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# Spectral properties of dissolved organic matter and their dependence on depth in artificially and naturally separated meromictic reservoirs

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> The spectral properties of the dissolved organic matter of the natural water of two parts of the reservoir artificially separated from the White Sea - the Kanda Bay: the sea reach and the meromictic Fedoseevsky reach. For comparison, data were analyzed for natural meromictic reservoirs - lakes Spruce, Tricolor, Lagoon on the Green Cape. Absorption spectra, fluorescence spectra and the dependence of the fluorescence quantum yield on the excitation wavelength in the range of the excitation wavelength of 250-500 nm were obtained for water samples from different horizons. The dependences of the wavelength of the emission maximum on the excitation wavelength are constructed and the value of the "blue shift"is calculated — the displacement of the maximum of the emission band in the short-wave direction. It is shown that the fluorescence intensity of dissolved organic matter in the Fedoseevsky Ples is higher than in the marine one, while the dependence of the fluorescence quantum yield on the excitation wavelength in both parts of the Kanda Lip is qualitatively similar, but differs in absolute values. In natural meromictic reservoirs, this dependence has a similar character, also differing in the absolute value of the fluorescence quantum yield, which indicates a different ratio of aromatic and aliphatic organic compounds. Thus, differences in the spectral and optical properties of the dissolved organic matter of two parts of an artificially separated reservoir and natural reservoirs isolated from the White Sea were revealed. The spectral and optical characteristics of the water column of sea bays, naturally or artificially separated from the main marine basin, can serve as an objective indicator of the trophic (ecological) state of the reservoir.

> Keywords: dissolved organic matter (DOM), natural water, absorption spectroscopy, fluorescence spectroscopy, fluorescence quantum yield.

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# Introduction

Dissolved organic matter (DOM), which is present in natural water of any type, plays a critical role in biochemical processes and affects the functioning of aquatic ecosystems [1–4]. Colored DOM of natural origin absorbs UV light and luminesces efficiently; its composition and concentration affect the optical properties of natural water. At present, absorption and fluorescence spectroscopy are used for its examination. Absorption and fluorescence spectra of natural water may be used for qualitative and quantitative characterization of DOM in environmental monitoring and remote sensing. For example, fluorescence and absorption spectroscopy experiments were performed in [3,5-9] to study DOM of natural water of the Kara Sea [3,5,6], the Laptev Sea [7], the Onega Bay of the White Sea [8], and freshwater lakes in Karelia [9]. Meromictic reservoirs (i.e., reservoirs with stable vertical stratification induced by the difference in densities of water layers) are of particular interest in research into the composition and distribution of DOM. Coastal reservoirs separated from the White Sea [10,11] may serve as an example here. Owing to the accumulation of dissolved and suspended organic matter and the active consumption of oxygen by heterotrophic microorganisms, anaerobic conditions are often established in their near-bottom layer. The study of DOM in different layers of coastal reservoirs at different stages of isolation from the sea provides a deeper insight into their evolution and is important for the development of methods for aquatic ecosystems monitoring.

This isolation may be natural (e.g., due to postglacial uplift of the coast, which is the case with coastal waters of the White Sea region [10,11]), artificial, or accelerated by anthropogenic intervention (e.g., due to hydraulic engineering). Natural meromictic lakes feature an upper aerobic layer of water and a lower layer with a higher density, which does not mix with the upper one. The transitional gradient zone between these two layers is called the chemocline. A small amount of dissolved oxygen is present in the upper

part of the chemocline, and massive growth of anoxygenic phototrophic bacteria may be observed in the lower part of the chemocline with anaerobic conditions if sunlight penetrates there [12].

The Kanda Bay at the head of the Kandalaksha Gulf of the White Sea was cut off from the main water area by two filter dams and is an example of an artificially separated reservoir. Its connection with the White Sea was disrupted at the end of 1916 when the first railway dam was built. Another dam, which was built in 1968, furthered the isolation [10,13,14]. In the present day, the Kanda Bay is a system of interconnected reservoirs that includes the outer sea reach; the middle reaches, wherein fresh and salt water masses coexist steadily; and a freshwater lake. They may be regarded as different stages of isolation from the sea [15,16]. Deep stagnant waters In the middle (meromictic) reaches are characterized by a significant concentration of dissolved hydrogen sulfide [15]. A disruption of the tidal regime translates into significant disturbances in benthic communities, including a disturbance of the composition of microbial communities [17]. It appears intriguing and relevant to examine the optical properties of DOM in water layers of different parts of the Kanda Bay in comparison with those of naturally separated meromictic reservoirs.

The aim of the present study is to establish the spectral and optical characteristics of DOM in natural water from the Kanda Bay of the Kandalaksha Gulf of the White Sea and compare them with similar characteristics of DOM in meromictic reservoirs of natural origin. Light absorption and fluorescence emission spectra were measured for this purpose at different excitation wavelengths for water samples collected from various depths in three reservoirs with different stages of natural isolation from the White Sea (the lagoon on Cape Zeleny and lakes Yelovoe and Tryokhtsvetnoe) and from two reaches of the Kanda Bay: the sea one and the meromictic Fedoseevsky reach. Dependences of the maximum of the DOM fluorescence emission band on excitation wavelength were also plotted, and the fluorescence quantum yield was calculated.

# Objects under study and water characteristics

Samples of natural water from meromictic reservoirs of natural origin located on the coast of the Kandalaksha Gulf of the White Sea were collected in September 2022 in the lagoon on Cape Zeleny and lakes Yelovoe and Tryokhtsvetnoe. At the time of sampling, the chemocline in the lagoon on Cape Zeleny occupied the 4.5-5.5 m layer. Water samples in the lagoon on Cape Zeleny were collected from the following horizons: 0, 1, 2, 3, 4, 4.5 m; the 5–5.9 m interval was sampled with a step of 0.1 m. Since the boundary of the aerobic zone in Lake Tryokhtsvetnoe was located at a depth of 2 m, the following horizons were chosen: 0, 1.5, 1.9, 2, 2.2, and 2.5 m. The boundary of the aerobic zone in Lake Yelovoe is located 3 m below the

surface; the depths of 0, 1.5, 2.9, 3, 3.1, and 3.3 m were chosen to be studied. The absorption and fluorescence spectra of DOM from these meromictic reservoirs were presented in [18,19]. The hydrochemical characteristics of water samples from lakes Tryokhtsvetnoe and Yelovoe (salinity, pH, oxidation-reduction potential ( $E_h$ ), and the values of fluorescence quantum yield at certain excitation wavelengths for DOM from these samples) were reported in [18]. Data on the concentration of dissolved oxygen,  $E_h$ , and chlorophyll and bacteriochlorophyll concentrations in unfiltered samples collected from various horizons in the lagoon on Cape Zeleny were provided in [19].

The sea reach of the Kanda Bay and the Fedoseevsky reach have different structures in terms of the depth distribution of water salinity (see the table). In the sea reach, salt water constitutes the greater part of the water column, but a layer of almost fresh water with a thickness below 1 m is present near the surface. At a depth of 1 m, the salinity increases sharply to 14–15%°, which is only 3% lower than the salinity in the near-bottom layer. The Fedoseevsky reach is separated from the sea by shallow rapids [15]. It became completely isolated from the sea after the construction of the railway dam in 1916. It differs significantly from the sea in its salinity distribution and has a two-layer structure with a pycnocline (a jump in water density) at a depth of 7-8 m [15]. The salinity below the pycnocline at a depth of approximately 11 m is as high as 14% (for comparison, this salinity level in the sea part is reached at a depth of just 1 m). The lens of salt water in the deepest part of the Fedoseevsky reach has probably been preserved for more than a hundred years [10,13]. In the central and eastern parts of the Fedoseevsky reach, hydrogen sulfide is present in the near-bottom layer [10,13,20,21].

Natural water samples were collected in the Kanda Bay in March 2023. The sampling points in both reaches were located above bottom depressions 14 m in depth. The depths of sampling and the temperature and salinity of water at these depths are listed in the table.

# **Research procedure**

In order to study DOM, water samples were filtered through nylon filters with a pore diameter of  $0.22 \,\mu$ m prior to spectral measurements. The absorption spectra of DOM in natural water (relative to distilled water) were recorded using a Solar PB2201 spectrophotometer within the 200–800 nm wavelength range with a scanning step of 1 nm. Spectra were measured at room temperature. The fluorescence emission spectra of DOM were measured with a Solar CM2203 spectrofluorimeter at fluorescence excitation wavelengths  $\lambda_{ex}$  varying from 250 to 500 nm in steps of 10 nm. These spectra were measured within the range from 260–515 to 700 nm (depending on the excitation wavelength: from 260 to 700 nm at  $\lambda_{ex} = 250$  nm and from 515 to 700 nm at  $\lambda_{ex} = 500$  nm) with a step of 1 nm. The excitation and detection wavelength ranges for

Sampling site	Sample number	Depth, m	<i>T</i> , °C	Salinity,%∘
Sea part		0.0	-0.23	2.48
of the Kanda Bay	1	1.0	+0.32	14.56
67° 07' 49.6" N;	2	3.0	0.58	15.82
32° 12' 02.0" E	3	5.0	0.30	16.55
	4	8.0	3.13	18.05
	5	11.0	3.87	18.32
	6	13.8	3.77	18.52
The Kanda Bay		0.0	-0.48	
Fedoseevsky reach	7	1.0	-0.06	3.3
67° 06' 23.8" N;	8	3.0	1.85	8.4
32° 10' 31.9" E	9	5.0	2.08	11.0
	10	8.0	1.65	11.6
	11	11.0	4.02	14.7
	12	12.0	5.24	15.6
	13	13.0	5.40	16.1
	14	13.8	5.26	16.6

Sampling horizons and physical and chemical characteristics of water

fluorescence emission spectra were chosen based on the available data on typical fluorescence bands for the humic and protein components of DOM [7,22]. The dimensions of entry and exit 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 internal filter effect as  $I = I_0 \cdot 10^{(D_{ex}+D_{em})/2}$  ( $D_{ex}$  and  $D_{em}$  are the optical densities at the wavelengths of fluorescence excitation and detection, respectively).

Data extracted from absorption spectra (optical densities at wavelengths of 250, 270, and 280 nm) were used as indicators to characterize DOM of natural water. Longer wavelengths were disregarded, since the corresponding optical density was low in samples from the sea part of the bay. The maximum of the DOM fluorescence emission band in the obtained fluorescence spectra was identified in order to calculate the dependence of its position on excitation wavelength, determine the so-called "blue shift," and compare them with similar parameters for natural meromictic reservoirs. The fluorescence quantum yield (FQY) is another informative parameter. In the case of dye molecules (and, consequently, colored DOM), the FQY value reflects the probability that a fluorophore will emit light on return to the ground state after excitation. It is defined as the ratio of the number of emitted photons to the number of absorbed photons [23]. DOM of natural water contains compounds fluorescing as a result of photon absorption and compounds that absorb light but do not fluoresce.

Therefore, it can be said that a high FQY is characteristic of DOM with a high concentration of aromatic groups. Note that the nature of fluorescence of humic compounds and DOM of natural water has not yet been clarified completely; therefore, "apparent fluorescence quantum yield" is a more correct term for the FQY of DOM, which is a combination of various organic compounds [18,24].

The FQY  $(\Phi)$  was calculated for DOM based on the fluorescence emission spectra and light absorption spectra using reference solutions with known FQY values (see, e.g., [23,25,26]). An aqueous solution of quinine sulfate was taken as a reference solution. The formula for calculation was as follows:

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

where  $\Phi$  is the fluorescence quantum yield of the sample; K and  $K_{qs}$  are the ratios of fluorescence intensity integrated over the spectrum to the optical density at the excitation wavelength for the sample and the reference solution, respectively; and  $\Phi_{qs} = 0.546$  is the fluorescence quantum yield of quinine-sulfate [25].

# **Results and discussion**

The measured light absorption spectra are shown in Fig. 1 (optical density D of the medium is plotted along the ordinate axis). It can be seen that the optical density decreases as wavelength increases upward of 205-210 nm.



**Figure 1.** Absorption spectra for DOM in water samples from the sea reach of the Kanda Bay (a) and from the Fedoseevsky reach (b) (sample numbering according to the table).



**Figure 2.** Dependences of the optical density of DOM at wavelengths of 250, 270, and 280 nm on depth for water samples from the sea reach (*a*) and the Fedoseevsky reach (*b*) and on salinity (*c*) (I — sea reach, 2 — Fedoseevsky reach).

A small "shoulder," which is located approximately at 260–270 nm, is typical of natural water [18] and forms due to the presence of phenolic groups or aromatic amino acids in DOM. No additional absorption peaks were noted in the spectra of DOM samples at wavelengths up to 800 nm. A similar pattern was observed in the light absorption spectra of water from natural meromictic reservoirs: lakes

Tryokhtsvetnoe and Yelovoe and the lagoon on Cape Zeleny [18,19]. The values of optical density D measured at fluorescence excitation wavelengths were then used to calculate the fluorescence quantum yield. Figure 2 presents the plots of dependences of the optical density of water at wavelengths of 250, 270, and 280 nm on depth and salinity. In the sea reach, the optical density decreases gradually with



**Figure 3.** Fluorescence emission spectra for four samples collected from the Kanda Bay sea reach at depths of 1 m(a) and 13.8 m(b) and from the Fedoseevsky reach at depths of 1 m(c) and 13.8 m(d). (Curves 1-16 correspond to  $\lambda_{\text{ex}} 250-400 \text{ nm}$  with a step of 10 nm; 17 - 420 nm, 18 - 450 nm, and 19 - 500 nm). The presented spectra are corrected for the internal filter effect.

depth as salinity increases. This is attributable to the fact that the concentration of DOM in sea water is lower than the one in surface (freshened) water. In the Fedoseevsky reach, an anaerobic zone with hydrogen sulfide extends downward from a depth of 11 m. It can be seen that the optical density starts increasing rapidly at this depth. The optical density at a maximum depth of 13.8 m is approximately 6 times higher than the density corresponding to a depth of 8 m.

Figure 3 shows the example fluorescence emission spectra. The fluorescence spectrum of DOM in the studied range of excitation wavelengths (250-700 nm) contains two overlapping bands: the "protein" UV band with its maximum in the region of 300-350 nm (corresponding to proteins or phenolic compounds) and the "humic" band in the visible spectral region with a maximum at 400-450 nm (corresponding to humic compounds). The spectral response of DOM in the studied water samples also features a signal associated with Raman scattering (RS) of light by water molecules. This signal has the form of a small peak at short wavelengths ( $\approx 270-350 \text{ nm}$ ), which merges

with the primary DOM fluorescence band as the excitation wavelength increases. A small peak at 330 nm is also observed in the spectra of samples collected from shallow depths in the Fedoseevsky reach. This peak is probably attributable to settling of microorganisms, which proliferate actively in surface water and produce a band of free amino acids (tyrosine, tryptophan) or fragments of proteins with these amino acids, on the filter. In addition, it is evident that the fluorescence intensity in the Fedoseevsky reach is higher than in the sea part of the bay. In the Fedoseevsky reach, an increase in fluorescence intensity with depth is also noticeable (the difference is more than 5-fold for depths of 1 m and 13.8 m at an excitation wavelength of 250 nm and more than 7-fold at an excitation wavelength of 500 nm). This is indicative of DOM accumulation in near-bottom water.

Figure 4 presents calculated dependences  $\Phi(\lambda_{ex})$  of the FQY of DOM on excitation wavelength for water samples collected from different depths of two reservoirs. (Note that the contribution of the Raman signal of water molecules



**Figure 4.** Dependences of the FQY (a) and the wavelength corresponding to the DOM fluorescence emission band maximum (b) on excitation wavelength (1 - sea reach, 2 - Fedoseevsky reach).



**Figure 5.** Variation of FQY values at excitation wavelengths of 250, 270, 310, and 340 nm for the sea reach (a) and the Fedoseevsky reach (b) with depth of the water layer.



**Figure 6.** Dependences of the wavelength corresponding to the DOM fluorescence emission band maximum (*a*) and the FQY (*b*) on excitation wavelength in natural meromictic lakes (1, 2 — lagoon on Cape Zeleny, depths of 1 and 5.9 m, respectively; 3 — Lake Tryokhtsvetnoe, 1.5 m; 4 — Lake Yelovoe, 1.5 m).

may be considered negligible in calculations of fluorescence quantum yields.) It can be seen that the FQY has a minimum at an excitation wavelength of  $\approx 290 \,\text{nm}$  and a slight "dip" at  $\lambda_{ex} \approx 360$  nm; its maxima are found at  $\lambda_{ex}\approx 340$  and  $380\,nm.$  The dependences of FQY values on excitation wavelength in two reservoirs are qualitatively similar, but the absolute FQY levels in the sea and Fedoseevsky reaches (and at different depths within the same reservoir) differ. The FQY for the Fedoseevsky reach is lower, since it contains a greater amount of DOM of terrigenous origin (with more intense fluorescence, but a lower quantum yield). It is known from [5,27] that the dependence of FQY of DOM in the coastal zones of the Arctic Ocean is non-monotonic: the FQY decreases from an excitation wavelength of 250 nm to 270-280 nm, starts growing as the wavelength increases further, forms a local maximum around 340 nm, and reaches maximum levels under excitation by light with a wavelength of 370-390 nm; at even longer wavelengths, the FQY decreases again. The presence of two maxima of FQY of DOM under excitation by light within the 340-355 nm and 370-400 nm ranges has also been reported in [24, 28-31]. The dependence for natural coastal meromictic reservoirs of the White Sea region is qualitatively similar and differs only in the absolute FQY values [18,19].

The depth dependences of FQY at wavelengths of 250, 270, 310, and 340 nm for each of the two reservoirs are plotted in Fig. 5. The FQY increases slightly with depth In the sea reach of the Kanda Bay; in contrast, the FQY in the Fedoseevsky reach starts decreasing at a depth of 11 m. A comparison with the depth dependence of the FQY at a wavelength of 340 nm in the lagoon on Cape Zeleny (see [19]) reveals their significant qualitative difference.

The dependence of wavelength  $\lambda_{max}$  corresponding to the spectrum maximum on excitation wavelength  $\lambda_{ex}$  was calculated based on the obtained fluorescence emission spectra. Example dependences of this kind for four samples (collected from the shallowest and greatest depths in the sea and Fedoseevsky reaches) are shown in Fig. 4, b. Dependence  $\lambda_{max}$  ( $\lambda_{ex}$ ) is non-monotonic:  $\lambda_{max}$  increases at fluorescence excitation wavelengths of  $250-270\,\mathrm{nm}$  and decreases as the wavelength grows further to 310 nm. The observed shift of the emission band maximum with a change in the excitation wavelength from 280 to 310 nm is a "blue" one (i.e., a shift toward shorter wavelengths). This is typical of humic compounds that are found in DOM [5]. With a further increase in the excitation wavelength, the emission maximum undergoes a shift toward longer wavelengths (within the measurement error). Comparing Figs. 4 and 6, one may note that dependence  $\lambda_{max}$  ( $\lambda_{ex}$ ) in natural meromictic reservoirs [18,19] does not differ qualitatively from the one obtained for the Kanda Bay. The only slight difference is in the magnitude of the "blue shift" of fluorescence emission spectra. This effect has already been noted in [18,32,33] for natural humic substances of marine, river, and soil origin and is indicative of heterogeneity of the composition of DOM fluorophores.

shift" The "blue magnitude calculated for represented the examined samples was by  $\Delta_1 = \lambda_{\max}(270) - \lambda_{\max}(310)$ and two parameters:  $\Delta_2 = \lambda_{max}(350) - \lambda_{max}(310).$  In samples from the Kanda Bay sea reach,  $\Delta_1=24\pm4\,\text{nm},\;\Delta_2=20\pm4\,\text{nm},$  and the magnitude of this shift is practically independent of depth. In samples from the Fedoseevsky reach,  $\Delta_1 = 23 \pm 4$  nm,  $\Delta_2 = 19 \pm 4$  nm, and the magnitude is also practically independent of depth. Thus, the differences between parameters  $\Delta_1$  and  $\Delta_2$  in these two reservoirs are within the measurement error. For comparison, the average  $\Delta_1$  and  $\Delta_2$  values in natural meromictic reservoirs (lakes Yelovoe and Tryokhtsvetnoe and the lagoon on Cape Zeleny) differ slightly both from those obtained for the Kanda Bay and between each other. The minimum magnitude of blue shift in natural reservoirs is also lower (12-13 nm).

# Conclusion

Spectral and optical characteristics of DOM of natural water from two parts of the Kanda Bay (sea reach and meromictic Fedoseevsky reach), which was separated artificially from the White Sea more than a hundred years ago, were examined. They were compared with the results of similar studies for DOM in meromictic reservoirs of natural origin (lagoon on Cape Zeleny and lakes Tryokhtsvetnoe and Yelovoe). Light absorption spectra and plots of the depth dependence of the optical density of water at wavelengths of 250, 270, and 280 nm were obtained. In the sea reach, the optical density decreases gradually with depth; in the Fedoseevsky reach, the optical density increases sharply in the anaerobic zone due to the accumulation of organic matter.

Fluorescence emission spectra of DOM were recorded, and the dependence of FQY on excitation wavelength was calculated for water samples collected from different depths in two reservoirs. The fluorescence intensity in the Fedoseevsky reach is higher than in the sea one, which is attributable to differences in the composition of DOM. The dependences of FQY on excitation wavelength in two reservoirs are qualitatively similar, but the absolute values differ. The FQY in the Fedoseevsky reach is lower than in the sea part of the bay. For comparison, these dependences for the lagoon on Cape Zeleny and lakes Tryokhtsvetnoe and Yelovoe are also qualitatively similar and differ in the absolute FQY value. The depth dependences of FQY at wavelengths of 250, 270, 310, and 340 nm were plotted for each of the two reservoirs. It was demonstrated that the FQY increases slightly with depth in the sea reach and starts decreasing in the anaerobic zone in the Fedoseevsky reach. These dependences were compared with a similar FQY dependence for the lagoon on Cape Zeleny, and their significant qualitative difference was noted.

Dependences of the wavelength corresponding to the fluorescence spectrum maximum on excitation wavelength

were also plotted, and the "blue shift" magnitude was calculated. Parameters  $\Delta_1$  and  $\Delta_2$  in two reaches of the Kanda Bay are virtually indistinguishable within the measurement error, but differ slightly from the corresponding values for the lagoon on Cape Zeleny and lakes Tryokhtsvetnoe and Yelovoe.

Thus, differences in the spectral and optical properties of DOM of two parts of an artificially separated meromictic reservoir and naturally separated reservoirs were identified. Absorption and fluorescence spectra revealed differences in the composition of DOM and in the stratification structure of reservoirs with depth. Spectral and optical characteristics of the water column of sea bays separated naturally or artificially from the main sea basin may serve as an objective indicator of the trophic (ecological) state of a reservoir.

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### **Conflict of interest**

The authors declare that they have no conflict of interest.

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