THE "BLUE SHIFT" OF EMISSION MAXIMUM AND THE FLUORESCENCE QUANTUM YIELD AS QUANTITATIVE SPECTRAL CHARACTERISTICS OF DISSOLVED HUMIC SUBSTANCES

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ABSTRACT

Humic substances (HS) play important roles in a variety of biogeochemical processes. Fluorescence spectra can be used for quantitative and qualitative characterisation of water-soluble humic substances (HS) and, in particular, of dissolved organic matter (DOM) in water. In this study, we measured the fluorescence spectra of natural HS (riverine, lake, marine water, and soil aqueous extractions) and commercial water-soluble HS under excitation from 270 to 355 nm. Natural water samples were filtered in two stages by micro- and ultra-filtration. After ultra-filtration, DOM was separated into two fractions depending on the size of the particles, for which fluorescence spectra were also obtained. The comparative analysis was made with a focus on the so-called "blue shift" (emission maximum shifts towards shorter wavelengths with increasing excitation wavelength) and the fluorescence quantum yield (QY) of samples of different origin. The fluorescence quantum yield under excitation at 355 nm for commercial HS (QY=0.008) was less than that for natural water DOM (QY=0.028), but more than for soil HS (QY=0.003). The low molecular weight fraction quantum yield was bigger than that for the colloidal fraction by 20-30%. The fluorescence quantum yield for natural water and for soil extractions HS increased with excitation wavelength, but the quantum yield did not depend on the excitation wavelength for commercial HS samples. Natural HS differed from commercial HS in higher heterogeneity of fluorophore composition as evidenced by the larger "blue shift" value and QY dependence on excitation wavelength.

INTRODUCTION

The goal of this study is to examine the spectral characteristics of humic substance (HS) from natural water and soil. HS plays an important role in biogeochemical processes. Up to 50% of dissolved organic matter (DOM) in natural water and about 12% of soil organic matter is HS (1). In spite of a two-century history of HS research (2,3), there are a lot of unsolved problems concerning its structure and properties. HS absorbs in the UV and in the visible (blue light) range of the spectrum. Therefore, natural water containing a large amount of HS has yellow colouring and soils with large amounts of HS are brown and black (4).

HS is particularly suitable for spectral research since its complex structure precludes analysis by chemical methods. Macromolecules of HS have a chaotic structure, which can be investigated by methods considering total ensemble properties such as luminescence spectroscopy. Moreover, HS is an interesting object for spectroscopy as an aggregate of interacting fluorophores. Such a macromolecular aggregate could vary in size from a few nanometres to micrometres.

HS dissolved in water has a maximum fluorescence in the visible range under excitation at UV wavelengths. Fluorescence spectra can be used for quantitative and qualitative characterisation of water-soluble HS fractions. The most extensively studied DOM fluorescence spectra are those of DOM in natural waters (5,6,7,8). The wide band of DOM emission has a fluorescence maximum in

the range 400-460 nm changing with excitation wavelength (Figure 1) and is more than 100 nm in width (9). From excitation wavelength λ_{ex} =270 nm up to about λ_{ex} =310 nm, which depends on the DOM type, the fluorescence maximum wavelength is essentially stable. At higher excitation wavelengths, one can observe a so-called "blue shift" of the emission maximum towards a shorter wavelength. At a further increase of excitation wavelength the emission maximum shifts towards a longer wavelength again. This behaviour of emission spectra is unique for natural water DOM and it reflects the heterogeneity of the fluorophore composition of HS from natural origin.

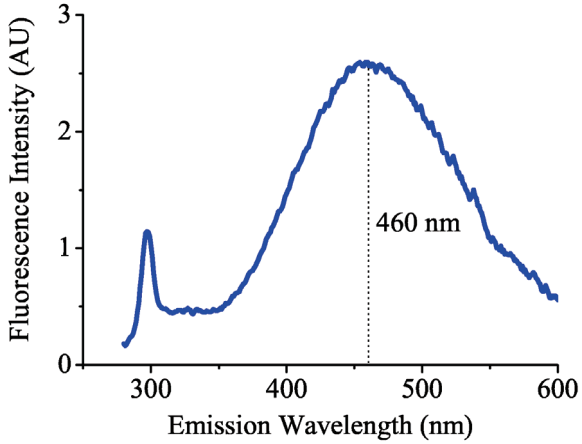

Figure 1: White Sea sample 5 fluorescence spectrum under excitation at 270 nm.

In publications (10,11,12) three basic bands of natural water HS fluorescence were identified based on the analysis of the excitation-emission matrix using the PARAFAC model (13). Every band has a typical excitation/emission wavelength. In an earlier report of Coble (6), these bands have been named as

- A (λ_{ex} =250-260 nm, λ_{em}=380-480 nm),
- M (λ_{ex}=290-320 nm, λ_{em}=380-420 nm) and
- C (λ_{ex}=330-350 nm, λ_{em}=420-480 nm).

They are found in all DOM fluorescence spectra and represent basic bands for aqueous soil extractions and samples of plant origin. These bands can be found in marine water DOM spectra, with fluorescence intensity drastically decreasing towards the open ocean. Coble assumes that the A and C fluorophores are of terrestrial origin, and the M fluorophore is of marine origin and is released by microorganisms that cause the biological degradation of HS.

The purpose of the present paper is to investigate the fluorescence spectral characteristics (such as emission maximum "blue shift" and fluorescence quantum yield) of natural and commercial HS under an excitation wavelength ranging from 270 nm to 355 nm. Absorption and fluorescence spectra were obtained for natural water (riverine, lake, marine) samples, for aqueous soil extractions, and for water-soluble commercial HS. Some natural water samples were micro- and ultrafiltered (DOM was separated into fractions according to its particle size). Fluorescence spectra were also obtained for colloidal and low molecular weight DOM fractions.

MATERIALS AND METHODS

Test samples were collected from natural water (riverine, lake, marine water), soil aqueous extractions, and commercial HS.

Water samples from the Baikal Lake and the Angara River were obtained in August 2007 and January 2009. Winter samples were obtained from ice-holes. Summer and winter water samples were collected from the surface. Marine water samples were obtained from the White Sea region in August 2008. They were taken from the Kandalaksha Gulf; samples 7 and 5 were from the surface of the Gulf and samples 2 and 4 were from the surface of flood plain lakes Upper and Sour-Sweet. Marine samples were micro-filtered with the membrane filter MF-200 (Dubna, Russia) with 200 nm pore size and ultrafiltered through cellulose acetate filter UAM-50 (Vladipor, Russia) with 5 nm pore size. The filtration was

performed under pressure (1.5 atm. of argon). DOM molecules passed through the filter membrane with pore size 5 nm make up a low molecular weight (LMW) fraction, and particles held by the filter make up a colloidal fraction with molecule sizes from 5 to 200 nm.

Soil samples were obtained from the surface (0-20 cm) of black-earth and sod-podzol soils, dried, and diluted with distilled water (1:4). After shaking and settling, samples were filtered with ashfree paper filters.

Commercial HS samples were dissolved in distilled water at 0,02 grams per litre. The following commercial HS were investigated: humic acid “Aldrich”, lignite “Sol-80” (China), “Lignohumate” (Russia), “BE-Dsol”(Canada), “Energen” (Russia), “Ion-14” (USA).

Emission fluorescence spectra were measured with a spectrofluorimeter “Solar CM 2203” under excitation at 270, 310 and 355 nm. The choice of excitation wavelengths was based on previous reports (14,15), as well as on expected spectral components of DOM. Absorbance spectra were measured with a spectrophotometer “Unico 2804” in the 200-700 nm range. Spectral measurements were made under room temperature in quartz cuvettes with 1 cm optical path length and 5 ml volume.

The emission maximum “blue shift” is described by two parameters Δ_1 and Δ_2 (Figure 2). The value Δ_1 is defined as the difference between fluorescence maxima under excitation at 270 and 310 nm ($\Delta_1=\lambda_{max}(270) - \lambda_{max}(310)$), and the value Δ_2 is defined as the difference between fluorescence maxima under excitation at 355 and 310 nm ($\Delta_2=\lambda_{max}(355) - \lambda_{max}(310)$), where λ_{max} is the emission maximum wavelength. The absolute error of fluorescence maximum detection is 2 nm; therefore the absolute error of maximum “blue shift” values is about 3 nm. The relative errors of fluorescence maximum “blue shift” values are 14% and 7% for 20 nm and 40 nm “blue shift” values, respectively.

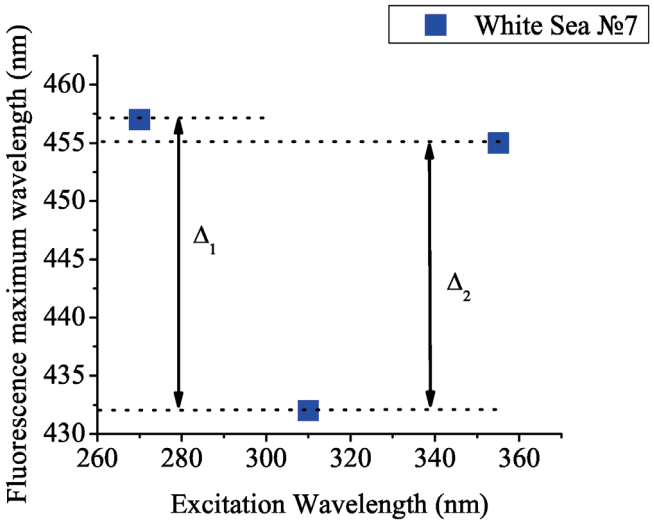

Figure 2: Fluorescence maximum wavelength dependence on excitation of marine water samples with schematic view of Δ_1 and Δ_2 values.

The fluorescence quantum yield for each excitation wavelength was calculated using a reference sample with a known quantum yield. Quinine sulphate solution was used as reference sample, because its fluorescence band is similar to the HS band, both in shape and in location of the maximum. The quantum yield of quinine sulphate dissolved in solution of sulphuric acid in water is 0.546 at a concentration of 0.05 mole per litre (16). A similar method for the fluorescence quantum yield calculation is described in Jobin Yvon’s guide (17). We estimate that the fluorescence quantum yield relative error is about 10%.

RESULTS

Emission maximum “blue shift”

All DOM fluorescence spectra under UV excitation show wide bands of HS fluorescence with a maximum depending on the excitation (Figure 3). Emission maxima under excitation at 310 nm

shift towards a shorter wavelength compared to excitation at 270 nm and 355 nm. This phenomenon was observed for all types of natural HS and is known as “blue shift”.

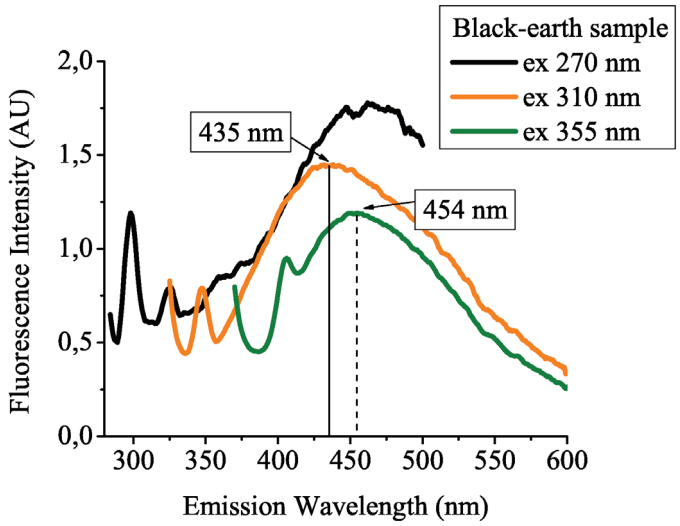

Figure 3: Emission spectra of soil aqueous extractions under excitation at 270,310 and 355 nm.

In contrast, commercial HS fluorescence maxima do not depend on the excitation wavelength, although the shape of spectra is not the same for different excitation wavelengths (see Figure 4).

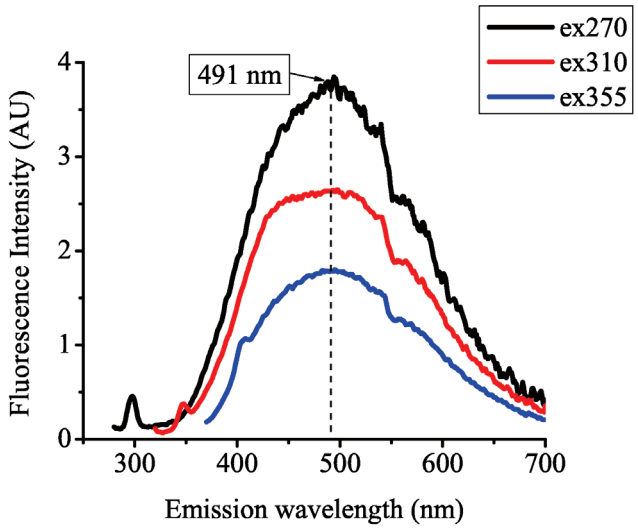

Figure 4: Emission spectra of commercial HS sample “Energen” under excitation at 270, 310 and 355 nm.

The observed “blue shift” values Δ_1 and Δ_2 are presented in Table 1. LMW fraction of DOM with a molecule size less than 5 nm has a greater “blue shift” value than DOM itself. LMW fraction Δ_1 and Δ_2 values are (125 ± 31)% and (131 ± 10)% of those values for DOM. The colloidal fraction usually has a smaller “blue shift” than DOM, but some samples have a greater colloidal fraction “blue shift”.

The fluorescence quantum yield

The fluorescence quantum yield value is 0.02-0.04 (2-4%) for natural water samples, 0.001-0.003 (0.1-0.3%) for soil aqueous extractions samples, and 0.004-0.012 (0.4-1.2%) for commercial HS samples (Table 1, Figure 5). The quantum yield of most natural HS increases with excitation transition from 270 to 355 nm. The fresh water spectrum for the Angara river sample has an additional protein and phenol band with maximum at 350 nm under excitation at 270 nm, which has a significant impact on the quantum yield (see Figure 6). The quantum yield of marine water samples from the White Sea has a monotone dependence on excitation for all samples (see Figure 7). For com-

mercial HS samples, the fluorescence quantum yield does not depend on the excitation wavelength.

Table 1: Emission maxima "blue shift" and the fluorescence quantum yield (QY) values for all investigated samples.

Sample	«Blue shift»		QY ·10^{-3}		
	Δ_1, nm	Δ_2, nm	λ_{ex}=270 nm	λ_{ex}=310 nm	λ_{ex}=355 nm
Natural water samples					
White Sea №2	24	27	14	21	33
White Sea №7	25	23	14	18	27
White Sea №5	24	18	10	15	24
White Sea №4	24	22	9	14	23
Danube River	23	24	27	35	49
Vorja River	22	24	23	33	32
Angara River	35	18	24	18	19
Baikal Lake	35	41	24	32	33
DOM fractions (averaged over 9 samples)					
DOM sample	24	22	12	17	26
Colloidal fraction (5…200 nm)	24	23	9	13	20
LMW fraction(< 5 nm)	30	29	16	25	31
Soil aqueous extractions					
Black-Earth	29	20	2	2	3
Sod-podzol	21	21	1	2	3
Commercial HS					
Humic acid Aldrich	No*	No	4	7	8
Sol-80 from lignite	No	No	12	11	11
Lignohumate	No	No	8	9	6
BE-Dsol from humalite	No	No	5	6	6
Energen from coil	No	No	9	9	8
Ion-14 from lignite	No	No	11	10	10

* «Blue shift» value < 5 nm

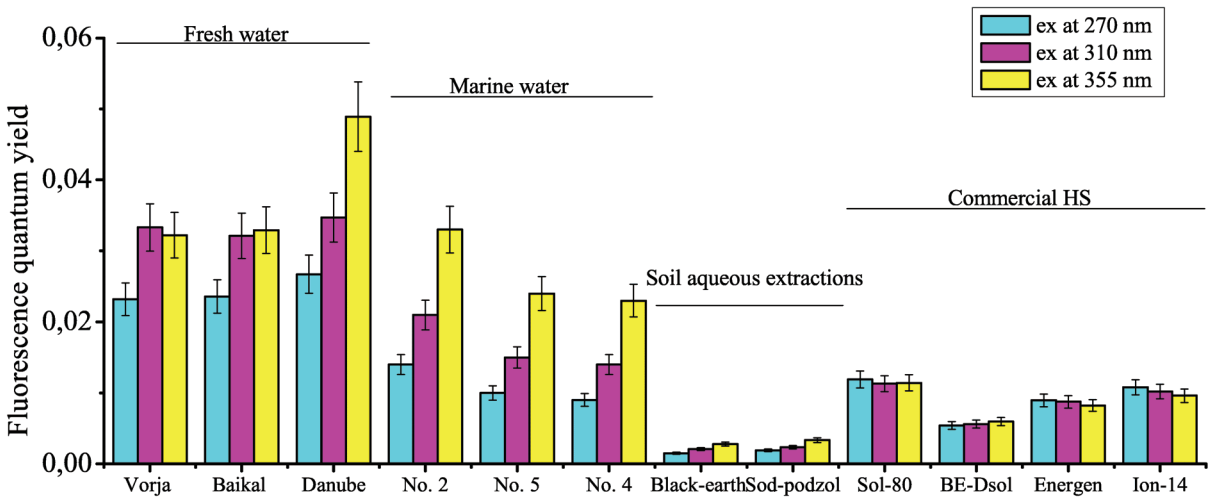

Figure 5: The fluorescence quantum yield of natural and commercial HS.

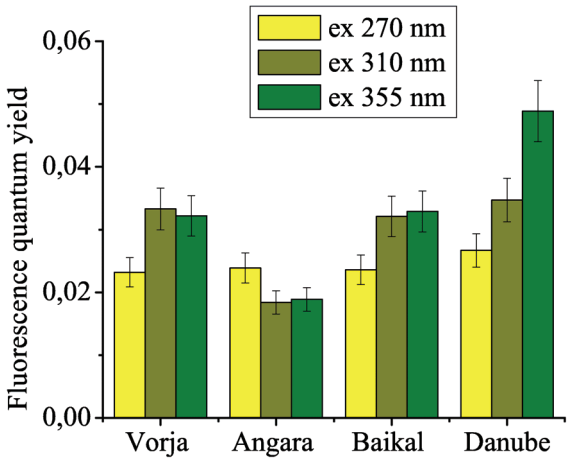

Figure 6: The fluorescence quantum yield dependence on excitation of fresh water samples.

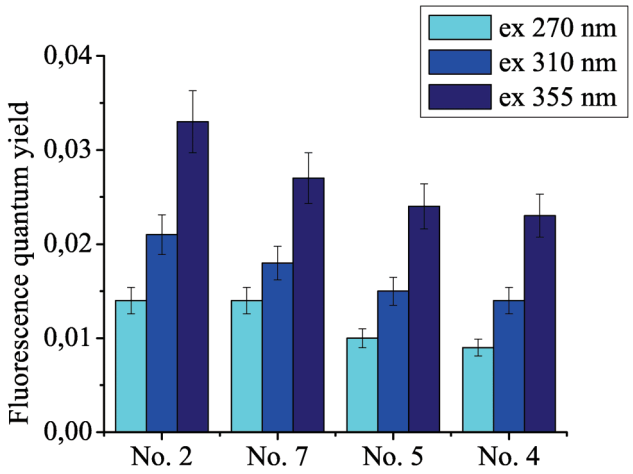

Figure 7: The fluorescence quantum yield dependence on excitation of White Sea samples.

DISCUSSION

The phenomenon of the emission maxima “blue shift” observed in our experiments can be explained by heterogeneity in composition of the HS fluorophore group. Specifically, the humic-like band is due to three overlapping bands A, M and C that are excited at 270, 310 and 355 nm respectively. Band A and C maxima are located in the 420 -480 nm range, and the band M maximum is in the range from 410 to 440 nm. The “blue shift” value reflects concentration ratios of fluorophore groups present in natural HS. As the “blue shift” increases, the relative concentration of the component rises, shifting the maximum towards a shorter wavelength.

The “blue shift” Δ_1 characterises the A and M components concentration ratio. Δ_1 reaches a maximum when component concentrations are equal, and there is no shift when only one component is present. Δ_2 similarly characterises the concentration ratio of components M and C. “Blue shift” Δ_1 and Δ_2 values are about 20…40 nm for natural water samples and for soil aqueous extractions. Since a “blue shift” was not observed for commercial HS, we take this as an evidence for its composition homogeneity. It appears likely that in these samples components A or C are present, while component M is absent.

The “blue shift” increases as the DOM particle size decreases. The colloidal fraction has the same (or smaller) “blue shift” value and the LMW fraction has a “blue shift” value that is 20…30% higher than that of DOM (see Table 2 and Figure 8). Consequently, one can assume that the three fluorophore groups have different particle (macromolecular) sizes. It is well-established that an increasing number of conjugated bonds in complex molecules leads to a shift of fluorescence maxima towards longer wavelengths (18). Thus, the shift towards a shorter wavelength is caused by the

shortening of the conjugated double bond chain. Therefore, the component M corresponds to smaller particle sizes in comparison with the two others components. Band M intensity increases for samples obtained from sites rich in microorganisms (6). Moreover, the fact that these band fluorophores have a relatively small molecular size is consistent with the idea that component M particles are more biologically degraded than the two other humic-like fluorescence components.

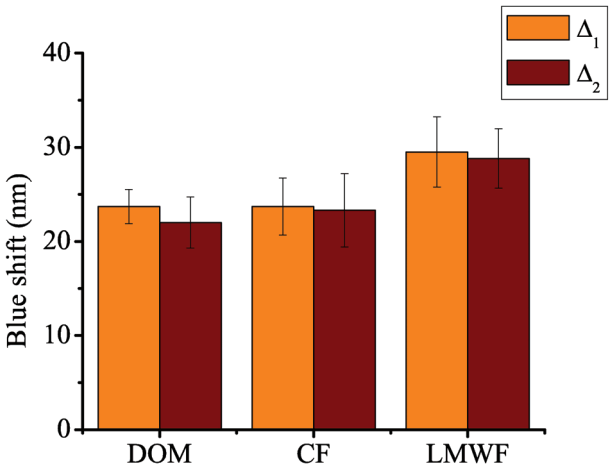

Figure 8: Emission maximum "blue shift" of DOM, its colloidal fraction (CF) and its low molecular weight fraction (LMWF).

It seems likely that group M fluorophores represent the products of relatively recent biological degradation in comparison to groups A and C of humic substances. A higher degree of HS degradation and relatively recent time of origin likely reflects smaller particle sizes. Over a long period of time, the products of biochemical degradation present in the aquatic environment can combine into supramolecular formations. There is an assumption that more mature DOM fluoresces at longer wavelengths (19). However, it is clear that the age of components A and C particles differ from that of component M particles. Consequently, the "blue shift" increases after HS ultra-filtration due to penetration of fluorescent components through the filter at a different ratio.

Figure 9 illustrates DOM and the dependence of the quantum yield of its fractions on excitation for natural water samples from the White Sea coast. Regardless of the particle size, the quantum yield increases with increasing excitation wavelength. We observe the following pattern in the quantum yield changes of DOM fractions: the colloidal fraction QY is less than the DOM QY and, in contrast, the LMW fraction QY is higher than that of DOM. This result may indicate three different fluorescent components that excite at 270, 310 and 355 nm having a different quantum yield, QY(A) < QY(M) < QY (C). The DOM quantum yield ratio is 10:14:22 for these three excitation wavelengths. It increases by 40% with an excitation rising from 270 to 310 nm and by 120% with an excitation rising from 270 to 355 nm. The LMW fraction quantum yield increases by 50% with an excitation rise from 270 to 310 nm and by 90% with an excitation rise from 270 to 355 nm. Thus, the fluorophore M quantum yield is 50% higher than that of fluorophore A, and the fluorophore C quantum yield is 40% higher than that of fluorophore M.

Table 2: Increase of spectral characteristics with decrease of macromolecule size from 200 to 5 nm for marine samples.

	Emission maximum "blue shift"		**Fluorescence quantum yield, $\cdot 10^{-3}$**		
	Δ_1, nm	Δ_2, nm	λ_{ex}=270 nm	λ_{ex}=310 nm	λ_{ex}=355 nm
DOM	24	22	12	17	26
LMWF	30	29	16	25	31
Increasing by (with macromolecule size decreasing from >200 nm to 5 nm)	25%	30%	40%	50%	20%

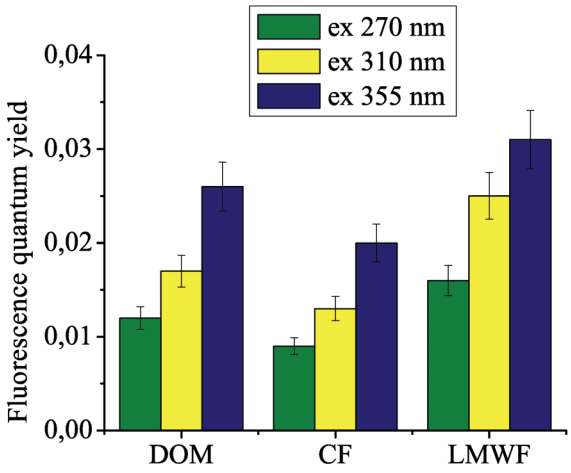

Figure 9: The fluorescence quantum yield of DOM, its colloidal fraction (CF) and its low molecular weight fraction (LMWF).

Figure 9 also illustrates that the QY depends on particle size. For example, the colloidal fraction QY under excitation at 355 nm amounts to 75% of the DOM quantum yield and the LMW fraction quantum yield is 120% of the DOM one (see Table 2). Presumably, this means that particles with a size below 5 nm fluoresce with high efficiency. The data presented for DOM and the quantum yield of its fractions confirm results obtained earlier in investigations on riverine (Moscow region) water (15).

CONCLUSIONS

The comparative analysis of the “blue shift” value and the fluorescence quantum yield was performed for the samples of different origin, as well as for molecular DOM fractions. The fluorescence quantum yield was found to be small; it was less than one percent for HS of soil origin (soil extractions) and 2-4% for HS of riverine and marine origin.

Based on our experimental data, we conclude that

- for all HS samples (marine and fresh water), fluorescence spectra show an emission maximum “blue shift” of about 20-30 nm along with a rising excitation wavelength
- the HS fluorescence quantum yield increases as the excitation wavelength changes from 270 nm to 355 nm. The quantum yield increases by 40-80% for fresh water HS samples depending on the type of the sample and by 100-150% for marine water HS samples
- both the fluorescence quantum yield and the “blue shift” depend on the HS particle size and increase after ultra-filtration of DOM. The fluorescence quantum yield increases by 30% on average with the average particle size decreasing from 200 nm to 5 nm. The “blue shift” increases by 20-30% for the low molecular weight DOM fraction with a particle size of 5 nm in comparison with the initial DOM
- such behaviour of spectral characteristics is consistent with the presence of three basic groups of fluorophores in HS samples as described in the literature. These groups of fluorophores differ from each other in the fluorescence quantum yield and in the average particle (macromolecular) size.
- natural HS is more heterogeneous than commercial HS, as evidenced by a more significant “blue shift” and fluorescence quantum yield dependence on the excitation wavelength.

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