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Research Article

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Tracking the Impact of Anthropogenic Inputs on Fluorescent Dissolved Organic Matter in the Gapeau River Using 3D Fluorescence Spectroscopy

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Abstract

The spatial and geographical variation of dissolved organic matter fluorescence signal is influenced by various factors, including biodegradation, photochemical degradation, and anthropogenic inputs. Understanding this variation is crucial for environmental studies such as pollution tracking and remote sensing of coastal waters. In this study, we used 3D fluorescence spectroscopy of excitation emission matrices (EEMs) coupled with parallel factor analysis (PARAFAC) to track the geographical variation of fluorescence signal along the urban Gapeau river in the PACA region of France. Two geographical sampling campaigns were conducted in August 2016 at 14 sampling points, resulting in 56 EEMs from nonfiltered and filtered samples. PARAFAC analysis revealed two components (C1 and C2), with C1 showing humic-like fluorescence and C2 showing protein "tyrosine"-like fluorescence. Both C1 and C2 increased at the wastewater treatment plant discharge point of La Crau city before decreasing with distance, indicating the impact of photochemical and biological degradation of dissolved organic matter. There were no significant differences between filtered and nonfiltered samples, indicating a negligible impact of particulate organic matter. The fluorescence signal at each sampling point can be used to determine mixing composition using a model developed elsewhere [1,2]. Future improvements to this work could include boat-based sampling campaigns and depth-profiling of the fluorescence signal to check for variability with depth.

Keywords: Fluorescence Spectroscopy; Dissolved Organic Matter; Parallel Factor Analysis (PARAFAC); Coastal Waters; Wastewater Treatment Plant; Photochemical Degradation

1. Introduction

Anthropogenic activities such as agriculture and urbanization have a significant impact on the fluorescent dissolved organic matter (FDOM) in rivers. Exponential population growth is expected to increase leading to increasing the impact on FDOM [3]. For example, agricultural practices such as the use of fertilizers and pesticides can increase nutrient levels in rivers, leading to an increase in FDOM. Urbanization can also contribute to higher FDOM levels in rivers due to increased runoff from impervious surfaces such as roads and buildings.

Additionally, human activities alter the composition and properties of FDOM in rivers. For example, wastewater discharges can introduce new types of organic compounds into rivers, which can alter the fluorescence properties of FOM. Land-use changes can also lead to changes in the types of vegetation and organic matter present in river systems, which can affect the composition of FDOM.

The analysis of dissolved organic matter (DOM) is essential for environmental monitoring, as it comprises a complex mixture of humic substances, including humic and fulvic acids, and non-humic substances [4]. These substances vary in molecular weight, water solubility, and reactivity [5,6]. In addition to the fact that DOM controls the contaminant transport in the environment [7].

Chromophoric dissolved organic matter (CDOM) is a significant component of natural organic matter (NOM) and a fraction of dissolved organic matter (DOM) that can absorb light in the UV-vis range which in turn includes Fluorescent organic matter (FDOM) in rivers and other aquatic environments [8,9]. FDOM is an important parameter to monitor because it can indicate the level of contamination and the quality of the water.Fluorescent dissolved organic matter (FDOM) is a subfraction of CDOM that can emit light in longer wavelengths after excitation by a shorter wavelength of light. FDOM is considered to be of great importance to the optical properties of surface coastal waters, and rivers are the main source of FDOM in these environments [10]. The migration of dissolved organic matter (DOM) from terrestrial sources, such as rivers, to receiving water bodies, such as coastal zones, has been demonstrated to have significant impacts on water quality [11].

Geographical differences in fluorescent dissolved organic matter (FDOM) in river water can result from natural processes and human activities, such as urbanization. Fluorescence spectroscopy is a frequently employed technique that measures the fluorescence intensity of liquid samples and solid-state luminescent materials using a specialized instrument known as a spectrofluorometer. The instrument fixes the excitation wavelength at a starting wavelength and then scans the entire emission wavelengths in small steps, typically 5 nm, before increasing the excitation wavelength and repeating the process. This technique provides valuable information on the composition and quality of DOM in aquatic systems, and it is particularly useful for identifying the presence of fluorescent organic components such as humic-like and protein-like substances.The resulting landscape is referred to as an excitation-emission matrix (EEM) [12,13]. Excitation emission matrices (EEMs) are a form of 3D fluorescence spectroscopy that enable the complete representation of the features of fluorescence spectra of a given environmental sample by exciting the sample at a given wavelength and measuring the fluorescence intensity at a longer emission wavelength [13,14]. The optical properties obtained through fluorescence spectroscopy can be used as indicators of the composition of dissolved organic matter (DOM) in aquatic ecosystems. This enables the identification of the sources and chemical characteristics of DOM, which can provide valuable insights into the biogeochemistry of aquatic systems[15,17].

Fluorescence spectroscopy is a technique with high sensitivity and selectivity that allows the measurement of concentrations below the detection limit without requiring sample extraction or destruction. The measured EEMs can be deconvoluted into several discrete components using Parallel Factor Analysis (PARAFAC), which indicates the relative pseudo-concentration of each component in every single EEM [15,18,19].

While several research works have investigated the geographical variations of fluorescence signal in rivers, there is still a research gap to be filled, particularly in the context of limnological and oceanographical studies. Accordingly, the present study focused on the geographical variations of fluorescence signal in the Gapeau river. The results of this study were used elsewhere (data not yet published) to calculate the mixing composition (river water, effluent wastewater, seawater) at each sampling location using our previously developed mixing composition models to distinguish natural and anthropogenic inputs of FDOM [1, 2]. The main research aim addressed in the manuscript is to study the geographical and spatial variations of fluorescence signal in the Gapeau river trajectory, from upstream of the La Crau city wastewater treatment plant (WWTP) to the coastal waters at the Gapeau river mouth, and to evaluate the impact of anthropogenic inputs from effluent wastewater on the river's FDOM content. Additionally, the study explores the hypothesis that sunlight-driven photodegradation occurs to anthropogenic FDOM from the WWTP.

2. Materials and Methods

2.1. Study Area

Gapeau River an important waterway situated in the southeastern France and it flows through a highly dense-population region and provides water for drinking, agriculture, and industry. Unfortunately, this Gapeau river is heavily contaminated by agricultural waste, urban development, and industrial activities.

The contamination of this Gapeau river has significant impact on the ecosystem and the aquatic organisms that depend on it, human health when it is used as a source of drinking water and the local economy as it can result in decreased recreational opportunities and creates a negative image of the region. Therefore, it is of crucial importance to track the impact of anthropogenic pollution in this river.Gapeau River is highly influenced by 13 wastewater treatment plants in its watershed. These treatment plants contribute to the anthropogenic organic pollution of the river's waters. La Crau city, with a population of 50,086 inhabitants, exerts the greatest influence on the pollution coming from its wastewater treatment plant WWTP. La Crau WWTP employs secondary and tertiary treatment technologies, which include activated sludge technology, sand filtration, prolonged aeration, and anaerobic sludge digestion. Gapeau river discharges into the Mediterranean Sea at the city of Hyeres.We undertook three excursions along the course of the Gapeau river, commencing 500 meters before the La Crau city WWTP and concluding at the leisure harbor "marina" of the Gapeau river in the coastal area of Hyeres city.

2.2. Sampling Sites

The sampling sites were selected based on field observations, land use variations and the existing literature on the Gapeau River, and after consulting with local experts. In addition to the fact that, those points correspond to the 15 points in the mixing ternary in roughly speaking [1,2]. These 15 points (as shown in Figure 1) in the ternary diagram of mixing between Gapeau river, WWTP Effluent and Seawater were produced in laboratory controlled-environment and several solar irradiation experiments were done to study the impact of sunlight-induced photodegradation on these mixtures as previously described in more detail elsewhere [1,2]. The rationale was that sampling those sites enables the validation of the multilinear regression models developed therein.

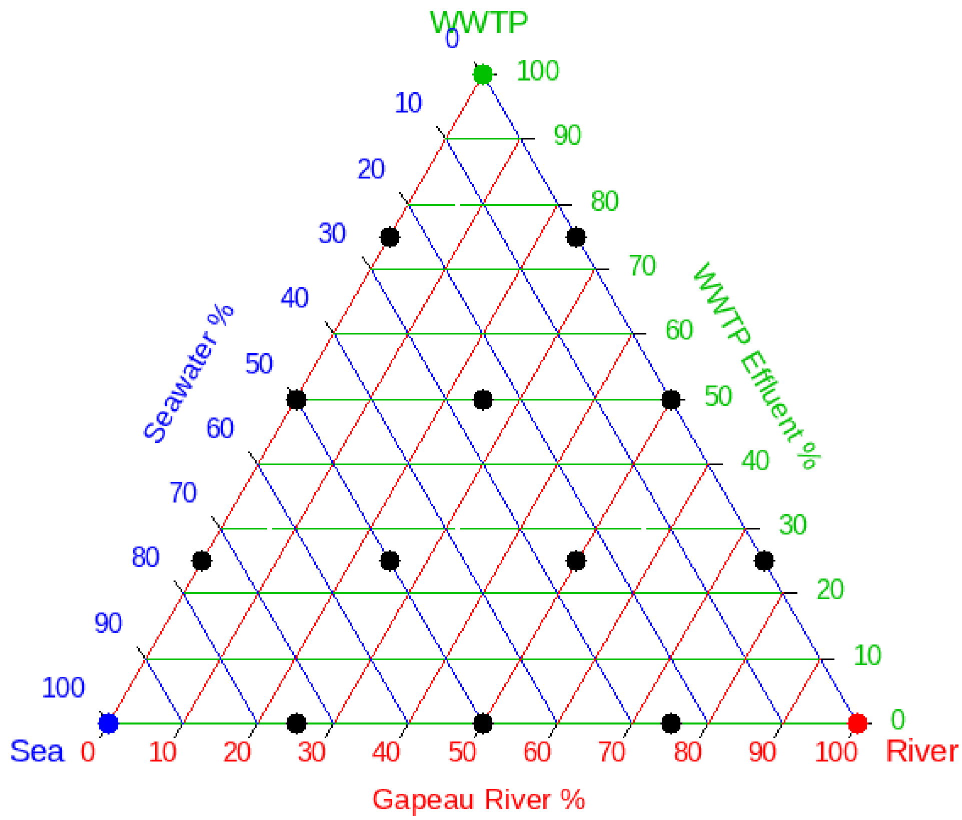


Figure 1: Ternary plot showing the proportion of three water sources: Gapeau river water (River), wastewater treatment plant effluent (WWTP), and Seawater (Sea). Each point on the plot represents a unique mixture that contains precisely the percentage indicated by the corresponding number on the ternary axes. For example, a point located at 50% River, 25 % WWTP effluent, and 25% SW represents a mixture that contains 50% Gapeau river water, 25% La Crau city WWTP effluent, and 25% seawater at Hyeres city. The plot provides a visual representation of the relative contribution of each water source in the mixtures studied. Source: [1, 2].

Four sites were chosen in the estuary mixing zone, one site was selected after the confluence of the Gapeau River and the tributary Réal Martin, and another site was chosen 500m upstream of La Crau WWTP. The GPS coordinates of the sampling sites along the Gapeau river is presented in Table 2, and they are shown in Figure 2. Fourteen samples were collected along the Gapeau river, from upstream of the tidal barrier dam to the estuary and the sea, during two cruises in August 2016. The sampling procedures involved using 60 mL bottles that were rinsed previously with deionized water and then rinsed with river water three times to ensure a representative sample. The first sample was taken using a one-liter bottle and handle stick to ensure homogeneity. The samples were taken from the top surface water layer (i.e. 50cm to 70cm water depth). The samples were collected at the same time of day then transported and stored under controlled conditions during each sampling campaign. Due to budget and time constraints, we were unable to collect replicate samples at each location. The exact dates of the sampling campaigns can be found in Table 1. Photos of the geographical sampling campaigns are shown in Figure 4.

Sampling Campaign	Date
1st one	August 19, 2016
2nd one	August 22, 2016

Table 1: Dates of sampling.

This table shows the dates of the sampling campaigns.

N°	Latitude	Longitude
01	43.151325	6.081343
02	43.145467	6.093158
03	43.152044	6.120395
04	43.15220	6.127978
05	43.149764	6.147565
06	43.146901	6.149686
07	43.131293	6.164386
08	43.125625	6.171226
09	43.121489	6.177349
10	43.120367	6.179271
11	43.118376	6.181620
12	43.114503	6.187077
13	43.111305	6.192815
14	43.103346	6.177326

Table 2: Latitude and longitude GPS coordinates of sampling sites.

This table shows the GPS coordinates of the sampling sites.

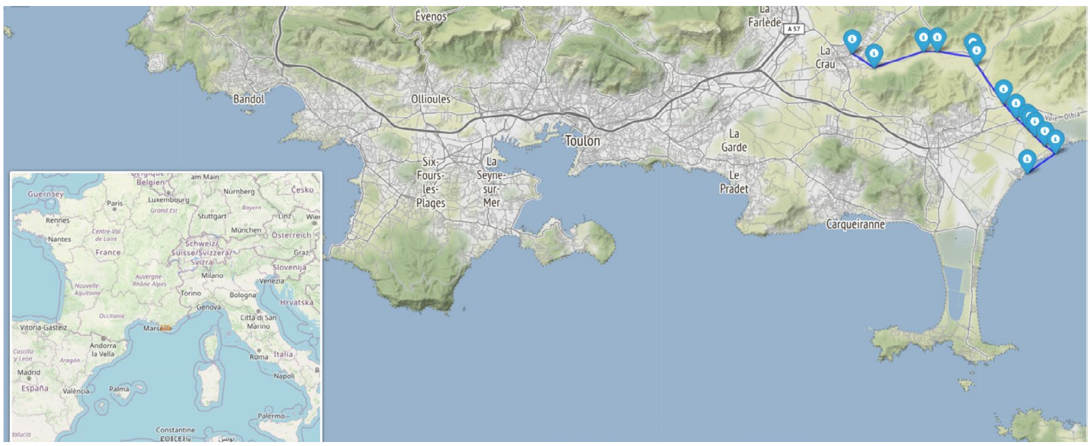


Figure 2: Sampling locations along the Gapeau river. The sampling locations span from upstream of the La Crau city wastewater treatment plant to the river discharge point in the sea at Hyeres city.

2.3. Measurement of Electrical Conductivity (EC) and pH in Geographical Water Samples Using YSI EXO Multiparameter Sonde

Electrical conductivity (EC) and pH were measured in situ using a YSI EXO multiparameter sonde at a depth of 1 cm of each collected sample from each sampling site from the Gapeau River. The YSI EXO model provided pH and EC measurements, and the brand and model used was YSI EXO2 with a pH and conductivity (EC) probe. The sonde was calibrated using a 4.01 pH buffer solution and a 12.88 mS/cm KCl solution, respectively. The pH meter was calibrated by immersing the probe in the buffer solution for 10 minutes prior to measurement, and the EC probe was calibrated using a 12.88 mS/cm KCl solution. The measurements of pH and EC were automatically temperature-corrected using a built-in temperature sensor and the manufacturer's algorithm. The measurements were taken at all samples from the sampling locations along the Gapeau River, from upstream of the La Crau city WWTP to the coastal waters at the Gapeau River mouth.

2.4. Analysis of Fluorescence Properties of Geographical Samples Using 3D Excitation Emission Matrix (EEM) Spectroscopy Coupled with Parallel factor analysis (PARAFAC).

After each sampling campaign along the Gapeau river, 3D fluorescence spectroscopy was performed on the samples to obtain the excitation emission matrix spectra (EEMs). Samples were collected from the sampling sites listed in Table 2 and transferred to a 16-cuvette sample holder without any filtration treatment. The cuvettes used in the study were cleaned with 10% nitric acid and deionized water before use to ensure accuracy in the measurements. The fluorescence intensity was measured using a Hitachi F-4500 spectrofluorometer at room temperature, with a PMT voltage of 700 V. The excitation emission matrix of fluorescence spectroscopy 3D EEMs were measured by scanning excitation wavelengths between 200 nm and 400 nm at 5 nm increments and emission wavelengths between 220 and 420 nm at 5 nm intervals, with a scan speed of 2,400 nm/min. The effect of particles on the fluorescence signal was investigated by filtering the samples during the second sampling campaign on August 22, 2016. The EEM datasets were analyzed using the Parallel Factor Analysis (PARAFAC) algorithm to decompose them into unique individual fluorescence components [20,21]. A 25 nm cutoff filter was used to remove Raman/Rayleigh scattering from the EEMs datasets [22]. The appropriate number of PARAFAC components was chosen and validated according to the CONCORDIA score, split-half analysis, and other methods [23-25].

3. Results and Discussion

The central research objective of this work was to investigate the impact of the wastewater treatment plant discharge on FDOM in the Gapeau River, and more specifically, to explore the geographical and spatial variations in FDOM content from upstream the La Crau WWTP to the coastal waters at the river mouth.Additionally, we wanted to evaluate the impact of anthropogenic inputs from effluent wastewater and explore the hypothesis that sunlight-driven photodegradation occurs to anthropogenic FDOM from the WWTP. The geographical sampling campaigns conducted aimed to investigate the effect of the mixing process on the fluorescence signal of the Gapeau river. The results showed that the fluorescence signal of the river was influenced by both

Figure 3: Schematic diagram of the experimental setup for EEMs measurements

	August 19, 2016	August 22, 2016
Temperatures	25řC/29řC	26řC/28řC
Precipitations	0 mm	0 mm

Data source: `https://www.historique-meteo.net/`

Table 3: Weather conditions during sampling.

natural and anthropogenic sources of dissolved organic matter (DOM). The fluorescence components identified by PARAFAC analysis were used to distinguish between the different sources of DOM. The results also showed that the fluorescence signal of the river was affected by photodegradation and biodegradation processes along its way to the sea. Overall, the study provides valuable insights into the variablity of FDOM along the Gapeau river and highlights the possiblity of using this geographical variability ,affected by sunlight-driven photodegradation, to predict the mixing composition of Gapeau river hence enabling the quantification of anthropogenic sources of FDOM (data not published).

3.1. Weather Conditions During Geographical Sampling Campaigns

During our sampling campaigns, we recorded the weather conditions that may have influenced the fluorescence signal of the Gapeau river.The weather conditions during the geographical sampling campaigns conducted in August 2016 are summarized in Table 3. It is noteworthy that the Hyères city region experienced no rainfall during the month of August, with precipitation values recorded at 0 mm.The average temperature during August in Hyères city is around 25 řC, as reported by meteo-france. Some photos taken during the two geographical sampling campaigns are shown in Figure 4. These weather conditions could have contributed to the degradation of FDOM in the river, particularly in the sections downstream of the La Crau WWTP. We did not conduct any statistical tests to evaluate the significance of the weather variables on the fluorescence signal, however, we acknowledge the potential influence of weather conditions on our study's research question. Specifically, the absence of rainfall or high temperatures may have affected the fluorescence signal of the Gapeau river. Future works can address the potential impacts of weather conditions on our results.

Figure 4: Photographs of the geographical sampling campaigns conducted in August 2016, showcasing the specialized sampling rod used during the campaigns and the tidal barrier dam in Gapeau river estuary.

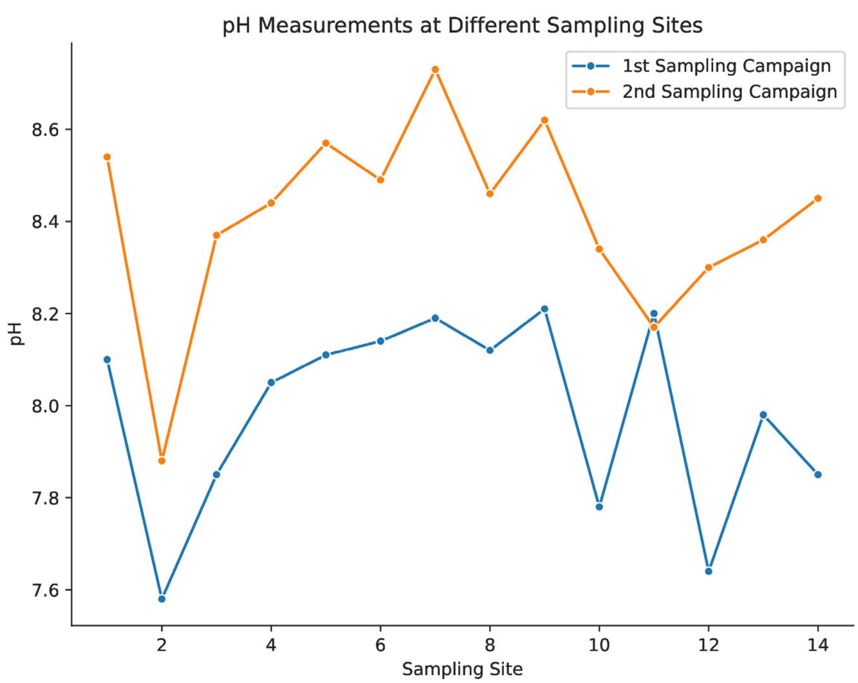


Figure 5: pH variation along the Gapeau river with sampling sites for the first (August 19, 2016) and second (August 22, 2016) geographical sampling campaigns.

3.2. pH Variation Along the Gapeau River

The pH values was measured at each sampling site along the Gapeau river, from the upstream side of the La Crau wastewater treatment plant to the l'Ayguade market on the beach in the city of Hyères where the river meets the sea. The pH values were plotted against sampling sites, as demonstrated in Figure 5.

pH is a key parameter that can affect the fluorescence signal of natural waters, and its measurement can help in understanding the biogeochemical processes in the Gapeau river. It has been shown that pH values influence the solubility and speciation of different chemical species in the water, such as dissolved organic matter (DOM), heavy metals and nutrients, which can affect the fluorescence properties of River water [26-28]. An example of factors that can significantly affect DOM fluorescence is its molecular weight and composition, as well as pH levels. Lower pH levels can cause a decrease in fluorescence intensity because of the protonation of functional groups [29,30]. Figure 5 shows the pH values measured during two sampling campaigns along the Gapeau river, with slight variations observed between the two campaigns. While the pH values during the second sampling campaign were slightly more basic than during the first campaign, the source of this increase remains unclear and requires further investigation. Additionally, the pH values at the second point in both graphs in Figure 5 were lower, indicating the discharge of La Crau WWTP into the Gapeau river. The pH values recorded after this discharge point increased due to the mixing process. The slight fluctuations in pH values observed at around 12 km (the tidal

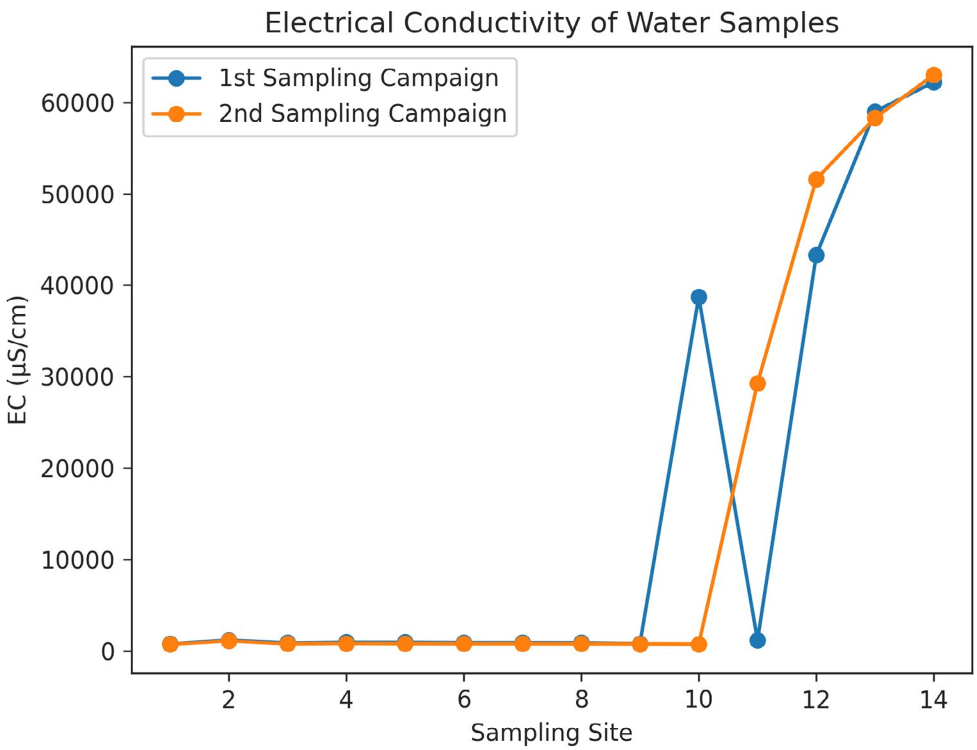


Figure 6: Variation in electrical conductivity (EC) in S/cm with sampling sites during the first (19-August2016) and second (22-August-2016) geographical sampling campaigns.

Barrier dam may be attributed to the mixing of freshwater and seawater, as well as the intrusion of seawater into the river. The pH measurements of the wastewater treatment plant discharge were consistent with the overall trend of decreasing pH values observed downstream from the discharge point.

3.3. Electrical Conductivity Variation Along the Gapeau River

The fluorescence signal of the Gapeau river can be influenced by Electrical conductivity since this physicochemical parameter can affect the properties of dissolved organic matter (DOM) (e.g. its solubility) in water [31]. The electrical conductivity (EC) of water samples collected from the Gapeau river during two sampling campaigns in August 2016 was measured and plotted against distance in kilometers. The data indicate a consistent trend in EC values from 0 to 12 km, with significant variations observed at the wastewater treatment plant discharge point and downstream from the tidal barrier dam.

The electrical conductivity of water samples collected from the Gapeau river during two geographical sampling campaigns conducted on August 19th and August 22nd, 2016, is presented in Figure 6. The EC values at the wastewater treatment plant discharge point were significantly lower than those observed downstream from the tidal barrier dam, likely due to dilution and mixing with freshwater.The data indicate a consistent trend in electrical conductivity from 0 to 12 km, except for the second point where the EC values were 1,177 µS.cm-1 and 1,090 µS.cm-1, respectively. These

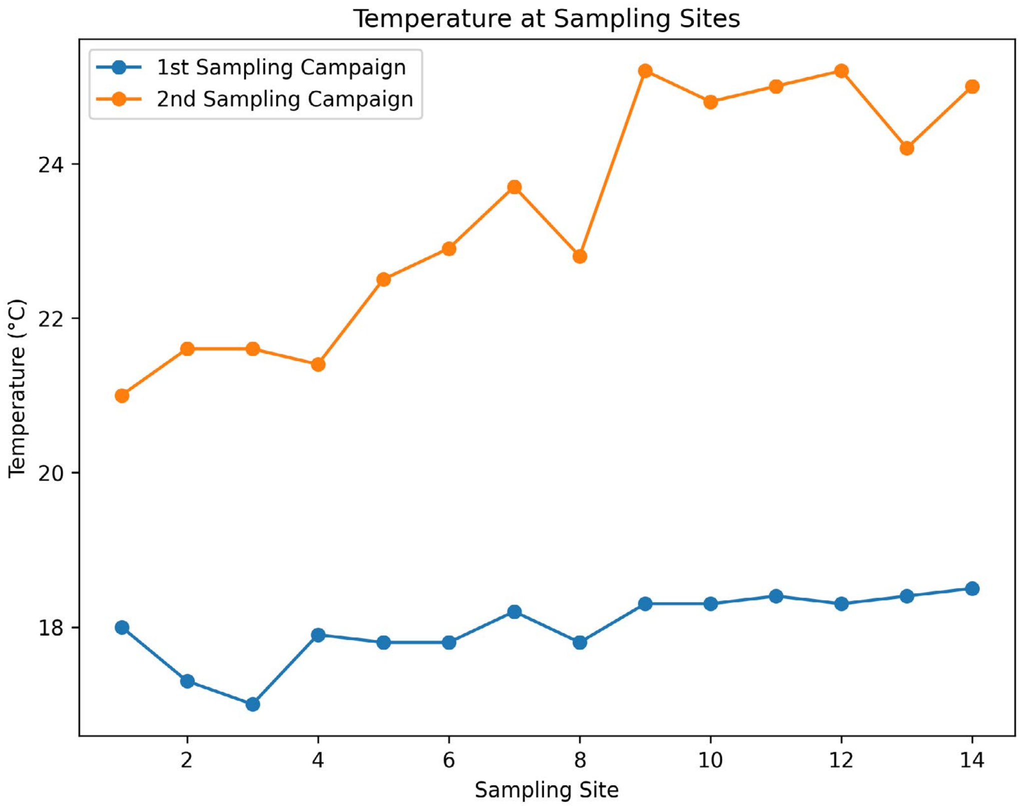


Figure 7: Water Temperature () Variation Along the Gapeau River During Two Sampling Campaigns

Values correspond to the discharge from the wastewater treatment plant, which was significantly lower than the electrical conductivity values found after the tidal barrier dam, located approximately 12 km from the starting point of sampling. The first point sampled during both campaigns was located approximately 500 m to 1 km upstream La Crau wastewater treatment plant and represents river water. From this point, the electrical conductivity gradually increased from 30,000 to 40,000 S.cm-1 and eventually reached about 60,000 S cm-1, which represents the electrical conductivity of seawater at lAyguade market on the beach. The increase in electrical conductivity after the tidal barrier dam is attributed to the intrusion of seawater into the Gapeau river mouth. The tidal barrier dam was constructed to limit the advancement of brackish water into river water and prevent seawater intrusion.

3.4. Water Temperature Variation Along the Gapeau River

Water temperature (in Celsius) was measured along the Gapeau River using YSI EXO multi-parameter sonde, which was also used to measure the other physicochemical properties like pH and electrical conductivity in situ. The data indicate a consistent trend in water temperature from the upstream side of the La Crau wastewater treatment plant to the l'Ayguade market on the beach in the city of Hyères where the river meets the sea for the 1st campaign and slight icrease of water temperature in the 2nd campaign.

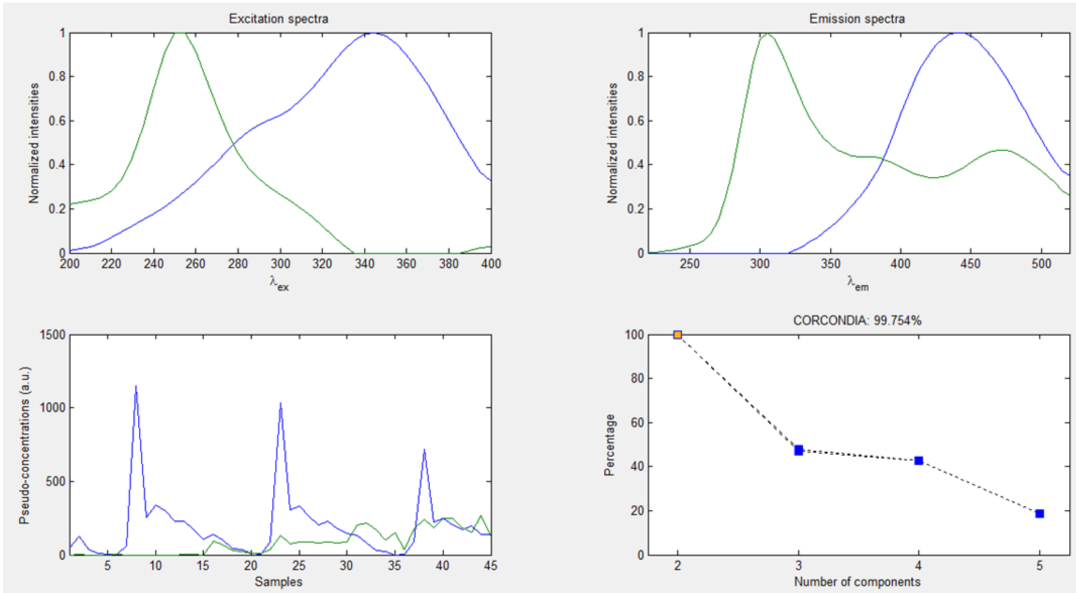


Figure 8: The Concordia graph is located in the lower right-hand corner, with the value of the concordance test for two PARAFAC components displayed above the graph (99.75%). Additionally, the loadings of the two PARAFAC components are presented.

Figure 7 illustrates the variation of water temperature (in Celsius) as a function of distance in km for the two geographical sampling campaigns. The data reveal a consistent trend in water temperature from the first to the second sampling campaign, with a slight decrease of only one degree Celsius during the first campaign and a significant increase of approximately 4 deg. Celsius during the second campaign. For the first sampling campaign, the mean temperature was 17.94 +/- 0.38 deg. Celsius, with very little variation in temperature, decreasing by only one degree Celsius and returning to its constant value. The sampling was carried out from 11:07 am to 03:30 pm. In contrast, during the second sampling campaign on August 22nd, 2016, the temperature increased with distance in kilometers, showing an increase of approximately 4 deg. Celsius. The observed increase in water temperature in the Gapeau River is in line with the air temperature range reported on https://www.historique-meteo.net/france/ provence-alpes-c-te-d-azur/hyeres/2016/, which represents the temperature of the surrounding air rather than that of the river water. The observed temperature variations may be related to the influence of solar radiation on the river, which could impact the rate and extent of photodegradation of FDOM. According to [16,17], temperature can affect the rate and extent of photodegradation of CDOM, which includes FDOM. Further research is necessary to gain a better understanding of the correlation between water temperature and FDOM photodegradation in the Gapeau river.

3.5. Results of EEMs/PARAFAC Analysis

EEMs dataset consisting of the 14 samples * 2 sampling campaigns* 2 filteration sate = 56 EEMs were collected using Hitachi spectrophotometer and deconvulted using PARAFAC Analysis into the underlying fluorescent components in order to track each fluorescence signal separately and to track the impact of WWTP on FDOM in the Gapeau River. The results of PARAFAC analysis are presented in the following figures.

CORCONDIA analysis was conducted to evaluate the core-consistency of the PARAFAC model on the EEMs datasets from the geographical sampling campaigns along the Gapeau River [23]. The analysis revealed a drop in core-consistency value from 100% to less than 20% between two and five PARAFAC components, respectively, which is much more lower than the acceptable threshold of 60%. However, the analysis showed a value of 99.75% for two components, indicating that a two-PARAFAC components model was appropriate, as shown in the lower right-hand graph of Figure 8.

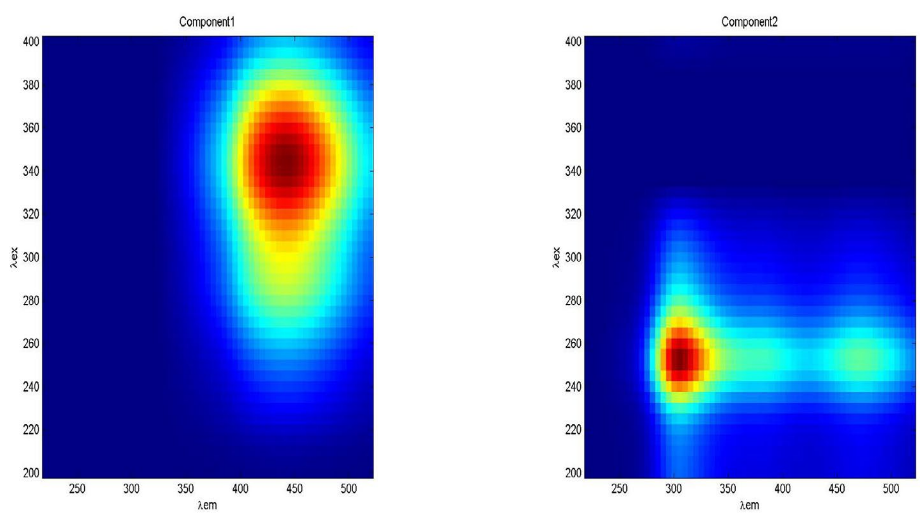
The lower left-hand graph of Figure 8 shows the pseudo-concentrations of the PARAFAC components in the EEMs datasets of samples of the geographical sampling campaigns. The peak in the graph is attributed to the pseudo-concentrations of PARAFAC components in WWTP sample EEMs during the second sampling campaign on August 22nd, 2016. This peak is lower in comparison to the first and second peaks in the same graph, indicating that it is related to the filtered samples EEMs of the same sampling campaign. This result is consistent with the results of the geographical variation of PARAFAC C1 component shown in Figure 10. These findings provide valuable insights into the sources and composition of organic matter in the water samples, which can be used to better understand the water quality of the Gapeau River.

Component	λEX/λEM (nm)	Description and References in literature
C1	345/440	- Peak C [13, 32]: Terrestrial origin - degradation of plant and animal debris (humic substances); Component 4 [24]: terrestrially derived organic matter
C2	255/305 (470)	- Peak B [13]: Tyrosine-like component; Component 5 [33]; Component 7 [34]

Table 4: Descriptions of the PARAFAC components, with comparison to relevant literature.

PARAFAC modeling was conducted on the EEMs datasets of the two geographical sampling campaigns along the Gapeau River trajectory till sea (August 19th and 22nd, 2016) after the removal of the 1st and 2nd order Rayleigh and Raman Scattering. The analysis successfully decomposed two components, which are presented in Figure 9 as contour plots with their corresponding loadings for both excitation and emission wavelengths.

The first fluorescent PARAFAC component, C1, showed an excitation maximum at 345 nm and an emission maximum at 440 nm, with a range of excitation-emission wavelengths (Ex=280-400 nm, Em=400-480 nm). Previous studies have associated this component with UVA humic-like fluorescent PARAFAC component and Peak C and peak α [8,35,36]. Additionally, the C1 component (Ex=280-400 nm, Em=400-480 nm) has been linked to terrestrial, anthropogenic, and agricultural sources [24,37]. The presence of C1 in the river indicates a considerable influx of terrestrial organic matter, as this humic-like component is typically associated with dissolved organic matter originating from plant material and soil organic matter decomposed in the watershed. It is likely that C1 is derived from the decomposition of these materials, highlighting the importance of terrestrial sources in the river's dissolved organic matter composition.



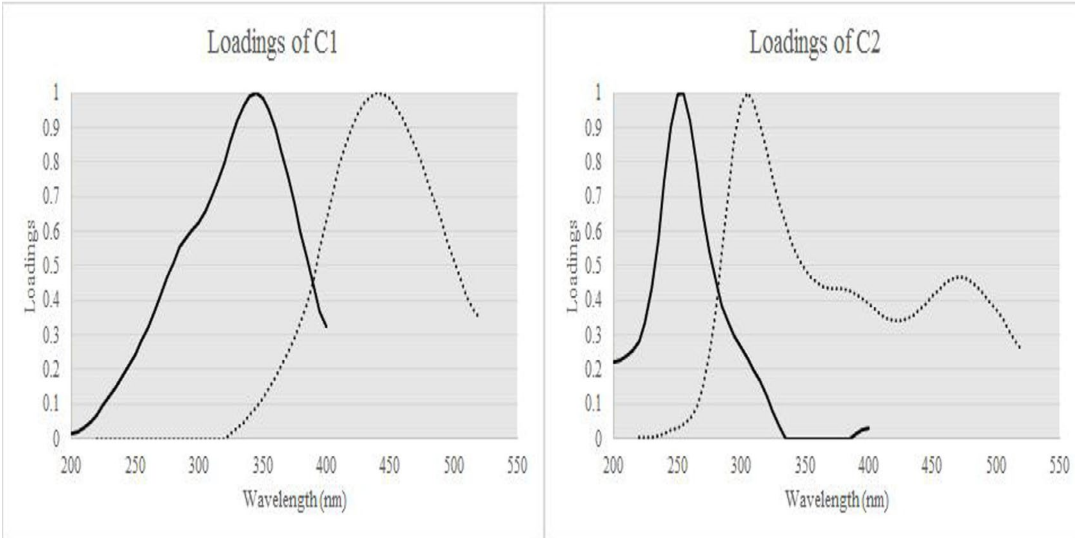


Figure 9: PARAFAC components (C1 & C2) with their respective spectral loadings of the excitation and emission wavelengths. The excitation and emission loadings are depicted as solid and dotted lines respectively.

The second fluorescent PARAFAC component, C2, showed an excitation maximum at 255 nm and an emission maximum at 305 nm, with a minor emission peak at 470 nm and a range of excitation-emission wavelengths (Ex=230-290 nm, Em=290-500 nm). Previous studies have associated this component with a tyrosine-like fluorescent component [33,34]. Additionally, this protein-like fluorescent component contains Peak B [13,32]. These findings provide valuable insights into the sources and composition of organic matter in the water samples, which can be used to better understand the water quality and ecosystem dynamics of the Gapeau River. C2 is a protein "tyrosine"-like component found in the river's dissolved organic matter, and it is usually associated with microbial sources. The presence of C2 indicates that there is a substantial input of microbial organic matter to the river, likely from the decomposition of microbial biomass within the river.

These findings have significant implications for environmental studies, particularly in pollution tracking and remote sensing of coastal waters. The fluorescence signal of C1 and C2 can be tracked to identify the sources of dissolved organic matter in the river and monitor the transport of pollutants downstream. Remote sensing of the fluorescence signal can also be used to assess the health of coastal waters and detect harmful algal blooms. In summary, these results provide valuable insights into the biogeochemistry of the Gapeau River and have essential applications in environmental monitoring.

3.6. Geographical Variation of PARAFAC Components

FDOM is a ubiquitous and critical constituent of coastal and riverine environments, and understanding its origins and makeup is vital for evaluating the condition and operation of these ecosystems [22, 38]. One significant source of FDOM in rivers is the discharge of WWTP [39-41]. Our research aimed to enhance our understanding of the effects of such discharge on the Gapeau River. However, the present study was conducted over a relatively short period of time (two sampling campaigns in August 2016) which is not enough to fully understand the seasonal and annual geographical and spatial variations in FDOM content and the impacts of wastewater treatment plant discharge , and accordingly, longer-term studies may be necessary.

3.6.1. Geographical Variation of PARAFAC Component 1 (C1)

Figure 10 illustrates the spatial variation of the first PARAFAC component (C1), which is associated with humic-like fluorescence, for the first (August 19th, 2016) and second (August 22nd, 2016) geographical sampling campaigns along the Gapeau River. It should be noted that the samples from the second campaign were filtered, and other EEM-PARAFAC methods were employed. The observed variations in the C1 component as a function of sampling sites can provide valuable insights into the sources and composition of organic matter in the water samples, thereby improving our understanding of the water quality dynamics in the Gapeau River.

As shown in Figure 10, the first PARAFAC component (C1) exhibits a peak at approximately less than 2 km, corresponding to the wastewater treatment plant discharge in the Gapeau River. This peak indicates a significant input of humic-like fluorescence (C1) from the wastewater treatment plant to the already existing background fluorescence of the river. The contribution of C1 decreases with distance in the three graphs in this figure, corresponding to the first and second (non-filtered, filtered dataset) sampling campaigns. This decrease in C1 contribution could be attributed to photodegradation

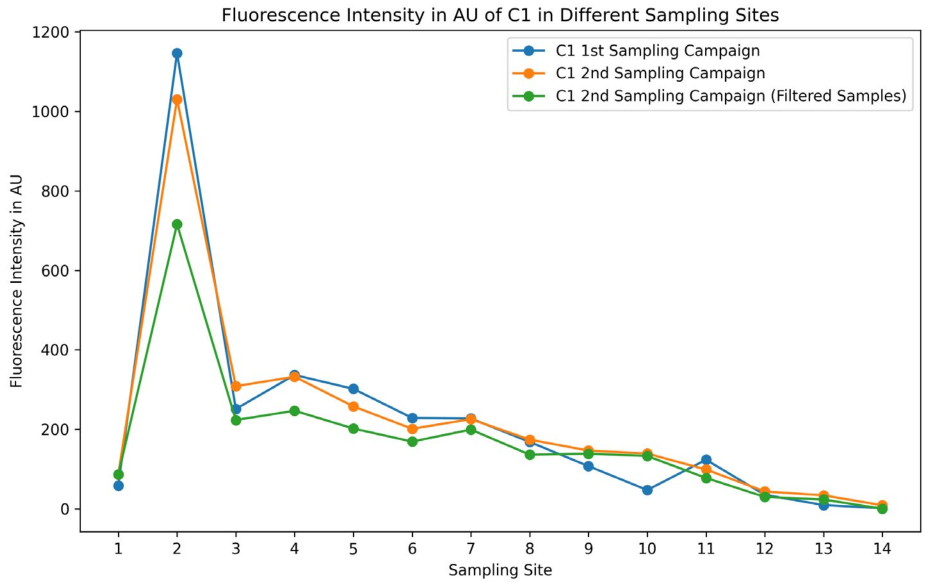


Figure 10: Geographical variation of PARAFAC component C1 along the trajectory of Gapeau river as a function of sampling sites.

and biodegradation processes, as well as the mixing process, which leads to dilution.These results suggest that photochemical processes are degrading the input of terrestrial organic matter to the river.

The pattern of graphs in the second sampling campaign (August 22nd, 2016) is similar, except for a lower contribution of C1 in the case of filtered samples, indicating the effect of filtration on the fluorescence contribution of C1 and suggesting that part of C1 contribution is due to particulate organic matter. The study's results suggest that the C1 contribution is stable from one day to the next, indicating that the processes affecting C1 are consistent and that there is an equilibrium in terms of fluorescence. These observations can help researchers better understand the sources and composition of organic matter in the Gapeau River's water samples and contribute to improved water quality management and ecosystem dynamics.

The study's results shed light on the geographical and spatial differences in fluorescence signal within the Gapeau River and the influence of anthropogenic inputs from wastewater treatment plants on the river's fluorescent dissolved organic matter (FDOM) content, which aligns with the primary research goal outlined in the manuscript.

The results suggest that the wastewater treatment plant is a significant source of humic-like fluorescence in the river, with the first PARAFAC component (C1) exhibiting a peak at less than 2 km downstream of the treatment plant discharge. The contribution of C1 decreases downstream with distance from La Crau WWTP discharge, indicating that photodegradation, biodegradation processes, and mixing lead to dilution of the wastewater treatment plant's FDOM input. These findings support the hypothesis that sunlight-driven photodegradation occurs to anthropogenic FDOM from the WWTP. The findings also suggest that photochemical processes are an important factor in degrading the input of terrestrial organic matter to the river, and this could be taken into account in future water quality monitoring. This is in agreement with a previous study which found similar results [42].

The study also highlights the effects of filtration on the fluorescence contribution of C1, suggesting that part of C1 contribution is due to particulate organic matter. Moreover, the stable evolution of C1 contribution from one day to the next indicates that the processes altering C1 contribution are stable and ultimately result in an equilibrium in fluorescence.

The investigation focuses only on the impact of effluent wastewater from the La Crau city wastewater treatment plant on the river's FDOM content. Other potential sources of FDOM, such as agriculture and urban runoff, were not considered. Future research should aim to explore the contribution of other sources of FDOM to the Gapeau River and their potential impact on the river's water quality .

3.6.2. Geographical Variation of PARAFAC Component 2 (C2)

The fluorescence signal from the protein "tyrosine"-like component is a crucial indicator of the presence of microbial organic matter in rivers and other aquatic systems. Its use can help track the biodegradation of dissolved organic matter (DOM) due to microbial activity. In the context of the Gapeau River, the analysis of this fluorescence signal provides significant insights into the river's biogeochemistry and has important implications for environmental monitoring and management. Figure 11 shows the geographical variation as a function of distance in kilometers (km) of the second PARAFAC component (C2), which is associated with protein "tyrosine-like" fluorescence, for the first (August 19th,

2016) and second (August 22nd, 2016) geographical sampling campaigns. It should be noted that the samples from the second campaign were filtered, and other EEM-PARAFAC methods were employed. These results provide valuable insights into the sources and composition of organic matter in the water samples and can be used to better understand the water quality of the Gapeau River.

The first sampling campaign indicated that the values of C2 contribution were zero except for the distance range of 8-13 km (Sampling Sites from 1 to 10), which suggested the presence of protein-like fluorescence before the tidal barrier dam, possibly due to microbial or biological activity. It should be noted that no protein-like signal was observed at the WWTP discharge point (2 km) during this campaign. At first glance, the variation of C2 (protein tyrosine-like fluorescence) for the first sampling campaign appeared to be chaotic. During the second sampling campaign on 22 August 2016, the filtration process resulted in an increase in the values of C2 contribution with distance, indicating the presence of an inner filter effect due to particulate matter. The filtered dataset showed a decreasing trend of C2 contribution with distance after the tidal barrier dam, while the non-filtered dataset showed an increase in C2 contribution (protein-like fluorescence) at the second point, corresponding to the sampling point of the wastewater treatment plant discharge point, followed by a decrease and approximately constant fluctuation until 12 km, which is the tidal barrier dam, after which it decreased rapidly until reaching the sea water at lAyguade market on the beach of Hyères city.

3.6.3. Geographical Variation of PARAFAC Component ratio (C1/C2)

Figure 12 illustrates the variation in the ratio between the first and second PARAFAC components (C1/C2) as a function of distance in kilometers (km) for the first (19 August 2016) and second (22 August 2016) geographical sampling campaigns. It should be noted that the second campaign employed filtered samples and different EEM-

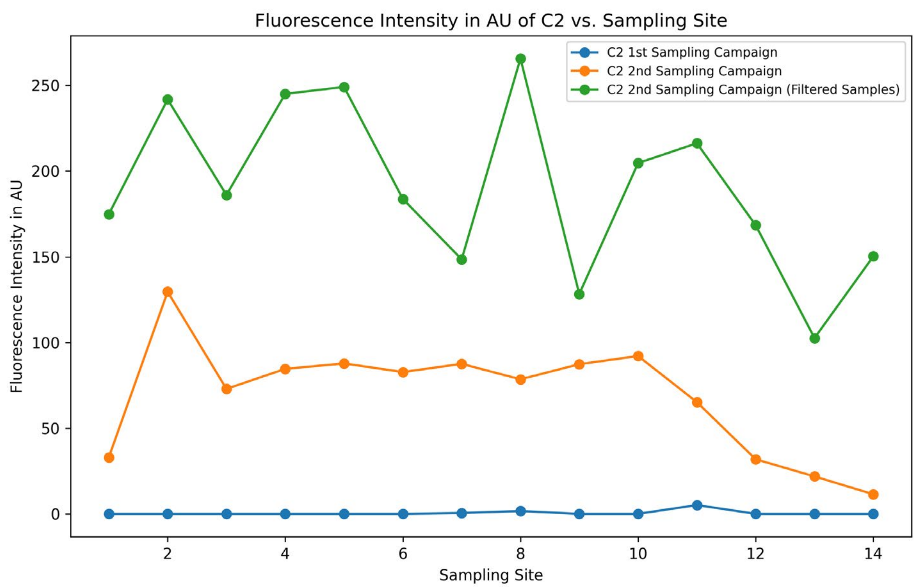


Figure 11: Geographical variation of the contribution of C2 along the trajectory of Gapeau river as a function of Sampling sites, starting from upstream La Crau WTTP all the way down to the sea sampling site at l'ayguade in Hyeres city.
PARAFAC analysis.

The study's first sampling campaign, conducted on August 19th, 2016, found that the C1/C2 ratio exhibited zero values before 8 km and after approximately 13 km, with a peak observed at 10 km, followed by a rapid decrease to zero at 12 km. This peak at 10 km suggests that the humic-like fluorescence (C1) dominates over the proteinlike fluorescence (C2) in this region. In the second sampling campaign, conducted on August 22nd, 2016, the C1/C2 ratio showed a similar trend as the variation of C1 contribution (both non-filtered and filtered dataset) with distance, indicating a higher proportion of humic-like fluorescence (C1) compared to protein-like fluorescence (C2). It should be noted that, the C1/C2 ratio decreased with distance, indicating an increase in the protein-like fluorescence (C2) of biological origin, rather than a dilution effect. This finding can be compared to previous one and the work in who found similar results in different aquatic environments [43,44]. This suggests a bacteriological production of protein-like fluorescence with distance, consistent with the predominance of fluorescence signals of biological origin observed with distance. Furthermore, the decrease in C1 with distance, as shown in Figure 9, is also consistent with this finding.

4. Conclusion

This study used excitation-emission matrices (EEMs) of 3D fluorescence spectroscopy coupled with PARAFAC analysis to investigate the geographical variations of fluorescence signal along the trajectory of the Gapeau river and track the impact of the discharge of anthropogenic DOM represented by La Crau city WWTP effluent on this signal. The results indicate a decrease in fluorescence intensity of C1 and C2 along the river's trajectory. This finding suggests that photodegradation of FDOM occurs due to sunlight exposure, which supports the null hypothesis.

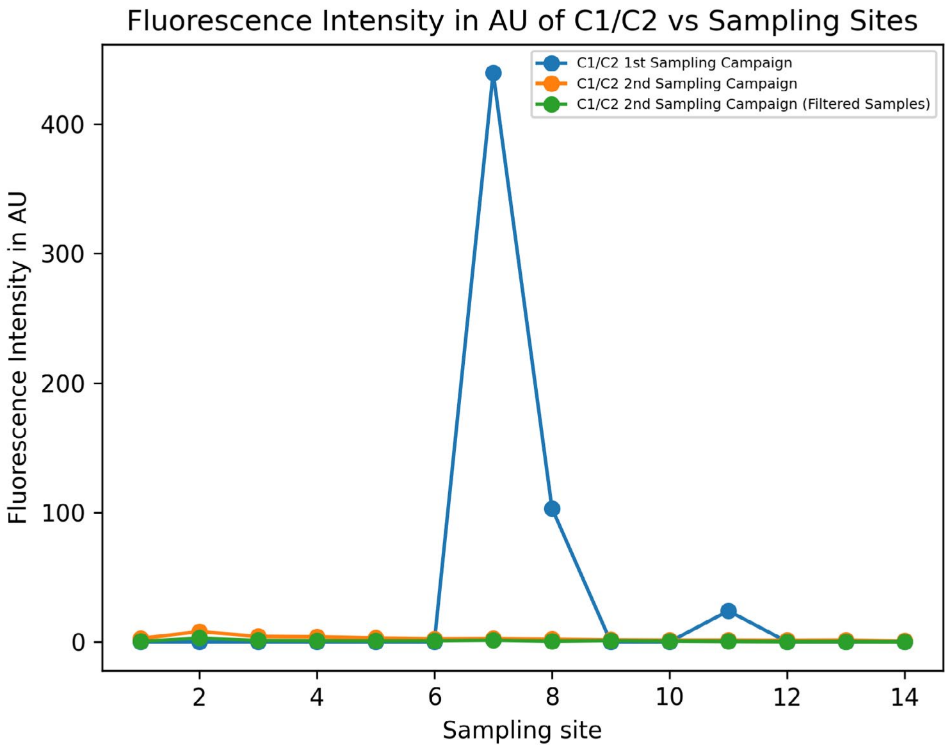


Figure 12: Variation of C1/C2 ratio along the trajectory of Gapeau river as a function of sampling sites from the 1st sampling site upstream La Crau WWTP all the way down to the sea sampling site at l'ayguade in Hyeres city. to sunlight exposure, which supports the null hypothesis.

These findings are used (data not published) in estimating the mixing composition of riverine samples from a specific region using a multilinear regression model developed elsewhere [1, 2]. This study is a real-world application for our previous studies [1, 2]. However, further research is required to study the spatial/geographical variations in different seasons to validate this hypothesis.

Overall, this study addresses the research gap on the geographical variations of FDOM in the Gapeau river starting upstream La Crau city WWTP discharge till the coastal zone where river meets the sea. The present work provides a crucial contribution to the field of water quality management, and the outcomes of this study can be utilized for further research and practical applications.

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Conflict of interest

The authors declare no conflict of interest

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