DOM fluorescence in centrifuged sea-water samples

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Received October 24, 2005

The fluorescence in centrifuged sea water samples has been studied to find out the mechanism of the fluorescent dissolved organic matter origination. The centrifuging causes evolving of a large amount of the dissolved fluorescent matter in the form of several gravitational fractions with different spectra of the excitation and fluorescence. The excitation spectra of centrifuged seawater samples change depending on the depth and the season. The applied centrifuging method allowed proving the existence of excitation and fluorescence bands in the short-wave spectra of the seawater samples. It was established that some layers of organic suspension are formed in the photic sea zone, and their origination is connected with the seasonal bioproductivity.

Introduction

Origination of the fluorescent dissolved organic matter (FDOM) in the sea medium is commonly connected with the yellow matter (as a part of the dissolved organic matter) produced in the sea water (SW) in the process of interaction between carbohydrates and amino acids, i.e., in reaction of the Mayer type. The fact that these reactions proceed immediately in the seawater is controversial enough since the synthesis of any matter can be successful only under all required standard conditions, that is practically impossible throughout the depth range of sea medium. The most favorable conditions for chemical reactions are in the sea photic zone. Densities of FDOM and SW are similar, therefore the natural FDOM sedimentation due to gravitation is practically absent and the matter molecules can travel only together with SW. Consequently, the maximal FDOM concentration could be observed in the photic zone of the sea. However, numerous experiments demonstrate that the photic zone is characterized by a lowered DOM fluorescence intensity (FI), which increases with the depth. Thus, the above-mentioned mechanism cannot explain the formation of the DOM FI vertical profile observed experimentally.

It is well known that the DOM concentration in cells of organic suspension can 100–10000 times exceed its concentration in the environment.[1] The detritus suspended in SW consists of carbohydrates (30%) and protein substances (>50%) having vitally important amino acids in their composition. Therefore, the detritus is a potentially huge reservoir of DOM and biogenic elements. Thus, all reagents necessary for the Mayer reaction can be found in the suspended organic matter in specific ambient conditions. Here their concentration is considerably higher than in the SW. The DOM is separated into the sea medium through the cell walls of organic suspension collapsed due to the aging and bacterial activity in the process of sedimentation.

Using the spectrofluorimetric method, we have studied FDOM resulting from the mechanical destruction of the organic suspension.

Instrumentation and procedure of investigation

Over several years, the SW sampling was carried out by us from the Black Sea depth up to 200 m. Prior to sampling, the vertical distribution of the SW density was measured by means of the ACITT hydrological probe and the profiles of the phytoplankton chlorophyll fluorescence intensity (FI) – by means of the submerged Variosens fluorimeter. The first procedure was aimed at determination of density jumps and possible density anomalies; the second one – at finding areas of maximal bioproductivity. A presence of fluorescent products in the organic suspension composition has been determined after centrifuging the SW samples. The centrifuging method was used in such investigations for the first time. The centrifuging accelerates the destruction processes in organic suspension and the washout of DOM, accompanied by separation of the sample content into gravitational fractions. These processes under natural conditions proceed at the organic suspension sedimentation in the sea medium.

The centrifuging was carried out in the Opn-8 medical centrifuge in plastic tubes (20 ml) at a rate of 8000 RPM during 30 min. After centrifuging, the SW sample was divided. The centrifuged sample (CS) top was taken by a pipette (part "a") and its bottom part "b" was drained off. Conditionally, "a" mainly contains a light fraction of the organic matter, and "b" – the heavy fraction and residues of the organic suspension. The fluorescence study in the initial sample, as well as in "a" and "b" was carried out at the specialized SDL-2 installation.

Experimental results and their discussion

As the long-term measurements have shown, the SW sample centrifuging leads to a significant increase in FI of DOM excluding the samples taken in December. This can be explained by the absence of

0235-6880/06/09 738-05 $02.00

organic suspension in the sea medium due to a low bioproductivity in this period. What about the organic suspension, earlier produced in the spring–summer–fall period, it either has totally destructed or sunk deeper to the moment.

The DOM FI after the centrifuging of samples taken from some sea depths much more exceeded the FI in the initial samples. The table presents the intensities in maxima of fluorescence bands of the initial (I) and centrifuged (I_a, I_b) SW samples normalized by the intensity of the spontaneous Raman water scattering (SRWS), as well as ratios of the centrifuged samples (CS) FI in parts "a" and "b" to the intensity in the initial sample ($k_a = I_a/I$ and $k_b = I_b/I$).

H, m	June					August				
	I	I_a	I_b	k_a	k_b	I	I_a	I_b	k_a	k_b
20	0.13	0.17	0.34	1.30	2.60	0.37	0.58	0.58	1.60	1.60
35	0.16	0.27	0.42	1.70	2.60	0.29	0.59	0.28	2.00	1.00
50	0.15	0.71	0.63	*4.70*	*4.20*	0.24	0.35	0.47	1.50	2.00
80	0.14	0.19	0.27	1.40	1.90	0.32	0.60	0.92	1.90	*2.90*
100	0.17	0.28	1.03	1.70	*6.10*	0.40	1.12	0.46	*2.80*	1.20
150	0.25	0.45	0.49	1.80	2.00	0.53	0.70	0.88	1.30	1.70
200	0.27	0.32	0.48	1.20	1.80	0.71	0.78	1.34	1.10	1.90

The DOM fluorescence of SW was measured when exciting June samples by the nitrogen laser radiation ($\lambda_{exc} = 337.1$ nm) and August samples by radiation passed through MDR-12 monochromator ($\lambda_{exc} = 330$ nm). As it follows from the table, the fluorescent matters evolved due to centrifuging can differ in gravitational size.

The abnormal FI increase of DOM in SW samples after centrifuging was observed in samples taken beneath the phytoplankton habitation zone and in the region free of pycnoclines and other density anomalies.

This FI increase cannot be explained only by the DOM redistribution over the gravitation sizes and adsorption of the fluorescent matters on the surface of organic suspension, but sooner by the presence in the sea media of some substances, the vital activity of which results in formation of specific fluorescent layers. These layers consist of organic suspension prone to the mechanical destruction and containing the increased FDOM concentrations. Most likely, the phytoplankton cells are the part of this suspension and the formation of the discovered layers in the sea medium is due to their simultaneous mass dying out after the full bloom.[2]

Studies of the centrifuged SW fluorescence spectra have shown that a new fluorescent matter originates in the SW solution and the maximum of its fluorescence band shifts by $\Delta\lambda = 30$ nm to the short-wave region relative to the fluorescence band maximum in the initial samples. These variations are connected with the depth of sampling. Figure 1 presents the fluorescence spectra of some SW samples before and after centrifuging.

Figure 2 presents the "a" and "b" excitation spectra, normalized to the maximum, for some CS taken in March.

There are spectral differences between different CS fractions at upper sea horizons. When approaching to a depth of 200 m, the FDOM composition becomes more homogeneous, as judged from the excitation spectra. The excitation band maximum is displaced to the long-wave region with the depth. When comparing the spectra of initial and centrifuged SW samples, we observe a similar tendency of shifting the maxima of the excitation and fluorescence bands to the red region, as well as coming together of the spectra with the depth. Most likely, this evidences about a terminating stage in the organic matter transformation and its conversion into the labile state. The obtained result agrees with data presented in Ref. 3, where it is shown that the formation and decomposition of organic matter, mainly, takes place in this depth range.

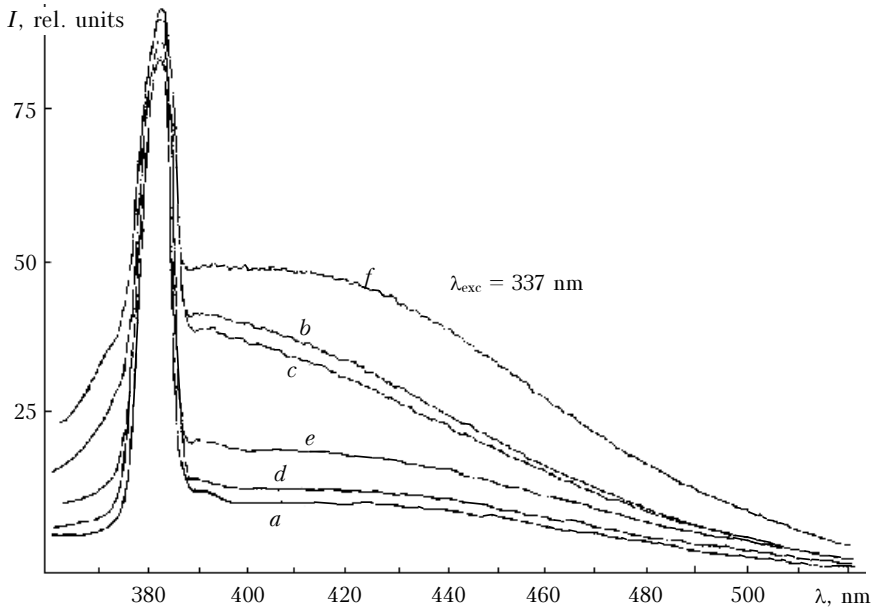

Fig. 1. Fluorescence spectra of the initial and centrifuged samples taken in June: curves *a*, *b*, and *c* correspond to the initial SW sample and to "a" and "b" parts of the corresponding centrifuged sample, respectively, taken at a depth of 50 m; curves *d*, *e*, and *f* mean the same for samples taken at a depth of 100 m.

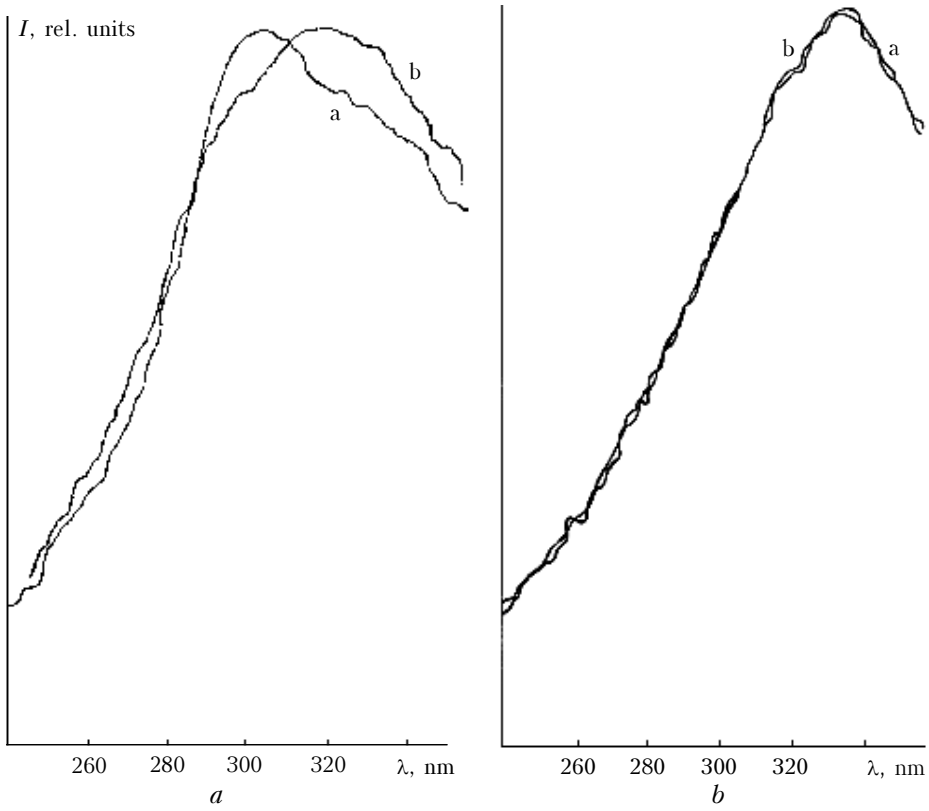

Fig. 2. The fluorescence excitation spectra normalized to the maximum (λ_{reg} = 420 nm) of parts "a" and "b" of the CS taken from 20 (*a*) and 200 m (*b*).

Our studies have shown that there are two maxima in excitation spectra of the CS: in 285–290 and 325–330 nm ranges and their intensities can be redistributed depending on the depth and the season (see Fig. 3).

Spectrofluorimetric studies of the centrifuged SW samples have shown the existence of the fluorescence excitation spectra in the short-wave spectral region of the initial samples, that was earlier debatable due to their weak intensity (see spectra of initial samples in Figs. 1*a* and 4*a*).

A joint fluorescence analysis for June and August has shown that excitation spectra in parts "a" and "b" of the CS taken from 50 m depth in June and from 80 m in August (see Fig. 4*a*) were identical and strongly differed from the spectra taken from other horizons (see Figs. 2 and 3*a*). According to the Variosens fluorimeter data, during the studying period this depth range was free of the living phytoplankton cells, as well as layers with density jumps and other density anomalies.

The spectrofluorimetric method has shown that in both cases in June and August we took the samples from the region of the same settling layer of the substance consisted of "dead" organic suspension formed in the sea medium everywhere after the phytoplankton mass dying off.

The absence of the density anomalies in this depth range has allowed estimation of the sedimentation rate of the layer. On the average, it is about 0.5 m/day. This corresponds to the rate of gravitation sedimentation of biogenic particles of 10 μm in size, which is typical for the half-dead phytoplankton and some species of the dead one.

The phytoplankton lifetime is about 3 days (Ref. 2). Its mass full bloom is followed by the rapid and mass death of cells settling then to the bottom. Based on the obtained sedimentation rate of organic suspension, it was easy to find the layer thickness of the dying cells produced after the monthly period of phytoplankton blossom, which turned to be about 15 m. Taking into account the depth discreteness of the sampling and the revealed location of these layers (due to centrifuging), we come to conclusion that in both cases the settling layer of organic suspension consisting of dead phytoplankton cells of approximately the same size was registered.

Similarly, the fluorescence excitation spectrum of part "a" for the CS taken from the 80 m depth in June, was identical to the part "b" taken from 100 m in August. Three maxima were distinguished in the both spectra: 295, 305, and 330 nm (Fig. 4*b*). This fact points to the presence in August of the organic suspension layer at a depth of 100 m, which was registered in June at a depth of 80 m. Sedimentation rate of the layer was lower, probably, due to a higher SW density in this depth range and to smaller cell sizes of the settling suspension.

Our spectrofluorimetric studies have shown that in different seasons with a year or longer periodicity there appears the organic suspension in the sea medium containing a fluorescent matter with identical

spectra of excitation and fluorescence. In particular, Fig. 5 presents the spectra of excitation and fluorescence of the parts "b" of SW samples taken at 35 and 50 m depths in February 1990, corresponding to the same spectra of the part "b" of the sample taken from 50 m depth in June 1987.

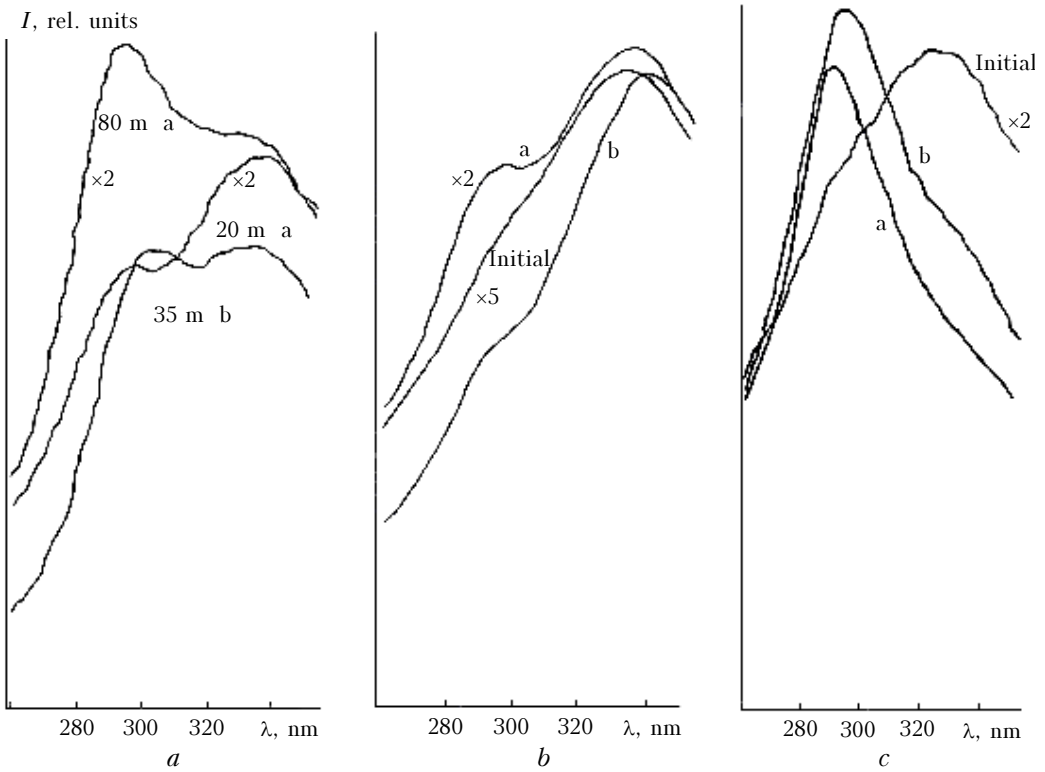

Fig. 3. The fluorescence excitation spectra (λ_{reg} = 420 nm) of the CS from 20, 35, and 80 m, taken in June (*a*); initial samples and parts "a" and "b" of the CS taken from 20 m in June (*b*) and August (*c*).

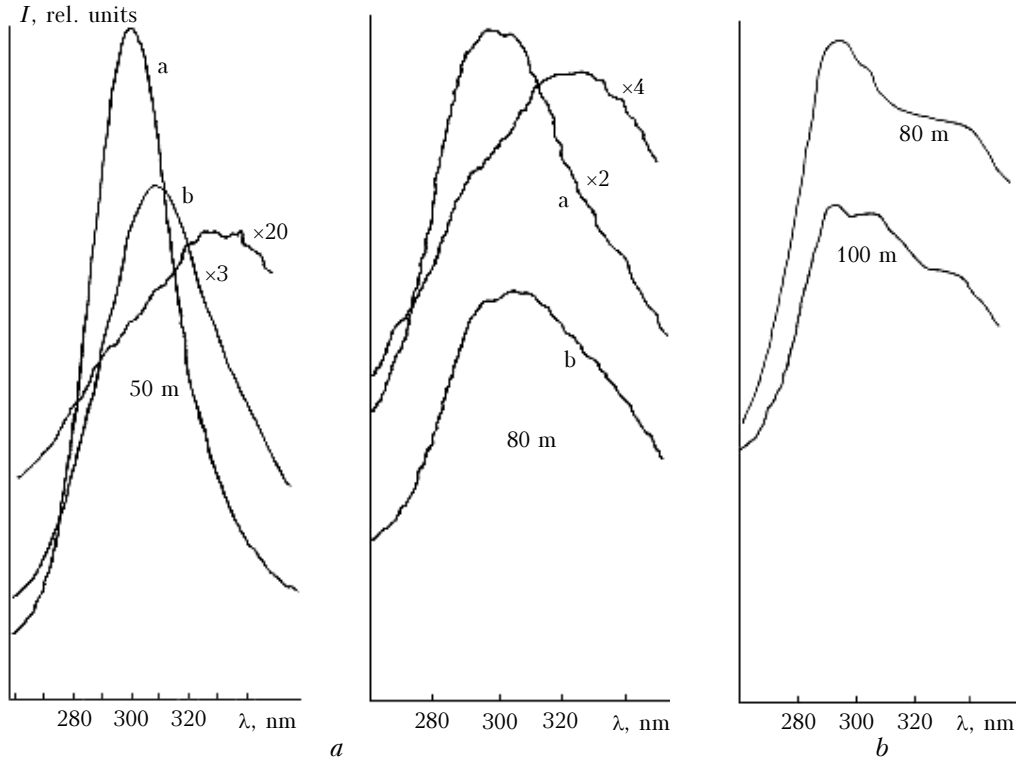

Fig. 4. The fluorescence excitation spectra (λ_{reg} = 420 nm) of the initial samples and parts "a" and "b" of the CS taken from 50 m in June and from 80 m in August (*a*); part "a" of the CS taken from 80 m in June and part "b" of the CS taken from 100 m in August (*b*).

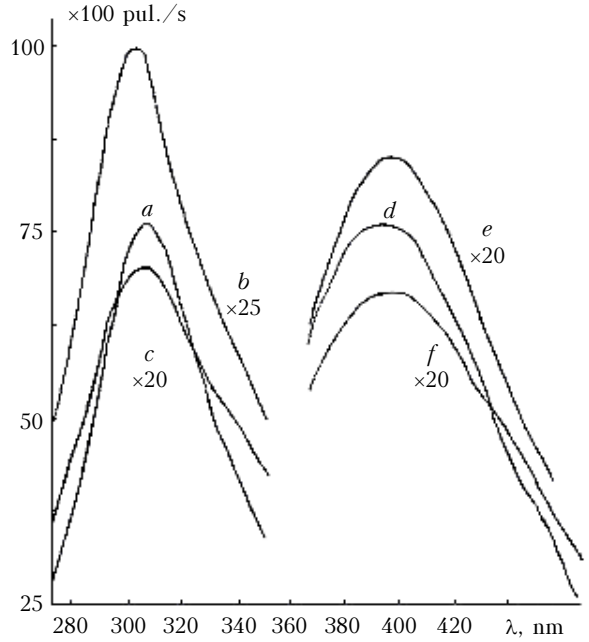

Fig. 5. Spectra of excitation (λ_{reg} = 420 nm) and fluorescence (λ_{exc} = 300 nm) of the part "b" of CS: CS, 50 m, June 1987 (*a, d*); CS, 35 m, February 1990 (*b, e*); SW, 50 m, February 1990 (*c, f*).

Such a coincidence evidences that if the same fluorophor groups are evolved from the centrifuged sea water samples in different seasons and years, they have one source related to a particular season.

This allows us to conclude that the appearance of organic suspension layers in photic sea zone is connected with seasonal bioproductivity.

The suspension is prone to mechanical destruction with liberation of the dissoluble fluorescent matters (DFM) differing in gravitational size and composition and significantly exceeding the FDOM content in the sea medium. At least six fluorophor groups can be distinguished in the dissoluble fluorescent matter, which have maxima in the fluorescence excitation band (λ_{reg} = 420 nm) at 295, 300, 305, 310, 330, and 335 nm. The DFM multicomponent composition, most probably, can be attributed to intermediate stages of the OM transformation or to season-dependent variety of primary production in the sea medium.

Due to biochemical transformations proceeding during sedimentation, the DFM in the organic suspension changes, its composition becomes stable and similar to the FDOM composition at great depths.

The presence of DFM different fractions in the organic suspension can be connected with integration of its particles with time or it can result from changes in sea water temperature-density characteristics affecting the sedimentation process of the organic suspension.

In the course of our investigations, we have followed the sedimentation of some layers consisting of unstable suspension and determining approximately their thickness. The submerged equipment *in situ* failed to recognize the layers; therefore, we cannot determine their power and the accuracy of sampling. The standard procedure of the depth-dependent sampling, probably, did not allow determining other similar layers. At the same time, we assume that the thickness of these layers is about 10–15 m and they are formed from dead cells of phytoplankton, which appear in sea water after the full bloom.

Because of aging and under the bacterial activity, the cell walls of organic suspension become weak and collapse. The content of OM due to diffusion is washed out outside enriching the sea environment with FDOM. The washout takes place during the organic suspension sedimentation in the field of positive gradient of the SW density, which, according to the Stokes formula, leads to a decrease in the sedimentation rate. Therefore, the organic suspension dwell time in the sedimentation area increases, that causes its decomposition accompanied by evolving the DOM into the sea medium.

The pressure increase with the depth and the fall of SW temperature favor the increase in DOM lifetime and its accumulation at lower horizons. Large suspension particles, settling intensively, favor the OM vertical transport, while small particles determine DOM concentration in sea water. Such mechanism of the FDOM origination and distribution in the sea medium is more realistic as compared to the Mayer chemical reaction, particularly, because it allows transporting DFM of low density to great depths, where proceeding of these reactions is highly conjectural. Further vertical FDOM distribution is determined by the sea water intrinsic dynamics.

References

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