Tutor/s

Dr. Jose Luis Beltrán Abadia
Departament de Enginyeria Química i Química Analítica

Grau de Química

Treball Final de Grau

Characterization of the dissolved organic matter (DOM) in the Besos river by molecular fluorescence. Influence of different water treatments.

Caracterización de la materia orgánica disuelta del acuífero del río Besós mediante fluorescencia molecular. Influencia de diferentes tratamientos de agua.

Dana Pierina Orlando Véliz

Junio 2021

Universitat de Barcelona

B:KC Barcelona Knowledge Campus
Campus d'Excel·lència Internacional

'Lo que sabemos es una gota de agua; lo que ignoramos es el océano'

Isaac Newton

En primer lugar, quiero agradecer a mi tutor el Dr. José Luis Beltrán Abadia, quien con sus conocimientos y apoyo me guio en todas las etapas de este proyecto; y por el tiempo empleado para avanzar de forma exitosa y conseguir mis objetivos.

También quiero agradecer a la *Universitat de Barcelona* por brindarme todos los recursos y herramientas que fueron necesarios para llevar a cabo el proceso de investigación. A la *Universitat Politècnica de Catalunya* por la oportunidad de esta colaboración.

De la misma forma, quiero agradecer a mi familia por ser los principales promotores de mis sueños, por su apoyo y confianza en mis decisiones. Gracias a mi madre que a pesar de estar a miles de kilómetros siempre está en cada paso importante de mi vida.

Por último, pero no menos importante, gracias a mis amigos, compañeros y personas especiales por el apoyo incondicional, por creer en mí y por darme fuerzas y ánimos cuando se veía todo muy difícil.

A todos ellos, muchísimas gracias.

REPORT

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1. SUMMARY

To guarantee water quality parameters for human consumption and industrial uses, it is treated to eliminate the largest portion of organic matter that derives from vegetation and aquatic organisms that are generally resistant to certain degradation processes.

Organic matter in water is made up of thousands of very diverse components, from macroscopic particles to dissolved macromolecules. The difficulty in removing this matter increases when the particles are dissolved.

The fraction of dissolved organic matter is called **DOM** and is divided into two groups: protein-like and humic-like substances. The complexity of this matter, especially of the second group, as will be observed with the treatments used, pose a great difficulty to be removed.

This study aims to evaluate the performance of different water treatments from the Besos river aquifer to eliminate dissolved organic matter (DOM), by using Fluorescence Emission-Excitation matrices (**EEMF**) as a monitoring technique due to the DOM fluorescent properties.

Subsequently, the DOM fraction has been characterized from the data processed with the **PARAFAC** tool. The maximum emission and excitation wavelengths allow the identification protein-like and humic-like fractions. However, it should be noted that we can only know its nature.

Keywords: water quality, DOM, EEMF, PARAFAC, Besos river.

2. RESUMEN

Para garantizar unos parámetros de calidad del agua para el consumo humano y usos industriales, ésta se trata para eliminar la mayor parte de materia orgánica originada por la vegetación y organismos acuáticos que generalmente son resistentes a ciertos procesos de degradación.

La materia orgánica en el agua está compuesta por substancias muy diversas, desde partículas macroscópicas hasta macromoléculas disueltas. La dificultad para la eliminar esta materia se incrementa cuando las partículas están disueltas.

La fracción de materia orgánica disuelta se denomina DOM y se divide en dos grupos: de tipo proteico y de tipo húmico. La complejidad de esta materia, especialmente del segundo grupo como se observará con los tratamientos empleados, supone una gran dificultad para su eliminación.

Este estudio tiene como objetivo evaluar el rendimiento de diferentes tratamientos de agua del acuífero del río Besós para eliminar la materia orgánica disuelta (DOM) empleando las matrices de Emisión-Excitación de Fluorescencia (EEMF) como técnica de monitorización debido a las propiedades fluorescentes que esta materia presenta.

Posteriormente, las fracciones de la DOM se han caracterizado a partir del tratamiento de los datos EEMF con la herramienta PARAFAC. Las longitudes de onda correspondientes a los máximos de emisión y excitación permiten la identificación de las diferentes fracciones de tipo proteico y húmico, sin embargo, hay que tener en cuenta que únicamente podemos determinar su naturaleza.

Palabras clave: calidad del agua, DOM, EEMF, PARAFAC, río Besós.

3. INTRODUCTION

The earth is formed for ¾ of water approximately of which 2.5% is fresh water; however, the major part is frozen in glaciers and only 0.025% it is suitable for human consumption.

From this minor percentage, it is necessary to guarantee a high quality, and for this reason a variety of methods have been developed, focused on the kind of matter to eliminate.

Organic matter is an undesirable component (suspended or dissolved) in water for human consumption which can cause health problems. For this reason, quality standards are fixed, being the cost of the different treatments an important parameter to study.

3.1. ORGANIC MATTER IN WATER

Natural water contains a complex mixture of organic material (NOM: Natural Organic Matter). NOM has a role in water potabilization processes because it contributes to the formation of disinfection by-products (DBP) and promotes microbial growth [1][2], giving the water a color, taste, and reek characteristic.

The knowledge of natural organic matter is insufficient which makes its treatment difficult [3]. NOM can be divided in two main types: particulate organic matter (POM) and dissolved organic matter (DOM), being POM easily removed by filtration.

3.1.1. DOM: Dissolved Organic Matter

The dissolved organic matter (DOM) is a heterogeneous mixture of organic components, originated from the degradation of animal or plant residues in different processes. It consists mainly of proteins, polysaccharides, and humic substances. These types of organic matter differ from their aliphatic and aromatic composition.

The DOM plays an important role in retention of nutrients, biological availability, mobility and reaction with metals ions and organic chemicals [4], being the humic fraction, which is the majority, responsible for this. Although the retention of nutrients is good for the microorganisms in the water, the other processes can affect our health.

3.1.1.1. Humic fraction

The humic fraction is a heterogeneous mixture without a certain composition and is the main component of DOM. Furthermore, it contains functional groups that can react easily with metals and organics pollutants [4]. This fraction is formed by humic and fulvic acids [5] and has a molecular weight between 1000-20000Da [6].

Humic acids are heterogeneous supramolecular compounds formed by small particles held together by non-covalent forces such as Van der Waals forces [7]. The compounds have aromatic nuclei with phenolic and carboxylic substituents (***Figure 1***) and are the major component of the humic fraction.

These organic acids are present in organic matter as his conjugated bases with a negative charge and they are soluble in water. Although, it precipitates in acid medium [5][7].

The carboxyl and phenolate functional groups allow complexing with metals such as Fe^{2+}, Ca^{2+}, Mg^{2+}.

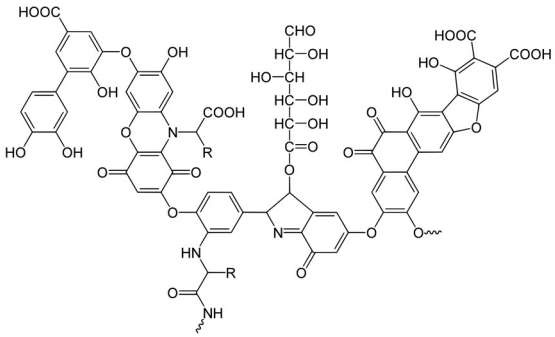


Figure 1. Example of a typical humic acid, with several of its components such as quinone, phenol, catechol, and sugars. By Yikrazuul – own work, public domain, via Wikipedia.

Fulvic acids are yellowish amorphous compounds that do not precipitate in acid medium and have a negative charge. Unlike humic acids, they have a lower molecular weight and in some conditions of pH and non-alkaline cation solutions, they experience flocculation [5].

3.1.1.2. Non-humic Fraction

The non-humic fraction consists of Biopolymers (BP), Building Blocks (BB), and Low Molecular Weight Neutrals (LMWN), and Acids (LMWA).

Biopolymers are macromolecules that are part of a lower portion of the hydrophilic fraction of DOM. Constituents within the fraction are polysaccharides, proteins, and amino sugars with a molecular weight >20000Da [6].

Building Blocks are molecules which contain reactive functional groups and are part of the hydrophilic fraction of DOM. Constituents within fractions are breakdown products of humic with a lower molecular weight than humic acids (300-500Da) [6][8].

Low Molecular Weight Neutral and Acids constituents within fraction alcohols, aldehydes, ketones, sugars (only for LMWN) and amino acids with a molecular weight <350Da. Unlike LMWA, LMWN has a low charge density, and this is the main characteristic for separation by chromatography [8].

3.2. CHARACTERIZATION OF DOM

The conventional DOM characterization techniques include total organic carbon (TOC) or UV absorption spectroscopy. However, both have limitations, as TOC only indicates the concentration of DOM but not its nature, and UV absorption spectroscopy allows the determination of the aromaticity of the substances.

Instead, the fluorescent properties of DOM allow them to be analyzed by fluorescence spectroscopy. This technique allows determining the concentration and nature of DOM, through excitation and emission fluorescent (EEM: excitation-emission matrices) and with Parallel Factor Analysis (PARAFAC).

Other techniques include HPSEC, this technique is used to separate the different fractions based on their molecular weights [8].

3.2.1. EEM fluorescence

The excitation-emission matrix fluorescence (EEMF) is a powerful tool to characterize DOM. In EEMF a series of emission spectra are obtained for a range of excitation wavelengths. The information collected can be represented as a 2D-contour map or 3D-graph.

Fluorescence is used for determining the DOM components in water because they have different excitation and emission wavelengths: humic substances have higher maximum emission than protein ones. These differences arise from the different fluorophore groups of each component.

The fluorescence of humic substances is mainly due to quinone groups. Protein-like fluorescence can be attributed to three amino acids: tryptophan, tyrosine, and phenylalanine. However, fluorescence is mainly attributed to tryptophan, which absorbs the longest wavelength and displays the largest absorption [9].

The following table indicates the characteristic excitation and emission wavelengths for the different DOM fraction [10]:

Fraction of DOM	Types of components	Excitation	Emission
Humic	**Humic-like (peak C)**	320-360	420-460
Humic	**Fulvic-like (peak A)**	230-260	400-480
Protein	**Tryptophan-like (peak T)**	275-285 / 215-237	320-380
Protein	**Tyrosine-like (peak B_2)**	275-310 /220-237	305-320

Table 1. Excitation/Emission wavelengths of the main fluorophores

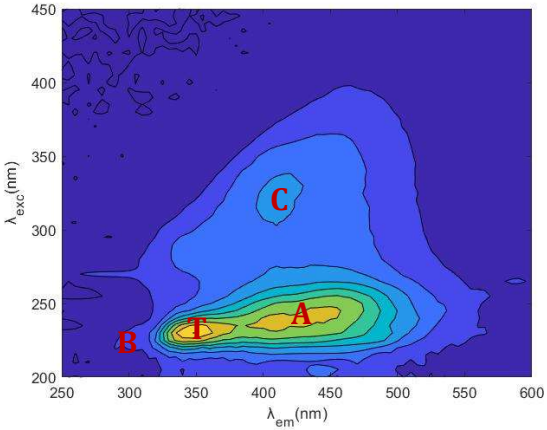


Figure 2. Location of the main EEMF peaks for groundwater

3.2.1.1 PARAFAC

PARAFAC is a multi-way decomposition chemometric method. The decomposition of the data is made into trials where each component consists of one score vector and two loadings vectors [11].

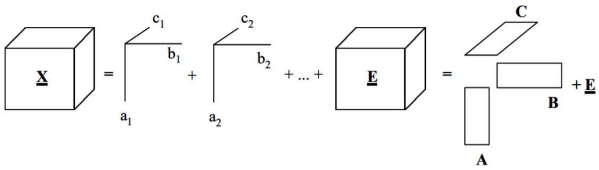


Figure 3. PARAFAC decomposition of a data array X (3D structure) in two-components [12]

From three-dimensional data matrices (EEM data: excitation and emission wavelengths excitation, and fluorescence intensity) can be obtained a model of a three-way array which is found by an iteration process, in order to minimize the sum of squares of the residuals, $\boldsymbol{e_{ijk}}$ in the model [11]:

$$\boldsymbol{x_{ijk}} = \sum_{\boldsymbol{f=1}}^{\boldsymbol{F}} \boldsymbol{a_{ij} b_{jf} c_{kf}} + \boldsymbol{e_{ijk}}$$

Another way to express the model is:

$$\underline{\boldsymbol{X}} = \sum_{\boldsymbol{f=1}}^{\boldsymbol{F}} \boldsymbol{a_f} \otimes \boldsymbol{b_f} \otimes \boldsymbol{c_f}$$

Where X is the three-dimentional (IxJxK) data matrix and $\boldsymbol{a_f}, \boldsymbol{b_f}, \boldsymbol{c_f}$ are the columns *f* of the loadings matrices A, B, and C, respectively.

When the experimental data contain several EEM spectra (three-dimensional data), the information obtained from the mathematical treatment decomposition consist of the relative concentration (SCORES) for each fluorescent compound in each sample, and the normalized emission and excitation (two LOADINGS) spectra of the components of the samples [11]. The fluorescence characteristics of the components (loadings) can be compared with those of corresponding to the fluorescent fractions of DOM (see ***Table 1***), allowing the identification of the different fluorophores of the samples.

3.2.1.2 EEMscat

The representation of EEM experimental data (***Figure 2, A***) shows intense diagonal bands that correspond to the first-order scattering of Rayleigh and Raman, and at a lower intensity those of the second order.

First-order Rayleigh scattering corresponds to the region where the emission wavelengths are equal to excitation wavelengths, while second-order appears when emission wavelengths are twice the excitation wavelength.

For Raman scattering, the first and second-order bands are mathematically described with the with the following equations [14]:

$$Raman_{1rt} = -\frac{\lambda_{exc}}{0.00036ex - 1}$$

$$Raman_{2rt} = -\frac{2\lambda_{exc}}{0.00036ex - 1}$$

Where ex in the incident excitation wavelength.

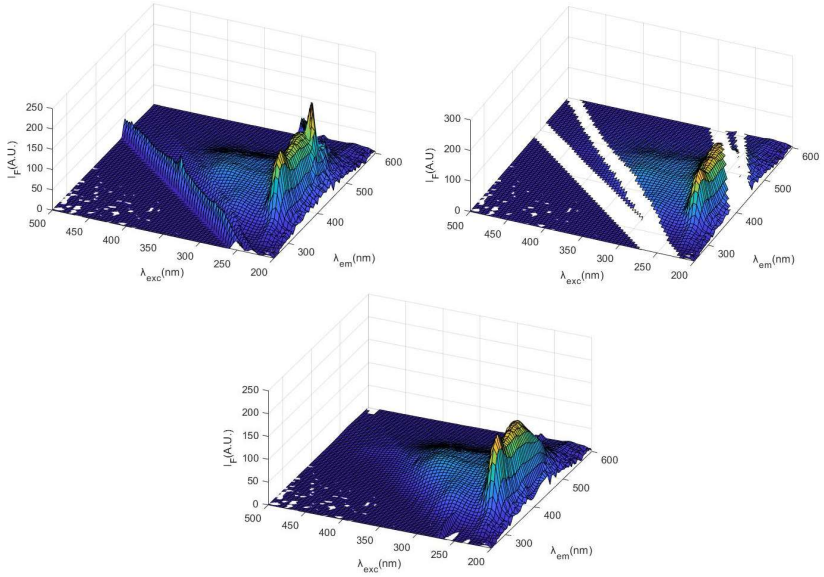


Figure 4. Groundwater sample before (A) and after (B) scattering removal. The contour map C represents interpolated values also after the scattering.

The first step in data treatment is to eliminate the scattering peaks, to obtain a correct description of the components present in the samples. The elimination consists of multiplying the data matrix by a 'change' matrix that contains 'NaN' (not a number) values in the dispersion position and *ones* in the other elements, this treatment is done using the ***EEMscat*** function under the MATLAB environment (***Figure 2, B***). In addition, this function presents the option to interpolate the values (***Figure 2, C***).

4. OBJECTIVES

The purpose of this project is to control the presence and elimination of dissolved organic matter (DOM) in groundwater of the Besos River by molecular fluorescence and subsequent a data analysis by PARAFAC. To achieve this main objective, the following aims have been established:

- Evaluate the use of EEM fluorescence as a simple and fast analytical method to monitor DOM and PARAFAC as a characterization tool.
- Study the nature of the DOM of groundwater from its maximum fluorescent excitation and emission.
- Testing of different procedures for DOM removal
- Determine which is the most effective method for each component from data analysis with PARAFAC.

5. MATERIALS AND METHODS

5.1. WATER TREATMENTS

This project is a collaboration between Universitat Politècnica de Catalunya (UPC) and Universitat de Barcelona (UB) to study the effect of different treatments to remove the dissolved organic matter (DOM) and determinate what is the most effective. The samples were filtered through nylon 0.45μm membrane before fluorescence measures. The treatments are:

- Treatments with activated carbon (1000 mg L^{-1}), and at different concentrations of sodium hypochlorite (0, 4 and 8 ppm)
- Treatments with zeolite (20g/l) at different pH ranges (5, 6, 7, 8, 9)
- Treatments with zeolite (20g/l) at different concentrations of sodium hypochlorite (0, 1, 2, 4, 6, 8, 18ppm)

Description of materials:

Activated Carbon: It is a carbonaceous material with high internal porosity. Due to its porosity, it can have surface area values of up to 2500m^2 g^{-1}, which gives it the characteristic of a highly absorbent material. This material purifies water by absorbing dissolved organic compounds.

Zeolite: Microporous mineral of volcanic origin with negative charge that allows the retention of heavy metals. Some physical or chemical treatment is applied to natural zeolite to activate it and improve its absorbent properties. Again, the removal of the DOM is by absorption.

Sodium hypochlorite: It is a strong oxidizing agent and is used for surface purification, odor removal, and water disinfection.

5.2. REAGENTS

Sodium hypochlorite 10%(w/v) technical grade by PanReac AppliChem

Hydrochloric acid 98% of analytical grade by Ricca Chemical

Sodium hydroxide 1M A.R. by SUPELCo 'EMPLURA'

Zeolite supplied by ZN AQUA with a pore size between 0.5-1.0mm

Activated Carbon, GC900 supplied by Chemivall with a surface area of 968.6 m^2g^{-1}

5.3. INSTRUMENTATION

1) pH meter HACH sensION + PH31, equipped with a glass electrode HACH 50 14T

2) Analytical balance Melter Toledo AT261

3) Beko fridge, model Logixx Frostfree

4) Cary Eclipse Fluorescence spectrophotometer using a PC running the Microsoft Windows 7 opening System

5) 1,000cm Hellma QS cuvette art. 101-10-40

5.4. FLUORESCENCE MEASUREMENTS

Fluorescence spectra were acquired using a Cary Eclipse Fluorescence Spectrophotometer with a 1cm Hellma QS cuvette at room temperature. The instrument settings were adjusted as follows:

- Excitation wavelength range: 200–500 nm (step 5 nm, with 5nm slit width)
- Emission wavelength range: 250–600 nm (step 5 nm, with 10nm slit width for groundwater and humic acid, 5nm for tyrosine and 2.5nm for tryptophan analysis)
- Scan rate: 3000 nm min^{-1}.

The spectrofluorometer was auto-zeroed before each analysis.

5.5. DATA TREATMENT

The mathematical treatment has been carried out using MATLAB 2020b (Marthworks, Inc., Natick, MA, U.S.A.) to generate a contour map after adjusting zero values.

First and second order Rayleigh and Raman scattering were eliminated with the ***EEMscat function*** (*Version* 3, 2013, Quality and Technology) using MATLAB. The data treatment with PARAFAC (Version 3.30, Quality and Technology) has been carried out over the EEMF matrices in which the first and second have been removed, even though the graphs are of the interpolated values.

6. RESULTS

6.1. TREATMENT WITH ACTIVATED CARBON

The samples treated were groundwater (G) for the Besos River with a TOC of 2ppm, humic acid (H), tryptophan (R) and tyrosine (Y); the last three samples in a concentration equivalent to 2ppm of TOC. The treatments consist in the application of different concentrations of sodium hypochlorite (4ppm and 8ppm), and later each sample was passed through in activated carbon. The assignment of the samples is compiled in the following table.

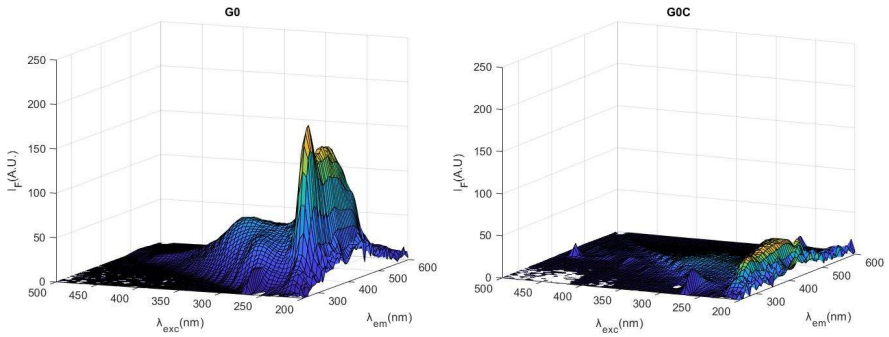
Table 2. Assignments of the samples

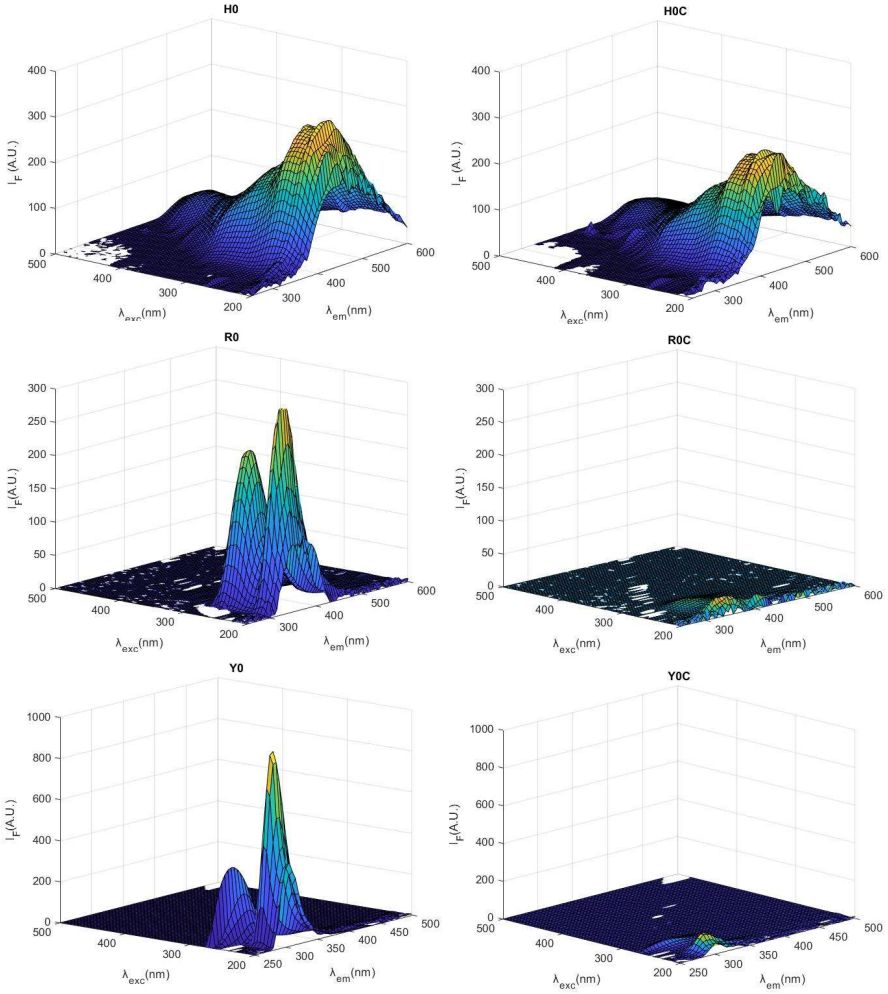
Activated carbon (mg/l)	NaClO (mg l^{-1})	Groundwater	Humic acid	Tryptophan	Tyrosine
0	0	G0	H0	R0	Y0
	4	G1	H1	R1	Y1
	8	G2	H2	R2	Y2
1000	0	G0-C	H0-C	R0-C	Y0-C
	4	G1-C	H1-C	R1-C	Y1-C
	8	G2-C	H2-C	R2-C	Y2-C

*The number after the letter means the concentration of sodium hypochlorite i.e. G-0 without NaClO, G-1 with 4ppm NaClO and G-2 with 8ppm

Figure 5 shows the EEM interpolated spectra without dispersion of different samples before (left) and after the activated carbon treatment (right).

Figure 6. Spectrum EMM of the samples treated with (right) and without (left) activated carbon.





According to the spectra, it can be determined that, in the first instance, the removal of the DOM with activated carbon is more effective in the protein-like samples (tryptophan (R) and tyrosine (Y)) followed by the groundwater (G) samples; and it does not cause an apparent change in the intensity of the humic acid samples, that is, the removal of this type of compounds with this treatment is not the most effective.

In the same way, the EMM spectra for the samples treated with a sodium hypochlorite concentration 8mg·l^{-1} have been represented (***Figure 6***) (because at this concentration the removal of the DOM is more effective than at 4mg·l^{-1} , this fact will be discussed later).

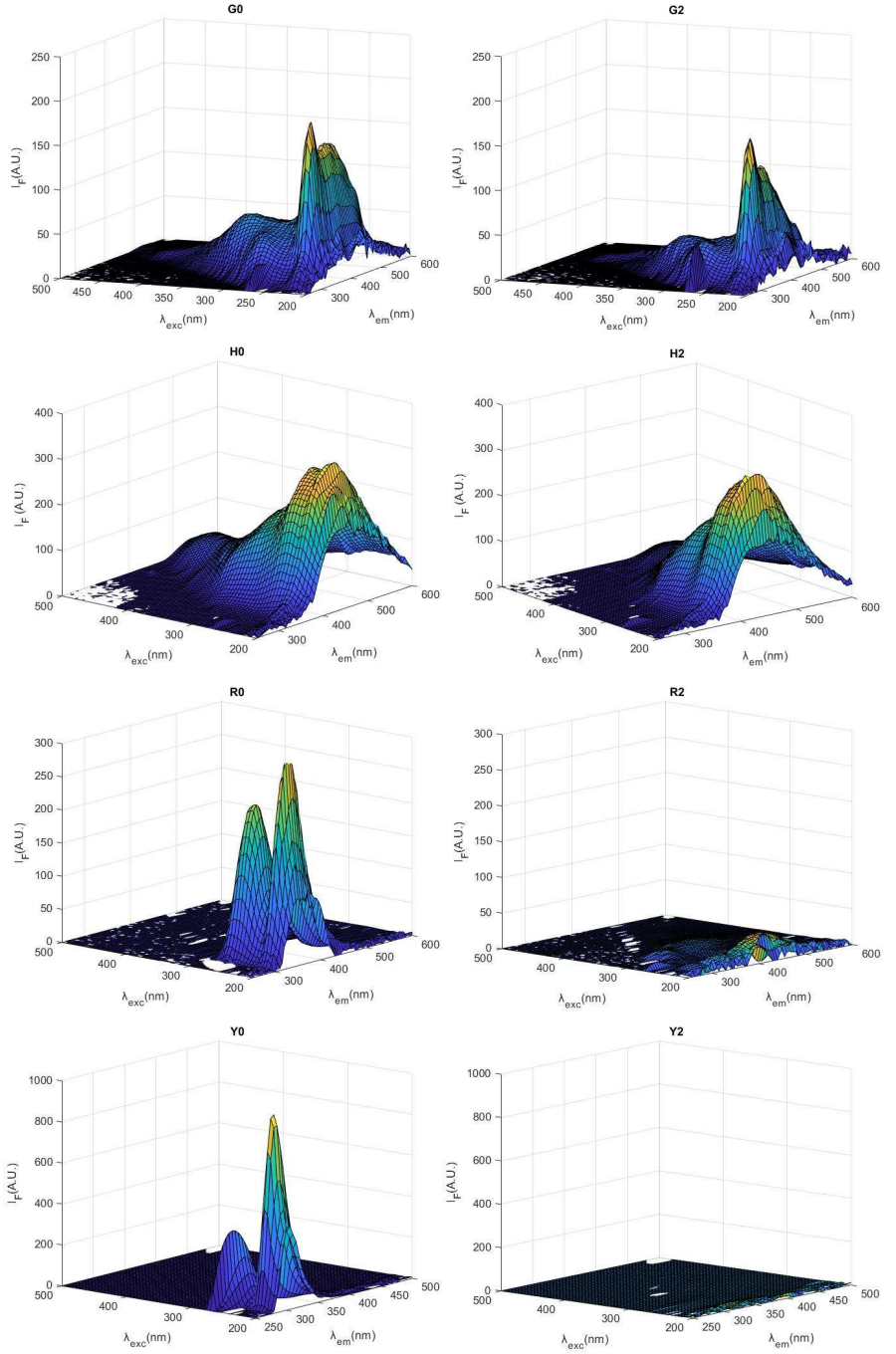


Figure 6. Spectrum EMM of the samples treated with (right) and without (left) NaClO 8mg·l^{-1}.

In this case, the treatment with 8mg·l^{-1} sodium hypochlorite shows an evident removal of the *protein-like* DOM, that is, from the tyrosine and tryptophan samples. In the groundwater and humic acid samples, no significant change in DOM intensity is observed.

The EMM spectra have allowed us to make a first estimate of the DOM removed with two of the applied treatments, but it does not give information on the characteristics of this organic matter.

Therefore, from the PARAFAC analysis, the DOM of each type of sample will be characterized and the intensity of each component after removal will be more accurately determined for each treatment used.

The samples have been characterized from the excitation and emission loadings of the PARAFAC analysis. For example, for the groundwater samples, the following excitation and emission spectra have been represented on the ***Figure 7***.

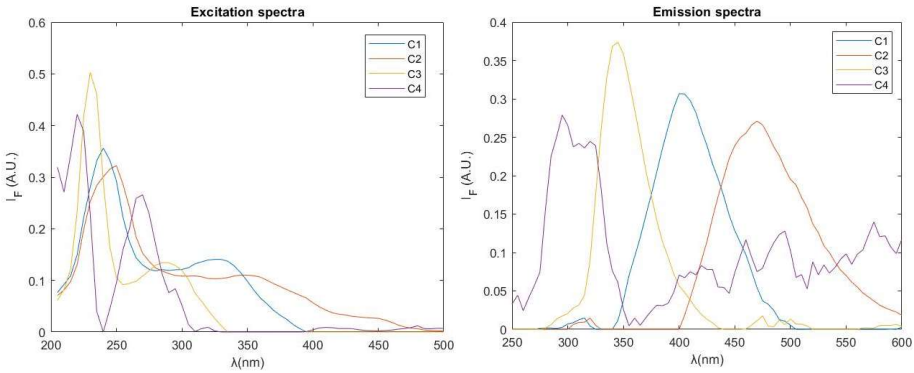


Figure 7. Excitation and emission loadings calculated by PARAFAC for a model of 4PC (variance (σ^2): 99.56%)

The four wavelengths of maximum emission show the presence of 4 components that are represented in the ***Figure 8***. According to their nature, these components are characterized as:

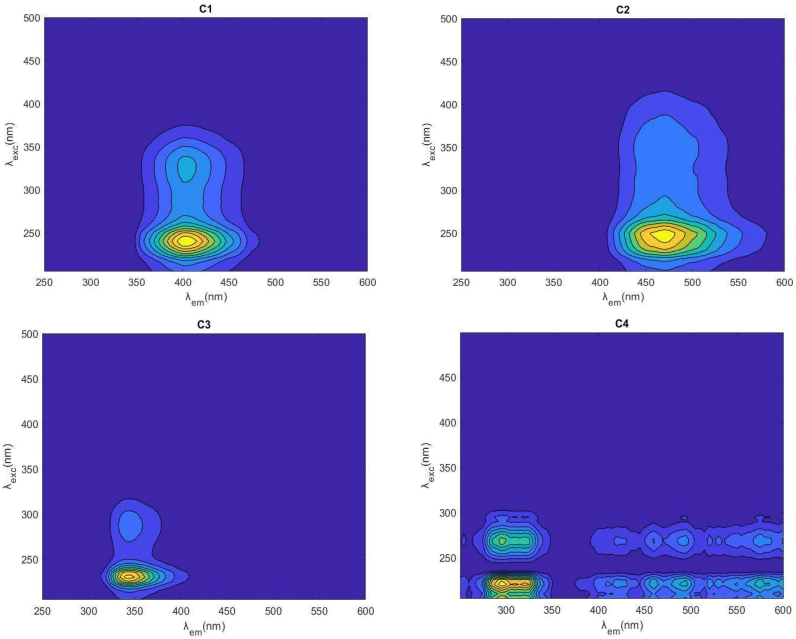


Figure 8. Contour map spectra of the different compounds of groundwater sample.

Compound 1 (C1) presents one maximum of emission at 405nm and two excitation maxima at 240nm and 325 nm. These peaks are assigned to fulvic substances (***peak A***)

Compound 2 (C2) presents one maximum of emission at 470nm and two excitation maxima at 245nm and 350 nm. These peaks are assigned to humic substances (***peak C***)

Compound 3 (C3) presents one maximum of emission at 345nm and two excitation maxima at 230nm and 290 nm. These peaks are associated to substances which have fluorophores like tryptophan. This compound is assigned as protein-like substances (***peak T***).

Compound 4 (C4) presents one maximum of emission at 295nm and two excitation maxima at 220nm and 265 nm. These peaks are associated to substances which have fluorophores like tyrosine. Therefore, this compound is also assigned to protein-like substances (***peak B***). The signal after to 350nm of emission, probably, is due to background noise or due to the presence of another component similar to tyrosine.

In the same way, the data for all types of samples were analyzed. ***Table 3*** shows the data for the maximum excitation and emission wavelengths of the components for each sample and the type of DOM.

Table 3. Excitation and Emission wavelengths of the compounds and characterization of each sample. The model for each sample is: G (4PC, σ^2:99.56%), H (4PC, σ^2:99.92%), R (2PC,σ^2:99.45%)[1], and Y (1PC, σ^2:99.78%).

	C1		C2		C3		C4	
	λ_{exc} (nm)	λ_{em} (nm)	λ_{exc} (nm)	λ_{em} (nm)	λ_{exc} (nm)	λ_{em} (nm)	λ_{exc} (nm)	λ_{em} (nm)
G	240 and 325	405	245 and 350	470	230 and 290	345	220 and 265	295
	Fulvic (peak A)		*Humic (peak C)*		*Tryptophan (peak T)*		*Tyrosine (peak B)*	
H	245 and 325	415	250 and 365	475	215 and 450	530	210 and 315	445
	Fulvic (peak A)		*Humic (peak C)*		*Humic (peak C)*		*Humic (peak C)*	
R	220 and 275	355	240 and 330	445	-	-	-	-
	Tryptophan (peak T)		*Humic (peak C)*					
Y	225 and 275	305	-		-	-	-	-
	Tyrosine (peak B)							

[1]For tryptophan, a 2PC model was chosen so that the explained variance was 99.56%

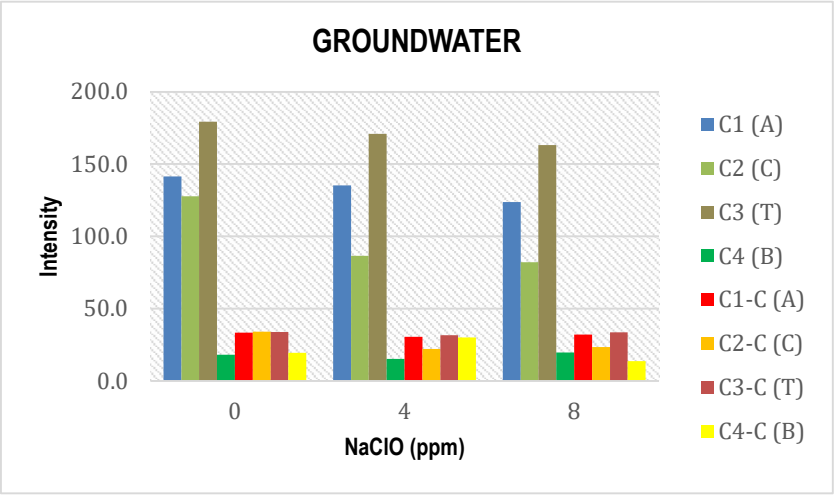


Figure 9. Maximum fluorescence intensity of each component for the groundwater

The maximum intensity of each component for groundwater samples at the different treatments is represent in ***Figure 9.*** The figure show that, peaks **A** and **T** are the highest fluorescence intensity.

Table 4. Maximum intensities for each component of the different samples and the %removal DOM calculated from these values. (***Appendix 1***: %removal for each compound)

		Maximum intensity				
	Treatment	**C1**	**C2**	**C3**	**C4**	**%Removal**
Groundwater	**G0**	141.5	127.6	179.3	18.2	**-**
	G1	135.1	86.5	170.8	15.1	-12.7
	G2	123.6	82.1	163.2	19.7	-16.7
	G0-C	33.3	34.0	33.9	19.5	**-74.1**
	G1-C	30.6	22.0	31.6	30.0	-75.5
	G2-C	32.1	23.5	33.7	13.7	**-77.9**
Humic Acid	**H0**	238.5	278.4	156.2	282.0	-
	H1	249.6	271.7	126.4	286.7	-2.2
	H2	244.3	262.6	118.2	285.2	-4.7
	H0-C	193.5	222.0	141.1	236.0	**-17.0**
	H1-C	228.0	235.6	109.5	257.8	-13.0
	H2-C	210.6	220.5	105.8	251.8	**-17.4**
Tryptophan	**R0**	318.9	4.9	-	-	**-**
	R1	158.5	18.6	-	-	-45.3
	R2	12.7	30.1	-	-	-86.8
	R0-C	23.1	0.8	-	-	**-92.6**
	R1-C	25.2	4.4	-	-	-90.9
	R2-C	4.3	7.4	-	-	**-96.4**
Tyrosine	**Y0**	4200.1	-	-	-	-
	Y1	523.8	-	-	-	-87.5
	Y2	62.3	-	-	-	-98.5
	Y0-C	424.8	-	-	-	**-89.9**
	Y1-C	76.6	-	-	-	-98.2
	Y2-C	41.7	-	-	-	**-99.0**

As observed in the EEM spectra, ***Figure 9*** indicates that the treatment with activated carbon (AC) and AC+NaClO 8mg·l^{-1} had the highest effect on the DOM; for groundwater samples, approximately a 74% and 78%removal (***Table 4***), respectively.

Furthermore, ***Table 4*** shows that the removal of *humic-like* compounds in the groundwater samples *(**peak A** (C1) and **C** (C2))* have a higher removal than in humic acid samples. Removal is probably more effective in groundwater samples because DOM components found in groundwater react more easily or by the presence of metals in these samples that act as catalysts.

On the other hand, the compound C4 of the groundwater samples (tyrosine-like-***peak B***) shows a slight increase in intensity in the treatment with activated carbon (Sample: **G0-C**) but does not occur in the tyrosine samples (Sample: **Y0-C**). Once more, this fact corroborates that the characterization of the DOM does not imply that the components present the same chemical behavior than substances with their similar fluorophores.

Tryptophan samples: ***Table 3*** show that this samples have an impurity because the second component is *humic-like*. And according to the intensities variation (***Table 4***), the concentration of this component slightly increases with increasing concentration of sodium hypochlorite (Noticed that the concentration of this component in the samples without treatment is minor, but when treating it with NaClO 8mg·l^{-1} it is approximately 2.5 times more than C1 under the same conditions). This impurity may correspond to a by-product of the redox reaction with NaClO.

Tyrosine samples: it is observed that the removal is effective in each treatment. The results show that these types of samples are more susceptible to sodium hypochlorite attack than tryptophan samples.

Humic acid samples, none of the treatments used seems to have a relevant effect on the elimination of DOM, compared to the removal percentages of the other samples.

6.2. TREATMENT WITH ZEOLITE AT DIFFERENT CONDITIONS

Zeolite is a material with negative change used in water treatments to retain metals. For this reason, the objective of this part is to determine if it also allows a good removal of the DOM by absorption.

In this part, the effect of various treatments with zeolite under different conditions will be studied. The samples treated were stirred for approximately 20 hours, the effect of the stirring time did not show a relevant change in the removal DOM with zeolite.

The sample is groundwater from the Besos River. The data shown in this part has been analyzed in the same way as for the *Treatments with activated carbon.* For this reason, the effect of each treatment on the removal of the DOM will be presented directly.

6.2.1. Treatments with zeolite at different pH

The groundwater samples were treated with zeolite (20g/l) and then was adjusted the pH to different values (pH used were 5, 6, 7 and 8; after stirring the samples for 20 hours the pH values had changed slightly).

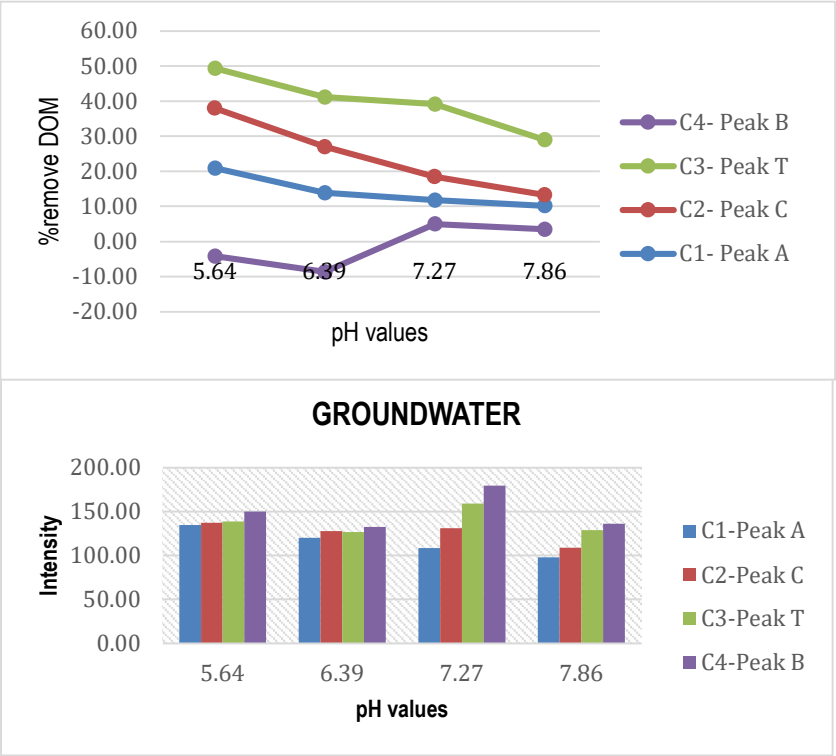


Figure 10. a) Percentages of DOM removal determined from the maximum emission intensities of each component. **b)** Maximum of intensity for each sample determined at their maximums of emissions and excitation wavelength. The wavelengths (λ_{exc}/ λ_{em}) are: C1 *240/405*, C2 *250/465*, C3 *290/345* and C4 *225/330.*

For a PARAFAC model of 4PC (σ^2:99.72%) was calculated the variation of maximum fluorescence intensities (***Figure 10, a***) on a basis of 100% for the groundwater samples treated at the same pH values.

Figure 10, a) indicates that at slightly more acidic pH values the remove is better. This occurs because zeolite at these pH values experiments a slight increase in pore diameter that will allow it to absorb the DOM more efficiently [13]. However, it should be noted that this elimination is effective for humic fractions (C1 and C2) and for tryptophan-like compounds (C3).

Figure 10, b) shows the intensity of each of these components. It is noted that, despite working with slightly acidic pH, differences compared to water pH (pH≈8) are not relevant, so making a pH adjustment would not be the most appropriate treatment to activate the zeolite.

6.2.2. Treatments with zeolite at different concentrations of sodium hypochlorite

The groundwater samples were treated with different concentration of NaClO (1, 2, 4, 6, 8, 18mg·l^{-1}) and with zeolite (20g/l).

Table 5 shows the intensities and the %removal DOM determined from the intensities of the 4 components (4PC model, σ^2:99.36%) of the different samples (on a basis of 100% for the groundwater with zeolite (20g/l))

Table 5. *Intensity variation after the treatments for the phreatic water (4PC, 99.36%) and the %removal calculated from these values. The wavelengths (λ_{exc}/ λ_{em}) are:* ***C1*** *240/405,* ***C2*** *235/345,* ***C3*** *250/470 and* ***C4*** *225/325.*

NaClO (mg/l)	C1 *(Fulvic-like)*	C2 *(Protein-like)*	C3 *(Humic-like)*	C4 *(Protein-like)*	%Removal
0	136.91	131.70	123.94	130.24	-
1	107.99	135.98	62.83	111.69	-20.0
2	72.69	120.37	45.04	112.15	-33.0
4	50.25	142.08	32.05	120.68	-34.0
6	45.11	124.67	29.52	111.70	-40.5
8	43.42	107.00	28.79	93.53	-47.8
18	37.23	85.58	22.66	59.83	-60.7

The results show that as the concentration of NaClO increases, the removal percentage of the total DOM also increases. For the humic fractions, this trend coincides; however, for the protein fractions it does not. This trend is not coincided for the protein fractions, probably, there is some type of interaction with the zeolite, generating by-products that cause an incorrect description of the model.

In addition, it is observed that the %removal does not change significantly when going from a treatment with NaClO 8mg·l^{-1} (48% removal) to 18mg·l^{-1} (61% removal), we determined that NaClO 8mg·l^{-1} is the most appropriate concentration in this treatment.

The zeolite +NaClO treatment has a lower removal percentage compared to the activated carbon+ NaClO.

10. CONCLUSIONS

In this study the EEM fluorescence has been used to evaluate the presence and elimination of dissolved organic matter during the different treatments in groundwater of the Besos River; and the use of PARAFAC as method to characterize the DOM.

The groundwater of Besos River is characterized of protein substances similar to tryptophan (*peak T*) and tyrosine (*peak B)*; and humic and fulvic substances *(peak C* and *A,* respectively*).* Peaks A and T showing the highest fluorescence intensity.

According to the results, activated carbon removes dissolved organic matter in a higher proportion than zeolite. Of all the treatments tested, treatments with activated carbon (AC) and AC+NaClO 8mg·l^{-1} present the highest DOM removal, 74% and 78%, respectively.

The percentages of total removal for the treatment AC for each compound were in the following order: peak T (-81%)> peak A (-76%)> peak C (-73%)> peak B (+7.50%). On the other hand, the treatment with AC+NaClO 8mg·l^{-1} the order was: peak C (-82%)≈ peak T (-81%)> peak A (-77%)> peak B (-25%). These percentages indicate that the removal is higher for the main compounds.

Finally, we can conclude that classify the components of a sample according to their main fluorophores similar to those of humic, fulvic, tryptophan or tyrosine; It does not mean that they present the same chemical behavior.

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11. ACRONYMS

NOM: Natural Organic Matter
BDP: Desinfection By-Products
POM: Particulate Organic Matter
DOM: Dissolved Organic Matter
LMWA: Low Molecular-Weight Acids
LMWN: Low Molecular-Weight Neutrals
TOC: Total Organic Carbon
EEMF: Emission-Excitation Matrix Fluorescence
PARAFAC: Parallel Factors Analysis
UV: Ultraviolet

APPENDICES

APPENDIX 1: VARIATION OF THE MAXIMUM INTENSITY FOR THE TREATMENT WITH AC

	Sample	C1 (%)	C2 (%)	C3 (%)	C4 (%)
GROUNDWATER	**G0-C**	-76.43	-73.37	-81.10	7.50
	G1	-4.46	-32.22	-4.73	-16.66
	G2	-12.62	-35.63	-8.97	8.57
	G1-C	-78.38	-82.72	-82.36	65.01
	G2-C	-77.30	-81.58	-81.23	-24.66
HUMIC ACID	**H0-C**	-18.88	-20.28	-9.63	-16.30
	H1	4.65	-2.41	-19.07	1.68
	H2	2.45	-5.70	-24.33	1.16
	H1-C	-4.40	-15.38	-29.88	-8.58
	H2-C	-11.68	-20.80	-32.25	-10.70
TRYPTOPHAN	**R0-C**	-92.76	-83.75	-	-
	R1	-50.29	280.97	-	-
	R2	-96.01	514.24	-	-
	R1-C	-92.10	-9.85	-	-
	R2-C	-98.64	51.33	-	-
TYROSINE	**Y0-C**	-89.89	-	-	-
	Y1	-87.53	-	-	-
	Y2	-98.52	-	-	-
	Y1-C	-98.18	-	-	-
	Y2-C	-99.01	-	-	-