Investigation into fluorescence of dissolved organic matter in photic zone water of the Black Sea

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The fluorescence of dissolved organic matter (DOM) in the seawater has been investigated in the photic zone of the Black Sea. The DOM excitation and fluorescence spectra in seawater samples have demonstrated that the composition of fluorescent DOM changes with depth and during a season.

The composition of organic matter in seawater is known to vary with depth. In particular, it was reported[1] that the dissolved organic matter (DOM) of deep layers (100–2000 m) in the Black Sea differs significantly from the organic matter in the physical-chemical properties in the upper 100-m layer. It would be quite natural if the florescence spectrum of DOM in seawater change with depth, as well as the DOM composition does. However, according to the literature data[2,3, and others] no changes were observed in DOM fluorescence spectra in the World Ocean. These data caused some doubts and initiated this study.

The aim of this work was to study the DOM excitation and fluorescence spectra in the seawater sampled from different depths mostly in the photic zone of the Black Sea, as well as seasonal changes in them.

Experimental instrumentation

In the experiment carried out, seawater was sampled at a distance of ~10 miles from the shoreline in the eastern part of the Black Sea in the zone free of anthropogenic impact. The sampling was carried out with the aid of a vinyl-plastic bathometer from a depth up to 200 m by the standard technique. According to B. Skopintsev (Ref. 4), the processes of formation and decomposition of organic matter mostly occur in this depth range. Then seawater samples were transported to the coast, and their fluorescence was studied on a setup assembled on the basis of an MDR-23 monochromator excited by a nitrogen laser (λ_{las} = 337.1 nm).

The measurements have shown marked changes with depth in the recorded fluorescence band of DOM in seawater of the Black Sea (395–465 nm). To estimate these changes, the intensities in the fluorescence band under study were compared with a spectral step of 10 nm for seawater sampled from depths of 15, 35, 100, and 150 m with those for seawater sampled from a depth of 200 m.

The coefficients of linear correlation obtained between the spectral distributions of fluorescence intensities in these samples appeared to be equal to 0.72 from the seawater samples from depths of 15 and 200 m, 0.85 for samples from 35 and 200 m, 0.96 for samples from 100 and 200 m, and ~1 for samples from 150 and 200 m. This estimate confirms that the profile of the seawater DOM fluorescence band changes with the depth. The analysis has shown that the change in the profile of the seawater DOM fluorescence band was caused by the visible shift of the DOM fluorescence band peak to the long-wavelength range by 20–35 nm with the depth.

The results obtained contradicted the literature data, and this initiated a more detailed study of the seawater DOM fluorescence band and the spectral range of its excitation at different depths and in different seasons.

The further investigation of the seawater DOM fluorescence was carried out at the SDL-2 setup. During measurements, the spectral width of slits of the MDR-12 excitation monochromator was set equal to 10 nm, while that of an MDR-23 recording monochromator was set equal to 3 nm. Before and after each measurement, a cell containing the samples under study was subjected to a standard chemical processing and then washed carefully by bidistilled water. The measurements were conducted in the photon-counting mode with the averaging over no less than 15 readings at every step of the scanning. The fluorescence band of a seawater sample from each level was recorded three times, and every time the sample in the measurement cell was replaced. The average spectral distribution was determined and then smoothed, and the results were output to the recording unit. The measurements have shown that spectral changes in the fluorescence intensity of seawater samples at repeating records did not exceed 5–10% and the shape of the fluorescence band remained unchanged. In this study, we carried out comparative measurements, and the excitation and fluorescence spectra were recorded without correction for spectral characteristics of the setup.

Results and discussion

To select the optimal wavelength for the excitation of seawater DOM fluorescence and the spectral range for the fluorescence recording, the DOM fluorescence band was studied depending on the excitation wavelength and the excitation band was investigated depending on the wavelength of

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recording of the DOM fluorescence. Figure 1 shows the results of investigation of DOM samples taken from depths of 50 and 100 m.

As it is seen from Fig. 1, the DOM fluorescence band narrows and its peak shifts to the long-wavelength region with the increase of the excitation wavelength. No marked changes were observed in the DOM fluorescence band starting from a wavelength of 440 nm and further to the red region at different excitation wavelengths. When the DOM fluorescence was recorded at $\lambda_{rec} = 400$, 420, and 440 nm, peaks were observed in the fluorescence excitation band at 325, 330, and 345 nm along with a small plateau at ~300 nm.

When the DOM fluorescence was excited by 300 nm radiation, a wide asymmetric fluorescence band consisting of at least several bands with the most intense one lying in the short-wavelength region was observed in the seawater sample taken from a depth of 50 m. At this excitation wavelength, pronounced peaks nearby 360, 380, and 400 nm were seen in the fluorescence spectrum of the seawater sample from a depth of 100 m.

At the excitation wavelength of 320 nm, the peak observed in the fluorescence band lied in a range 400–405 nm. This fluorescence spectrum included also a band with a peak at 420 nm. These bands overlap due to their close position, and because the band peak at 400 nm is more intense than the band with the peak at 420 nm, the latter peak is poorly visible in the fluorescence spectrum.

At the excitation by 340 nm radiation, the fluorescence band with a single peak at 420–425 nm was observed. It is responsible for the conservative part of the fluorescence band of seawater DOM present at large depths in the Black Sea and, according to the literature data, in water of other seas and oceans as well.

The investigation confirms a multicomponent composition of fluorescing DOM (FDOM) in seawater, which may be caused by the simultaneous presence of its products being at different stages of biochemical transformation.

The presence of a band in the short-wavelength part of the DOM fluorescence suggested that the study of seawater DOM fluorescence excited by a nitrogen laser ($\lambda_{exc} = 337.1$ nm) and by longer wavelength radiation is not fully correct. The peak of the spontaneous Raman scattering (SRS) band of water far exceeds the seawater DOM fluorescence in intensity, and upon the excitation by a nitrogen laser ($\lambda_{SRS} = 380$ nm) it falls within the discovered short-wavelength part of the DOM fluorescence spectrum with a peak near 385–390 nm, while water SRS from longer wavelength radiation overlaps the main spectrum. Due to the low (compared to SRS) intensity of the DOM fluorescence, this overlapping does not allow one to conduct high-quality measurements and to obtain the complete information about the seawater DOM fluorescence spectrum.

Thus, the study of the profile of the DOM fluorescence band at different excitation wavelengths has revealed that to investigate the seawater DOM fluorescence, it is necessary to use the short-wavelength exciting radiation (280–300 nm). In this case, it is possible to record radiation of a larger number of fluorophore centers present in the composition of seawater DOM. With this excitation, studying the changes in the fluorescence band with depth and in different seasons, it is possible to follow changes occurring in the composition of seawater FDOM.

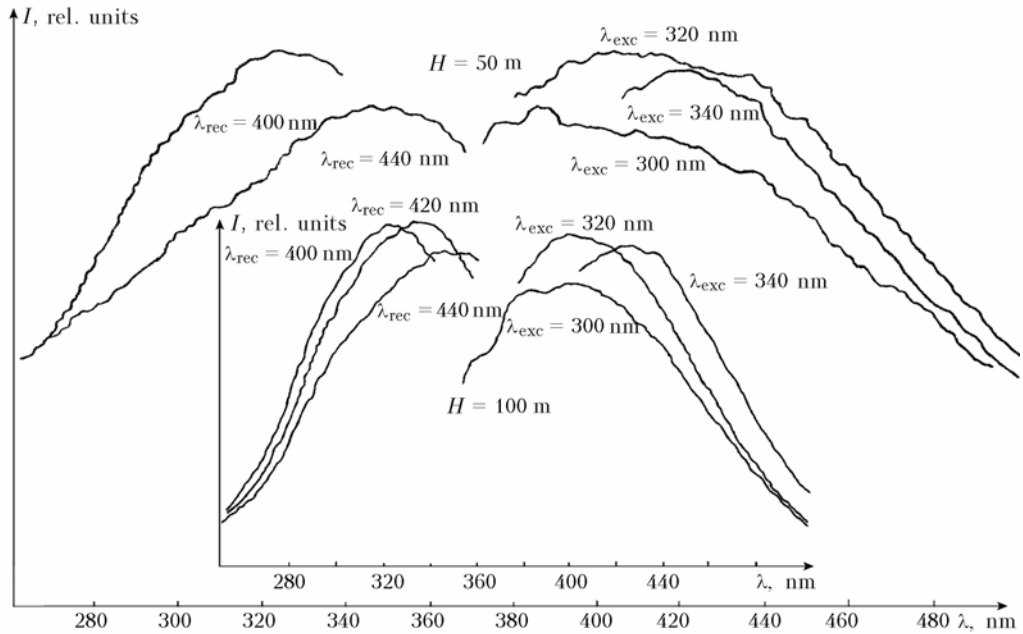

Fig. 1. Excitation and fluorescence spectra of seawater samples from depths of 50 and 100 m for different recording and excitation wavelengths.

As an example, Figure 2 shows the fluorescence spectra measured at λ_{exc} =300 nm in seawater samples from depths of 50 and 200 m, normalized to maxima. This figure also shows the difference between these fluorescence bands obtained by subtracting one spectrum from another. The difference demonstrates clearly the change in the DOM fluorescence band with depth.

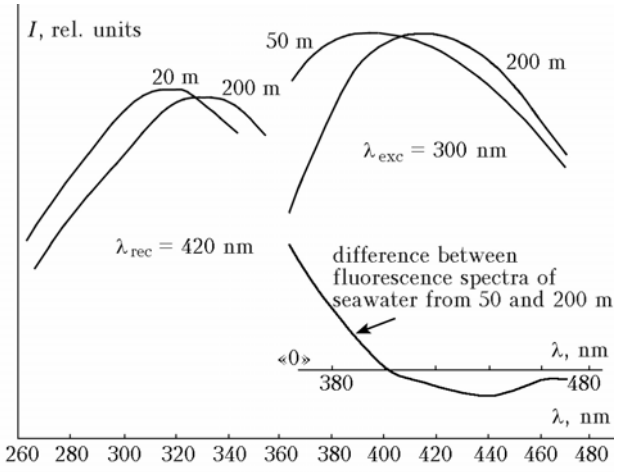

Fig. 2. Excitation and fluorescence spectra of seawater samples from different depths of the Black Sea.

One can see from Figs. 1 and 2 that the composition of FDOM in the marine photic zone includes a substance fluorescing in the short-wavelength spectral range and having a fluorescence peak near 380–390 nm. The intensity of its fluorescence in this range exceeds the intensity of the peak nearby 420–425 nm observed as an unclear plateau in the conservative fluorescence band of the seawater DOM.

The investigations conducted at the SDL-2 setup have confirmed the earlier information that the peak of the seawater DOM fluorescence shifts to the long-wavelength range. It has been found that this shift can achieve 20–35 nm (see Fig. 2). The similar shift was also observed in the band of fluorescence excitation (see Fig. 2). As the depth increased from 20 to 200 m, the shift achieved 15–20 nm.

The shifts observed can be mainly explained by the presence of instable products with a short lifetime in the composition of FDOM of the marine photic zone. According to the corresponding short-wavelength fluorescence band, these products are albuminoidal substances. Their concentration in the photic zone is much higher than at large depths. The decrease in the concentration of albuminoidal substances with depth causes the decrease in the fluorescence intensity in this spectral range and, as a consequence, the shift of the visible fluorescence peak to the long-wavelength range.

The found short-wavelength fluorescence of seawater DOM suggests soundly the presence of fluorescing albuminoidal substances in the marine photic zone. Thus, using the fluorometric technique, it is possible to study the distribution and changes in the concentration of these substances in the marine environment depending on the depth, season, and year.

The presence of a positive correlation between proteins and fluorescence of DOM in seawater in upper sea layers was reported in Ref. 5. However, the intensity of DOM fluorescence was measured by an immersible fluorimeter in the long-wavelength range of DOM fluorescence (> 450 nm), rather than in the short-wavelength part corresponding to the range of protein fluorescence, which makes the conclusions purely hypothetic.

As a result of many-year investigations, seasonal changes have been found in the fluorescence excitation spectra of seawater DOM in the marine photic zone. Thus, in the excitation spectra of seawater sampled in April down to a depth larger than 65 m, the peak at ~300 nm prevailed over the peak nearby 330 nm, whereas in June the peak at 330 nm was dominant. In August, the dominant peak was observed at 320 nm and a plateau near 290 nm was seen. Figure 3 shows the excitation spectra of seawater sampled from a depth of 20 m in different months.

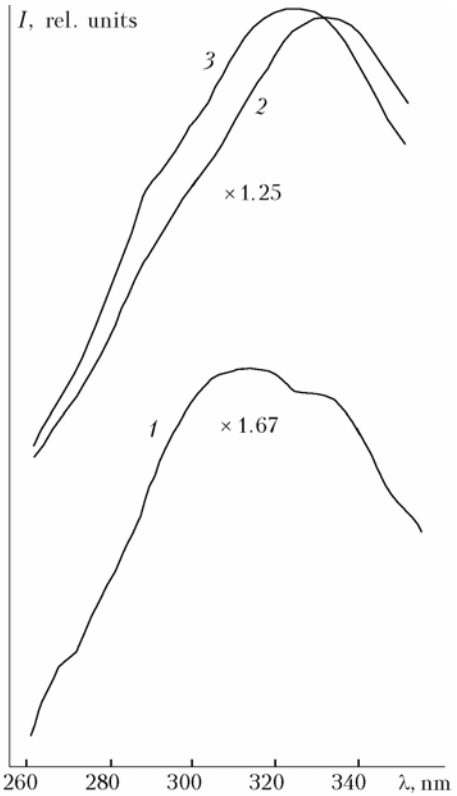

Fig. 3. Excitation spectra of seawater sampled from a depth of 20 m in different months: (*1*) April, (*2*) June, and (*3*) August.

The investigations carried out have allowed the following conclusions.

1. The fluorescence spectra of seawater DOM in the marine photic zone include two main bands with

peaks at 385–400 nm and at 420 nm, corresponding to the excitation band peaks at 300 nm and nearby 320–330 nm.

2. With the depth, the peaks of the fluorescence spectrum and the excitation spectrum shift to the long-wavelength range. This visible bathochromic shift in the spectra is explained by the disappearance of the short-wavelength fluorescence excitation band along with the short-wavelength DOM fluorescence band excited by it with depth and is caused by the decrease in the concentration of albuminoidal substances in the marine environment with the depth.

3. Seasonal changes have been found in the fluorescence excitation spectra of DOM in the marine photic zone. These changes are indicative of changes in the composition of FDOM of the marine environment during a year, which may be attributed to the period of phytoplankton bloom, seasonal changes in bioproductivity and species composition of primary production of seawater.

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