Tutor
Dr. José Luis Beltran Abadía
Departament de Química Analítica

Grau de Química

Treball Final de Grau

Characterization of dissolved organic matter in water by using spectrometric and chromatographic techniques.

Caracterització de la matèria orgànica dissolta en aigua mitjançant tècniques espectromètriques i cromatogràfiques.

Albert Pons Cejalbo

Juny de 2016

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

"Education is the most powerful weapon which you can use to change the world"

Nelson Mandela

En primer lloc vull agrair al meu tutor, el Dr. José Luis Beltran, tot el temps que m'ha dedicat i tot el que m'ha ensenyat, a més d'estar disponible sempre que l'he necessitat.

Vull agrair també a l'Eva tota l'ajuda i la seva companyia durant tots aquests mesos al laboratori.

Finalment, agrair també a tots els familiars i amics que han estat al meu costat i m'han fet créixer com a persona durant aquests 4 anys.

A tots vosaltres moltes gràcies.

REPORT

CONTENTS

1. SUMMARY

Water as an essential resource to life, requires the best sanitary quality possible for human consumption. Dissolved organic matter (DOM) is present in surface water and groundwater; due to their organoleptic characteristics and the possibility of forming by-products in various stages of purification, it must be eliminated in treatment plants.

There are different processes for removing the DOM, which affected its effectiveness due to the concentration and characteristics of the DOM. Therefore, to optimize the water purification processes is very important to know these parameters in the different stages.

For this reason, in this project the different components of the DOM (such as humic and fulvic fraction, or protein fraction) have been characterized at different stages of the purification process. The samples come from the treatment plant to the river Llobregat located in Sant Joan Despí.

The characterization was carried out by using excitation-emission fluorescence (EEM) and size exclusion liquid chromatography (HPLC-SEC) with fluorescence detection, which allowed us to obtain information about the nature of the fractions and their molecular weight.

Keywords: Dissolved Organic Matter (DOM), water treatment, Excitation-emission molecular fluorescence (EEM), Size exclusion liquid chromatography (HPSEC)

2. RESUM

L'aigua com a recurs imprescindible per a la vida requereix de la millor qualitat sanitària possible per al consum humà. La matèria orgànica dissolta (DOM), es troba present tant en aigües superficials com subterrànies. A causa de les seves característiques organolèptiques i a la possibilitat de formació de subproductes de desinfecció en les diferents etapes de purificació, ha de ser eliminada en les plantes de potabilització.

Hi ha diferents processos d'eliminació de la DOM, que veuen afectada la seva eficàcia a causa de la concentració i característiques de la DOM. Per tant, a l'hora d'optimitzar els processos de purificació de l'aigua, és de gran importància conèixer aquests paràmetres en les seves diferents etapes.

Per aquest motiu, en aquest projecte s'han caracteritzat les diferents fraccions de la DOM (tals com la fracció d'àcids húmics i fúlvics, o la fracció de proteïna) en les diferents etapes del procés de purificació. Les mostres analitzades provenen de la planta de tractament d'aigües del riu Llobregat localitzada a Sant Joan Despí.

La caracterització s'ha realitzat mitjançant les tècniques de fluorescència molecular d'excitació i emissió (EEM) i la cromatografia líquida d'exclusió (HPLC-SEC) amb detecció fluorimètrica, que ens han permès obtenir informació sobre la naturalesa de les fraccions així com del seu pes molecular.

Paraules clau: Matèria Orgànica dissolta (MOD), Tractament d'aigües, Fluorescència molecular d'excitació i emissió (EEM), Cromatografia líquida d'exclusió (HPSEC).

3. INTRODUCTION

The water is an indispensable element for life. This natural resource, increasingly scare, has an irregular distribution in our world and the water accessibility changes due to the human activity. For this reason, it is necessary use water treatment plants to remove all the harmful substances. Most of water for human consumption comes from surface water.

Natural organic matter (NOM) is a heterogeneous mixture of complex organic matter, including humic substances, amino acids, lipids, amino sugars, proteins, and polysaccharides. NOM in waters is derived from plants or terrestrial (allochtonous) and algal (autochthonous) sources and it is captured in contact with the soil during the tour river.

The understanding of NOM is crucial because this one affect the health of natural systems through light attenuation, nutrient availability, and toxic contaminant transport. Furthermore, due to water quality problems and stricter regulations for drinking water quality, more efficient water purification methods are needed. Thus, the requirements for more efficient removal of NOM necessitate more knowledge the organic matter present. [1]

According the size, these substances can be classified in two types: particulate organic matter (> 0,45μm) and dissolved organic matter (DOM, below 0,45μm). In this research, dissolved organic matter has been studied.

3.1. DISSOLVED ORGANIC MATTER

In the aquatic environment, DOM has a significant effect on the biogeochemical processes, particle stability, transport and metal complexation in the aquatic systems. Therefore, it is often utilized as a control parameter in design of the water treatment process as well as others such as water treatment efficiency and the formation of disinfection by-products (DPBs). These substances are oxidized very slowly and their solubility in water may vary with pH.

There are two different types of DOM:

- Humic substances
  - Humic acids
  - Fulvic acids
  - Humin
- Non-humic substances

3.1.1. Humic Substances (HS)

Humic substances are very important components of soil that affect physical and chemical properties and improve soil fertility. In aqueous systems, like rivers, about 50% of the dissolved organic materials are HS that affect pH and alkalinity. HS affect biological productivity in aquatic ecosystems, as well as the formation of disinfection by-products during water treatment. [2]

Humic substances are complex and heterogeneous mixtures of polydispersed materials formed by biochemical and chemical reactions during the decay and transformation of plant and microbial remains (a process called humification). Efforts to characterize humic substances with respect to structure and functionality often rely upon sophisticated methods that are applicable to pure substances, but whose applicability to complex mixtures such as humic substances is less certain. [3][4]

Humic substances can be divided into three main fractions in function of pH: humic acids (HA or HAs), fulvic acids (FA or FAs) and humin.

3.1.1.1. Humic acids:

Humic acids (HAs) are formed for a mixture of weak aliphatic and aromatic organic acids which are not soluble in water under acid conditions but are soluble in water under alkaline conditions. Humic acids consist of that fraction of humic substances that are precipitated from aqueous solution when the pH is decreased below 2.

On average 35% of the humic acid molecules are aromatic, while the remaining components are in the form of aliphatic molecules. The molecular size range of HAs is approximately 10,000 to 100,000 Da.

The presence of carboxylate and phenolate groups gives the ability to form complexes with ions such as Mg^{2+}, Ca^{2+}, Fe^{2+}, Al^{3+}, etc. [5]

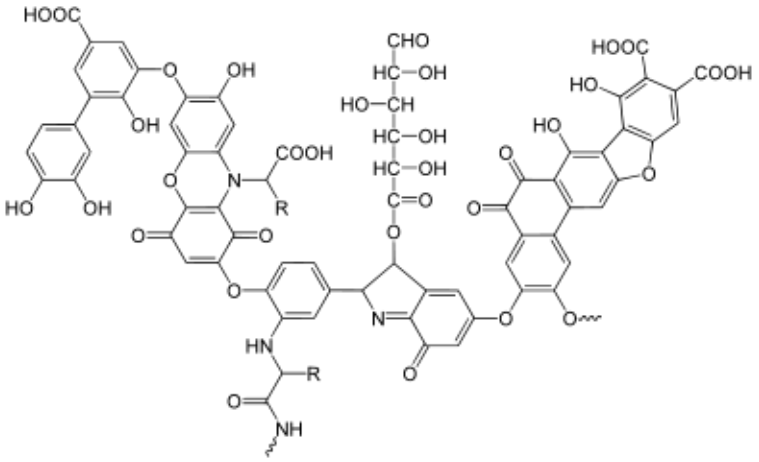

Figure 1. Example of a typical humic acid. [2]

3.1.1.2. Fulvic acids:

Fulvic acids are a mixture of weak aliphatic and aromatic organic acids which are soluble in water at all pH conditions (acidic, neutral and alkaline).

Their composition and shape is quite variable. The size of FAs is smaller than HAs, with molecular weights which range from approximately 1,000 to 10,000 Da.

On the other hand, the oxygen content of FAs is twice that of HAs. They have many carboxyl (-COOH) and hydroxyl (-OH) groups, thus FAs are much more chemically reactive. The ion exchange capacity of FAs is more than double that of HAs, owing to the total number of carboxyl groups present.

Fulvic acids are the most natural effective carbon containing chelating compounds known.[6]

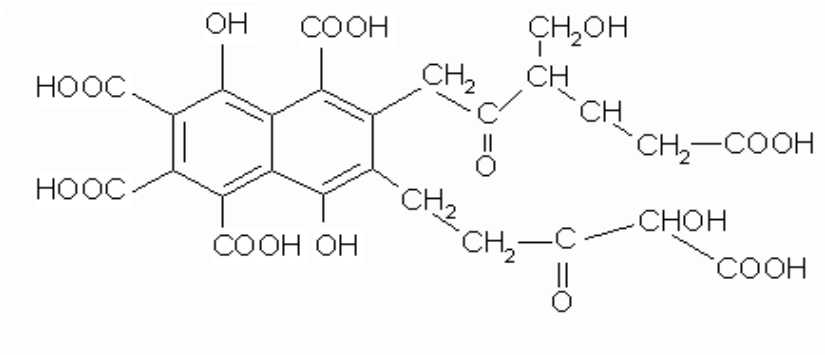

Figure 2. Example of a typical fulvic acid [7]

3.1.1.3 Humin:

The humin portion of humic substances is not soluble regardless of solution pH. Humin has the greatest molecular weight of any humic substance and is extremely resistant to degradation. Because these substances are not soluble, the study of humin is irrelevant.

3.1.2. Non-Humic Substances

The non-humic substances make up the remaining fraction and consist of hydrophilic acids, proteins, amino acids, and carbohydrates. The non-humic fractions have lower aromatic character and molecular sizes lower than HS.

3.2 PROBLEMATIC OF DOM:

The presence of DOM in waters can produce some aspects that are important to control in the water treatment process.

3.2.1. Formation of trihalomethanes:

Trihalomethanes (THMs) are compounds derived from methane, where three hydrogen atoms are replaced by halogens atoms, typically chlorine, bromine or even a combination of both.

THMs are a group of disinfection by-products including chloroform, bromodichloromethane, dibromochloromethane, and bromoform ($CHCl_3$, $CHCl_2Br$, $CHClBr_2$, and $CHBr_3$, respectively).

They are formed when water is disinfected with chlorine and the dissolved chlorine reacts with DOM, as humic acids and inorganic species in the water. [8]

In animal studies, it has been found that chloroform is carcinogenic in high doses and the other THMs are mutagenic. Royal Decree 1400/2003 and European standards of water quality for human consumption set a maximum allowable concentration of THMs of 100µg/l. [9]

For this reason, is very important to decrease the DOM concentration in waters prior the disinfection water process.

3.2.2 Fouling:

To remove unwanted substances, filtration membranes are used. These membranes remove particulate and microbial contaminants.

The principal problem of these membranes is the fouling. Fouling is the accumulation of unwanted material on solid surfaces to detriment of function. It is a major obstacle to the widespread use of this technology.

Membrane fouling can cause severe flux decline and affect the quality of the water produced. Severe fouling may require intense chemical cleaning or membrane replacement. Periodically, the membranes are washed using the backwash technique.

Backwashing is performed with a reversed flow pushed from permeate side to the feed side of a membrane. The reversed flow dislodges the deposits from membrane pores and decreases the fouling on the external side. Backwashing typically requires a working flux at least two times higher than the typical filtration flux. It should be executed carefully to avoid membrane damage. [10]

This increases the operating costs of a treatment plant. Then, knowing and decreasing the concentration of DOM during the water process could improve the fouling.

3.3. CHARACTERIZATION METHODS:

For the optimization of the water treatment process is necessary know the DOM in the different parts of the treatment plant. The samples will be determinate by EEM fluorescence and HP-SEC chromatography.

3.3.1. Fluorescence

Fluorescence is a spectrochemical method in which the analyte molecules are excited by irradiation at a certain wavelength and the emitted radiation is measured at a different wavelength. The main advantage of the fluorescence techniques compared to other methods such as UV-Vis, is the better sensibility and selectivity. The specific wavelengths of excitation and emission are the characteristics of a particular molecular conformation, called the fluorophore. These fluorophores are useful to describe the structural composition of humic materials. [11]

Excitation-emission matrix (EEM) fluorescence spectroscopy is a sensitive and small sample volume analysis method without sample pre-treatment prior to analysis. [12]

EEM has been suggested as a powerful tool to characterize aquatic DOM which could detect the specific fluorescent fractions in organic matter from different sources with much higher sensitivity. In the table 1, are the major fluorescence peaks for water samples.

Range of excitation (nm)	Range of emission (nm)	Component type	References
270–280	310–320	Tryptophan-like, protein like	Coble (1996) and Baghoth et al. (2009)
270–285 (220–235)	340–360	Tryptophan-like, protein like	Coble (1996), Spencer et al. (2007), Baker et al. (2008), Hudson et al. (2008), and Baghoth et al. (2009)
320–350	400–450	Fulvic-like	Spencer et al. (2007) and Baker et al. (2008)
310–320	380–420	Humic-like (marine humic-like)	Coble (1996) and Baghoth et al. (2009)
330–390	420–500	Humic-like	Coble (1996), Spencer et al. (2007), and Baghoth et al. (2009)

Table 1. Major fluorescence peaks for water samples. (Adapted from Matilainen et al, 2011) [13].

Traditional "peak-picking" method for EEM has identified humic-like (A, C) and protein-like fluorescence peaks (T) using simple excitation-emission wavelength pairs of the fluorescence peak locations.

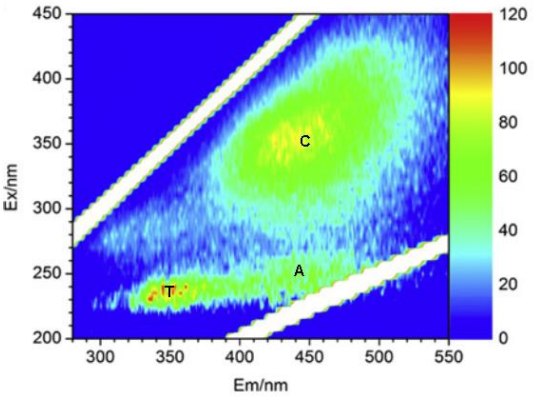

Figure 3. HPLC/HPSEC-FLD with multi-excitation/emission scan for EEM interpretation and dissolved organic matter analysis. [14]

3.3.2. HPLC-SEC with FLD or DAD:

Size exclusion chromatography (SEC) is also known as gel filtration, gel permeation or molecular sieve chromatography. SEC may be used to fractionate and characterize substances according to size. The sieving medium is a porous gel.

The actual speed of movement of each component in a mixture is dependent on the ease with which molecules can pass into the gels and be retarded. When molecules are smaller than the pore diameter will have more probability of penetrating the gel and will pass through the column more slowly. Molecules with diameter much larger than the pore size will have less probability to penetrate the gel particles and will be excluded from the gel and will pass through the column unimpeded. Intermediate size molecules can pass into some of the gel particles but compared to very small molecules, a greater proportion of the intermediate size molecules will be outside the gel at any time. [15]

This method allows separating the different fractions of DOM according the size, and then detecting the fractions using a spectroscopy method like fluorescence detector (FLD) or Diode array detector (DAD).

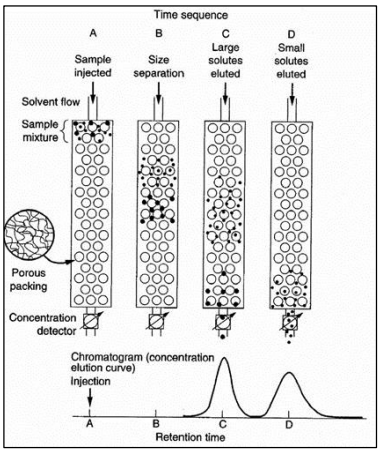

Figure 4. Development and detection of size separation by SEC. [16]

4. OBJECTIVES

The main objectives of this project are:

- Characterization of the several fractions of DOM (e.g., humic-like or protein-like) by using excitation-emission molecular fluorescence (EEM)
- Separation of the different molecular weight fractions by using size exclusion liquid chromatography (HPLC-SEC).
- Evaluation of the DOM's removal effectivity in the different points of the water treatment plant.
- Evaluation of metals effects on the DOM's characterization.

5. EXPERIMENTAL SECTION

This section covers the experimental procedures and instrumental parameters of the techniques needed for the samples characterization, as well as a brief description of the assessed parameters and the used resources in the whole process.

5.1. WATER SAMPLES

All water samples have been taken from different parts of the Drinking Water Treatment Plant (DWTP) at Sant Joan Despí.

5.1.1. Origin

Seven different water samples were analysed:

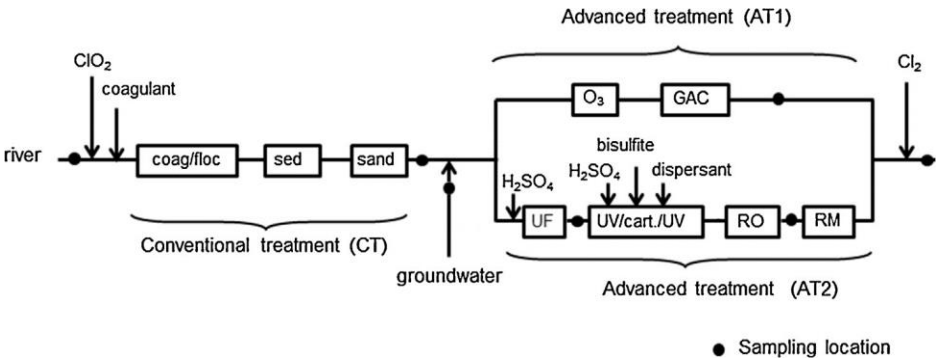

Figure 5. Flowchart of the DWTP of study with sampling locations. [17]

- Crude water: Corresponds to the water samples arriving to the treatment plant.

- Groundwater: The plant incorporates groundwater from its aquifer at the

Sand filter output. This water is previously analysed.

- Sand Filtration: Water obtained after the sand filtration process.

- Activated Carbon filtration (GAC): Water obtained after the activated carbon filtration. A granulated activated carbon is used.

- Ultrafiltration (UF): Water obtained after ultrafiltration process.

- Reverse Osmosis (RO): Water obtained after the reverse osmosis process.

- Output of the plant: Water obtained after all the treatment process.

5.1.2. Pre-treatment

All samples were filtered through 0,45µm nylon membrane filters on site and stored in glass vials at 4ºC prior to measurement.

5.2. REAGENTS AND MATERIAL

- Ammonium acetate (≥98%, Merck)
- Acetonitrile (≥99,9%, Panreac)
- EDTA (Merck)
- Triton x-100 (Merck)
- Brij 35-P (Fluka)
- Bovine serum albumin (≥98%, Sigma Aldrich)
- Humic acid (ash~20%, Sigma Aldrich)
- Tryptophan (≥98%, Sigma Aldrich)
- Tyrosine (≥98%, Sigma Aldrich)
- PEG/PEO calibration kits (Agilent technologies)
- Milli-Q water (Millipore)

5.3. INSTRUMENTS AND EQUIPMENT

5.3.1. EEM Fluorescence

The excitation-emission fluorescence spectra (EEM) for the different water samples were obtained an SLM-Aminco Series 2, model AB2, Luminiscence Spectrometer, equipped with 10 mm quartz cells operating in the radio mode.

EEM analysis were conducted at a scan rate of 30 nm/sec, at 5 nm and 1 nm intervals for excitation (Ex) and emission (Em) wavelengths, respectively. Ex and Em slits were set at 2 nm. The excitation and emission wavelength ranges were from 200nm to 400nm (Ex) and 280nm to 500nm (Em). The voltage photomultiplier was set to 720 V.

5.3.2. Chromatographic conditions

The system used consists of a chromatograph Agilent Series 1100 HPLC (Agilent Technologies) equipped with a degasser (G1322A), quaternary pump (G1311A), automatic injector (G1329A) diode array detector (G1315D) and a fluorescence detector (G1321A). All modules belong to the 1100 series, except the automatic injector and the diode array detector that belong to the 1200 series.

The Agilent ChemStation software (B.04.02 SP1) was used for instrument control and data acquisition.

HPLC-SEC has been the main method used for the DOM separation and characterization. However, in some cases, C18 column was used to expand and compare results.

	SEC	C18
Mobile phase	Ammonium acetate (10mM)	Ammonium acetate (10mM)/Acetonitrile
Elution mode	Isocratic elution	Gradient elution
Flow rate	1mL/min	1mL/min
Volume injection	80µL	10µL
Elution time	15 min	15 min
Detector	FLD	FLD and DAD

Table 2. Instrumental parameters of chromatographic study

5.3.2.1. SEC

The chromatographic separation was carried out with a PL Aquagel-OH Mixed-M SEC column (7,5 x 300mm) with 8µm of particle size and a molecular weight range between 1.000 to 500.000 (g/mol). A precolumn PL Aquagel-OH Guard (50 mm x 7,5 mm, 8 µm) has been used to protect the separation column. The chromatographic method used was previously optimized in the working group. The solution of ammonium acetate (10mM) was used as the mobile phase at a flow rate of 1mL/min in isocratic conditions. Table 2 indicates the chromatographic conditions.

The fluorescence detector was set to excitation/emission wavelengths according with the values obtained from the EEM spectra. They were (as Ex/Em): 230/340, 280/340, 230/430 and 280/430 nm.

5.3.2.2. C18

The chromatographic separation was carried out using a Kinetex C18 column (50 x 4.6 mm) with 2.6µm of particle size and 100Å of pore size. The experimental conditions are indicated in Table 2 and Table 3 (gradient elution).

Time (min)	% Acetonitrile	% Ammonium acetate	Flow rate (mL/min)
0	20	80	1
4	70	30	1
5	90	10	1
10	95	5	1
15	95	5	1

Table 3. Parameters of gradient elution

5.3.3. Molecular weight calibration curve

The calibration curve allows the correlation between the retention time with the molecular weight, when using SEC column. Agilent PEG/PEO calibration Kits were used. These hydrophilic polymers are suitable for both aqueous SEC and organic GPC using the majority of polar organic solvents. Polyethylene oxide (PEO) kits are available in high molecular weights, while the Polyethylene glycol (PEG) kits cover the lower molecular weight range.

Before use, the calibration kits were dissolved with 2mL of milli-Q water and then were shaken gently, for an hour to get a completely dispersion of the polymer solution. May be susceptible standards polymer to degradation the solutions should be stored in a cool, dark place and used within 48 hours.

PEG/PEO calibration kits were detected with SEC column and FLD detector. Chromatograms were recorded at the wavelengths of (Ex/Em) 280/280. The injection volume was 100 µL and the other parameters were the same described before.

6. RESULTS AND DISCUSSION

6.1. FLUORESCENCE STANDARDS

According the literature and the information provided by the water treatment plant, water often contains fluorescence substances. These substances are certain proteins, amino acids (such as Tyr or Trp) and humic substances.

With this information, the BSA, TYR, TRP and humic substances were used as standards.

Tyrosine and tryptophan are amino acids with a MW of 181 and 204Da, respectively. BSA is a protein with a MW of 66.000 Da. Finally, HA (described before) have a MW between 10.000-100.000 Da.

The standards were measured by Excitation-Emission fluorescence spectroscopy. The obtained spectra for the different substances are given in the following figures:

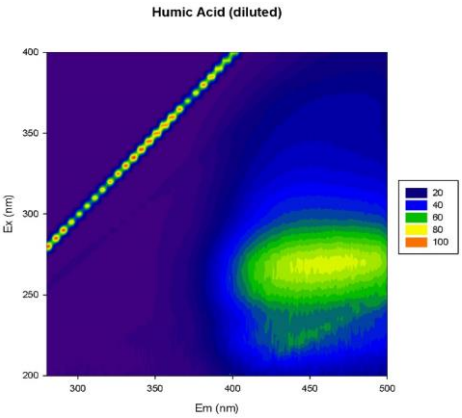

Figure 6. EEM spectrum of HA standard

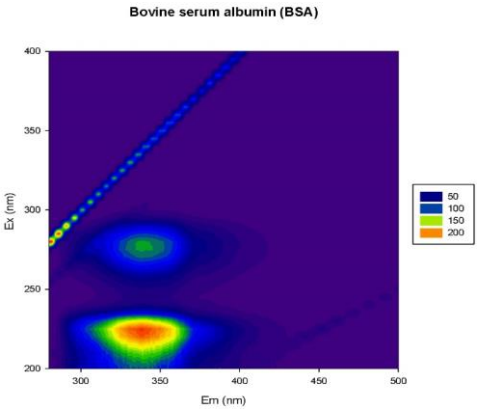

Figure 7. EEM spectrum of BSA standard

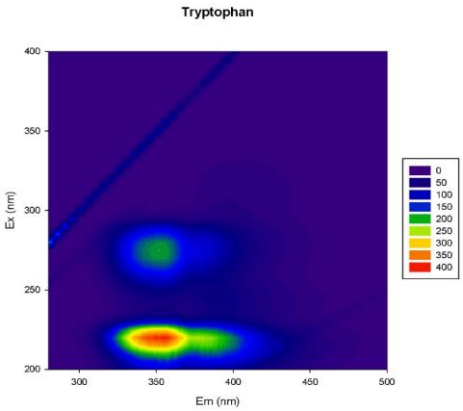

Figure 8. EEM spectrum of Tryptophan standard

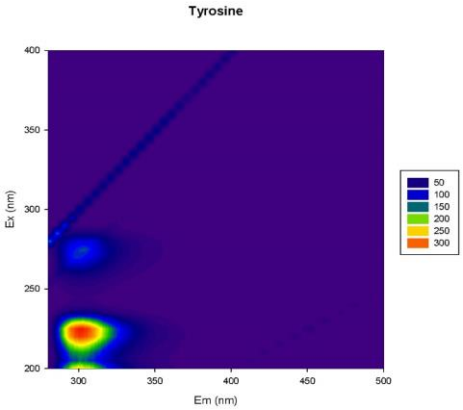

Figure 9. EEM spectrum of Tyrosine standard

In humic acid spectrum (figure 6), one peak was observed at λex= 230nm and λem= 430nm. On the other hand, in BSA spectrum (figure 7), two different peaks were observed. The first one is present at λex=230nm and λem=340nm and the second is present at λex=280nm and λem= 340nm.

Finally, in amino acid spectra (figure 8-9) two peaks were obtained in also both cases. Tryptophan peaks were observed at (Ex/Em) 230/340nm and 280/340nm, and tyrosine peaks were observed at (Ex/Em) 230/300nm and 280/300nm.

This information will allow comparing standards results with the samples studied.

The analyses of the standards were carried out also by HPSEC-FLD, obtaining the following chromatograms.

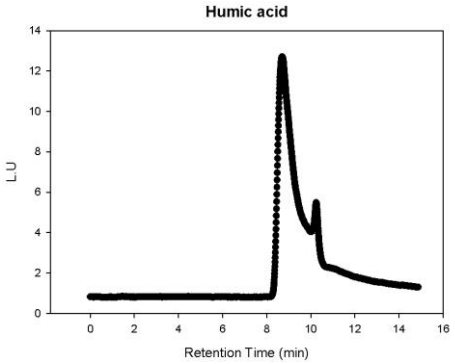

Figure 10. Chromatogram of HA standard

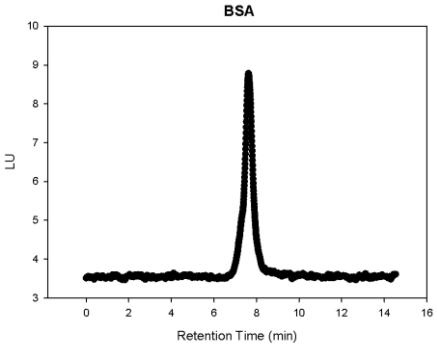

Figure 11. Chromatogram of BSA standard

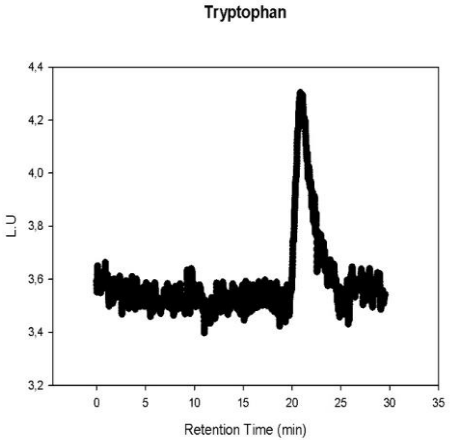

Figure 12. Chromatogram of Tryptophan standard

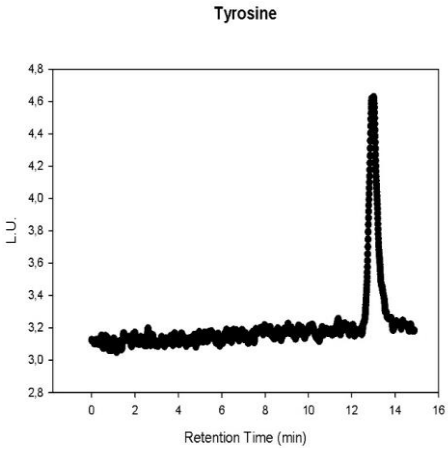

Figure 13. Chromatogram of Tyrosine standard

Each substance provides a single peak in chromatogram, except humic acid that provides two fractions.

In SEC chromatographic columns, the substances with higher molecular weight have lower retention times. Therefore, BSA elutes first because of the biggest size, being the HA's the following.

Looking the two amino acids, theoretically it would have to elute first the tryptophan and subsequently tyrosine. However, tyrosine elutes at 12 min and tryptophan at 23 min. That may be due to molecular interactions between the column and tryptophan, retaining it longer.

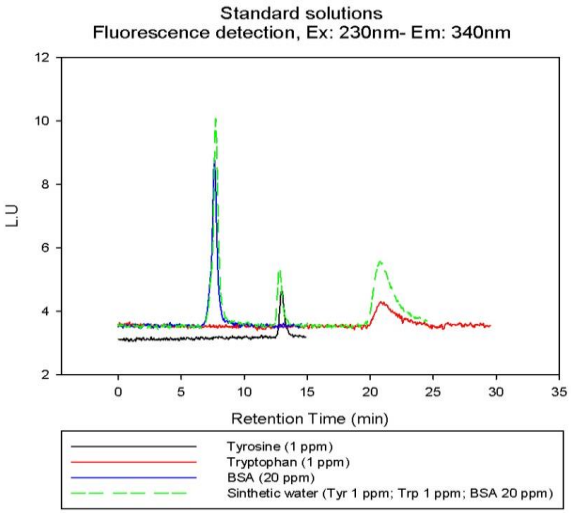

Figure 14. Chromatogram of standards solutions and synthetic water

6.2. EEM FLUORESCENCE

During this work, samples of different days were analyzed in the laboratory. In all of them the results were very similar. So, only a few results will be discussed and showed. The next samples are representative of the treatment water process.

EEM spectra of the differents samples are shown below:

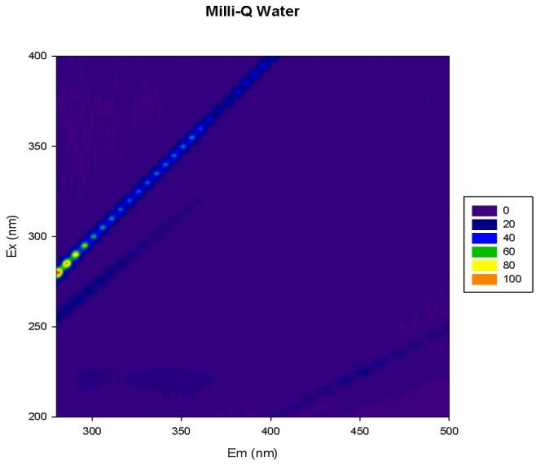

Figure 15. EEM fluorescence spectrum of Milli-Q water

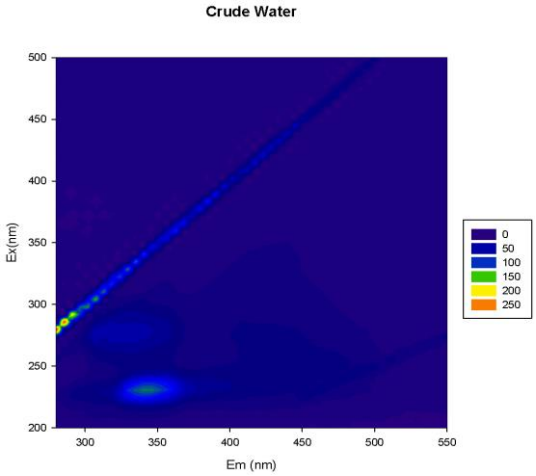

Figure 16. EEM fluorescence spectrum of Crude water

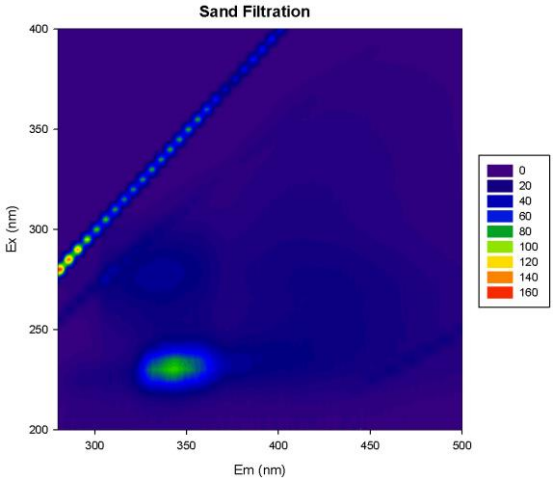

Figure 17. EEM fluorescence spectrum of Sand filtration

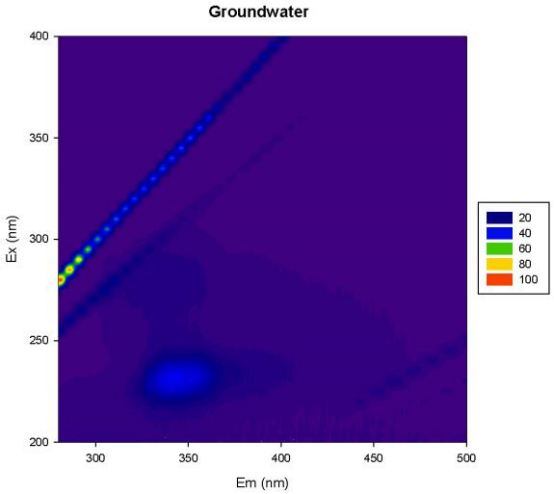

Figure 18. EEM fluorescence spectrum of Groundwater

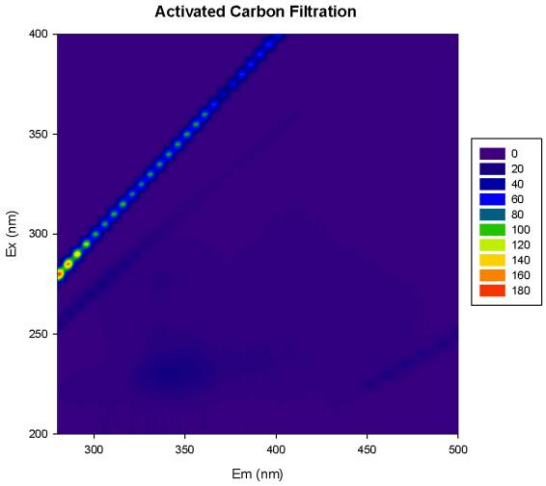

Figure 19. EEM fluorescence spectrum of activated carbon filtration

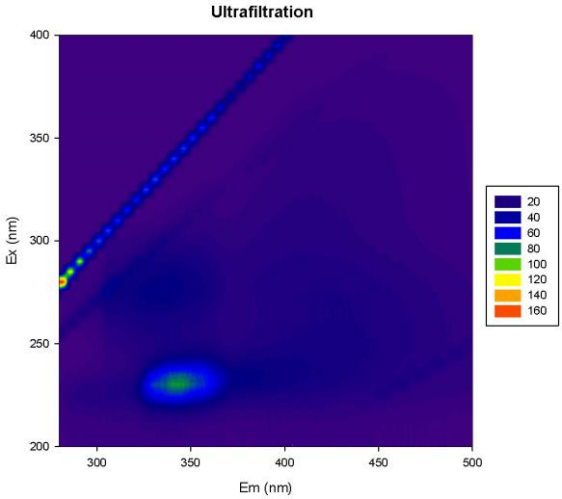

Figure 20. EEM fluorescence spectrum of ultrafiltration

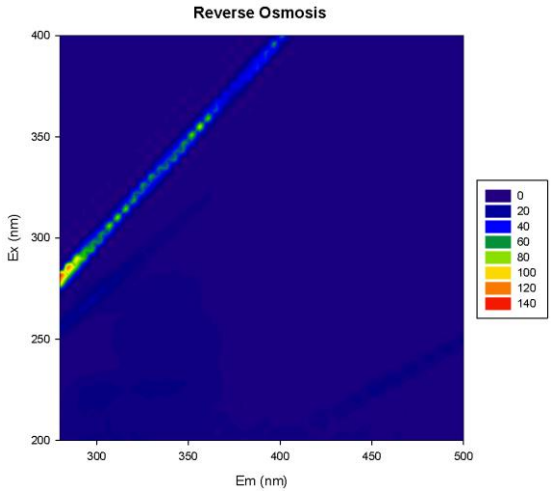

Figure 21. EEM fluorescence spectrum of reverse osmosis

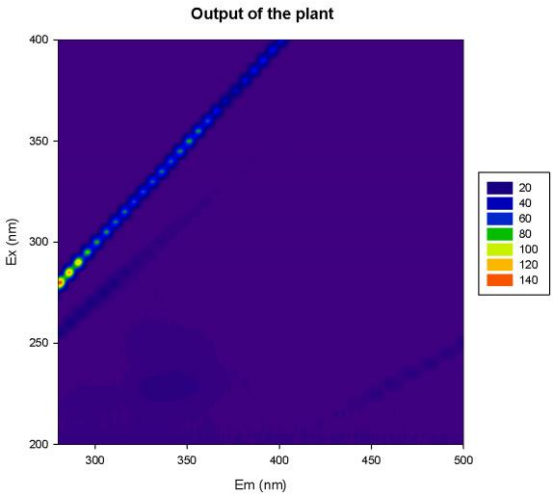

Figure 22. EEM fluorescence spectrum of Output plant

The first 3-D Fluorescence spectrum (Figure 15) corresponds to a sample of Milli-Q water. Pure water has two clear scatter peaks: Rayleigh and Raman. The first is due to direct scattering of the incident light and therefore occurs at the same wavelength as the excitation. The water Raman peak is, however, a result of non-elastic scatter. A fraction of the incident photons lose energy because of the vibration water molecules and the photon emitted at a higher wavelength than the incident light. The energy loss in water has a fixed frequency of approximately 3400 cm^{-1}. The Raman peak has a relatively low intensity and is often over shadowed by the fluorescence of even moderate concentrations of fluorophores that fluoresce at these wavelengths. [18]

The EEM fluorescence spectra (figure 16-22) show the qualitative variety of the organic matter during the water treatment process. In the first case (crude water), two main peaks have been detected (corresponding to two fluorophore groups). The first one is present at ex=230nm and em=340nm and the second is present at ex=280nm and em=340nm.

Two more peaks, with lower intensity, have also been detected. Due to their minor intensity its more difficult to see them on the spectra. The first one is detected at ex=230nm and em= 430nm and the second at ex=280nm and em=430nm. These peaks have been widely identified in previous studies.

Peaks with an emission wavelength of 340nm are associated with the protein-like fluorophores. On the other hand, peaks obtained with an emission wavelength of 430nm are related with the humic-like fluorophores. [14]

In table 4, the characteristics of the EEM fluorescence peaks for the different water samples are indicated.

	λ(Ex/Em) = 230/340 (*)	λ(Ex/Em) = 280/340 (*)	λ(Ex/Em) = 230/430 (**)	λ(Ex/Em) = 280/430 (**)
Crude Water	82.28	30.94	24.97	23.3
Sand Filtration	85.75	30.89	20.41	19.98
Groundwater	35.85	11.14	5.38	3.54
Activated Carbon Filtration	22.45	7.86	6.41	5.06
Ultrafiltration	84.08	30.79	18.58	19.03
Reverse Osmosis	8.01	4.08	1.87	1.34
Plant outlet	11.32	3.74	2.48	1.21

Table 4. Maximum intensity peaks obtained from the different EEM spectra

* Protein-like; ** Humic-like

First of all, it should to differentiate the results taken by the groundwater samples because they come from another part of the treatment plant. Thus, it is necessary to considerate this sample as an independent sample.

The data shown in table 4 indicates a decrease in the peaks intensity throughout the treatment process. The Crude water, gives intensity around 80 luminescence units (LU) in the protein zone and around 30 LU in the humic substances zone. At the end of the process, fluorescence intensity decrease to values between 10 and 2 LU respectively. The reduction of the intensity during the treatment clearly demonstrates the treatment process performance efficiency.

Some of the treatment processes induce a DOM increase in water; this was noted after sand filtration. The effect of sand filtration can be limited by overloaded or defective filtration units which could be one contributing factor for the peak increase. It has been noted that DOM enables the micro-organisms to grow in the treatment unit or distribution system. Thus, exposure to light, bacterial growth and degradation on edges of sedimentation tanks when water has been standing for a long time, may be the cause for the DOM increase.

It should be taken into account that, ultrafiltration and output's plant cases, obtain higher final values due to the water samples. These samples are the result of a mix between two different waters taken from distinct parts of the plant.

When talking about ultrafiltration samples it is the result of groundwater and sand filtration waters. On the other hand, the two different samples mixed are (AT1) and (AT2).

Reverse Osmosis is the process which obtains better elimination of DOM. After this process, DOM is almost non-existent. This fact shows that RO process is the most effective one.

On the other hand, UF is a less effective process to remove DOM. It is unknown if the day when the sampling process was done, the ultrafiltration filters were obstructed, but a continuous and large use of them could be the reason of a remarkable decrease of their functions.

6.3. INCREASE IN DOM'S FLUORESCENCE

As shown in some EEM spectra, some substances are difficult to detect due to the low intensity of the signal. To ensure the detection of all organic matter present in the different samples, it seeks a way to increase the intensity of the peaks.

In the literature, some surfactants have been used to improve fluorescence. For this work two surfactants substances were used, the neutral surfactants Triton x-100 and Brij 35. [19]

These substances form micelles at higher concentrations than critical micelle concentration (c.m.c). DOM, in presence of micelles, penetrates into the apolar zone, reducing quenching effects. In that way shall be achieved the increase of the fluorescence intensity.

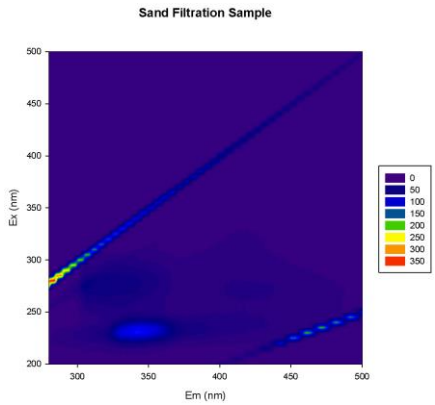

Figure 23. EEM spectrum of Sand Filtration Sample

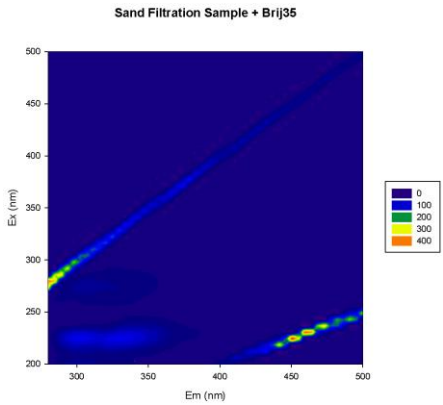

Figure 24. EEM spectrum of Sand Filtration Sample with brij35 addition

However, the results were not favourable, because the increase in the fluorescence emission is mainly due to the fluorescence of surfactants, both for Brij 35 and Triton X-100.

As can be seen from figure 25, the peaks increased before are the same fluorophore groups of the surfactant.

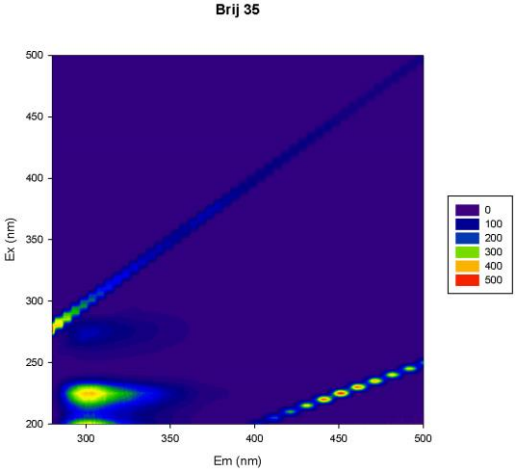

Figure 25. EEM spectrum of Brij-35

Consequently, the increase of the fluorescence peaks has not been achieved using the surfactants.

6.4. HPSEC ANALYSIS

With HPSEC analysis, the compounds present in water samples can be roughly identified to different MW range. Particle size is an important characteristic, affecting their removal in water treatment plants. With the size exclusion column, DOM species with shorter retention times are of higher apparent molecular weight (MW). The column used for the HPSEC-FLD analysis is previously calibrated with known MW standards (PEG/PEO). (See experimental section, page 20)

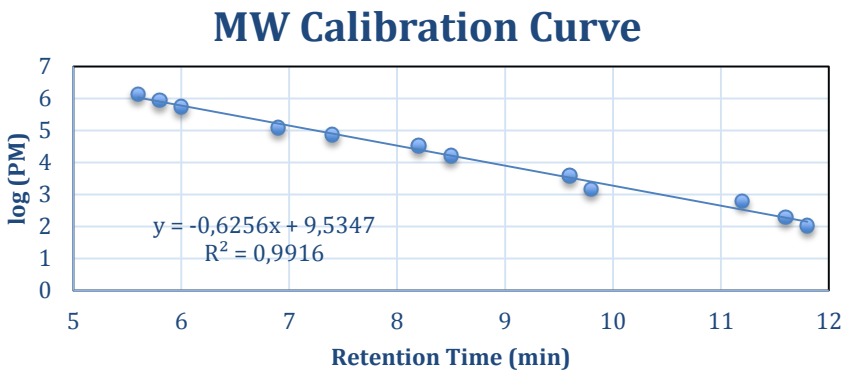

Figure 26. Molecular weight calibration curve (log (MW) vs Retention time)

The calibration curve shown in figure 26 has been used to estimate the MWs of the fractions obtained in the chromatograms of the water samples.

	Retention Time (min)	Log MW	MW (Da)
Fraction 1	8,8	4,029	10.700
Fraction 2	9,15	3,810	6.500
Fraction 3	9,8	3,404	2.500
Fraction 4	10,8	2,778	600

Table 5. Estimated MWs of the different fractions obtained from the molecular weight calibration curve

Crude water chromatograms are shown below:

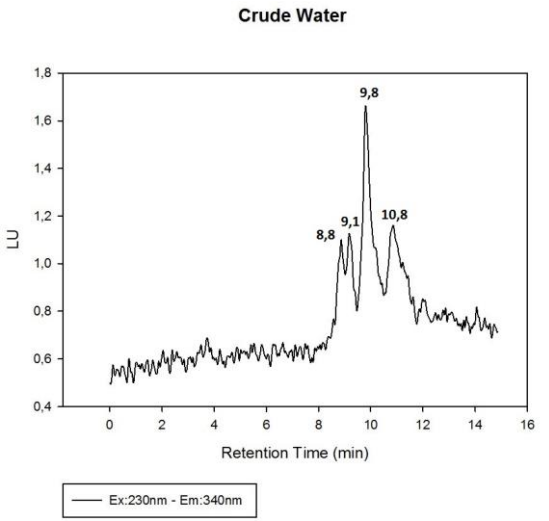

Figure 27. HPSEC Chromatogram of Crude Water at λ(Ex/Em) = 230nm/340nm

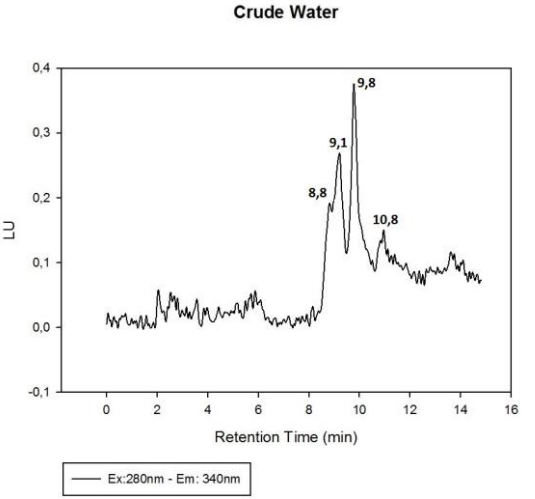

Figure 28. HPSEC Chromatogram of Crude Water at λ(Ex/Em) = 280nm/340nm

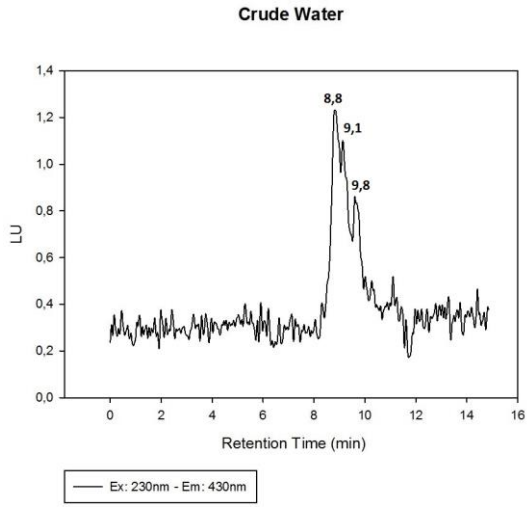

Figure 29. HPSEC Chromatogram of Crude Water at λ(Ex/Em)= 230nm/430nm

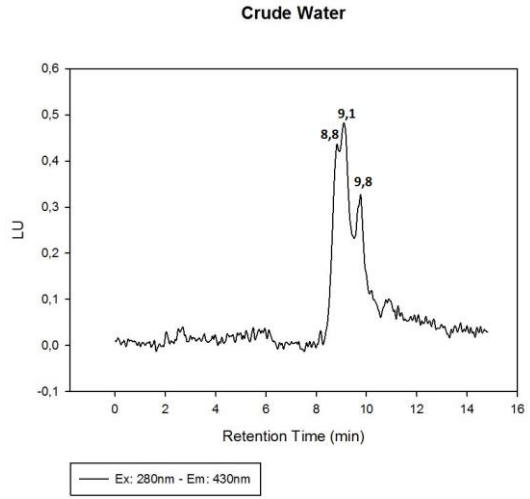

Figure 30. HPSEC Chromatogram of Crude Water at λ(Ex/Em)= 280nm/430nm

It has been observed that the lower MW fraction, with a retention time (RT) of 10,8min, is only present at the λ(ex/em) of proteins (em=340). For lowers RT, 8,8; 9,15 and 9.8 minutes respectively, the fractions with higher MW are present both in proteins and SH excitation-emission wavelengths.

While it may appear unusual that fractions detected as humic-like in EEM fluorescence are also observed in the HPSEC chromatograms at protein-like emission (340), should be taken into account that at this point there is a maximum. Although it doesn't mean that minor intensity signals can't be detected in other zones of the spectra.

The HPSEC chromatograms provide sufficient information to characterize the different fractions.

The first case to study is the fraction obtained at 10,8 minutes. With a help of the calibration curve (figure 26), this fraction has an apparent MW around 600Da. This MW corresponds to proteins-like, tryptophan or tyrosine. Moreover, the fact that is only observed at em=340nm (protein zone) confirms the previous theory.

A second fraction with a retention time of 9,8 minutes were observed. This corresponds to a MW around of 2500 Da. In figures 29-30 is possible to see a diminution of this peak in the humic zone. Due to this and the lower MW is possible to consider their fraction as a protein-like fraction.

Finally, the remaining fractions were obtained at retention times of 8,8 and 9,1 minutes. These peaks correspond to a MW around of 11000Da and 6500Da, respectively. Thus, is possible to consider the fractions as humic-like fractions. Comparing the RT of the samples with the standard of humic substance (figure 10) a similarity is observed confirming the supposition indicated before.

The EEM spectra of the different fractions, as also the corresponding chromatograms (figure31-34), indicate the decrease of DOM throughout the treatment process.

In particular, the fractions are remarkable, with the exception of RO and the output of the plant. In both cases DOM is almost non-existent.

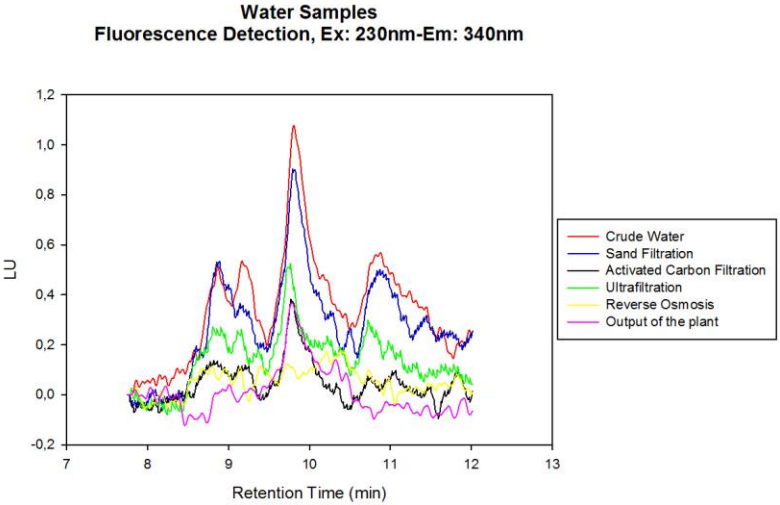

Figure 31. Evolution of DOM fractions during the treatment process. HPSEC-FLD Detection (λex/em = 230/340 nm)

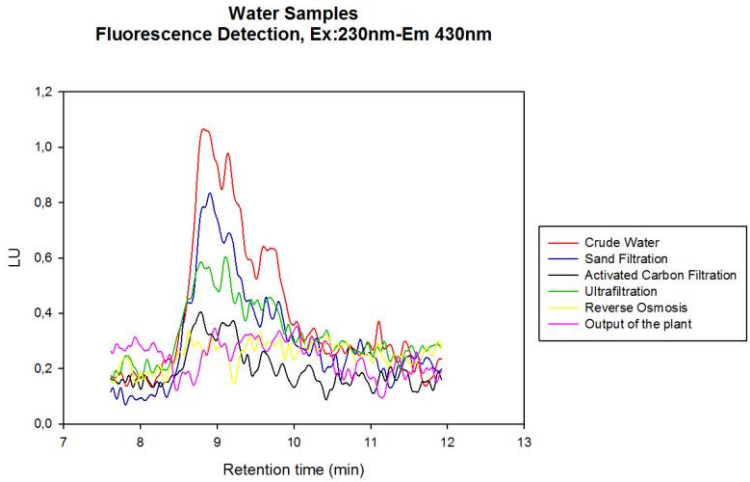

Figure 32. Evolution of DOM fractions during the treatment process. HPSEC-FLD Detection (λex/em = 230/430 nm)

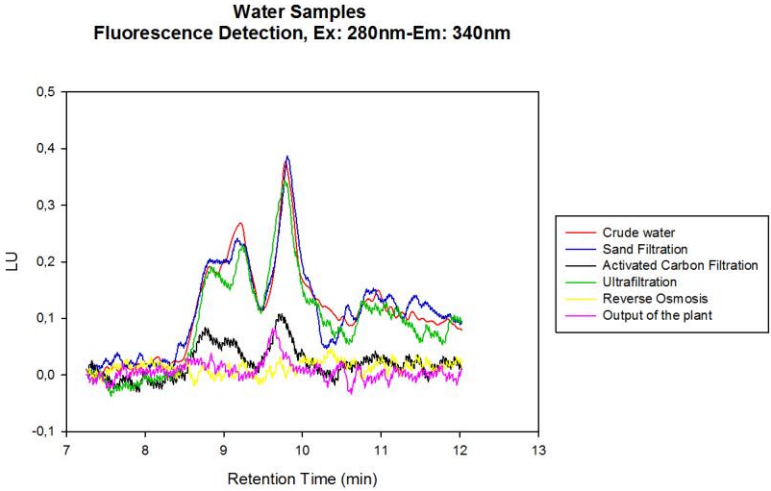

Figure 33. Evolution of DOM fractions during the treatment process. HPSEC-FLD Detection (λex/em = 280/340 nm)

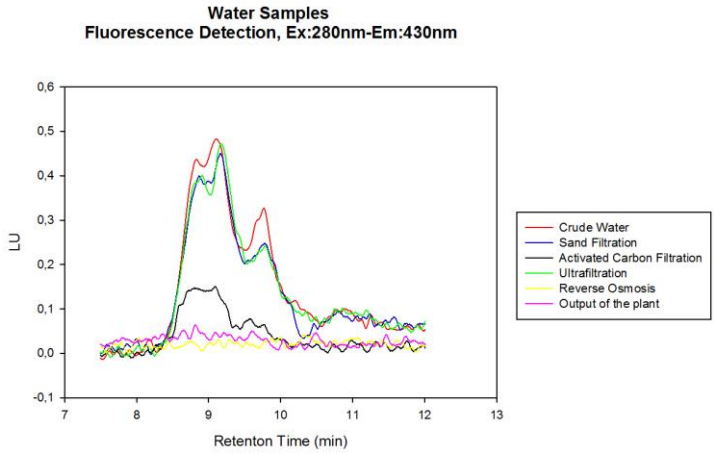

Figure 34. Evolution of DOM fractions during the treatment process. HPSEC-FLD Detection (λex/em = 280/430 nm)

6.5. EFFECT OF METALS IN WATER SAMPLES:

The metals in contact with DOM tend to act as bridges between the different substances, forming bigger complexes. This fact can interfere in the correct characterization of DOM by using fluorescence. [20]

Surface water often contain some quantity of metals, being iron the most abundant. Moreover, in DWTP of Sant Joan Despí, there is a flocculation process in which aluminium salts are used. Due to other studies it is known that the presence of iron, decrease the peaks intensity, however aluminium presence increases it. In this part, the possible interference of these metal ions in the DOM characterization has been studied.

In order to carry out this research, two tests have been conducted:

- From one hand, a test with EDTA has been conducted. EDTA as a complexing agent: in presence of metals, it will form complexes freeing it from the union with DOM. The complex formation with EDTA allows to molecular disaggregation, and it will affect in the quantity of fluorophores groups and that's the reasons of the fluorescence increase.
- On the other hand, the iron and aluminium effects have been separately tested, with the addition of these metal ions. In both cases the concentrations of metals were 10ppm.

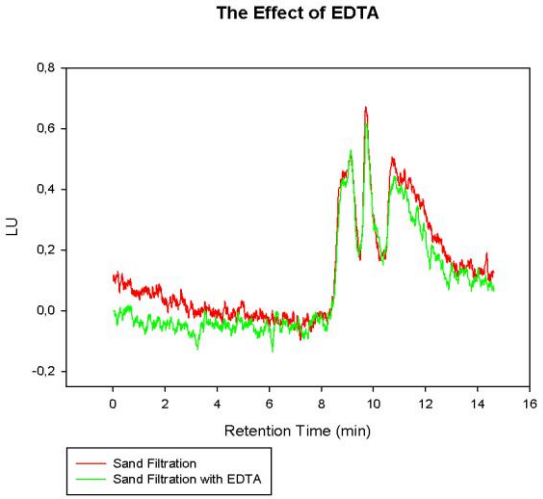

Figure 35. Effect of EDTA in Sand Filtration Sample

In the first case, the EDTA presence doesn't produce any substantial change in the peaks intensity, as it is shown in figure 35. This fact informs us that the metals quantity present in our samples is small.

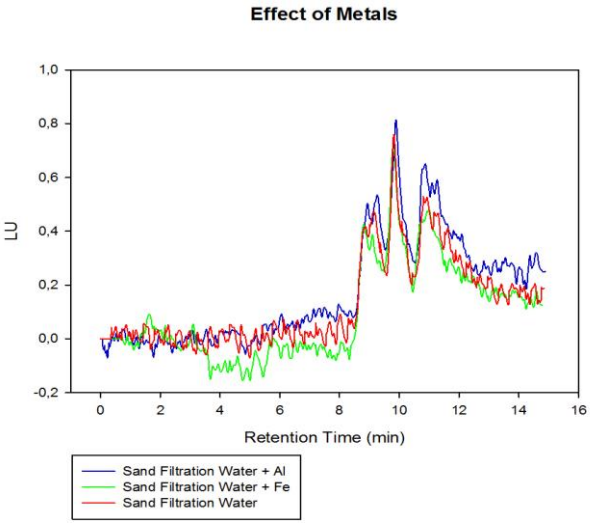

Figure 36. Effect of metals in Sand Filtration sample with Al^{3+} and Fe^{3+} addition

However, when aluminium and iron are added into a sand filtration sample a small variation is detected; in the case of Iron addition the peaks increase their intensity. Besides this, the aluminium addition decreases the peaks intensity. In both cases the change in the fluorescence intensity is minimum. Thus, the conclusion is that the effect of these metal ions is not relevant at these concentrations.

7. CONCLUSIONS

- By using EEM fluorescence the presence of humic-like and protein-like substances have been detected. Humic-like fractions have been detected at λ(Ex/Em) = 230/430nm and 280/430nm. Protein-like fractions have been detected at λ(Ex/Em) = 230/340nm and 280/340nm.

- HPSEC-FLD has been allowed to complement the information obtained with EEM spectra. The result has been the separation of 4 DOM fractions with different MW: two with high MW (between 11000 and 6500 Da), related with HS; another one with intermediate MW (approx. 2500 Da) and the last one with low MW (below 600 Da), both assigned to "protein-like" fractions.

- Thanks to the operational ease, low volume required and minimum pre-treatment necessary, HPSEC-FLD could be considerate as an attractive option for DOM's characterization in function of MW.

- Throughout the different purification processes, the correct removal of DOM in the plant of Sant Joan Despí has been demonstrated. In the different spectra, a diminution of DOM has been observed.

- RO is the most effective process removing DOM in DWTP. Furthermore, UF is less effective in removing DOM, compared with RO and GAC.

- The presence of Al^{3+} and Fe^{3+}, at concentrations of 10ppm, has been irrelevant on the characterization of the DOM.

8. REFERENCES AND NOTES

1. Gjessing, E., Egeberg, P. and Hähedal, J. 1999. Natural organic matter in drinking water- the NOM-typing project, background and basic characteristics of original water samples and NOM isolates. Environment International. 25:145-159
2. Leenher, J.A., Croué, J.P. Characterizing Dissolved; Environmental Science and Technology, 2003.
3. International Humic Substances Society, Natural organic Matter Research, 2007
4. J. Cornejo, M. C. H. (1996). Interaction of Humic Substances and Soil Clays., Humic Substances in Terrestrial Ecosystems (pp. 595–624).
5. E. Tipping, 1994. A chemical equilibrium model and computer code for waters, sediments, and soils incorporating a discrete site/electrostatic model of ion-binding by humic substances. Pp. 973–1023.
6. Masashige Yamauchi, et al. (1984). "Total synthesis of fulvic acid" (23). J. Chem. Soc., Chem. Commun. pp. 1565–6.
7. Jacques Buffle, France L. Greter, Werner Haerdi, “Measurement of complexation properties of humic and fulvic acids in natural waters with lead and copper ion-selective electrodes”, 1977.
8. S. (2010). Control of Trihalomethanes in Wastewater Treatment. Florida water resources journal.
9. European Communities (Drinking Water) (No. 2) Regulations 2007 (S.I. 278 of 2007) Hua, G., & Yeats,
10. Kimura, K., Hane, Y., Watanabe, Y., & Amy, G. (2004). Irreversible membrane fouling during ultrafiltration of surface water, 38, 3431–3441.
11. Douglas A. Skoog, F. James Holler, S. R. C. (2008). Fluorescence spectroscopy. In Principles of Instrumental Analysis (Vol. 1, p. 15A–15C).
12. Her, N., Amy, G., McKnight, D., Sohn, J., & Yoon, Y. (2003). Characterization of DOM as a function of MW by fluorescence EEM and HPLC-SEC using UVA, DOC, and fluorescence detection. Water Research, 37(17), 4295–4303.
13. Anu Matilainen , Egil T. Gjessing , Tanja Lahtinen , Leif Hed , Amit Bhatnagar , Mika Sillanpää, “An overview of the methods used in the characterisation of natural organic matter (NOM) in relation to drinking water treatment”, 2011.
14. Li, W. T., Xu, Z. X., Li, A. M., Wu, W., Zhou, Q., & Wang, J. N. (2013). HPLC/HPSEC-FLD with multi-excitation/emission scan for EEM interpretation and dissolved organic matter analysis. Water Research, 47(3), 1246–1256.
15. S.Tayyab., W. Qamar, W. I. (1991). Interdisciplinary Biotechnology Unit Aligarh Muslim University Aligarh 202002, UP India. Biochemical Education, 19(3), 149–152.
16. Adapted from A. M. Striegel, W. W. Yau, J. J. Kirkland, and D. D. Bly. Modern Size-Exclusion Liquid Chromatography- Practice of Gel Permeation and Gel Filtration Chromatography, 2nd Edition. Hoboken. N.J. (2009).
17. Gibert, O., Lefèvre, B., Teuler, A., Bernat, X., & Tobella, J. (2015). Distribution of dissolved organic matter fractions along several stages of a drinking water treatment plant. Journal of Water Process Engineering, 6, 64–71

18. A. J. Lawaetz and C. A. Stedmon, "Fluorescence Intensity Calibration Using the Raman Scatter Peak of Water," Appl. Spectrosc. 63, 936-940 (2009)
19. Bagazgoitia, F. J., Garcia, J. L., Diéquez, C., Weeks, I., & Woodhead, J. S. (2015). Effect of surfactants on the intensity of chemiluminescence emission from acridinium ester labelled proteins. Journal of Bioluminescence and Chemiluminescence, 2(3).
20. Park, J. H. (2009). Spectroscopic characterization of dissolved organic matter and its interactions with metals in surface waters using size exclusion chromatography. Chemosphere, 77(4), 485–494.

9. ACRONYMS

AT1	Advanced Treatment 1
AT2	Advanced Treatment 2
BSA	Bovine Serum Above
CT	Conventional Treatment
CMC	Critical Micelle Concentration
DAD	Diode Array Detector
DOM	Dissolved Organic Matter
DPBs	Disinfection By-Products
DWTP	Drinking Water Treatment Plant
EEM	Excitation-Emission Matrix
Em	Emission
Ex	Excitation
FA	Fulvic Acids
FLD	Fluorescence Detector
GAC	Granular Activated Carbon
HA	Humic Acids
HPSEC	High Performance Size Exclusion Chromatography
HS	Humic Substances
LU	Luminescence Units
MW	Molecular Weight
NOM	Natural Organic Matter
PEG	Polyethylene Glycol
PEO	Polyethylene Oxide
RM	Remineralized

RO	Reverse Osmosis
THMs	Trihalomethanes
Trp	Tryptophan
Tyr	Tyrosine
UF	Ultrafiltration
UV-Vis	Ultraviolet-Visible

APPENDICES

APPENDIX 1: PLANT DESCRIPTION

The DWTP of study is located in Sant Joan Despí (Barcelona, Spain) and has a nominal capacity of 5.3m^3/s, supplying a population of over 1million people in the metropolitan area of Barcelona. The raw water used by the DWTP comes from the Llobregat River (and occasionally its aquifer).

The treatment applied in the DWTP includes a conventional treatment (CT) comprised of pre-chlorination, coagulation/flocculation, subsequent sedimentation and sand filtration, followed by an advanced treatment (AT1) comprised of ozonation, granular activated carbon (GAC) filtration and post-chlorination.

The need to improve water quality beyond that achieved by CT plus AT1 prompted the installation of an additional membrane-based advanced treatment (AT2) run in parallel to AT1. AT2 treats half of the sand filtered water and consists of ultrafiltration (UF), ultraviolet (UV) irradiation, cartridge filtration, reverse osmosis (RO) and remineralization (RM) units.

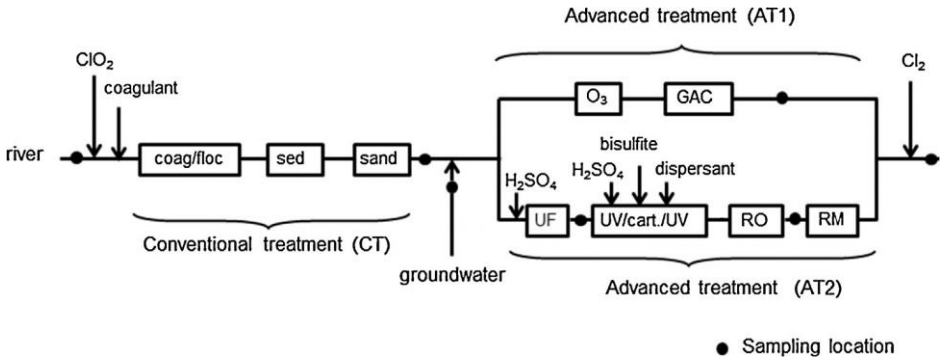

Figure 37. Flowchart of the DWTP of study with sampling locations. [17]

The processes and subprocesses that take place in the DWTP for the purification of surface water and groundwater of the Llobregat River are described below:

-Pre-chlorination: Pre-chlorination is carried out by dispensing in-situ generated ClO_2 into the collected river water for the purposes of disinfection and pre-oxidation of certain metals and NOM.

- Coagulation: It is carried out by the addition of $Al_2(SO_4)_3$ to enable the separation of flocs by gravity. The dosage of coagulant (typically in the range of 60–110 mg/L) is readjusted automatically depending on the water quality and the flow to be treated.

- The sedimentation phase takes place in static decanters, for about two hours. Flocculant polydiallyldimethylammonium chloride (poly-DADMAC) was sporadically added when the water quality required it. During the sedimentation phase two parallel processes are carried out: the sedimentation of coagulated matter and the decanting of clarified water. In these static decanters, the water flows upwards and they operate while maintaining a layer of stabilized sludge at a certain height in the decanter, where new small particle clusters bind and agglomerate with the pre-existing ones, whereas the ones that have reached a certain size and weight form a sediment at the bottom. To ensure that the sludge is removed and the sludge layer is kept at a certain height, regular purging operations are performed.

-Sand Filtration: After sedimentation, water passes through some open sand filters to remove traces of particles and certain microorganisms. These sand filters are backwashed in order to restore their initial permeability.

-Groundwater Sample: The underground water from the aquifer of the Llobregat River is incorporated at the sand filtering output. This underground resource makes it possible to cover demand in the event of not having other resources, or for optimizing the quality of the treated water, by diluting the water coming from the previous treatment phase. Although the underground water has saline levels equal to or higher than those of the surface water, it is of better quality.

- Ozonation: Once entered in AT1, water is ozonated with in-situ generated O_3 for its biocidal and oxidizing effects. The mixture of oxygen and ozone is diffused in the contact chambers, through which the water circulates, by porous diffusers. The ozonation results in a break-down of DOM into smaller and lighter fragments.

-Granular activated carbon (GAC) filtration: The ozonated water is then filtered through GAC in 20 filters to remove organic compounds through adsorption. It also retains metallic oxides (iron, manganese, nickel, etc). Part of the carbon filter absorption capacity is lost as it is used, and so its capacity must be monitored to ensure the correct elimination of the contaminants. When the active carbon filter is saturated with organic matter and loses its absorption capacity, a regeneration process is applied in special ovens. The granular activated carbon filters, like the sand filters, require counter-current cleaning when the filters become clogged.

-Ultrafiltration: The diverted sand-filtered water is acidified with H_2SO_4 and passed through 0.02µm-pore size UF membranes, for the elimination of bacteria and also possible remaining suspended matter.

-UV radiation/Cartridge filtering/UV radiation: Prior to RO, ultrafiltered water undergoes a pre-treatment consisting of UV radiation (to eliminate viruses that have survived the previous phases); the addition of H_2SO_4 (to lower pH and avoid undesirable precipitation on RO membranes), bisulphite (to eliminate traces of oxidants) and a dispersant (to prevent the crystallization of the salts present in the water). Then, cartridge filtering is carried out (with selectivity of 5µm) to provide additional protection to the RO membranes in the event of leaks during the UF phase.

- Reverse Osmosis: The RO process constitutes a total barrier to viruses and bacteria. It also eliminates practically all organic and inorganic compounds present in the water, and achieves conductivities and total organic carbon levels that are very low. High-pressure pumping allows the ultra-filtered water to pass through two of the osmosis phases through which it has previously passed for treatment in the RO plant. Since water that is practically salt-free is produced, subsequent remineralization is needed to ensure that the water is not aggressive.

-RM: Finally, water is remineralized in some calcite filters with optional CO_2 injection and finally chlorinated with Cl_2 before water distribution into the network.

Treated water from both AT1 and AT2 are blended before distribution.