Fluorescence emission
Dissolved substances
Estuaries
Conservative behaviour

Émission de fluorescence
Substances dissoutes
Estuaires
Comportement conservatif

Comparative study of dissolved fluorescent matter in four West-European estuaries

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ABSTRACT

Filtered waters from four European estuaries are studied directly after filtration for their fluorescence properties, using the same procedure with corrections for scattering. A high similarity in the spectral characteristics (position of emission maximum, width at mid-height) is found for each estuary from upstream to downstream, and in comparison between the four estuaries. In each estuary, fluorescence intensities decrease linearly when salinity increases. These observations are correlated with the geochemical behaviour of the organic fluorescent matter in estuaries.

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RÉSUMÉ

Étude comparative du matériel fluorescent dissous dans quatre estuaires européens

Les eaux de quatre estuaires européens sont étudiées en fluorescence directement après filtration, en utilisant toujours la même procédure et en effectuant des corrections pour l'effet de diffusion. Une grande similarité est observée pour les propriétés spectrales (position du maximum d'émission, largeur à mi-hauteur), le long de chaque estuaire d'amont en aval et d'un estuaire à l'autre. Les intensités de fluorescence sont inversement proportionnelles à la salinité pour chaque estuaire. Ces observations sont corrélées au comportement géochimique de la matière organique fluorescente dans les estuaires.

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INTRODUCTION

The first studies of fluorescent matter dissolved in the marine environment were carried out by Kalle (1949-1956), Postma and Kalle (1955), and Van Andel and Postma (1954). Fluorescence is caused by a large group of dissolved organic compounds (Laane, Koole, 1982, and references cited in this paper).

With the understanding that the behaviour of fluorescence in estuarine waters is conservative, *i.e.* the rate of decomposition or production of the fluorescent matter is slow or non-existent compared to the flushing-time of the estuary (Duursma, 1974; Laane, 1982 *a*), fluorescence measurements have been used by several authors to study the mixing of river water with sea water in different estuaries (Kalle, 1949-1956; Zimmerman, Rommets, 1974; Willey, Atkinson, 1982; Dorsch, Bidleman, 1982).

In the present work, fluorescence measurement is again used to study the behaviour of the fluorescent matter in four Western European estuaries. However, in at least two important aspects, the technique used is different from those reported in the literature. Following the method of Ewald *et al.* (1981-1983), careful attention has been given to the effect of Rayleigh, Raman and Tyndall scattering on all of the recorded fluo-

rescence spectra. This technique permits precise delineation of the shape of the fluorescence spectra, and thus makes it possible to compare the spectral characteristics of the fluorescence emission of different estuaries. Christman (1970), Laane and Koole (1982) and Gienapp (1979) observed that the emission spectra of different natural waters are very similar. Larson and Rockwell (1980) summarized the reported fluorescence maxima of different natural waters, ranging from 410-510 nm. A shortcoming in these studies was that all spectra were recorded on different instruments and were not corrected for the spectral sensitivity factor of the equipment used. Only Gienapp (1979) described "true" fluorescence spectra of which the maximum wavelengths were around 450-460 nm (excitation 367 nm).

To the author's knowledge, the present work is the first in which identical methodology and the same instrument have been applied to samples from various estuaries, situated in a region geographically as large as that extending from the Netherlands to Southern France. The results obtained can thus be directly compared, and the behaviour or properties of the fluorescent matter that might be of general nature can readily be elucidated.

MATERIAL AND METHODS

Sample locations

The estuaries studied are all situated in the western part of Europe, namely the Gironde Estuary, the Loire Estuary, the Rade de Brest, all three on the Atlantic coast of France, and the Ems-Dollart Estuary between the Netherlands and Germany (Fig. 1).

Sampling and sample storage

Water samples from the Gironde Estuary were collected with a 10 l capacity Niskin bottle during a helicopter cruise. The bottle was lowered from this helicopter to a depth of 2 m below the surface. Samples were filtered on the same day as they were collected with precombusted (450°C, 2 hours) Whatman GF/C glass fibre filters. The filtered samples were then stored in precleaned glass bottles and kept in the dark at 4°C before analysis in the laboratory at Bordeaux.

Water samples from the Loire Estuary and the Rade de Brest were collected with a 2 l capacity amber glass bottle lowered to a depth of 0.5 m below the surface from oceanographic ships. All samples were filtered and stored in 500 ml glass bottles in a manner similar to that applied to the Gironde samples. During transfer from Nantes and Brest to Bordeaux, the samples were kept in darkness in portable ice boxes at 4°C.

Water samples from the Ems-Dollart Estuary were collected with a pump on a boat at 1.5 m below the surface, similarly filtered with precombusted Whatman GF/F glass fibre filters and stored in 500 ml capacity polyethylene bottles at 1°C temperature before analysis. The transfer of samples to Bordeaux was also effected in an ice-box.

Fluorescence experiments

Fluorescence spectra were recorded with a Hitachi Perkin-Elmer MPF-3 spectrofluorometer equipped with a R 106 photomultiplier, using 1 × 1 cm quartz cells, thermostated at 25°C. Measurements were made directly on filtered samples without any further pretreatment. Excitation wavelength was 370 nm and the emission wavelength used for intensity measurements was 460 nm. Emission spectra were scanned from 380 nm to 580 nm. The raw spectra were corrected for Tyndall scattering due to organic and inorganic colloïds and for Raman scattering due to water molecules (Parker, 1968). Scattered light intensity was simulated using a solution of non-fluorescent glycogen. The concentration of glycogen is so adjusted that it has the same intensity of Rayleigh-Tyndall scattering at 370 nm as that of the sample (Ewald *et al.*, 1983). The intensity of the fluorescence of the samples was determined from the corrected spectra at 460 nm emission wavelength, and expressed in cm unit (height on the recorder paper) using the same sensitivity range for each sample. These intensities were corrected for the time-fluctuations of the lamp by using the Raman band of pure water as internal standard. Intercalibration carried out by two of the authors (P. B. and R. L.) between the Physico-chemical Oceanography Group in Bordeaux and the Netherlands Institute of Sea Researchs in Texel on 30 samples indicates that 1 cm of this unit is equivalent to 0.72 in mF1, which is the unit of fluorescence intensity defined by Kalle (1956) and following researchers

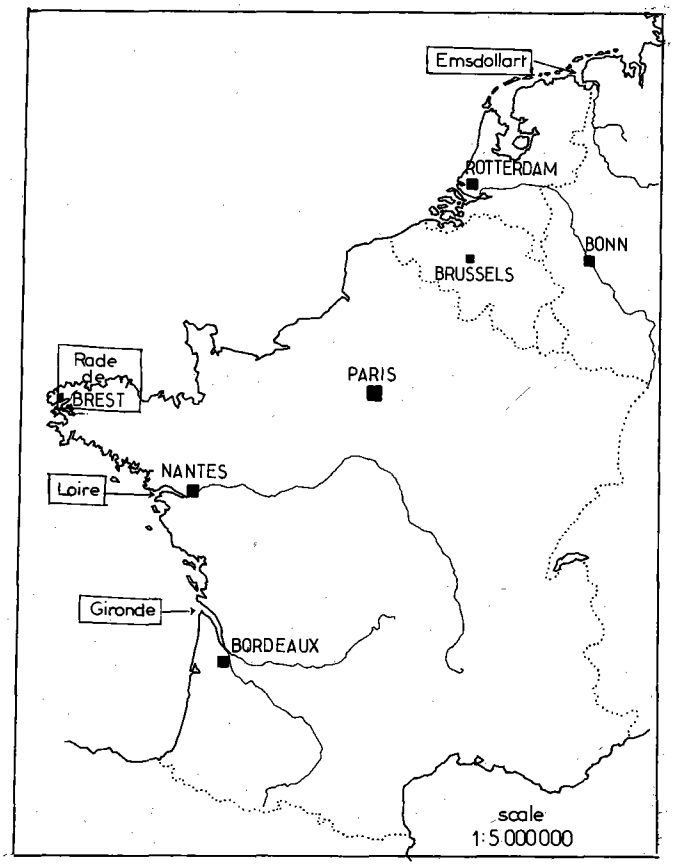


Figure 1
Geographical location of the 4 estuaries studied.
Situation géographique des 4 estuaires étudiés.

(Duursma, Rommets, 1961; Duursma, 1974). "A solution of 1 mg Quinine Bisulphate in 1 l of 0.01 N H_2SO_4 has a fluorescence intensity of 700 mFL" (Duursma, 1974). The results presented in this paper are expressed in these two units. The corrected spectra were in turn normalized for their intensity using a Hewlett Packard 9821 A computer. The normalized spectra were then examined for their individual characteristics such as shape, half height width (HHW) and the position of the emission maximum (PEM).

Readers are referred to earlier published works for details of the method used (Ewald *et al.*, 1983). The reproductibility of this method in terms of spectral characteristics has been assessed by performing 18 individual analyses of a sample from the Ems-Dollart Estuary. In each analysis, a fresh sample and a new solution of glycogen were used. The result is shown in Figure 2. With regard to the shape of the spectra, it can be seen that the difference between the intensities of individual spectra and of the average spectrum is large only in the region between 390 nm to 430 nm, *i.e.* just before the position of the maximum at 445 nm. The deviation can be as high as 16% because in this region the slope of the spectrum is very steep while the intensity is very much influenced by Raman scattering. Around and beyond the maximum, deviation of the individual spectrum from the average is small, except towards 580 nm, where the difference becomes large again relative to the average because of the low values of the intensity.

Besides the shape, other two important properties of the spectra are the PEM and the HHW. Statistically, the average values of PEM and HHW are 445.1 nm and 94.7 nm. The standard deviation (2 σ) for the 95% confidence limit is 2.4 nm for PEM and 2.8 nm for HHW. These values have been used as a criteria to determine the similarity or dissimilarity of each family or each group of fluorescence spectra from different estuaries.

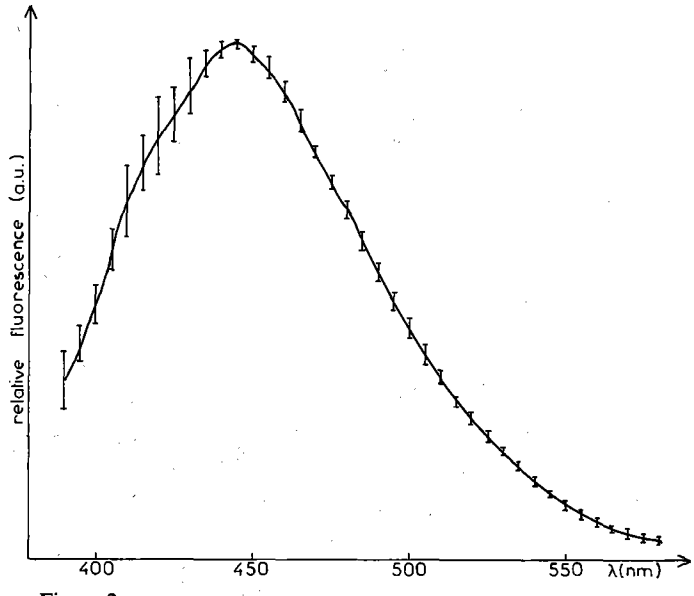


Figure 2

Average fluorescence spectrum of 18 *analyses on one sample from the Ems-Dollart estuary.*

Spectre de fluorescence moyen de 18 mesures effectuées sur un échantillon de Ems-Dollart.

RESULTS AND DISCUSSION

Spectral characteristics

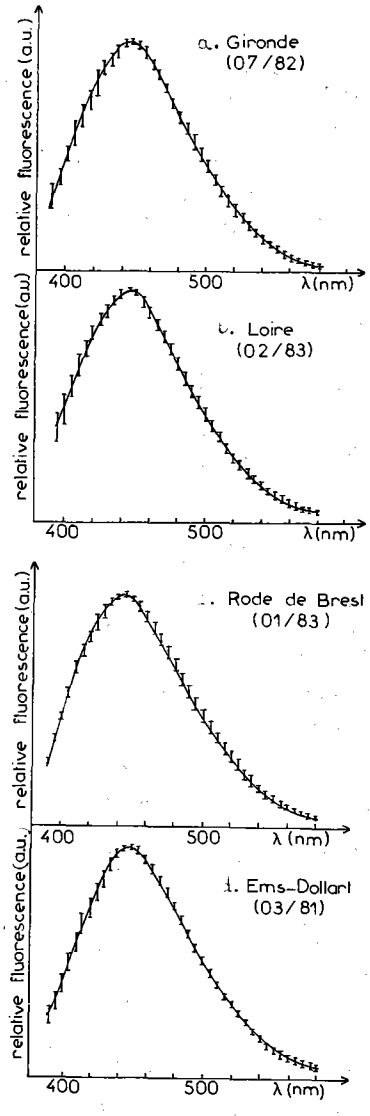
The results are examined first for each estuary separately and then to compare one estuary with another.

For a particular estuary and for a particular month, all samples along the estuary give very similar spectra. The representative average fluorescence spectrum of the 4 estuaries studied is shown in Figure 3.

Figure 3

Average fluorescence spectra of the different samples along each estuary:
a) Gironde (July 1982*);*
b) Loire (February 1983*);*
c) Rade de Brest (January 1983*);*
d) Ems-Dollart (March 1981*).*

Spectres de fluorescence moyens des différents échantillons prélevés le long de chaque estuaire:
a) Gironde (juillet 1982);
b) Loire (février 1983);
c) rade de Brest (janvier 1983);
d) Ems-Dollart (mars 1981).



The PEM of each spectrum is mostly within the 2.4 nm criterium value from the average PEM of the group, as indicated by their respective 2 σ values (Table 1). An exception is for the Ems-Dollart Estuary in October 1981, for which a 2 σ value of 3.2 nm is obtained, and for the Loire in May 1983 with 2.7 nm.

The HHW of the spectra is also very similar for each group. The 2 σ deviation values from the average HHW are mostly less than the 2.8 nm criterium value except for the Loire Estuary, for which the 2 σ value is 3.2 nm (Table 1).

The similarity of the fluorescence characteristics from upstream to downstream in a particular estuary could probably be attributed to the similarity of the fluorescent material present in the waters along the estuary.

Table 1

Average values of PEM (position of emission maximum) and HHW (half height width) of the fluorescence spectra for the 4 estuaries, with mention of the 2σ Values.

Valeurs moyennes de la position du maximum d'émission et de la largeur à mi-hauteur des spectres de fluorescence des 4 estuaires, avec mention des valeurs de 2σ.

Sample location	Time of sampling	Number of samples	PEM $\bar{x}$	PEM 2σ	HHW $\bar{x}$	HHW 2σ
Gironde Estuary	July 1982	33	444.4	2.4	94.4	3.1
Loire Estuary	February 1983	9	445.0	2.1	93.5	3.2
	May 1983	9	444.0	2.7	94.3	2.3
Rade de Brest	October 1982	10	444.6	2.0	92.7	2.3
	January 1983	10	444.8	1.7	94.6	2.4
Ems-Dollart Estuary	March 1981	11	448.2	2.2	94.5	2.1
	October 1981	6	445.0	3.2	94.6	2.4

Similarity of the spectral characteristics is also important for comparison of their spectral intensities along each estuary, because without such a similarity, intensities cannot be compared.

When the average PEM and the average HHW of all four estuaries are mutually compared, further similarity is again found (Fig. 3). With the exception of the average PEM value of 448.2 nm of the Ems-Dollart Estuary in March 1981 (Table 1), the difference between the smallest average PEM (Loire May 1983, 44.0 nm) and the largest one (Loire February 1983 and Ems-Dollart October 1981, 445.0 nm) is only 1.0 nm. This difference is below all the 2 σ values within each estuary and also below the 2.4 nm criterium value.

The difference between the smallest average HHW (Rade de Brest, October 1982, 92.7 nm) and the largest average HHW (Gironde July 1982, 94.6 nm) is only 1.9 nm, which is again below the 2.8 nm criterium value.

These similarities in the average PEM and HHW values are very surprising in view of the differences in the geographical locations, climate, vegetation and aquatic environment among these estuaries. Other studies using the same experimental procedures carried out on fulvic acids extracted from several aquatic environments (Berger *et al.*, 1983) have shown that a shift of the PEM of about 14 nm is observed due to the geochemical origin of the samples. This shift is much more important than the difference reported here between the natural samples from the four estuaries studied. In this case, it could be suggested that the most important source of fluorescent matter would be essentially the same in the four estuaries, or that the mixture of dissolved end products of humification would be the same.

Spectral intensities

The results of the spectral intensity measurements plotted against salinity is shown in Figure 4. The most important result to be noted is that in all estuaries, regardless of the season, for salinity higher than $1^0/_{00}$, fluorescence intensity is always inversely and linearly related to salinity (see also Table 2). This means that during its passage through the estuaries, the fluorescent matter behaves conservatively. It also indicates that the rivers are the major source of the fluorescent matter in each estuary with the mixing processes as the sole agent governing their distribution there. This result is in good agreement with recent works published by Mantoura and Woodward (1983) and by Ittekot (1982), and leads to the conclusion that precipitation and flocculation processes are probably not quantitatively important for the complex fluorescent material at the continent-ocean interface. Model studies in simulated estuarine conditions carried out by Preston and Riley (1982) have produced the same result.

For the Gironde, the Loire and the Rade de Brest, this is the first time that such conservative behaviour is reported. With regard to the Ems-Dollart Estuary, earlier studies by Laane (1982 *b*) had already revealed the linear relationship between fluorescence and salinity in this estuary. This author notes further that in summer, at high salinities, a slight increase in fluorescence is observed in the Ems-Dollart Estuary that cannot be explained solely on the basis of river discharge. A similar slight increase in summer is also observed in the Rade de Brest (Ilahude, unpublished data). Whether these increases are due to the biological production of fluorescent matter is as yet difficult to ascertain. Conclusions concerning the production or decomposition of fluorescent matter can only be drawn if more of the chemical and physical factors involved in the fluorescence intensity are studied.

Another fact to be noted is the large variation of fluorescence intensity from one estuary to another, especially at the river end of these estuaries. The inten-

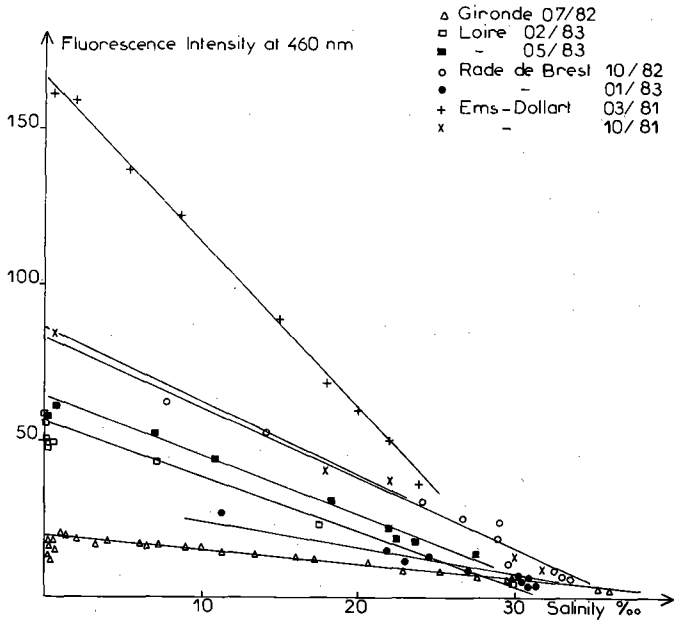


Figure 4

Fluorescence intensities versus salinity (fluorescence intensities are measured at 460 nm after correction for scattering and expressed in cm, using the same sensitivity range for each sample).

Relation entre les intensités de fluorescence et la salinité (les intensités de fluorescence sont mesurées à 460 nm après correction pour la diffusion et exprimées en cm, ramenées à la même sensibilité pour tous les échantillons).

Table 2
Linear regression values (%) of fluorescence versus salinity in the 4 estuaries studied.
Calculs de régression linéaire des graphes intensités de fluorescence fonction de la salinité.

		$Y = Ax + B$				Calculated Intensity at $30\,^{0}/_{00}$	
Estuary	Date	A	B	r	n	cm	mFL
Gironde Estuary	July 1982	—0.48	19.8	0.993	22	5.4	3.9
Loire Estuary	February 1983	—1.72	55.1	0.992	6	3.5	2.5
	May 1983	—1.92	64.1	0.995	8	6.5	4.7
Rade de Brest	October 1982	—2.27	83.0	0.983	10	14.9	10.7
	January 1983	—0.85	31.6	0.983	10	6.1	4.4
Ems-Dollart	March 1981	—5.31	166.0	0.999	9	6.7	4.8
Estuary	October 1981	—2.36	85.2	0.996	5	14.4	10.4

(r: correlation coefficient. n: number of samples used for the calculation).

sity in the Ems-Dollart Estuary can attain 166 cm, which is 8 to 10 times that of the Gironde with a value as low as 20 cm (Fig. 4).

The high values in the Ems-Dollart Estuary can probably be attributed to the fact that the Ems runs through an old peat area from which it takes up fluorescent matter. The exceptionally high average PEM value of 448.2 nm noted earlier could probably be attributed to certain chemical differences in the structure of fluorescent molecules. The low values in the Gironde could probably be caused by the low river discharge in the month of July, with few rainfalls.

Near the sea, at the end of the estuaries, fluorescence intensity values are generally low, approching that of the open ocean (Duursma, 1974). In all four estuaries, the calculated values at $30\,^{0}/_{00}$ salinity are comparable, being 3.5 to 14.9 cm (2.5 to 10.7 mFl; Table 2). In the ocean, Duursma (1974) has reported values of 0.5 to 3.0 mFl for the surface and 0.5 to 1.5 mFl in the deeper layer. It is difficult to compare strictly these results with the values reported here; nevertheless, the latter seem to be in good agreement with those published in the literature.

Another important feature disclosed during the present study is the abnormally low values of fluorescence intensity at salinity $0.5\,^{0}/_{00}$ or less. This feature is found especially in the Gironde Estuary, and to a lesser degree also in the Loire and in the Ems-Dollart (Fig. 4). In the Rade de Brest, this phenomenon is not observed since all the sampled examined originated in the estuarine zone with salinity over $7\,^{0}/_{00}$. Cadée and Laane have, moreover, observed changes in fluorescence intensities in the freshwater tidal compartment of the Ems-Dollart. They have enumerated five possible reasons for this phenomenon, but have not found any decisive explanation (Laane, 1982*c*). In mixing processes in estuaries, a parameter is considered as conservative if its value measured in the estuary fit well with the theoritical dilution curve (Burton, Liss, 1976). The determination of this curve is possible only if the two limit values of the considered parameter are known, *i.e.* the riverine limit and the marine limit. Since the ocean is more chemically homogeneous than the rivers, the marine limit is generally more easy to determine. Figure 4 shows that the marine value is not as different from one estuary to another as the riverine limit, which may change with seasons, climate, characteristics of soils and vegetation, etc. For each estuary, it is probable that terrigenic inputs entering the system are not constant, even over a short period. This could be one explanation of the non-linearity observed in the salinity range lower than $1\,^{0}/_{00}$.

CONCLUDING REMARKS

The results of the present study indicate that corrections of the fluorescence emission spectra due to Tyndall and Raman scatterings are very important in fluorescence studies in the environment. When this correction is taken into account, differences and or similarities in the spectral characteristics of fluorescent matter of different origin can be revealed.

The high similarity in spectral characteristics (PEM and HHW) suggests that, despite the different geographical locations and geochemical environments, dissolved fluorescent matter seems to have the same type of origin in the four estuaries.

The linear relationship of fluorescence with salinity shows that freshwater is the main source of fluorescent matter. According to Mantoura and Woodward (1983), the conservative behaviour of dissolved organic carbon in estuaries indicates that any microbial degradation, chemical flocculation or adsorption processes do not affect the flux of dissolved organic material through macrotidal turbid estuaries of the temperate zone. This new approach supposes a constant input of organic matter from continental origin to the coastal zone of the oceans, contributing to their fertility. Mantoura and Woodward's results are corroborated by the results reported here.

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Loire samples were obtained during cruises organized by the pluridisciplinary group GRECO-I.C.O. from the French Centre National de la Recherche Scientifique.

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