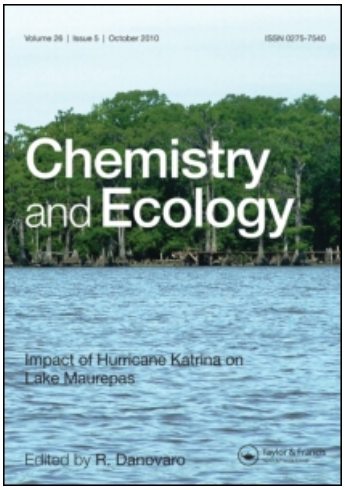

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Fluorescence excitation-emission matrices showed the spectral signature of dissolved organic matter (DOM) downstream in the River Têt at all seasons corresponding to humic substances with maximum fluorescence emission $\lambda em = 420–460$ nm for excitations of $\lambda ex = 340–360$ nm and the occasional presence of tryptophan, tracer of anthropogenic pressure, spectroscopically identified by $\lambda ex/\lambda em = 310/350$ nm. A factorial discriminant analysis, performed using the parameters selected ($\lambda ex/\lambda em$ pairs of wavelength), succeeded in a better discrimination of seasons than stations, and clearly showed the presence of two fluorophores. Fluorophore 1, with two absorption bands: $\lambda ex = 260–320$ nm and 330–390 nm for $\lambda em = 440–500$ nm, and Fluorophore 2, with one absorption band: $\lambda ex = 300–360$ nm for $\lambda em = 410$ nm, which are attributed to humic acid (HA) and fulvic acid (FA), respectively. Variations of DOM relative contents downstream in the River Têt, according to seasons and stations, showed high amounts of DOM (pedogenic character) along the river in the humid period, with the highest value obtained in the mountain section. In contrast, in summer, the DOM contents were low (aquagenic character). Moreover, DOM presents a relatively constant composition with a percentage of FA ranging from 40% at the mountain station to 48% at the mouth river, whatever the season.

Keywords: riverine water; natural dissolved organic matter; humic substances; tryptophan; fluorescence; emission-excitation matrices; factorial discriminant analysis

1. Introduction

Enhanced by the collective awareness of climatic change and global warming, the problems of water quality are a great challenge for many countries. In order to prevent water pollution, to promote water sustainable use, to protect their environment and to improve the conditions of aquatic ecosystems, the European Community Council Directive 2000/60/EC requires Member States to draw up management plans for each water use (surface waters, ground waters, transient and coastal waters), based on an assessment of the sources of contamination that are likely to affect water quality.

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Water quality determines its suitability for a particular use based on selected physical, chemical, and biological characteristics. Natural processes and human activities may affect water quality. At present many constituents (nutrients, trace elements, xenobiotics) considered not harmful to human health can be determined by complex and painstaking methods with increasing accuracy.

All of the natural organic compounds are part of the so-called humus [1], i.e. dissolved organic matter (DOM) which can be divided into two groups: (i) nonhumic substances, and (ii) humic substances [2], and can be considered the most important parameter in aquatic environments [3].

Lixiviation and leaching processes on the watershed explain the presence of DOM in water bodies, i.e. humic substances (HS), which can be functionally separated into humic acids (HA), fulvic acids (FA) and humin (or kerogen, H) according to their solubility in alkali (whole HS and HA, apart from H), and/or in alkali and acids (FA) [4].

This study concerns the dissolved fraction of DOM which could be of an allochthonous or pedogenic [5] origin, coming into riverine waters from organic horizons of the soils of the watershed, or an autochthonous or aquagenic (ibidem) origin from biological activity in the water column [6–8].

One of the great interests in studying aquatic humic substances is their ability to be physically or chemically associated with natural or anthopogenic pollutants in the input of wastewaters to rivers [9] or on coagulation techniques in water treatment [10].

The fluorescence technique provides specific qualitative information on fluorophores present in the organic matter. We selected this method in order to perform a rapid screening of DOM contents in aquatic environments and for its ability to detect directly on a raw filtered sample the presence of DOM overlaid with low levels of anthropogenic compounds. This fast, non-destructive, specific, sensitive and precise method has been widely applied to study DOM from different aquatic environments: sea water [11–13], estuarine water [14], marine sediment pore water [15–19], riverine water [20–23], lake water [24,25] and waste water [26].

Fluorescence spectrometry leads to a data set corresponding to the whole emission spectra for each excitation wavelength, referred to as excitation-emission matrice (EEM), which has been widely used for various water environments [27–34]. Three-dimensional fluorescence, or EEM, allows the description of the yellow-coloured water organic matter (gelbstoff or HS) or their HA and FA fractions [35,36]. Especially in wastewaters, protein-like substances were described through the fluorescence properties of aromatic amino acids, mainly of tryptophan [37].

The aim of the present work is to characterise DOM from a Mediterranean coastal river, the River Têt, by the fluorescence properties of its HA and FA fractions in order to check the hypothesis that these parameters could distinguish the seasons and the stations. This hypothesis will be tested by factorial discriminant analysis.

2. Materials and methods

2.1. *Study area*

The River Têt is the most important coastal river in the Pyrénées-Orientales region in southern France (Figure 1). Its watershed is located in the northern part of the eastern Pyrénées and it drains an area of about 1380 km^2. The River Têt and many of its tributaries originate high on the slopes of the Pyrénées. The River Têt flows about 80 km in a northeasterly direction and flows into the Mediterranean Sea near Canet-en-Roussillon.

The waters of the upper part of the Têt basin are influenced throughout the year by annual snowfall and maintain the major flow, whereas in summer the lower part of the stream does not contribute significantly to the flow of the mainstream Têt.

According to the topography (Figure 1), the Têt profile presents two regions: the upper one drains a very steep slope covered by the alpine and subalpine vegetation stages, and the lower

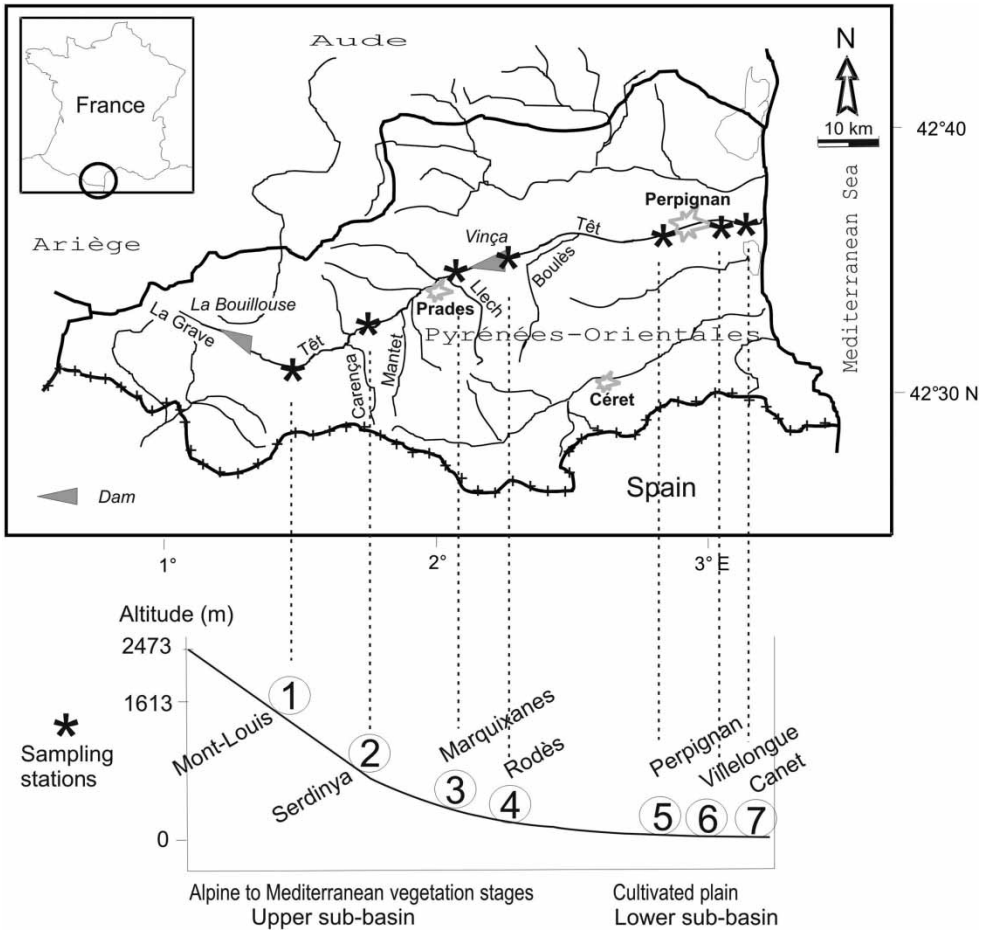

Figure 1. Location of the stations along the River Têt (Pyrénées-Orientales, France).

one corresponds to the mountainous and mediterraneous vegetation stages which gradually merge into the coastal vegetation.

The bioclimatic features of our sampling area and time-period can be clearly expressed by the graphical representation (Figure 2) called an 'Ombrothermic Diagram' [38] with the graphical conditions for Mediterranean bioclimates of Rainfall $= 2\times$ Temperature (°C). In these conditions and for the Mediterranean region (rainfall concentrated in Autumn–Winter), the intersection area between the rainfall and temperature lines represents the dry period. Figure 2 shows a long and characteristic period of dryness for Perpignan, extending from February–March and from April–August, while for Mont-Louis the dry period is limited to the month of August.

The station locations and numbers are shown in Figure 1: stations are listed according to the name of the closest village. Stations 1 and 2 are situated in the mountainous section of the river, stations 3 and 4 are situated in the upper part of the plain, respectively upstream and downstream of the dam of Vinça, station 5 is situated in the centre of the city of Perpignan, while stations 6 and 7 are situated respectively between Perpignan and further downstream, close to Canet-en-Roussillon. Pollution along the mainstream Têt is only of an anthropogenic nature and is concentrated downstream in the urban zones, which are essentially the city of Perpignan and the seaside resort of Canet-en-Roussillon.

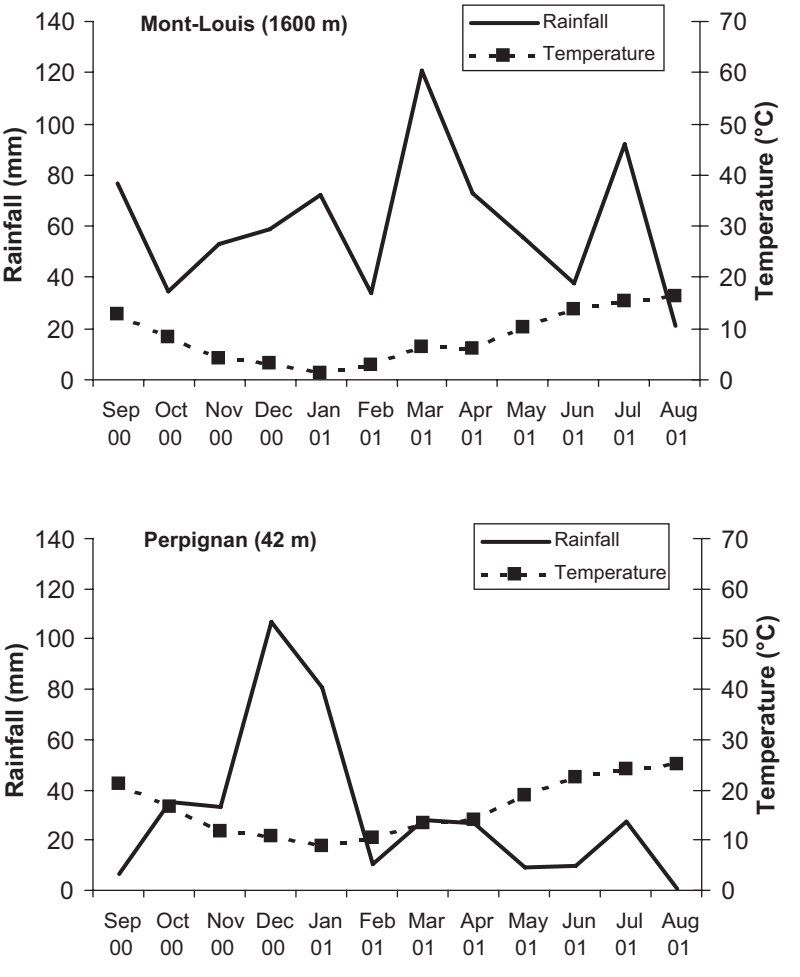

Figure 2. Ombrothermic diagrams of Mont-Louis (mountain section) and Perpignan (plain section).

2.2. *Sampling procedure*

Natural waters were collected along the River Têt in 2000 and 2001 during a sampling schedule, in October 2000 and January, April, June and August 2001, for all stations from the high altitude in Mont-Louis (station 1), Serdinya (station 2), Marquixanes (station 3), Rodès (station 4), to the low altitude in Perpignan (station 5), Villelongue (station 6) and Canet (station 7) (Figure 1). Water samples were collected seasonally in 10 L poly-ethylen jerrycans and stored immediately in a refreshed container before filtration on glass fiber filters (GF/F Millipore) previously heated at 450 °C during 4 h. Filtrates were stored in the dark, in a refrigerated room at 4 °C before spectral analysis was performed within 48 h after sampling.

2.3. *Chemical composition of DOM*

2.3.1. *Determination of dissolved organic carbon (DOC) and total nitrogen (TN)*

DOC and TN analysis used the 'NPOC/TN method', incorporating a Shimadzu TOC/TN Analyser with a platinised alumina catalyst under ultra-pure oxygen flow. Samples were acidified with 3%

HCl 2 M and sparged for 3 min at 150 mL·min^{-1} with ultra-pure oxygen to remove inorganic carbon from samples prior to the measurements. The mean of two to three injections of 100 μL is reported for every sample and precision, described as a coefficient of variance (C.V.), was <2% for each replicate injection.

2.3.2. *Determination of absorbance*

DOM in natural waters that has different proportions of functional groups and aromatic rings shows different molar absorptivities as a function of wavelength, ranging from 180–330 nm. UV-visible absorbance measurements were performed on a Safas spectrophotometer between 200 and 1000 nm, equipped with two quartz cells (1 cm path length), with distilled water as a blank. Determination of absorbance was reported at 254 nm (Abs_{254}), which represents the absorption of aromatic compounds [40].

2.3.3. *Origin and aromaticity of DOM*

Elemental analysis of DOC, TN and C/N ratio was used to discriminate the aquagenic (autochthonous) from the pedogenic (allochthonous) origin of organic matter. C/N values range from 60–70 in Arctic soils to around 10 in temperate grassland soils [41] and from 5 to 9 for riverine natural humic substances [42].

The evaluation of aromaticity of DOM was performed using a Specific UV Absorbance at 254 nm ($SUVA_{254}$ expressed in L·(mg C·m)$^{-1}$) calculation: UV absorbance measured at 254 nm to DOC concentration (mg C·L^{-1}) ratio [43].

2.4. *Spectrofluorometry*

Spectroscopic analysis of water samples was performed using a SAFAS flx spectrofluorometer with a xenon excitation source. The slits were set to 10 nm for excitation and emission. In order to remove any fluctuations in instrumental conditions, the fluorescence intensities were corrected by the Raman intensity.

2.4.1. *Humification index*

Humification of organic matter is closely related to environmental conditions and a humification index can be used to investigate the transformations of compositional characteristics of FA and HA fractions. Emission fluorescence spectrometry is a simple and efficient method to determine a humification index (HIX). For determination of the HIX, fluorescence emission spectra ranging from 265–800 nm were recorded at the excitation wavelength of 254 nm. For samples showing absorbances at 254 nm less than 0.3, HIX was calculated by dividing the fluorescence intensity in the (435–480) nm region by the total intensities in the (300–345) + (435–480) nm regions [44].

2.4.2. *Excitation-emission matrices*

Excitation-emission matrices were obtained recording fluorescence emission spectra from 250–800 nm at 2 nm steps, for excitation wavelengths ranging from 230–400 nm, with increments of 10 nm. Fluorescence intensities are expressed in arbitrary units (a.u.), all spectra being recorded with the same sensitivity and photomultiplicator adjustment. Emission spectra are corrected for Raman scattering bands. Data is represented in two-dimensional contour plots using Minitab® software.

Table 1. Fluorescence intensities retained for factorial discriminant analysis: λex/λem pairs of wavelengths (nm), flanking the maximum of fluorescence emission intensity in our spectra.

λex (nm)					λem (nm)		
			260	300	320	350	*Trypt.*
		300	320	330	360	410	
260	300	320	330	360	390	440	*D*
					260	460	*O*
	300	320	330	360	390	470	*M*
					390	500	

2.5. *Factorial discriminant analysis*

The factorial discriminant analysis (FDA) is a linear analysis which leads to a generalised PCA concerning the barycenters of the classes. FDA runs in two steps: the first is a PCA applied to the variables, and the second discriminates the classes. Our initial data set is composed of 700 observations of fluorescence emission intensity (FI, arbitrary units) obtained for 20 variables (λex/λem pairs of wavelengths) (Table 1) measured at 7 stations (classes) for each of the following 5 months: October (Autumn), January (Winter), April and June (Spring) and August (Summer) (classes).

Our goal was to test if the fluorescence parameters, determined as λex/λem pairs of wavelengths, could characterise the fluorophores HA and FA of DOM and allow us to: (i) discriminate the seasons; and (ii) discriminate the stations along the river flow, and to visualise the observations on a 2-dimensional map which shows clearly how separated these groups are.

The analysis of EEMs and emission spectra suggested the hypothesis that the characteristics of fluorescence could present qualitative variations according to seasons and probably to stations. These variations could eventually be due to the relative proportion of HA and FA in the organic inputs to the river related with seasonally variable climatic conditions.

Factorial discriminant analysis was achieved using Addinsoft® XLStat-Pro® software.

3. Results

3.1. *Chemical composition of DOM*

According to the climatic characteristics of the Mediterranean area, two contrasted situations can be distinguished. In the upper basin (station 1), a snow-thawing period (Spring) associated with rainfall events and a baseflow period (Summer). In the lower basin (stations 5 and 7, selected according to their respective location in the city centre and the Têt mouth), Autumn is the rainy period while Summer is characterised by a strong dryness. Results of DOC, C/N, Abs_{254} and $SUVA_{254}$ are summarised in Table 2 in relation to the described situations.

The higher values of DOC were reached at station 1 during the snowmelt period and at station 7 during rainfall events. For low water level, DOC values decreased to their minimum in the plain area at station 5. These values are in the same range as other values measured in natural water localised in the Western part of France [45].

In the upper basin (station 1), the origin of organic matter inputs was mainly pedogenic, as revealed by high C/N, especially during the snowmelt period (38.4). For the other parts of the river, organic matter stemmed from mixed origin with low C/N which decreased to 1.5 at station 5 (baseflow).

Table 2. Compositional characteristics of DOM in contrasted environmental conditions.

Condition - Station	DOC mg·L^{-1}	C/N	Abs$_{254}$	SUVA$_{254}$L·(mg C·m)$^{-1}$
Snow thawing – Station 1	7.0	38.4	0.216	3.1
Rainfall – Station 5	2.4	2.7	0.081	3.3
Rainfall – Station 7	4.0	3.2	0.101	2.5
Baseflow – Station 1	1.8	7.4	0.062	3.3
Baseflow – Station 5	1.6	1.5	0.045	2.8
Baseflow – Station 7	2.4	1.7	0.064	2.7

SUVA$_{254}$ is an indicator of aromaticity of aquatic humic substances in assessing general chemical composition of DOC [43]. Along the Têt river, DOM presented low variations of aromatic compound concentrations (2.7–3.3). These values are in good agreement with other results in riverine waters [43,45]

3.2. *Humification index*

HIX values (Table 3) decreased from the mountain (0.97) to the plain (0.69) sections. Humification of DOM was higher during the snowmelt and rainfall periods than during the dry period. These results are in good agreement with those presented by [44].

3.3. *Modifications of spectra according to season and station*

3.3.1. *EEMs contour plot representation*

EEMs data show spatial variations depending on station location from the high altitude to the plain course of the River Têt. EEMs contour plots of DOM show an extended fluorescence area characterised by a large range of excitation wavelengths (250–400 nm) and emission wavelengths (260–600 nm), with a maximum of fluorescence emission λem = 420–460 nm for excitations λex = 340–360 nm. Independent of the station and the season, the maximum fluorescence emission peak at λem = 390–455 nm for excitations ranging from λex = 340–360 nm was a constant characteristic. At a few stations, in the urbanised zones, a second emission fluorescence peak at λex/λem = 310/350 nm appeared with a variable intensity according to season. These two situations are illustrated in Figure 3.

At stations 1 and 2, and during all seasons, EEMs data show a large emission fluorescence peak in the range λem = 390–455 nm for a range of λex = 340–360 nm (Figure 3A, example of station 1, April 2001). These results could correspond to other reports in the literature for fresh waters: λex/λem = 340/448 nm for river waters [29]; λex/λem = 260/450 nm or 260/434 nm

Table 3. Humification index (HIX) values in contrasted environmental conditions.

Condition – Station	HIX
Snow thawing – Station 1	0.97
Rainfall – Station 5	0.94
Rainfall – Station 7	0.90
Baseflow – Station 1	0.83
Baseflow – Station 5	0.76
Baseflow – Station 7	0.69

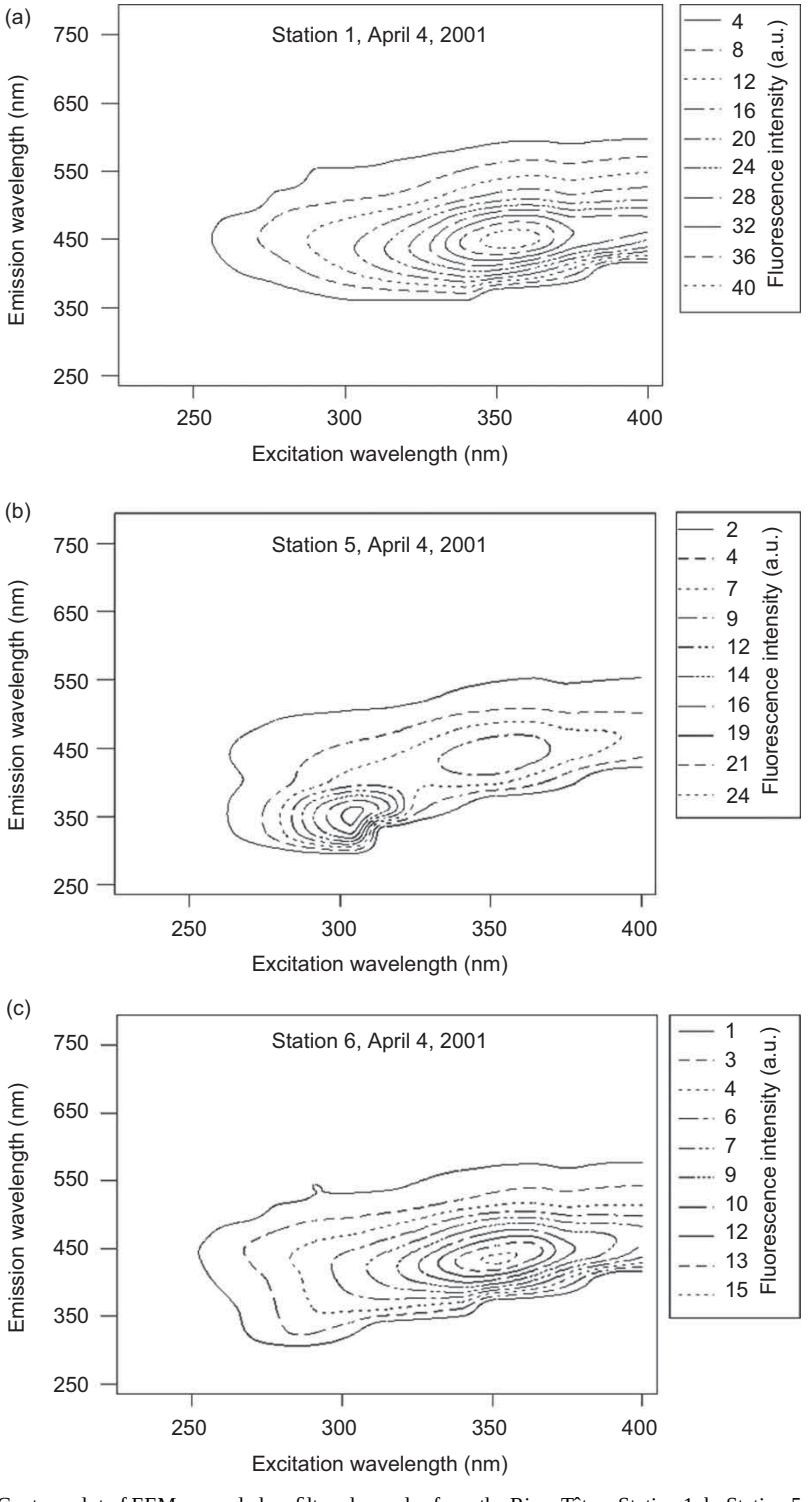

Figure 3. Contour plot of EEMs recorded on filtered samples from the River Têt. a: Station 1; b: Station 5; c: Station 6, in April 2001.

for riverine waters in the Orinoco River [46] and $\lambda ex/\lambda em = 240–260/420–445$ nm [47]; also in riverine waters, other wavelengths were recorded: $\lambda ex/\lambda em = 305/430$; $320/430$; $320/510$ and $460/520$ nm [48]; in estuary waters, $\lambda ex/\lambda em = 350/450$ nm were reported [49].

At station 5, EEMs contour plots show an intense peak in the region of $\lambda ex/\lambda em = 310/350$ nm (Figure 3B). During the same period, at stations 6 and 7, EEMs contour plots present similar peaks for the same ranges of $\lambda ex - \lambda em$, with a slight extra peak in the respective regions $\lambda ex/\lambda em = 300/350$ nm (Figure 3C, example of station 6, April 2001).

3.3.2. *Spectroscopic analysis*

3.3.2.1. *Fluorescence of DOM in riverine water.* As observed in EEMs (Figure 3), the large emission peak at $\lambda em = 420–460$ nm recorded for Têt's DOM presents (Figure 4A) a maximum of fluorescence emission at $\lambda ex/\lambda em = 350/450$ nm and a shoulder at 520 nm.

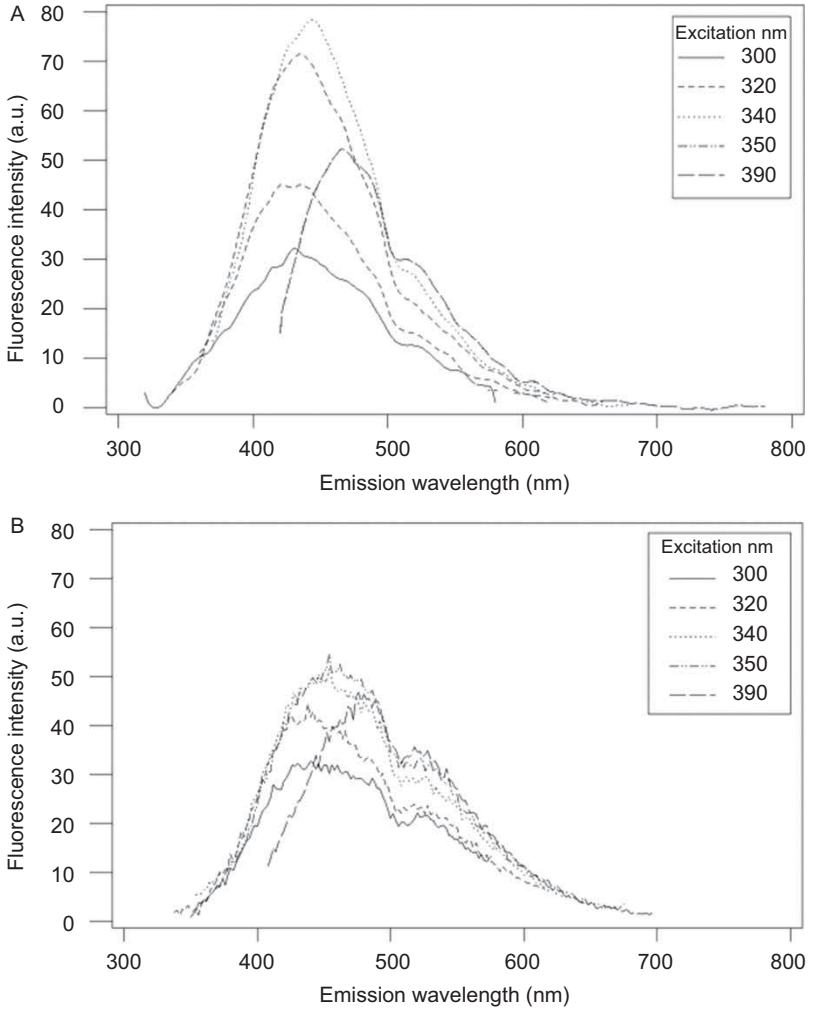

Figure 4. Comparative fluorescence emission spectra for five excitation wavelengths between (A) a Têt water sample (Station 1 in June 2001), and (B) a solution of humic acid from Aldrich.

Complexity of DOM fluorescence observed in EEMs can be better described with emission spectra (Figure 4A) at five different excitations. The shape of these spectra changes according to excitation. Below $\lambda ex = 350$ nm, the emission maximum is around $\lambda em = 440$ nm and over $\lambda ex = 350$ nm; maximum emission is close to $\lambda em = 470$ nm. The spectra present a large shift of 30 nm of the maximum of fluorescence emission, indicating at least two fluorophores for DOM. Apart from excitation, the shoulder at 520 nm is constant. The drift of emission maximum (440–470 nm) highlights the complexity of the DOM fluorophore. The changes observed for the maximum fluorescence spectra with different wavelengths indicate a complex mixing of fluorophores. DOM is also known to be a complex mix involving several fluorophores [50].

In order to validate our readings on fluorescence emission spectra of DOM referred to as humic acids, we have analysed in the same instrumental conditions a sample of commercial humic acid (HA technical grade) supplied by Aldrich (Figure 4B), as described in [35]. Comparison between Figure 4A and B highlights similar characteristics of emission spectra with comparable shape, shift, $\lambda ex/\lambda em$ maxima and the constant shoulder at 520 nm.

3.3.2.2. *Tryptophan fluorescence and anthropogenic pressure.* At the stations near urbanised zones, particularly at station 5, the EEMs contour plot (Figure 3B) shows a well individualised peak with a maximum intensity at $\lambda em = 350$ nm for $\lambda ex = 300$ nm. Many authors described similar values which they have identified as the amino acid tryptophan or sometimes as tryptophan-like or protein-like compounds, with slight differences in the pair $\lambda ex/\lambda em$ values [12,50–52]. These data show very little variability of fluorescence emission maxima, 340–350 nm, which could depend on tryptophan environment, while the larger excitation domain, 220–280 nm, reflects the width of the absorption band.

In order to control the spectral interpretation of our measurements, we compared, in the same experimental conditions, the tryptophan fluorescence emission spectra in river water with those recorded for BSA solution (Albumin Bovine 96% from Sigma) and authentic tryptophan solution (L-Tryptophan ultra 98% from Sigma) (Figure 5).

The spectra (Figure 5) do not allow us to assert if tryptophan is free or under a combined form (BSA or other proteins) in Têt riverine DOM.

Tryptophan fluorescence intensities, when detected, reflect the domestic impact of treated wastewater discharges in riverine waters [53].

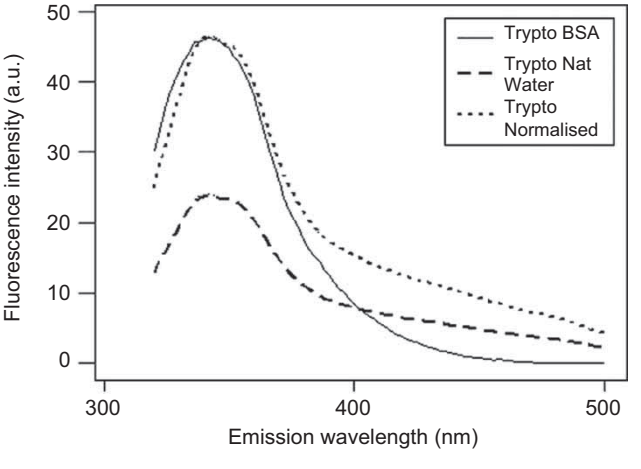

Figure 5. Comparative fluorescence emission spectra of a Têt water sample with presence of Tryptophan (Station 5, Trypto Nat Water) with a solution of Bovine Serum Albumine (BSA) in purified water (Trypto BSA), and with a solution of tryptophan in purified water (normalised to BSA solution at $\lambda em = 350$ nm, Trypto Normalised), at $\lambda ex = 300$ nm.

4. Discussion

Spectroscopic analyses of River Têt waters led us to select λex/λem pairs of wavelengths which characterise DOM (Figure 4A) and anthropogenic pressure (Figure 5). These λex/λem pairs of wavelengths were used as parameters in factorial discriminant analysis to discriminate seasons and stations with respect to the fluorescence signature of DOM.

4.1. *Factorial discriminant analysis: fluorescence signature of DOM*

FDA is used here for the prospective investigation of properties of fluorescence intensities depending on sampling stations and seasons. This factorial method leads to a spatial representation of the groups of observations we have previously determined, i.e. seven groups for the sampling stations and four groups for the seasons (5 months). A similar statistical approach was adopted in [54].

The independent variables (also called *parameters* or *categorical predictors*) defined from our data set (Table 1) correspond to the fluorescence intensity for λex/λem pairs of wavelengths selected on the maximum and on either side of the peak of fluorescence emission in the records of Figures 4A and 5. Values of λem refer respectively to the maximum fluorescence emission of tryptophan (Trypt., 350 nm) and of DOM, also called humic substances (in the range 410–500 nm). The choice of the λex/λem selected pairs of wavelengths characterises the shift of the spectra (Figure 4A).

4.1.1. *Discrimination of seasons*

Factorial analysis of the data (cf. § 2–5) indicates that 98% of the variance is explained by the first two axes. The last two axes provide very poor information for parameter classification (2 to 0%), therefore only the first two axes will be used for data interpretation.

The result of FDA shows a clear discrimination between the four seasons (Figure 6A), very well defined by distinct and narrow areas. April and June are almost completely superposed and are representative of Spring. The first axis represents 70% of the total information while the second axis represents 28%. Axis 1 clearly separated Winter, Spring and Summer, and axis 2 increases the discrimination of Winter and clearly discriminated Autumn.

4.1.2. *Discrimination of stations*

Factorial analysis of the data (cf. § 2–5) indicates that 86% of the variance is explained by the first two axes. The dispersion of cumulative percentage of variance until the sixth axis indicates some variability of the distance of observations to axes. The last four axes provide poor information for parameter classification (6 to 2%). Therefore, here again only the first two axes will be used for data interpretation.

Axis 1 represents 67% of the total information while axis 2 brings 19%. Axis 1 distinguishes clearly stations 6 and 7 from the five other stations, while axis 2 separates stations 6 and 7, and station 1 from stations 2, 3, 4 and 5 (Figure 6B).

The result of FDA highlights a good discrimination for stations 1, 6 and 7 with a narrow area, in comparison to stations 2 and 4 which have a more extended area with a limited intersection (Figure 6B). Stations 3 and 5 also present a narrow area with a high overlapping.

In conclusion of the FDA, the parameters related to DOM are discriminating enough to separate the four seasons. We assume that the composition of DOM, and more precisely the balance between the two fluorophores, varies along the seasonal cycle, with relation to the erosion of the

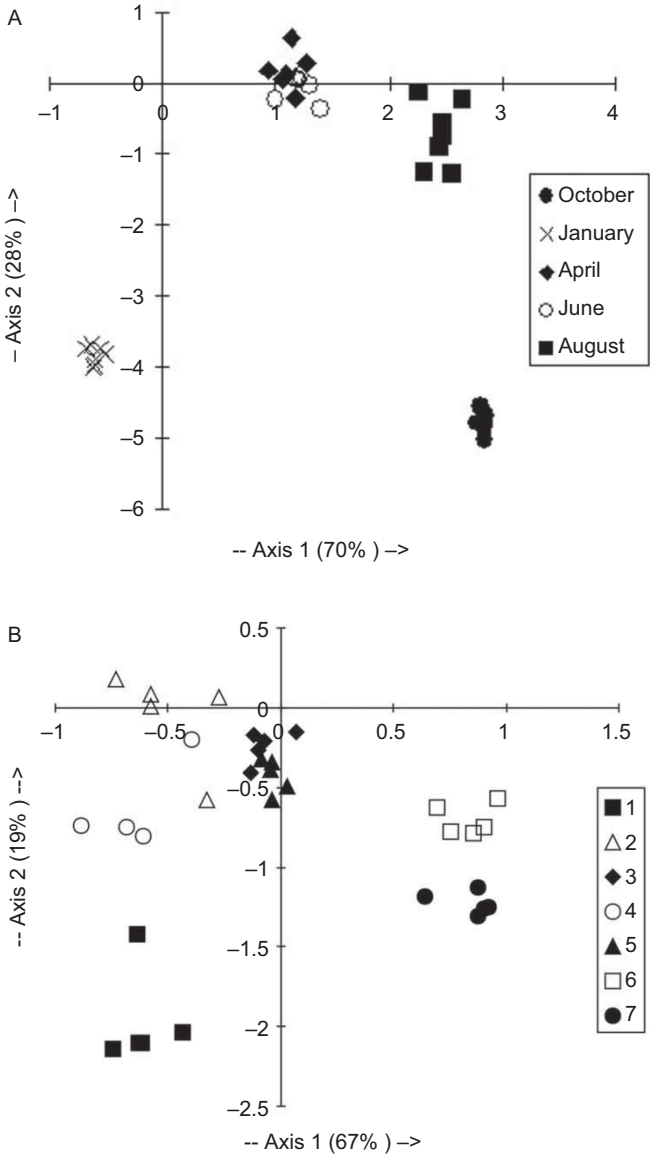

Figure 6. (A) Discrimination of the four seasons (5 months) plotted according to axes 1–2 of the four seasons group. (B) Discrimination of stations plotted according to axes 1–2 of the seven stations group.

watershed. Moreover, these seasonal variations are combined with the anthropogenic pressure, which becomes more important downstream from station 1 to station 7.

4.1.3. *Spectral characterisation of two fluorophores of DOM*

According to the correspondence chart (Figure 7), the second axis clearly discriminates the parameters (excitation/emission wavelengths) which characterises the fluorescence of tryptophan (Trypt.) around 0.0, and the fluorescence of DOM around −0.5. Regarding the first axis, there is no marked difference between tryptophan and DOM plots (0.40–0.60). In order to study the distribution of DOM parameters, we enlarged the scatter of points which could indicate

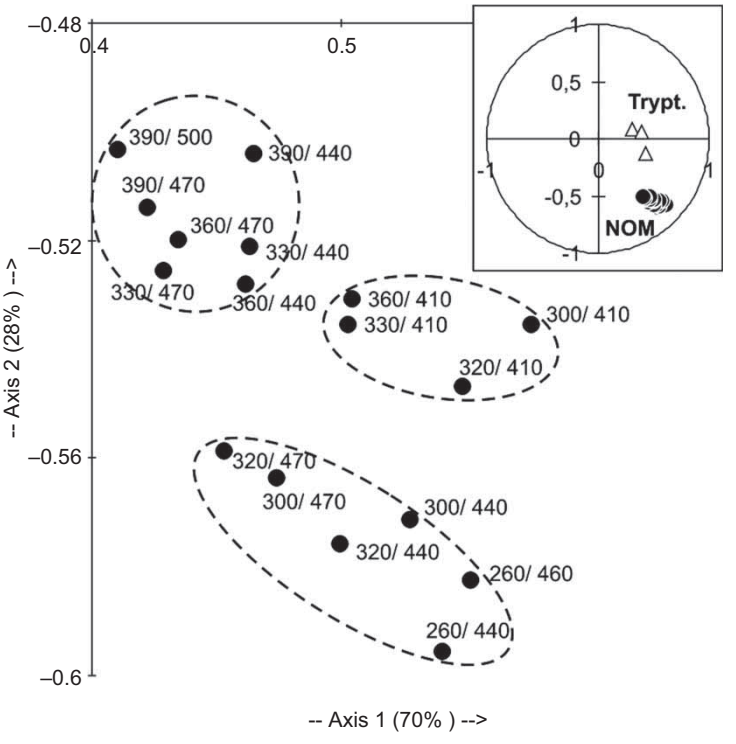

Figure 7. Correspondence chart of the parameters: distribution of λex/λem pairs of wavelengths corresponding to tryptophan (Trypt.) and to natural organic matter (NOM), with enlargement of NOM plots (which show the discrimination of fluorescence characteristics of the two fluorophores of NOM).

whether correlation exists between parameters. Considering the λex/λem pairs of wavelengths, we observed that parameters should be separated into three groups. The parameters with the higher emission wavelengths, 440–500 nm, correspond to two groups of excitation wavelengths: 260–320 nm and 330–390 nm. This situation should indicate the presence of a unique fluorophore species with two absorption bands. Another group of λex/λem pairs is characterised by an emission at 410 nm with excitations ranging between 300–360 nm which corresponds to a second fluorophore. The observed shifts of fluorescence emission maxima for DOM according to excitation, correlate with the above observation indicating the presence of at least two fluorophores (Figure 4A).

The important variations in the λex/λem values observed in the following references [29,46–49] are essentially due to differences in the apparatus setup and also in the different approaches of analyses by the authors, which leads to a complex nomenclature.

Our spectroscopic study of DOM from River Têt associating EEMs contour plot representation, spectral analysis and FDA clearly shows the presence of two fluorophores (Figure 7). Fluorophore 1, characterised by two bands of absorption (lower and higher excitation wavelengths: 260–320 nm and 330–390 nm, respectively) for an emission of fluorescence ranging from 440 to 500 nm, could correspond approximatively to the so-called 'humic-like' by authors. Fluorophore 2 (λex = 300–360 nm/λem = 410 nm) could correspond approximately to the so-called 'fulvic-like', described as 'Component 2' by [55] using a recent application of the method PARAFAC: a multi-way decomposition method which is a generalisation of principal component analysis to higher order arrays. This method allows the separation of HA and FA from several components in EEMs recordings from marine waters [56]. Nevertheless, FDA applied to fluorescence parameters was able to discriminate the spectral characteristics of the two fluorophores of DOM.

4.2. *Spatio-temporal distribution of DOM*

4.2.1. *Distribution of fluorescence intensities according to seasons and stations*

Fluorescence emission intensity (FI) being proportional to the amount of fluorophore at a given excitation and emission, we compared the DOM maximum values of FI recorded at $\lambda ex/\lambda em = 360/440$ nm, characteristic of humic substances, for each station during the 4 seasons (Figure 8). In Winter, FI values were low and constant along the river with a mean value of 32.5 a.u. In Autumn and Spring, the highest values of FI were recorded at station 1 (120.5 a.u. in October, 93.5 a.u. in April and 88.2 a.u. in June, respectively). In Summer, station 7 showed an increasing value of 84.6 a.u. Whatever the season was, the lowest variations of FI were observed at stations 4 and 5 and ranged from 25.4–33.9 a.u. So, we selected stations 1, 5 and 7 as the most representative three stations for the FI variations in the River Têt.

4.2.2. *Distribution of relative amounts of DOM according to seasons and stations*

In the mountain section, station 1 is characterised by a humid period with precipitation (Autumn and Spring), snow (winter) and snow thawing (Spring) (Figure 2). During this period, the leaching of soils in the watershed brings high inputs of humic substances to the river, so FI values reach their highest levels (FI up to 120.5 a.u.) (Figure 8). These high inputs of DOM are confirmed by high DOC contents ($7.0\,mg{\cdot}l^{-1}$), and high C/N values (38.4), which correspond to pedogenic material with high aromaticity ($SUVA_{254} = 3.1\,L{\cdot}(mg\,C{\cdot}m)^{-1}$) (Table 2). Moreover, this material presents a high humification index of 0.97 (Table 3).

During the baseflow period, there is no leaching and consequently low inputs of humic substances to the river (FI = 38.2 a.u.). These inputs are characterised by low DOC contents ($1.8\,mg{\cdot}L^{-1}$), and low C/N values (7.4) which correspond to low pedogenic material inputs with similar aromaticity ($SUVA_{254} = 3.3\,L{\cdot}(mg\,C{\cdot}m)^{-1}$) (Table 2). This material presents a relatively low humidification index (HIX = 0.83) (Table 3).

The plain section highlights two contrasted period between rainfall events (Autumn) and hard dryness (Summer) (Figure 2). At station 5, the inputs of DOM are lower compared to the mountain section and decreased from the humid to the dry period. The variations of DOC, C/N (Table 2) and FI (Figure 8) are in agreement with this reduction of DOM contents, whose aquagenic fraction

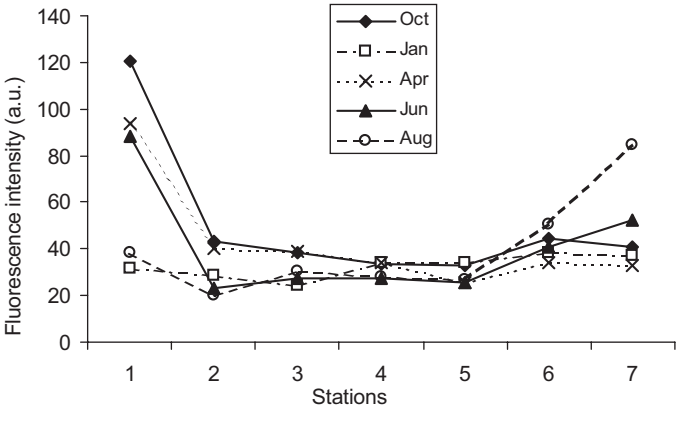

Figure 8. Variations of NOM relative contents downstream of the River Têt according to seasons and stations from fluorescence maximum intensities recorded at $\lambda ex = 360$ nm and $\lambda em = 440$ nm (with all other analytical conditions identical).

increases (C/N from 2.7 to 1.5). We can observe that in humid conditions, the DOM has similar aromaticity ($SUVA_{254} = 3.3$ L·(mg C·m)$^{-1}$, Table 2) and humification index (HIX = 0.94, Table 3) to the mountain section of the river, in relation to erosive processes. In contrast, during strong dryness periods, the nature of DOM changed with lower aromaticity ($SUVA_{254} = 2.8$ L·(mg C·m)$^{-1}$, Table 2) and humification (HIX = 0.76, Table 3) in relation to low terrigenous inputs.

At station 7, the DOM contents increase compared to station 5. In the rainfall period, the humic substances (FI = 41.2 a.u., Figure 8), DOC and C/N (4.0 mg·L^{-1} and 3.2 respectively, Table 2) reflect the influence of erosion of soil in addition with the output of the treatment plant of Canet. During the low water period, without erosion, the values of FI (84.6 a.u., Figure 8), DOC and C/N (2.4 mg·L^{-1} and 1.7 respectively, Table 2) decrease and represent the inputs of the river baseflow and the output of the treatment plant of the seaside resort overpopulated during Summer. The aromaticity is quite constant ($SUVA_{254} = 2.5$ and 2.7 L·(mg C·m)$^{-1}$, Table 2) independently of the period, but is lower than in station 5. The humification index is lower than in station 5 and decreases from the rainfall to the dry period from 0.90 to 0.69, respectively. These variations in nature and contents of DOM could be explained by the outputs of the treatment plant of Canet, whose efficiency is irregular throughout the year.

4.2.3. *Relative percentage of DOM fluorophores*

Generally, DOM in water samples exhibits low levels of emission fluorescence intensities. Therefore it is necessary to select the best conditions of excitation for observing the maximum fluorescence emission and for investigating the complexity of the constitutive compounds.

In order to estimate the relative percentages of the two fluorophores (FA and HA), we used the maximum values of emission fluorescence intensities of DOM recorded at $\lambda ex = 360$ nm. Accordingly with the FDA results (Figure 7), which determine the $\lambda ex/\lambda em$ pairs of wavelengths: $\lambda ex/\lambda em = 360/410$ nm (FI_{410}) for FA and $\lambda ex/\lambda em = 360/470$ nm (FI_{470}) for HA, the relative percentage of FA (FA %) was calculated as follows :

$$FA\% = FI_{410}/(FI_{410} + FI_{470}) \quad \text{at } \lambda ex = 360\,\text{nm}.$$

This calculation is based on the maximum of fluorescence emission, which best represents the whole characteristics of the highly polymerised humic substances of water DOM, including FA and HA fractions.

In these conditions at all seasons, the percentages of FA range from 40% at station 1 to 48% at station 7. So, the qualitative balance of FA/HA in DOM appears to slightly increase from the mountain section to the mouth of the River Têt and during the year.

These spatial variations of DOM composition show the same trend as the humification index (Table 3): a decrease of the humification from the mountain to the plain section.

This observation differs from that of [10], which suggests that DOM composition could change according to seasonal conditions.

5. Conclusion

This work allowed us to study the spatio-temporal variations of DOM in a coastal Mediterranean river, the Têt, using its fluorescence properties. The fluorescence technique was able to detect, directly on a filtered sample, the two main fluorescent constituents of DOM and low levels of anthropogenic pressure.

The EEMs contour plots showed the constant signature of natural DOM along the river and the variable occurrence of tryptophan, indicating the anthropogenic pressure.

Factorial discriminant analysis was a good classification method applicable to fluorescence data. It allowed us to confirm our assumption that temporal and spatial variations could be separated by means of the fluorescence emission intensities of DOM.

Futhermore, FDA coupled with spectral analysis confirmed the presence of two fluorophores in DOM. The first fluorophore presents two bands of absorption (lower and higher excitation wavelengths: 260–320 nm and 330–390 nm, respectively) for an emission of fluorescence ranging from 440–500 nm, corresponding to HA. The second fluorophore (λex = 300–360 nm/λem = 410 nm) corresponds to FA.

Our analyses emphasise clear spatio-temporal variations of DOM and highlight its qualitative and quantitative variations along the river. Two contrasted periods are distinguished: a humid period with rainfall events in Autumn (mountain and plain sections) and snow thawing in Spring (mountain section), with high amounts of DOM, and a dry period in Summer (mountain and plain sections) with lower amounts of DOM.

The results of fluorescence are in agreement with those of chemical composition. In the mountain section, the DOM inputs to the river mainly have a pedogenic origin, with a high aromatic character and a high humification index. In contrast, in the plain section, the aquagenic character of DOM increases while both the aromaticity and humification decrease.

These results confirm those of fluorescence, which established small qualitative variations of DOM composition: FA percentage ranging from 40% (station 1) to 48% (station 7) for all seasons.

The fluorescence technique is non destructive and causes no loss of material, and appears to be an efficient tool for determining the compositional characteristic of humic substances (FA and HA fractions) in riverine DOM.

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