	Bridgeman et al (2011) Zhao et al (2016) Baghoth (2011)

In the Santa River, the differentiation between humic-like and protein-like substances show that the very high DOC and FDOM values in Conococha are from humic origin mainly. In contrast, the PARAFAC-EEMS method associates variations in DOM concentration throughout the River course to fluctuations in protein-like components mainly, suggesting fluorescence of protein-like components as an indicator of anthropogenic influences on water quality.

2.5 Discussion

Studying DOM dynamics in two different glacierized watersheds of the tropical Andes provides an opportunity to compare the strengths and limitations of available measurement methods. DOC measurements carried out with the catalytic oxidation method have proven to be reliable in different environments (Mostofa et al., 2013; Sugimura and Suzuki, 1988). However, measuring organic carbon instead of the organic matter can create discrepancies in results from other DOM estimation methods that might be difficult to quantify. As a bench top laboratory method, it is not a direct on-site measurement and it requires sample pre-treatment due to the effects of air and light, among others. Additionally, proteins are known to be difficult to oxidize (Rogowska-Wrzesinska et al., 2014) creating possible biases in DOM dynamics assessment. In our study, some of the discrepancies with the other tested methods as those observed in the lowest sections of the Rio Santa might be due to these limits.

The fluorometer equipped with a FDOM/CDOM sensor is a fast and efficient way to assess the DOM state at the watershed scale. FDOM profiles agree with DOC in the Shullcas watershed and in the first section of the upper Santa watershed, suggesting this method could be used alone for DOM dynamics characterization. The other studied areas, however, show some limits. The sensor wavelength covers a fraction only. This area of the FDOM spectra mainly characterizes humic-like substances. The limits associated with the use of such narrow spectrum FDOM sensor at our study sites were observed at spots with relatively low DOC concentrations but with higher concentrations of protein-like substances. This was the case for samples S1 and L1 in the Shullcas watershed and P1, Q1, Q2, Q3, Q4 in the Santa watershed.

Other limits arise from the sensitivity of FDOM to stream temperature, pH and turbidity that require monitoring those characteristics in parallel to the FDOM and eventually applying some corrections to measurements.

The PARAFAC-EEMS method allows differentiation between humic-like and protein-like fluorescent substances and provides an estimate of DOM concentration through the F_{max} values. In the present study, PARAFAC-EEMS provides key information for interpreting DOC and FDOM results. This was the case for understanding the difference in DOC and FDOM concentrations in proglacial lakes. In the Shullcas River, the fluctuation of the total fluorescence correlates well with the DOC concentration. Indeed, the use of this method alone could have been sufficient to conduct the DOM dynamics assessment for that watershed. This is not the case in the upper Santa River where differences in DOM variations between the three tested methods have been observed on several occasions.

The combination of DOC, FDOM and fluorescence spectroscopy measurements allow us to capture and understand DOM variations in both watersheds. The lack of sewage treatment systems in urban areas has been clearly traced by the combined methods. Through the study, the Shullcas watershed has a clear tendency of DOM concentration to increase as the water flows from the proglacial lakes to the urbanized area of Huancayo. The upper Santa DOM has a different behavior as natural and anthropogenic loads of DOM get diluted by the confluence with numerous tributaries flowing from the Cordillera Blanca highlands. Results from the study do not allow identifying a unique method that would provide as much information as the three combined. The PARAFAC-EEMS is the method that could be seen as the most valuable for understanding DOM dynamics at the watershed scale. Discrepancies with other methods for the upper Santa River indicate that further studies are required prior to being able to recommend using it as a standalone solution.

2.6 Conclusions

Comparing the performance measurement of DOC non-dispersive infrared detection, fluorescence spectroscopy and on-site fluorometry to assess DOM dynamics in two glacierized watersheds of the tropical Andes allows us to identify the origins and magnitude of DOM loads. Results show that in general the Santa River presents higher DOM concentrations than the Shullcas River with the exception of the measurements in the Huancayo city that presented particular high concentrations. Comparing the strengths and limits of the methods individually has shown:

- The PARAFAC-EEMS method isolated 3 components in the Shullcas basin and 4 in the upper-Santa basin. Identifying the components using previous literature studies allows us to characterize the different fractions of DOM that fluoresce. Further research is needed to link total fluorescence to DOM concentration before making this method robust enough to study the DOM dynamics throughout a watershed.
- The performance of the instruments used in this research suggests that even if FDOM represents only a fraction of DOM, it captures dynamics that are comparable to those of DOM
- The FDOM/CDOM *in situ* sensor used in the study has a bandwidth that is mainly situated in the humic part of the fluorescent fraction. Therefore, care must be taken when using such sensor for DOM dynamics study as the measurement might miss protein-like FDOM that is found among others, in wastewater. Other FDOM sensors on the market may present different spectral coverage. FDOM sensors comparison in similar conditions as in the present study still needs to be performed.
- DOC measurements are a good reference for DOM dynamics. It is however recommended to keep in mind that DOC is a carbon-specific fraction of DOM and concentrations might differ in a considerable way.

2.7 Acknowledgements

This research is supported by the Geochemistry and Geodynamics Research Centre (GEOTOP) of Quebec, the Natural Science and Engineering Research Council (NSERC) of Canada and the National Science Foundation (NSF). The authors want to thank Eng. Jesus Gomez, former Director of the Huascarán National Park, and Alejo C. Rapre, Director of the Autoridad Nacional del Agua office of Huaraz for their active support of the research.

CONCLUSION

This research consisted of the evaluation of dissolved organic matter loads in two watersheds located in the Andean region by applying two different fluorescent dissolved organic matter measurements: on-site fluorometry and fluorescence spectroscopy coupled with a parallel factor analysis. The objective was to test the performance of the two analytical techniques and compare them with a conventional measurement of DOC at a watershed scale, where the concentration of DOM is highly dynamic considering the glacierized headwaters and the impact of the cities downstream.

From this study we obtained analytically speaking and environmentally speaking conclusions:

Analytically speaking, this study responds to the need of testing the mentioned methods experimentally on the field without a controlled environment. Therefore, this research represents a new approach towards the understanding and the comparison of the performance of fluorescence-based techniques that have been recognized as promising tools in the last decades. This provides information towards identifying weaknesses and strengths in the application of these techniques, useful for further development and improvement of the tools available. This is of great importance for the near-future of water quality monitoring, especially where DOM concentration and composition varies drastically as observed in the tropical alpine watersheds studied here. It also serves to new users as a tool to understand the experimental difference in results within the techniques used. This is useful as it provides enough information so that the right equipment(s) is/are used.

Environmentally speaking, the state of the rivers in the Andean region is critical, especially considering the water use for all the human activities and the impact they generate. The lack of sewage treatment systems is evident in the values obtained from the measurements carried out with the three different techniques. Concentrations of DOM increased drastically after points of strong anthropogenically activity. This is alarming since the decrease in water availability in response to the glacier retreat in the region indicates a decrease in meltwater

contribution. Therefore, the DOM concentration is expected to continue to increase. Particular attention should be given to protein-like FDOM that demonstrates to respond to microbial activity.

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