23

Spectral-Luminescent Characteristics of Dissolved Organic Matter in Meromictic Water Bodies of the Kandalaksha Bay of the White Sea

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The optical properties of dissolved organic matter (DOM) of natural water have been studied by fluorescence spectroscopy and absorption spectroscopy. Samples of natural water were taken as objects from different depths of the Tryokhtsvetnoe and Yelovoe meromictic lakes. Dependences of the quantum efficiency and maximum of fluorescence emission on the excitation wavelength were obtained for the first time in a wide range of excitation wavelengths (250−500 nm) for different layers of stratified water bodies. It is shown that the dependence of maximum of the fluorescence emission band on the excitation wavelength and the dependence of the fluorescence quantum efficiency on the excitation wavelength in both lakes and at all studied depths have similar behavior, however, the absolute value of quantum efficiency differs in different water layers. For example, fluorescence quantum efficiencies were from 1.4% to 2.4% at an excitation wavelength of 340 nm. The similar behavior of the fluorescence intensity dependence on the excitation wavelength is explained by the common origin of DOM fluorophores in the under-study layers of stratified lakes. Different values of the fluorescence quantum efficiency are due to the difference in the proportion of aromatic compounds in the composition of DOM and are related to the difference in the hydrochemical characteristics of water at different layers.

Keywords: dissolved organic matter, humic substances, natural water, fluorescence spectroscopy, fluorescence quantum efficiency.

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Introduction

It is known that natural water always contains a certain amount of dissolved organic matter (DOM), which plays a significant role in natural biogeochemical processes [1,2]. The optical properties of natural water depend on its composition and concentration having effect on the functioning of aquatic ecosystems [3]. The DOM of natural origin effectively absorbs UV light and luminesces; therefore, fluorescence spectroscopy and light absorption spectroscopy are currently widely used to study it [4–9]. Absorption and fluorescence spectra of natural water can be used for qualitative and quantitative characteristics of the organic matter in natural water, for example, the DOM fluorescence signal intensity is used in environmental monitoring and remote sensing to estimate its concentration in natural water [10]. Currently, the research is underway to study optical properties of DOM in the northern regions, including arctic and subarctic zones. Thus, in [11–14], the waters of bays of the Laptev, Kara and White Seas were studied. However, the amount of data on DOM in the natural waters of these regions is currently rather limited.

Meromictic water bodies, i.e. water bodies with a stable vertical stratification, which arises - due to the difference in the density of water layers, are of particular interest from the point of view of studying the composition and distribution of DOM [15,16]. In this case, an anoxic zone usually appears in the bottom water, at the upper interface of which an irruption of anoxygenic phototrophic bacteria takes place with a sufficient amount of penetrating sunlight. An example of such water bodies are coastal water bodies that have separated from the White Sea, where the density stratification is a result of the overlapping of sea water by fresh runoff [15,17]. The study of DOM in such water bodies is important for understanding the evolution of oxygen-deficient aquatic ecosystems and for environmental monitoring. This is relevant in the context of the increase in the number and area of waters with near-bottom anoxia and sea areas with the so-called „dead zone“ in the World Ocean due to the climate warming. The study of natural water DOM in different layers of stratified water bodies is important for the development of methods for environmental monitoring of water bodies with sulfide anoxia, which can occur naturally or in the case of anthropogenic pollution in different water bodies, including relict water bodies in the Arctic region.

The purpose of this work is to compare the spectral and luminescent properties of DOM in natural water in two meromictic water bodies with sulfide anoxia on the White Sea coast: Tryokhtsvetnoe and Yelovoe lakes where the distribution of hydrological characteristics differs over depth, and as a result, differences in the chemical composition and optical properties of DOM are possible. For this purpose, the light absorption spectra and fluorescence emission spectra with different excitation wavelengths were measured in water samples taken at different layers, the fluorescence quantum efficiency and the dependence of the fluorescence emission wavelength maximum on the excitation wavelength were calculated.

Studied objects and measurement method

Natural water samples were taken in September 2022 in two meromictic lakes: Tryokhtsvetnoe and Yelovoe, located on the coast of the Kandalaksha Bay of the White Sea. Meromictic lakes feature an upper layer of water with a lower density that is subject to circulation, and a lower layer of higher density that does not mix with the upper one. The transitional gradient zone between these two layers is called the chemocline. It is of special interest to compare the spectral-luminescent properties in the aerobic zone, in the upper part of the chemocline, where there is still a small amount of dissolved oxygen, and in the lower part of the chemocline with anaerobic conditions.

To analyze the spectral-luminescent properties and their dependence on depth in each of the lakes, six samples were taken from several layers. The interface of aerobic zone in the Tryokhtsvetnoe lake has been at a constant depth of 2 m for many years [15,18,19], therefore the following layers were selected for the study: 0 m — surface water, 1.5 m (the aerobic zone above the chemocline), 1.975 m and 2.000 m (upper interface of the chemocline), 2.225 and 2.450 m (lower, anaerobic part of the chemocline). The position of interface of the aerobic zone in the Yelovoe is also constant; it is located at a depth of 3 m [15,18,19]. Depths 0 and 1.500 m (aerobic zone), 2.875 and 3.000 m (upper part of the chemocline), 3.100 and 3.325 m (lower, anaerobic part of the chemocline) were selected for the study. The accuracy of sampling at a certain layer was ensured by using a sampler of in-house design, which is a „comb“ of syringes with a volume of 5 ml with their sampling holes located at a distance of 2.5 cm from each other [20]. Accurate positioning at a given depth is ensured by markings on the cable used to lower the sampler under water, the vertical position of the sampler due to the weight attached to the bottom of the sampler, and the float that fixes the upper reference point. To study DOM, the spectral measurements were preceded by water samples filtering through nylon filters with a pore diameter of $0.22\,\mu$m.

DOM absorption spectra of natural water were recorded at room temperature relative to distilled water by a Solar PB2201 spectrophotometer in the wavelength range of 200–800 nm with a scan step of 1 nm. DOM fluorescence emission spectra were measured by a Solar CM2203 spectrofluorimeter at fluorescence excitation wavelengths λ_{ex} from 250 to 500 nm with a step of 10 nm, the spectra were recorded in the range from 270–515 nm (depending on the excitation wavelength) up to 700 nm with a step of 1 nm. The excitation and recording wavelength ranges for the fluorescence emission spectra were chosen based on the known information about the typical fluorescence bands for the humic and protein components of DOM [10,21]. The dimensions of inlet and outlet slits of the monochromator were 5 nm. Both types of spectra were measured using standard quartz cells with an optical path length of 1 cm. The measured fluorescence spectra were corrected for the effect of the internal filter as follows:

$$I = I_0 \cdot 10^{D_{ex}+D_{em}/2},$$

where D_{ex} and D_{em} are fluorescence values, respectively).

Φ, the fluorescence quantum efficiency of DOM, was calculated from the fluorescence emission spectra and absorption spectra using the reference solution method [22–24]. An aqueous solution of quinine sulfate was taken as a reference solution. Calculations were performed by the following formula:

$$\Phi = \Phi_{qs}\,\frac{K}{K_{qs}}$$

where Φ is fluorescence quantum yield of the sample, K/K_{qs} is the ratio of fluorescence intensity integrated over the spectrum to the optical density at the excitation wavelength for the sample (reference solution), $\Phi_{qs} = 0.546$ is the fluorescence quantum efficiency of quinine-sulfate [25].

Results and discussion

The measured light absorption spectra are shown in Fig. 1 (values of the optical density of the medium D are plotted on the ordinate axis). It can be seen from the figure that starting from 205–210 nm the optical density decreases with increasing wavelength with a small „shoulder“ observed near 260–270 nm, which is typical for the natural water [26] and due to the presence of phenolic groups or aromatic amino acids in the DOM sample. No other absorption peaks in the spectral range up to 800 nm were noted in DOM samples. Some difference in the absorption spectra at the same depth for the two lakes can be explained by the fact that the saline water layer begins higher in the Yelovoe lake compared to the Tryokhtsvetnoe lake (table). The absorbance values D measured at fluorescence excitation wavelengths are then used to calculate the fluorescence quantum efficiency.

Fig. 2 shows examples of the obtained fluorescence emission spectra in samples from the surface water layer and from the chemocline (2 m for the Tryokhtsvetnoe lake and 3 m for the Yelovoe lake). In this range of

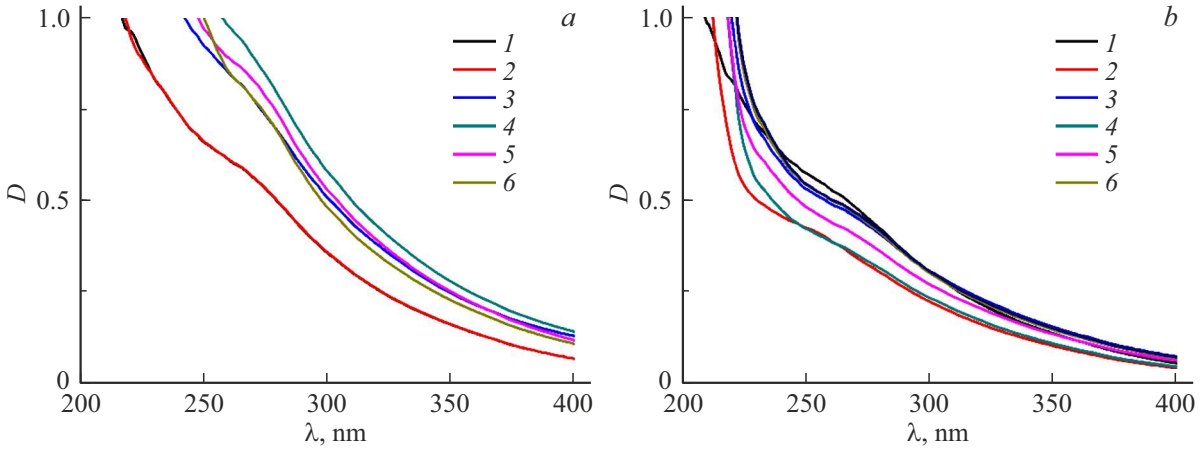

Figure 1. Light absorption spectra in DOM samples (*a*) from the Tryokhtsvetnoe lake: layers 0 m (*1*), *2* — 1.500 m, *3* — 1.975 m, *4* — 2.000 m, *5* — 2.225 m, *6* — 2.450 m and (*b*) from the Yelovoe lake: horizons 0 m (*1*), *2* — 1.500 m, *3* — 2.875 m, *4* — 3.000 m, *5* — 3.100 m, *6* — 3.300 m.

Hydrochemical characteristics of water samples from the Tryokhtsvetnoe (TR) and Yelovoe (Yel) lakes: salinity (*S*), pH, oxidation -reduction potential (*Eh*) and FQE values at a certain excitation wavelength for DOM from these samples

Water body, depth (m)	*S* %	pH	*Eh*, mV	Φ_{290}, %	Φ_{340}, %	Φ_{400}, %	Water characteristics before filtering
TR, 0	0.2	6.9	153	0.92±0.05	1.74±0.09	1.63±0.08	Brown
TR, 1.500	0.2	6.9	163	0.85±0.04	1.67±0.08	1.59±0.08	Brown
TR, 1.975	0.3	6.7	165	0.79±0.04	1.40±0.07	1.26±0.06	Yellow, mouldy odor
TR, 2.000	0.9	6.3	100	0.83±0.04	1.51±0.08	1.60±0.08	With green sulfur bacteria, Hydrogen sulfide
TR, 2.225	3.5	6.8	−316	0.89±0.04	1.61±0.08	1.89±0.09	With green sulfur bacteria, Hydrogen sulfide
TR, 2.450	8.5	6.8	−349	0.95±0.05	1.74±0.09	2.00±0.10	With green sulfur bacteria, Hydrogen sulfide
Yel, 0	0.3	7.5	116	0.94±0.05	1.76±0.09	1.64±0.08	
Yel, 1.500	7	7.5	161	1.33±0.07	2.43±0.12	2.85±0.14	
Yel, 2.875	17.9	7.4	98	1.39±0.07	2.44±0.12	2.61±0.13	Light green without Hydrogen sulfide
Yel, 3.000	18	7.2	−190	1.32±0.07	2.05±0.10	2.03±0.10	Muddy-green, with green sulfur bacteria, hydrogen sulfide
Yel, 3.100	18.5	7.0	−317	1.16±0.06	1.75±0.09	1.78±0.09	With green sulfur bacteria, hydrogen sulfide
Yel, 3.3000	20	7.0	−366	1.01±0.05	1.61±0.08	1.61±0.08	With green sulfur bacteria, hydrogen sulfide

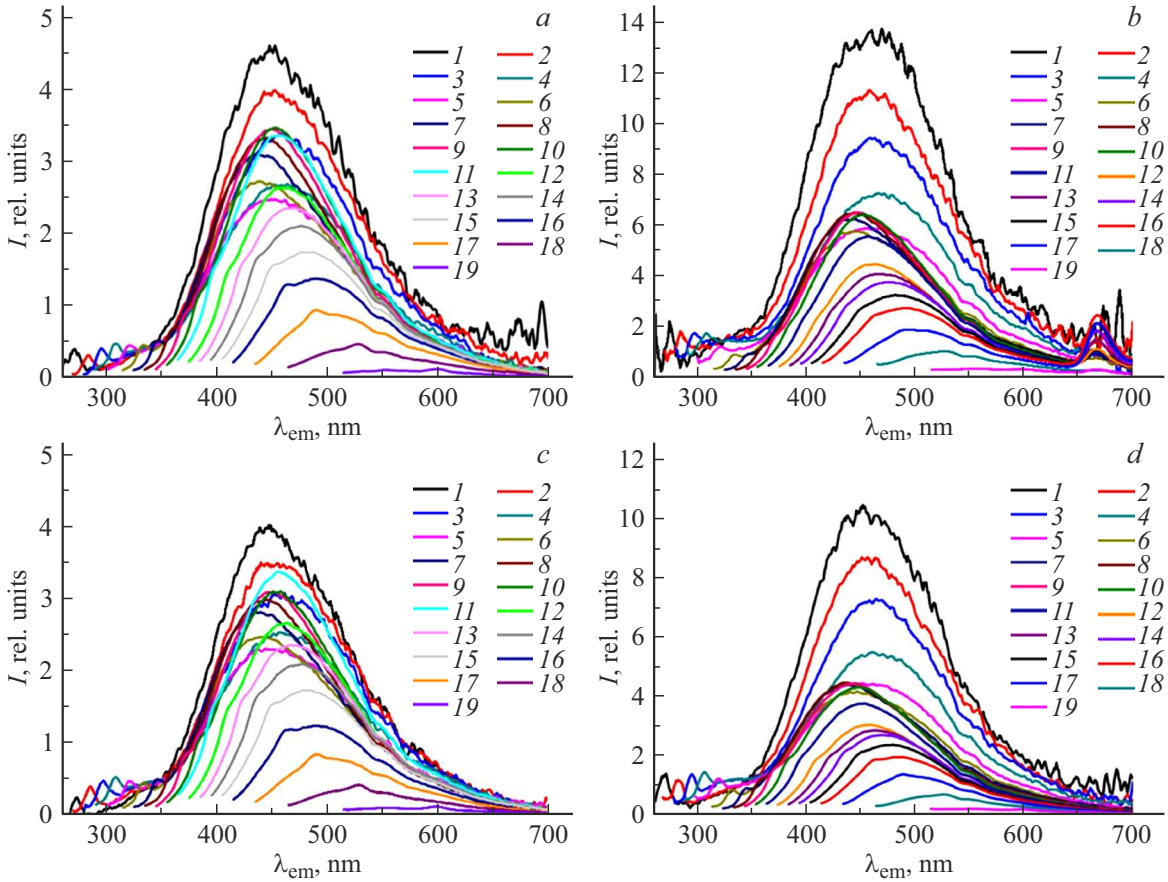

Figure 2. Fluorescence emission spectra of DOM in the Tryokhtsvetnoe lake: layers 0 m (*a*) and 2 m (*b*); and in the Yelovoe lake: layers 0 m (*c*) and 3 m (*d*). Curves from *1* to *16* correspond to λ_{ex} 250–400 nm with a step of 10 nm, *17* — 420 nm, *18* — 450 nm, *19* — 500 nm. The presented spectra are corrected for the effect of the internal filter.

excitation wavelengths, the DOM fluorescence spectrum consists of two overlapping bands: an UV band with a maximum in the region of 300–350 nm (corresponding to phenolic compounds or aromatic amino acids) and a luminescence in the visible spectral region with a maximum at 400–450 nm (corresponding to humic compounds) [21]. Humic compounds are organic acids with carboxyl and phenolic groups, which are formed in various ways, both as residual products of DOM degradation and through condensation. The position of maximum and the profile of the humic fluorescence band depend on the composition and origin of the DOM and can be used to classify the types of natural water based on its spectral properties.

In the spectral response of the studied samples at fluorescence excitation wavelengths of 250–320 nm, a signal of Raman scattering (RS) of light by water molecules is noticeable in the form of a small peak at short wavelengths ($\approx$ 270–350 nm), which merges with the main DOM fluorescence band as the excitation wavelength increases.

For the water taken from the chemocline region with a massive growth of green sulfur bacteria, after filtration, a weak fluorescence band was sometimes observed with a maximum at 670 nm (Fig. 2, *b*). This fluorescence cannot be due to the presence of algae, cyanobacteria, or extracellular chlorophyll in the sample, as fluorescence measurements made on samples prior to filtration showed no chlorophyll-containing microorganisms in water samples from the chemocline zone. However, green sulfur bacteria, anoxygenic phototrophs, are present in the water before filtration, which contain bacteriochlorophyll *d* (Tryokhtsvetnoe lake, Yelovoe lake) or bacteriochlorophyll *e* (Yelovoe lake) in their chlorosomes. It is likely that at a high concentration of bacteria, bacteriochlorophyll from destroyed cells could get into the filtrate during the filtration of a natural water sample from the chemocline zone and cause the presence of this fluorescence band.

Then, using the obtained spectra, the dependences of the wavelength of the spectrum maximum λ_{max} on the excitation wavelength λ_{ex} were calculated. Results are shown in Fig. 3. It can be seen that the dependence has a non-monotonic behavior, in all the studied samples at an excitation wavelength of 250–270 nm λ_{max} it increases, then decreases to 310 nm. The shift of the fluorescence emission band peak towards shorter wavelengths with a change in the excitation wavelength from 280 to 310 nm corresponds to the so-called „blue shift“, which is typical

for humic compounds that are part of DOM [11,26]. At the same time, in the studied samples, the magnitude of this shift is almost independent on the depth within the measurement error, but differs somewhat in the two lakes. The phenomenon of the „blue shift" of emission spectra was previously noted in samples of natural humic substances of marine, river and soil origin [23,27,28] and indicates the heterogeneity of the composition of fluorophores of natural humic substances.

With a further increase in the excitation wavelength, the emission peak shifts to the long wavelength region. The maximum intensity of fluorescence emission I_{max} with an increase in the excitation wavelength λ_{ex} also changes nonmonotonically: it decreases in the range of $\lambda_{ex} = 250-290$ nm, then increases at 300 -340 nm and decreases again at $\lambda_{ex} = 350-500$ nm.

It should be noted that all dependences shown in Fig. 3 are nearly repeating each other in both water bodies at all depths, despite the noticeable difference in the hydrological characteristics of water (water salinity varied from 0.2 to 20, and pH varied from 6.3 to 7.5).

Along with the traditional optical parameters calculated from the DOM absorption and fluorescence spectra, the fluorescence quantum efficiency (FQE) is an informative parameter. FQE for dye molecules is the probability that a fluorophore will emit light when returning to the ground state after being excited by light, and is defined as the ratio of the number of emitted photons to the number of absorbed photons [22,29]. The DOM composition of natural water includes both the compounds emitting fluorescence as a result of photon absorption and the compounds that absorb light but do not fluoresce. In addition, fluorescence in DOM molecules can take place due to the transfer of energy from light-absorbing chromophores to fluorophore groups of both intramolecular and intermolecular types. The nature of the fluorescence of humic compounds and DOM in natural water is unclear so far. Therefore, for a DOM, which is a set of various organic compounds with a huge structural and compositional diversity, it is more correct to call the FQE as the apparent quantum efficiency of fluorescence [30]. Separately, it should be noted that the contribution of the Raman signal of OH-groups of water molecules is small: for the studied samples of natural water DOM fluorescence prevailed in the spectrum and the area of the RS line was not more than 4–5% of the fluorescence band area, at the same time the contribution of the RS line in the spectrum decreased with increasing excitation wavelength. Therefore, when calculating the FQE, the contribution of the RS can be considered negligibly small.

Fig. 4 shows the calculated dependences of the FQE of DOM on the excitation wavelength $\Phi(\lambda_{ex})$ in water samples from different depths of two water bodies. It can be seen that the FQE has a minimum at the excitation wavelength of ≈ 290 nm, as well as a small minimum at $\lambda_{ex} \approx 360$ nm, maxima are observed at $\lambda_{ex} \approx 340$ and 380 nm. In both water bodies, the dependences of FQE on the excitation wavelength have similar behavior but the absolute value of FQE differs significantly in different water layers. This is especially noticeable for the samples from the Yelovoe lake, where there is a greater spread compared to the Tryokhtsvetnoe lake. Also, it can be seen that the maximum FQE values in these lakes differ from each other. All this can be caused by differences in the composition and concentration of dissolved substances in these lakes (which is also reflected in salinity, oxidation-reduction potential), as well as in the composition of the microorganism community.

Let us compare the FQE values of the DOM of water in the two studied meromictic lakes and their dependence on the excitation wavelength with the same characteristics for the DOM of another origin. The FQE in the water from the Mediterranean Sea and from the Atlantic Ocean at the excitation wavelength of 355 nm (1.18 and 1.06%, respectively) is higher compared to the FQE in the surface water of the Rhine and Rhone rivers (0.91 and 0.96%) [31]. Fluorescence FQE of DOM of sea and river natural water is from 2 to 5% when excited at wavelengths of 270, 310, or 355 nm; humic substances have their FQE varying between 0.1 and 0.4% with the excitation at the same wavelengths [23,26], and the FQE of soil fungi metabolites can be as high as 7% [26]. The colloidal organic matter of natural water has lower FQE values compared to low-molecular fractions of DOM [20]. The FQE of DOM of natural water and soil extracts significantly depends on the excitation wavelength, its values increase with excitation wavelength increasing from 270 to 355 nm. On the contrary, the FQE of commercial humic acids and preparations made of carbon material is lower and almost independent on the excitation wavelength of up to 355 nm [28,32]. In [33], the dependence of the FQE of DOM on the excitation wavelength has been presented for the first time using the example of sea, river, and estuary water of southern Florida, the Amazon River, the Tamiami River, and the Suwannee River. Later, in a number of studies, dependences of the FQE of DOM on the excitation wavelength in the waters of the coastal zone of the Atlantic Ocean [34], the Norwegian Sea [30] and river water [35] have been presented. A study of fulvic acids and humic acids in the Suwannee River is published in [36]. In all cases, the dependence of the FQE of DOM on the excitation wavelength has two peaks at 340–355 and 370–400 nm. Measurements for DOM in the coastal zones of the Arctic Ocean confirmed the nonmonotonic dependence of the FQE on the excitation wavelength in a wide wavelength range from 250 to 500 nm: The FQE first decreases from the excitation wavelength of 250 nm to 270–280 nm, and then grows, showing a shoulder or a local maximum at about 340 nm, has maximum values at 370–390 nm with a further drop in values towards longer wavelengths [11,37].

Surface water DOMs in the Tryokhtsvetnoe and Elovoye lakes are characterized by FQE equal to 1.7–1.8% at an excitation wavelength of 340 nm, which is typical for

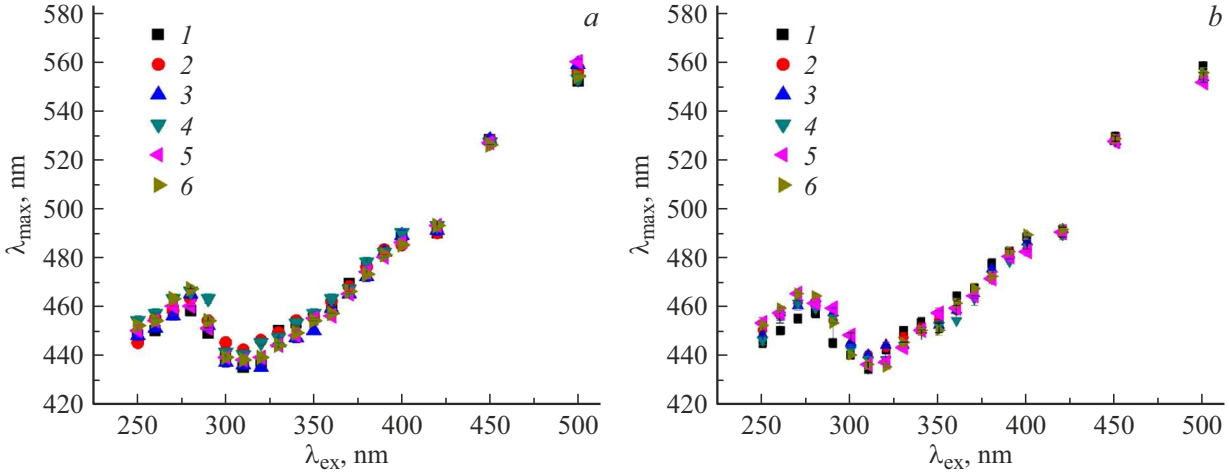

Figure 3. Dependences of the DOM fluorescence emission band peak on the excitation wavelength in Tryokhtsvetnoe (*a*) and Yelovoe lakes (*b*). The samples are numbered in the same manner as in Fig. 1.

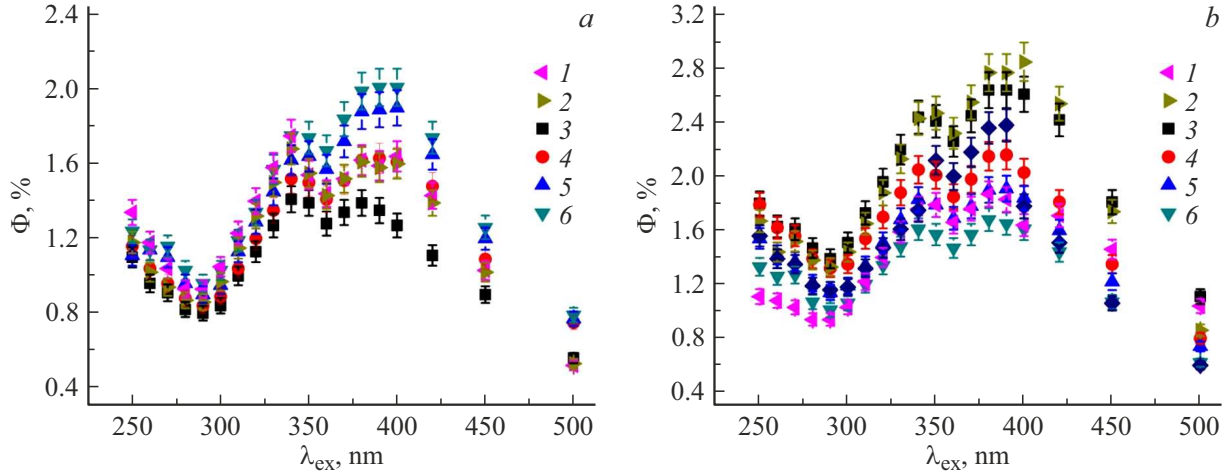

Figure 4. Dependences of the DOM fluorescence quantum efficiency on the excitation wavelength in (*a*) Tryokhtsvetnoe and (*b*) Yelovoe lakes. The samples are numbered in the same manner as in Fig. 1.

fresh water DOM with a moderate content of humic substances. The FQE values decrease near the upper interface of the chemocline in the Tryokhtsvetnoe lake (1.4−1.5%) but increase in the Yelovoe lake (2.4%). Such a multidirectional trend can be explained by the difference in the hydrochemical characteristics of water and the microbial community in these lakes above the chemocline: in the Yelovoe lake the halocline (salt water interface) is higher than in the Tryokhtsvetnoe lake. As the depth increases in the chemocline zone in both lakes, the FQEs equalize to values of 1.6−1.7%, and the dependence of the FQE on the excitation wavelength remains similar in shape at all layers, but FQE values increase at more long-wavelength excitation (> 390 nm) compared to the excitation with UV light. This trend is especially noticeable in the Tryokhtsvetnoe lake. The similarity of fluorophores, which give a humic fluorescence band in the emission spectrum of DOM of natural water from different water bodies and from different layers, is not a surprising fact because earlier, using the example of DOM of different origin in freshwater bodies (Suwannee River in Canada, Onega lake and Vodoprovodnoe lake in the Karelian part of Russia) it was shown that regardless of the origin (river or lake DOM), geographical location and different molecular size of DOM, several similar types of humic-type fluorophores were found in the samples, isolated using SEC-HPLC chromatography [38].

Thus, the FQE values obtained in this study and their dependence on the excitation wavelength are, in general, confirmed by research publications. What is new is the comparison of the FQE values and their dependence on the excitation wavelength at different layers with different water characteristics in the stratified water bodies of the White Sea coast and the explanation of the differences found.

Conclusions

This study analyzed spectral and luminescent properties of DOM in natural water from two meromictic lakes on the coast of the Kandalaksha Bay of the White Sea: Tryokhtsvetnoe and Yelovoe lakes. Water samples for the study were taken from the aerobic part of the water bodies, the upper part of the chemocline and from its lower, anaerobic part. Light absorption spectra were measured in the wavelength range of 200−800 nm, as well as fluorescence emission spectra with excitation wavelengths of 250−500 nm. From the spectra obtained, the dependences of the fluorescence emission band maximum on the excitation wavelength, as well as the dependence of the FQE on the excitation wavelength were calculated.

It is shown that the dependences of the fluorescence emission band maximum on the excitation wavelength in both lakes at all studied depths are similar in nature, a minimum in the region of 310 nm is observed, which corresponds to the „blue shift“ of the DOM fluorescence band (shift of the maximum to the short-wavelength region). Dependencies of the quantum efficiency of fluorescence on the excitation wavelength in two lakes also have a similar behavior (peaks at 340 and 370−390 nm), however absolute quantum efficiency of fluorescence is considerably different in water layers sampled from different depths, which may be a consequence of the difference in structural characteristics of DOM in these lakes.

Studies of this kind are important for understanding the mechanisms of formation of the optical properties of DOM in natural water of various origins, studying the habitat conditions of microbial communities inside the water column, as well as for understanding the evolution of meromictic water bodies and for developing methods for environmental monitoring of water bodies with sulfide anoxia, including relict water bodies of the Arctic region.

Conflict of interest

The authors declare that they have no conflict of interest.

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