Using excitation-emission matrix fluorescence to evaluate the performance of water treatment plants for dissolved organic matter removal

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ABSTRACT

This study is aimed at assessing the performance of water treatment plants (both wastewater and drinking water treatment plants) for dissolved organic matter (DOM) removal using excitation-emission matrix fluorescence (EEMF) as the monitoring technique.

The influent from the wastewater treatment plant (WWTP) of Burgos (Spain) is characterized from the presence of protein-like peaks (T1 and T2) and humic-like peaks (A and C), T2 and A showing the highest fluorescence intensity. The percentages of total removal in the effluent were in the following order: peak T1 (65%) > peak A (45%) > peak C (34%) > peak T2 (26%). The humic-like peaks were the most removed at the primary sedimentation stage, whereas peak T1 was by far the most removed in the biological reactor. Protein-like peaks T1 and T2 experienced a slight increase in the final effluent in comparison to their fluorescence at the previous stage (the exit of the biological reactor), an increase that can be explained by the release of SMP (soluble microbial products) from the biomass in the secondary clarifier. A poor correlation was obtained between peak T2 fluorescence and COD, BOD (r^2 = 0.34-0.38).

The natural water from the Úzquiza reservoir in Burgos (Spain) is characterized by the only presence of humic substances: a majority peak A (fulvic-like) and a weak peak C (humic-like). The whole fluorescent DOM was removed by coagulation-flocculation but a low fluorescence peak T2 appeared at the final stage, coming from protein-like SMPs released by the biomass attached to the filters.

Keywords.

fluorescence, excitation-emission matrix (EEM), urban wastewater, drinking water, water treatment plants, organic matter removal.

1.- INTRODUCTION

Fluorescence is a versatile analytical technique that has been widely used for the analysis of dissolved organic matter (DOM) in natural waters (rivers, sea, etc) (Markechová et al, 2013; Shutova et al, 2014) and urban wastewaters (Bridgeman et al, 2013; Cohen et al, 2014; Guo et al, 2018; Li et al, 2020; Xiao et al, 2020; Yu et al, 2020). Aquatic DOM is highly heterogeneous and consists of a great variety of organic compounds, from small molecules such as acetic acid to high molecular weight macromolecules (humic substances, proteins, fatty acids, polysaccharides, etc) (Esparza-Soto et al, 2011; Huang et al, 2015; Komatsu et al, 2020; Yu et al, 2015).

Among the different fluorescence techniques, excitation-emission matrix fluorescence (EEMF) is currently the most complete tool to characterize DOM. The matrix can be represented either in the form of a 2D-contour map or a 3D-graph, thus allowing a quick location of the main peaks of the sample at a glance. EEMF, in which a series of emission scans are collected for a range of excitation wavelengths, is a fast (the collection of a spectrum in the region λ_{ex} x λ_{em}: 220-450 x 300-550 nm usually takes 10-12 min.) and simple technique (pre-treatment normally consists of a filtration of the sample and dilution, if necessary) (Qian et al, 2017; Rodríguez-Vidal et al, 2020).

It is frequent the use of sophisticated mathematical tools (such as PARAFAC: Parallel Factor Analysis) to study EEMF spectra; however, the traditional "peak-picking" method has been used in this study since it is faster, more user-friendly and provides satisfactory results for the identification of the typical fluorophores usually present in waters (Yin et al, 2020). Actually, some studies have shown that monitoring a few individual peaks at the target coordinates (λ_{ex}/ $\lambda_{em)}$ can provide basically the same information as monitoring the whole EEMF (Murphy et al, 2011). Additionally, this work is aimed at exploring the applicability of EEMF to routine measurement in water treatment plants (both, DWTPs and WWTPs) and these plants usually demand fast and simple analytical techniques; PARAFAC is a time-consuming method usually used in research studies, not in routine measurements in treatment plants, and requires a high degree of analytical expertise. Moreover, once the influent of a specific plant is characterized (either natural water or wastewater), the main peaks remain basically the same (obviously they vary in intensity from sample to sample) and the pick-picking method provides a simple and fast tool to monitor changes in the concentration of the main DOM fluorophores, that is what most DWTPs and WWTPs require.

The EEMF peaks found in both natural and wastewaters can be classified into two main groups (λ_{ex}/ λ_{em}, see Figure 1) (Rodríguez-Vidal et al, 2020):

- <u>Humic-like peaks</u>: peak C (humic-like: 320-360/420-460), peak A (fulvic-like: 230-260/400-480), peak M (290–310/370-420).

- <u>Protein-like peaks</u>: peak T_1 (tryptophan-like: 275-285/320-350), peak T_2 (tryptophan-like: 215-237/340-381), peak B_1 (tyrosine-like: 275-310/305-320), peak B_2 (tyrosine-like: 220-237/305-320).

Protein-like T_1 and T_2 are usually the majority peaks in domestic wastewaters, associated to anthropogenic and microbially derived organic matter (Li et al, 2014; Yu et al, 2013). However, the humic-like peaks A and C are habitually the most abundant fluorophores in freshwaters. Both peaks are related to the presence of humic substances (humic and fulvic acids, the latter being predominant in natural waters) and can have both an allochthonous (soil organic matter and terrestrial plants washed away by rainwater) and an autochthonous origin (microbial processes in water) (Rodríguez et al, 2014). Actually, a significant presence of protein-like peaks in freshwaters is usually attributed to anthropogenic pollution coming from wastewater discharges (Borisover et al, 2011; Yu et al, 2016). Peak M, originally defined as "Marine humic-like" indicates the presence of organic compounds from recent biological activity (soluble microbial humic-like material) (Ishii and Boyer, 2012; Murphy et al., 2011; Yu et al., 2014).

In the wastewater field, EEMF has been principally applied to effluent characterization in wastewater treatment plants (WWTPs) and the effect of effluent discharge on natural waterbodies (Yang et al, 2014, 2015). Influent (raw wastewater) characterization is less frequent but very few studies have focused on DOM fluorescence behaviour throughout the internal stages of a WWTP, not only at the beginning (influent) and at the end of the treatment (effluent).

EEMF can provide researchers with valuable information about the chemical composition of DOM and track its changes in quantity and quality throughout the stages of the wastewater treatment (Yang et al, 2015). Therefore, one of the aims of this work is to study the behaviour of the main fluorescence peaks (humic-like peaks A and C, protein-like peaks T1 and T2) through the treatment sequence in a conventional WWTP: influent (raw wastewater) → primary clarification → biological reactor → effluent (treated wastewater).

Similarly, the whole sequence of treatment in a drinking water treatment plant (DWTP) has been studied, assessing the behaviour of DOM fluorescence through the main stages of the plant: raw natural water → coagulation-flocculation-sedimentation → filtration → treated water (drinking water). As in the case of WWTPs, few studies have focused on this goal.

2.- MATERIAL AND METHODS.

2.1. Types of wastewaters.

Wastewaters were sampled at the urban WWTP of Burgos (Spain), from March to May 2018 (six samples in total, once a fortnight). Four sampling points were chosen in this study: Influent (raw wastewater), Primary Clarification exit, Biological Reactor exit (activated sludge supernatant) and Effluent (final treated water after secondary clarification). These are the most critical points in a WWTP.

Drinking water samples were collected at the DWTP of Burgos (Spain), from February to April 2018 (six samples in total, once a fortnight). Four sampling points were chosen in the plant to be studied: Reservoir water (raw water), Coagulation-flocculation exit (settled water), Sand Filters exit (filtered water) and Treated water (final water after chlorine disinfection at low dosage as a residual disinfectant). The main disinfection is carried out using ozonation at the head of the plant.

Samples were adjusted to pH 7 using dilute HCl or NaOH and filtered in order to avoid turbidity interferences (Hudson et al, 2008).

2.2. Fluorescence measurements.

Fluorescence spectra were acquired using a spectrofluorometer (Varian Cary Eclipse) with a 1-cm cuvette at room temperature (20-22°C). The instrument settings were adjusted as follows: excitation wavelength range: 220-450 nm (step 5 nm), emission wavelength range: 350-500 nm (step 1 nm), scan speed: 600 nm/min. The spectra recorded in the spectrofluorometer were then arranged in the form of an excitation-emission matrix (EEM) using QtiPlot software to generate three-dimensional contour plots of fluorescence intensity as a function of emission and excitation wavelengths. The spectrofluorometer was auto-zeroed before each analysis. Fluorescence intensity variation in triplicate measurements was less than 9%. Fluorescence intensities were normalized to Raman units (R.U.) using the Raman peak of Milli-Q water at λ_{ex}=348 nm (Yang et al., 2015). The instrument stability was checked by measuring the Raman emission at λ_{em}=395 nm, which averaged 29.1 ± 1.7 intensity units (n=22). Inner-filter effects were corrected in wastewater samples by absorbance spectroscopy (McKnight et al., 2001; Ohno, 2002); this correction was not necessary in drinking water samples since inner-filter effects are negligible in this kind of samples. Fluorescence "quenching" effects due to the presence of metal ions (mainly iron) are not expected in domestic wastewater samples due to the high amount of DOM and the very low concentration of these ions (Baker et al, 2003).

Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD) of wastewater samples were measured by WWTP staff using standard methods.

3.- RESULTS

3.1. – Wastewater treatment plant.

Figure 2 shows the EEMF spectra for the main stages of the WWTP of the city of Burgos (Spain): Influent (raw wastewater, Fig 2.a), Primary Settlement exit (Fig 2.b), Biological Reactor (activated sludge) exit (Fig 2.c) and Effluent (final treated water, Fig 2.d). The location of the peaks ($\lambda_{ex}/\lambda_{em}$) and their fluorescence intensities (Raman units) are shown in Table 1. All six wastewater samples (from March to May 2018) showed the same behaviour (Fig. 2 corresponds to the April 12th sample) and quantitative data (fluorescence intensities, percentages of removal, etc) in Table 1 and Figures 3-4 correspond to average values for the six samples (individual data for each one of the six samples are shown in Table S1, supplementary online material).

The influent (Fig. 2a) is characterized by the presence of the protein-like peak T_2 and the fulvic-like peak A as majority peaks (Rodríguez-Vidal et al, 2020). The protein-like peak T_1 and the humic-like peak C also appear but with lower fluorescence intensity. That is to say, the fluorescence is controlled by the presence of protein-like substances (proteins, peptides, amino acids) and fulvic acids, the latter coming from the drinking water used in houses and which is the basis of the subsequent wastewater. Throughout the sequence of treatment the fluorescence intensity of all the peaks decreases, the percentages of total removal in the effluent (Fig 2.d and Fig 3) following this order: peak T1 (65% removal) > peak A (45% removal) > peak C (34% removal) > peak T2 (26% removal). These results are in line with others reported in the literature (Riopel et al, 2014; Yang et al, 2014, 2015).

A careful examination of the partial removals at each stage can help explain the abovementioned results (see Fig 3). Some interesting conclusions can be drawn: humic substances show the greatest removal at the primary settlement stage (32.5% for the humic-like peak C and 27.2% for the fulvic-like peak A). Humic substances are high molecular-weight macromolecules that are readily amenable to removal by coagulation-flocculation-sedimentation (Cohen et al, 2014; Xiao et al, 2018; Yang et al, 2015; Yu et al, 2014). Humic acids (peak C) have a higher molecular weight than fulvic acids (peak A), which is in accordance with their greater removal by sedimentation. However, both peaks A and C show the lowest removal in the biological reactor (24.2% removal for peak A and 0% removal for peak C), indicating their low biodegradability (Rodríguez et al, 2011). The peak showing the greatest removal by biodegradation is by far peak T1 with a 64.9% removal, whereas peak T2 shows a similar removal (25.5%) to peak A (24.2%). Protein-like substances are known to be readily biodegraded in biological reactors (Gao et al., 2020; Liu et al, 2011; Park et al, 2010), but it is interesting to note the different removal of the protein-like peaks T1 and T2 in the biological reactor,

indicating a different composition and reactivity towards biodegradation. In fact, some authors (Meng et al, 2011) refer to T1 as "tryptophan protein-like substances" and to T2 as "aromatic protein-like substances". Some authors propose the use of T1 fluorescence as an indicator or the biological treatment efficacy (Cohen et al, 2014).

Humic substances (peaks A and C) do not show any significant change in the effluent in comparison with the biological reactor; however, protein-like peaks T1 and T2 do slightly increase their fluorescence intensities in the effluent (11.1 % for peak T1 and 6.2% for peak T2). This result might seem at the beginning a bit strange, but it can be explained by the release of SMP (soluble microbial products) from the biomass in the secondary clarifier; these SMP are mostly of hydrophilic character (Quaranta et al, 2012) and are composed of proteins, polysaccharides, amino sugars and small organic acids (Jarusutthirak et al, 2007; Ni et al, 2010; Okamura et al, 2009). They constitute the majority of EfOM (Esparza-Soto et al, 2011; Jacquin et al, 2017) and have their origin in both, extracellular polymeric substances (EPS: biopolymers located on cell surface) and intracellular constituents released following cell lysis (Li et al, 2013).

Since both protein-like peaks T1 and T2 increase their intensities from the biological reactor to the final effluent, it can be reasonably argued that SMP contribute to both of them (Yu et al, 2013). However, there is no agreement in the literature regarding this issue: some authors only include T1 as the sole peak coming from a biological origin (SMP) (Chen et al, 2003) whereas others indicate that tyrosine-like peak B1 (not found in our wastewater) has an exclusively microbial origin (Yu et al, 2015). Other authors (Maie et al, 2007) proposed that peak T1 can be separated into two constituents: high molecular weight proteinaceous materials and humic associated phenolic moieties with small molecular weight. Recent studies have shown that proteins can be encapsulated by humic substances, indicating a possible combination between them through electrostatic attraction or hydrophobic effects (Li et al, 2014; Rodríguez-Vidal et al, 2020).

Another interesting fact is that some peaks shifted their location (λ_{ex}/ λ_{em}) from the influent to the effluent (see Table 1). Concerning protein-like peaks, Peak T1 shows a red-shift (towards longer wavelengths) in both, λ_{ex} and λ_{em} (from 280/355 nm to 290/365 nm), Peak T2 having a mixed shift (from 225/359 nm to 230/353 nm). That means that the composition of protein-like peaks is not the same in the influent as in the effluent: this fluorescence is dominated by protein moieties mainly coming from food and human waste degradation in the influent whereas it is mostly composed of SMP coming from microorganisms in the effluent. Regarding humic-like fluorescence, a red-shift is also observed for peak A (from 225/423 nm to 230/424 nm) and peak C (from 320/420 nm to 330/424 nm), which indicates a change in the composition of humic substances too. In the specific case of humic substances, a red-shift is related to a greater presence of hydroxyl, alkoxyl, carbonyl and amino

groups in the humic macromolecule and to a higher degree of humification and aromaticity of the humic substances (Rodríguez et al, 2014; Wang et al, 2009). There is also evidence in the literature that a part of the humic substances present in the effluent has a microbial origin too (SMP) (Esparza-Soto et al, 2011; Galinha et al, 2011; Riopel et al, 2014; Wang and Zhang, 2010; Yu et al, 2015).

As a result of the different removal of the peaks in the wastewater treatment, the relative composition of the effluent also differs from that of the influent (see Fig. 4). Even though the two major components in both cases (influent and effluent) remain the same (peaks T2 and A), the relative abundance of protein-like peaks significantly changes: peak T2 relative abundance in the influent (31.6 %) rises up to 40.5 % in the effluent whereas peak T1 decreases from 24.5 % to 14.5 %. The variation in the relative abundance of humic-like peaks A and C is negligible.

As far as wastewater fluorescence is concerned, another topic of interest studied in the literature is the evaluation of potential correlations between protein-like peaks (T peaks) and typical parameters indicative of organic matter content in urban wastewaters, such as biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total organic carbon (TOC). There is no agreement in the literature regarding this issue: some authors reported good linear correlations for wastewaters, such as Bridgeman et al, 2013 (BOD/T1: r = 0.92 and COD/T1: r = 0.85), Lee and Ahn, 2004 (COD/T1: $r^2 > 0.9$), Reynolds, 2002 (BOD, COD and TOC/T1: r = 0.93), Reynolds and Ahmad, 1997 (BOD/T1: $r^2 = 0.89$-0.94), whereas other authors reported moderate correlations (Hudson et al, 2008: BOD/T1: r = 0.71, TOC/T1: r = 0.76, BOD/T2: r = 0.47 and TOC/T2: r = 0.50) and even no statistically significant correlations (Riopel et al, 2014: TOC/T1: $r^2 = 0.093$-0.238; Vasel and Praet, 2002: COD and TOC/T1: $r^2 = 0.4$). In our study, we obtained poor correlations for the whole set of samples (influents and effluents): BOD and COD/T2: $r^2 = 0.34$-0.38.

3.2.- Drinking water treatment plant.

Figure 5 shows the EEMF spectra for the main stages of the DWTP of the city of Burgos (Spain): reservoir water, coagulation-flocculation-sedimentation exit (settled water), sand filtration exit (filtered water) and final treated water. All six samples (from February to April 2018) showed the same behaviour (Fig. 5 corresponds to the March 15th sample). Individual data for each one of the six samples are shown in Table S2 (supplementary online material).

As shown in the figure, the reservoir water fluorescence is characterized by the only presence of humic substances: an intense peak A and a weak peak C, with no presence of protein-like peaks. The raw water has a low organic content (about 2 mg/L DOC), which is reflected in the very low peak A

fluorescence (roughly 0.3 R.U.). Peak C is known to be susceptible to UV-induced photodegradation whereas peak A is relatively resistant to that process (Markechová et al, 2013). This fact could explain the majority presence of peak A in the reservoir water. Good correlations between humic-like peaks and DOC (Bieroza et al, 2010; Shutova et al, 2014) and also between humic-like peaks and disinfection byproduct (DBP) formation (Markechová et al, 2013) are reported in the literature.

The coagulation-flocculation-sedimentation stage virtually removes the whole of the fluorescent organic matter (see "settled water" in Fig. 5), a situation maintained during the filtration stage. Humic-like peaks are known to be readily removed via coagulation processes (Baghoth et al, 2011; Gone et al, 2009; Sanchez et al, 2013; Yang et al, 2015) whereas protein-like peaks are more amenable to biodegradation (Huang et al, 2011).

More interestingly, peak T2, which was no present in the previous stages, appears in the treated water. This can be explained by the fact that sand filters have been proved to be biologically active and the biofilm attached to the filters can release SMP containing protein-like moieties, which are responsible for the appearance of T2 fluorescence (Bieroza et al, 2010; Lavonen et al, 2015).

4.- CONCLUSIONS.

This study has evaluated the usefulness of EEM fluorescence as a simple and fast analytical method to monitor DOM during the treatment sequence of wastewater and drinking water treatment plants.

The urban wastewater from the WWTP of Burgos (Spain) is characterized for the presence of protein-like peaks (T1 and T2) and humic-like peaks (A and C), T2 and A showing the highest fluorescence intensity. The percentages of total removal in the effluent were in the following order: peak T1 (65% removal) > peak A (45% removal) > peak C (34% removal) > peak T2 (26% removal). The behaviour of the peaks was different at each stage: the humic-like peaks were the most removed at the primary sedimentation stage (32.5% and 27.2% removal for peaks C and A, respectively), whereas peak T1 was by far the most removed in the biological reactor (64.9% removal) followed by peak T2 (25.5% removal) and peak A (24.2% removal). It is interesting to note that peak C showed no apparent biodegradability at this stage. Protein-like peaks T1 and T2 experienced a slight increase in the final effluent (11.1% for peak T1 and 6.2% for peak T2) in comparison to their fluorescence at the previous stage (the exit of the biological reactor); this increase can be explained by the release of SMP (soluble microbial products) from the biomass in the secondary clarifier. Most peaks showed a red shift (towards longer wavelengths) in the effluent compared to their initial location in the influent, indicating a change in the composition of the fluorophores. As a result of the different removal of the

peaks in the treatment, peak T2 increased its relative abundance in the effluent in comparison to the influent (from 31.6% to 40.5%) whereas peak T1 abundance decreased from 24.5 % to 14.5 %. The potential correlation between peak T2 fluorescence and the traditional COD and BOD parameters was also evaluated in this study, obtaining a poor correlation in both cases ($r^2 = 0.34$-0.38).

The performance of drinking water treatment plants was also evaluated in this study using EEMF. The natural water from the Úzquiza reservoir in Burgos (Spain) is characterized by the only presence of humic substances: a majority peak A (fulvic-like) and a weak peak C (humic-like), with no presence of protein-like peaks. The whole fluorescent DOM was removed by coagulation-flocculation but a low fluorescence peak T2 appeared at the final stage (treated drinking water), coming from protein-like SMP released by the biomass attached to the filters.

This study has demonstrated that EEMF is a useful and user-friendly technique to track DOM changes in water treatment plants, allowing a rapid response to potential problems in the treatment.

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Table 1. Main peaks identified in the wastewater of Burgos WWTP

	T_1	T_2	A	C
Influent (raw wastewater)	280/355 (2.97)	225/359 (3.83)	225/423 (3.43)	320/420 (1.87)
Primary Settlement exit	280/360 (2.62)	225/358 (3.57)	225/421 (2.50)	320/417 (1.26)
Biological Reactor exit	290/359 (0.92)	230/352 (2.66)	230/423 (1.90)	330/419 (1.27)
Effluent (final treated water)	290/365 (1.02)	230/353 (2.82)	230/424 (1.89)	330/424 (1.23)

λex / λem (Fluorescence Intensity). λ in nm, Fluorescence Intensity in Raman units (R.U)
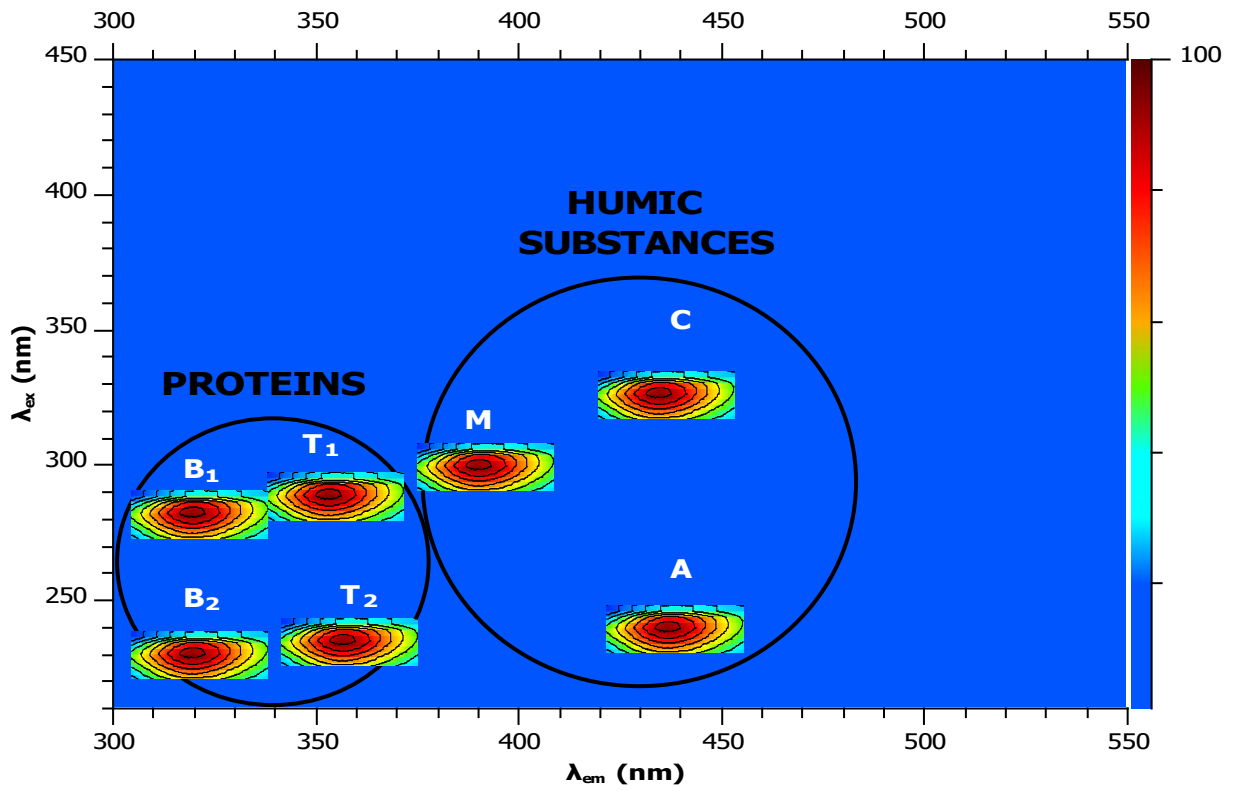
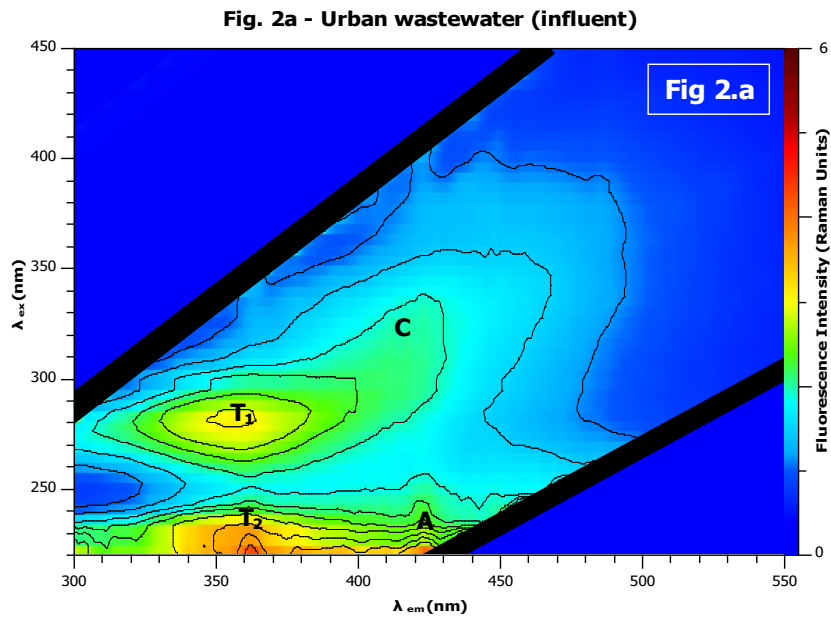Figure 1. Location of the main EEMF peaks for natural and wastewaters.
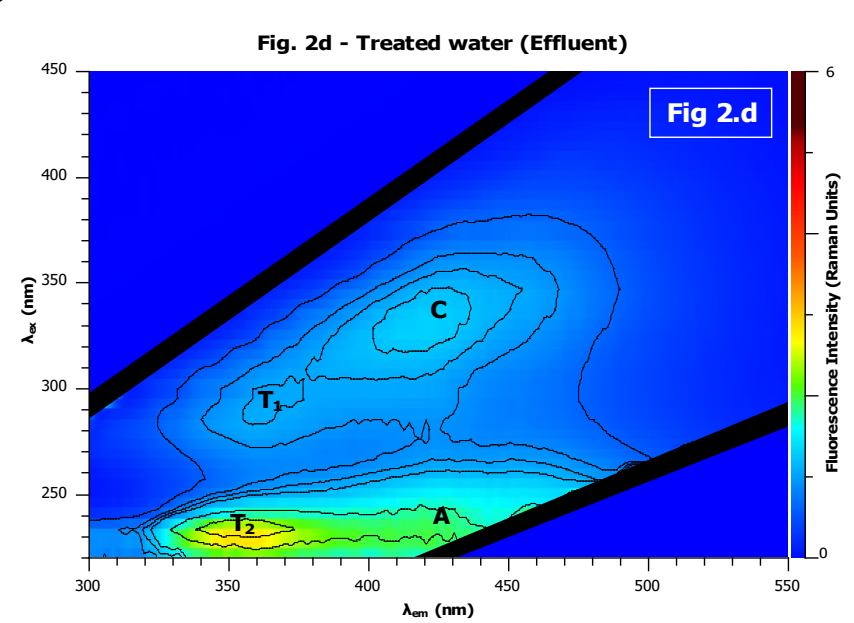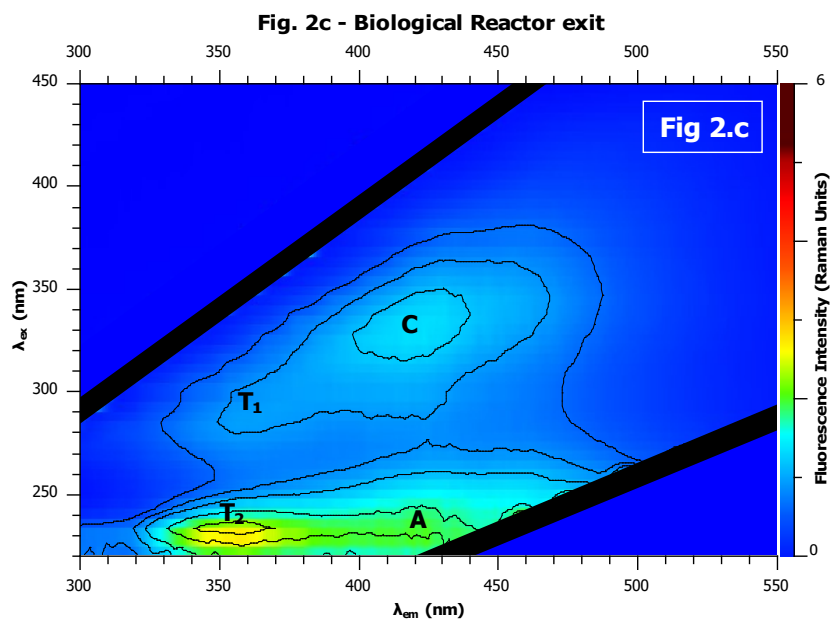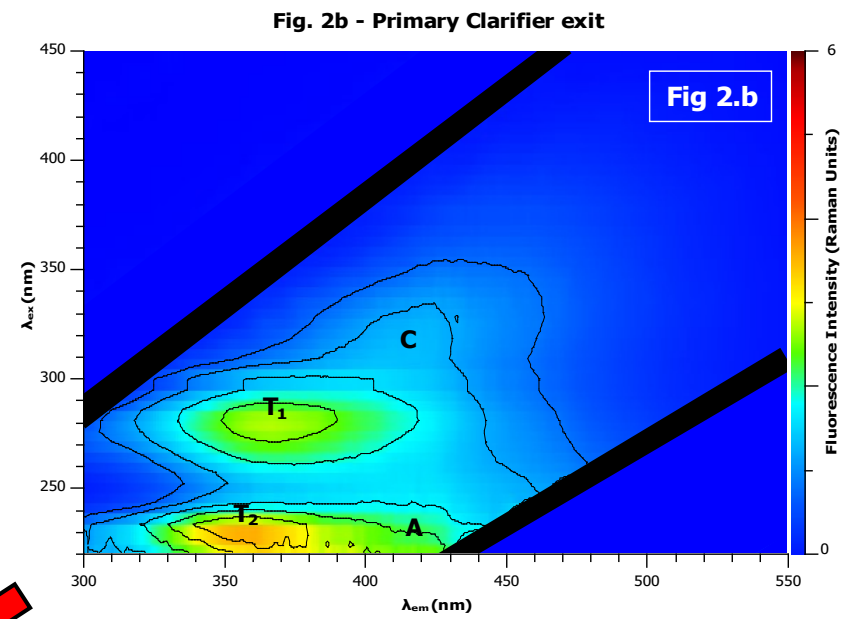
Figure 2. EEMF spectra for the main stages of the WWTP of Burgos (Spain)
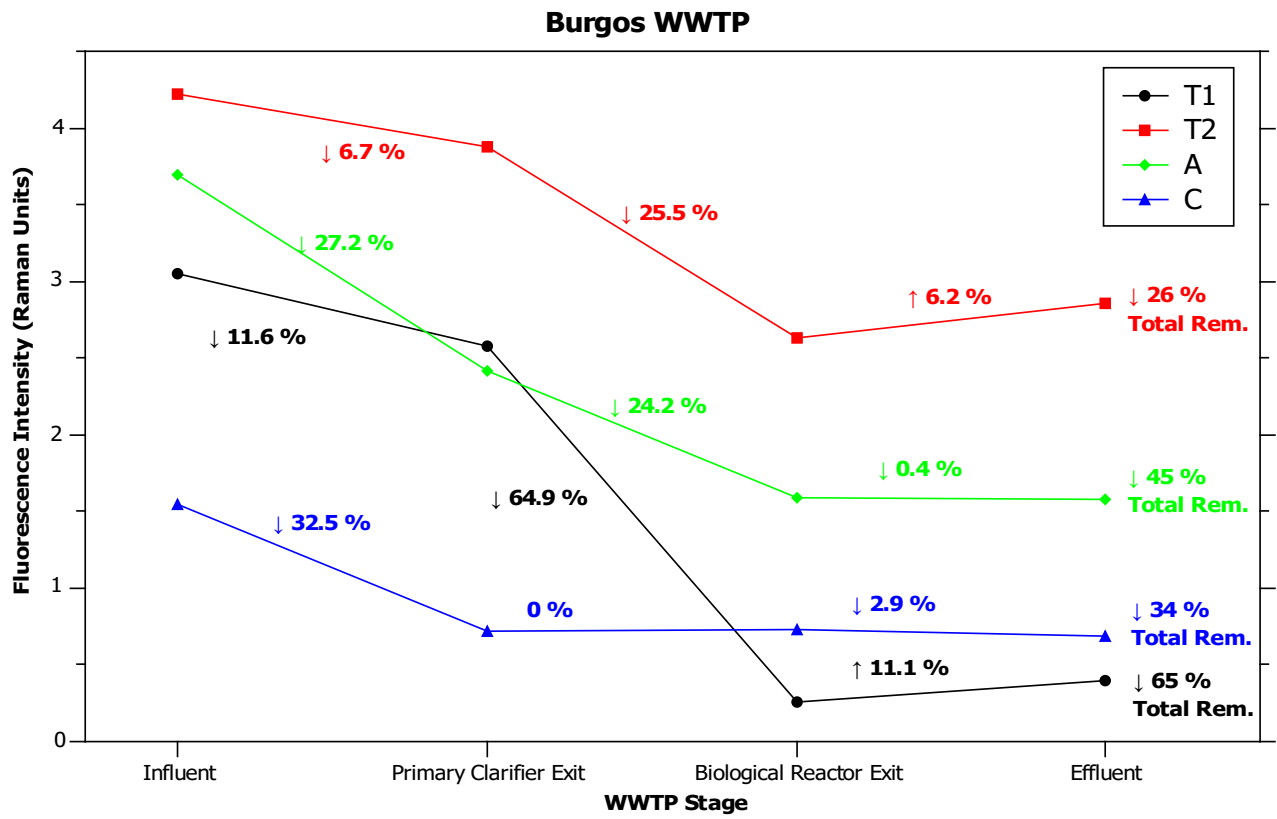
Figure 3. Fluorescence removal percentages at each stage of the WWTP of Burgos (Spain)
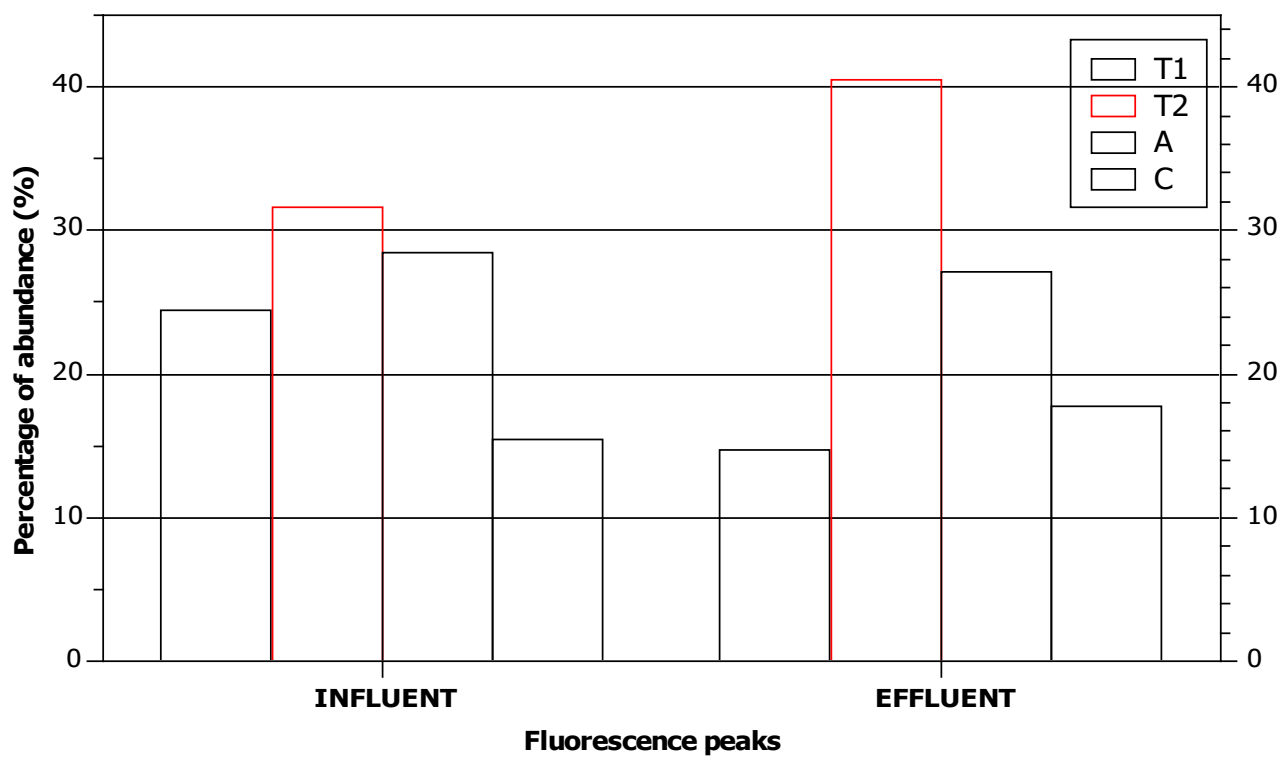
WWTP BURGOS

Figure 4. Comparison of the abundance percentage of the fluorescence peaks in the influent and effluent of the WWTP of Burgos (Spain).
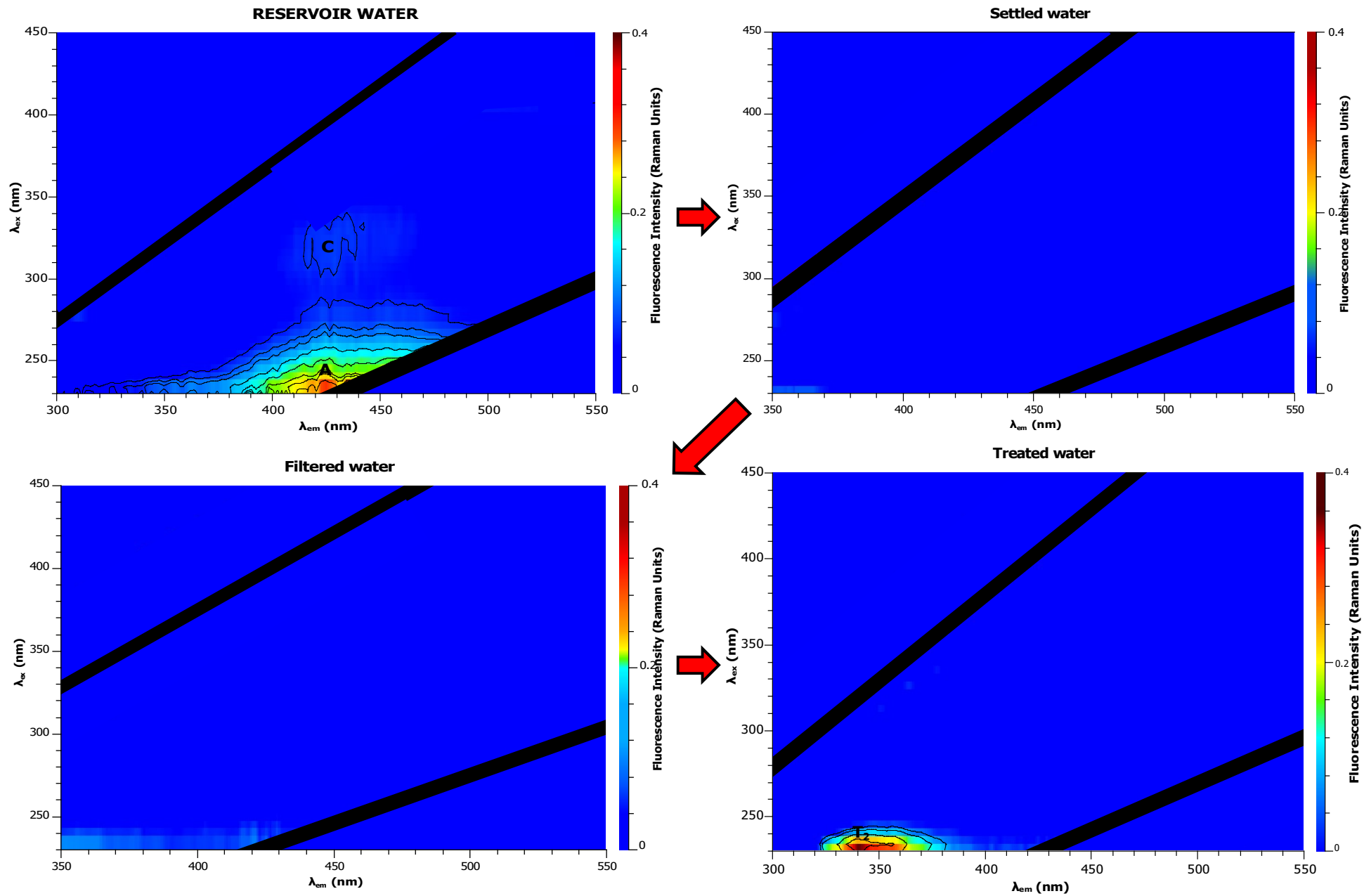
Figure 5. EEMF spectra of the different stages of the DWTP of Burgos (Spain)