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Tracing the Variability of Dissolved Organic Matter Fluorescence in the East China Sea in the Red Tide Season with use of Excitation-emission Matrix Spectroscopy and Parallel Factor Analysis

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Abstract

From the end of March to the end of May, 2011, five cruises were carried out to survey the red tide occurrence in the Zhejiang coast of the East China Sea where the red tides occurred each spring and there was a trend for community succession from diatoms to dinoflagellates. Using Excitation-Emission Matrix Spectrum(EEMs) combined with Parallel Factor Analysis (PARAFAC) examine the fluorescent components feature of dissolved organic matter (DOM) sampled from the East China Sea in the red tide season. Three fluorescent components were identified by PARAFAC, including tyrosine-like component C1(230,280/320), tryptophan-like component C2(240,305/355) and humic-like component C3(270,340/480). The result showed that the fluorescence intensity of C1 was relatively high and changed along with the succession of red tides, besides, the weak correlation coefficient with salinity and the particularity of its source suggested that phytoplankton activity was the important factor in fluorescence intensity change of C1. The fluorescence intensities of component C2 and C3 were relatively low and changed not very significant, but its good linearity with salinity indicated that the terrestrial input was the important sources of two components during the algae dispersion. Lower Fluorescence Index (FI) (<1.4) also tested the terrestrial distribution. Nevertheless, correlation coefficient with salinity was slightly decreasing showed the effects of biological activity had increased during the outbreak of dinoflagellate. Higher (>0.8) Biological Index (BIX) and lower Humification Index (HIX) (<2) inferred that biological activity intensively in the red tide season in the East China Sea would contribute the CDOM in the water.

Keywords: Dissolved organic matter; Excitation-emission matrix spectroscopy; Parallel factor analysis (PARAFAC); The East China Sea

Introduction

Chromophoric dissolved organic Matter < CDOM > is an important part of the dissolved organic matter (DOM) and plays an important role in marine photoreactions [1-6] and the biogeochemistry of biogenic elements [7]. Spectroscopic techniques can provide information about the source and composition of the DOM present in a natural abundance, thereby eliminating the need for isolating or concentrating it prior to analysis [8,9]. There are notable differences in CDOM composition in different water environments. The highest humic-like CDOM concentrations in estuarine and coastal areas are usually observed in freshwater and estuaries due to river runoff [10], and decreases in coastal and offshore waters [11]. Amount of protein-like CDOM were monitored in the algae bloom water and changed with the bloom process [12,13].

The East China Sea is the red tide-frequent-occurrence area and its eutrophication water area is the first of the four China Sea area, which threatens fisheries, public health and economic. According to statistics, since the 1990s, the number of the red tides in this region accounted for over 50% of the total in China, and the frequency increased. Meanwhile, red tides in East China Sea also presented the characteristic of the duration lengthening and area expanding. Its occurring time is concentrated in the April and May annually [14-16].

The red tide and subsequent succession are resulted from a combination of biological, chemical and physical factors. Chromophoric dissolved organic Matter < CDOM > plays an important role in the biogeochemistry of biogenic elements. In this paper, using EEMs combined with PARAFAC traced the fluorescent components variation of dissolved organic matter. The type, distribution and origin of the fluorescence dissolved organic matter were discussed. Besides, the relationship between organic matter and red tide succession was examined, which provided theoretical basis for a progressive research on red tide occurrence mechanism.

Materials and Methods

Study area

The investigation area located at 28-30.5°N and 122-123.5°E in the East China Sea. This water area is greatly influenced by the Changjiang dilute water (CDW), the East China Sea Coastal Current (ECSCC), the Taiwan Warm Current (TWC) from Taiwan Strait, and a branch of the Kuroshio Current [17]. The Yangtze River (or Changjiang River) is the fifth largest river in the world with huge amounts of freshwater discharge (9.24×10^{11} yr^{-1}). The fresh water discharge has significant seasonal variation with a minimum in May to October and a maximum in November to April. The CDW is divided into two branches out of the estuary; one extends southward along the coast of Zhejiang province and another northeastward directing at the Cheju Island [18,19]. The relative strength of these two branches varies seasonally. The southward

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branch was stronger in autumn and winter, and the northward branch was stronger in spring and summer.

Sample collection and preparation

Samples were collected in 5 cruises from sampling stations and sample times are shown in Figure 1. Diatom bloom occurred during March 31 to April 2, then diatom began to dispersion and 8 um size unknown algae appeared in part of the survey area in April 9 to April 10. Afterwards, most of the diatom dispersion and about 8um size unknown algae dominated in April 19 to April 20. Soon, dino flagellate began to bloom in May 13 to May 15 and out broke in May 25 to May 27. Surface, middle water samples which chlorophyll a was maximal, and bottom water samples (30-60m) were collected. The water samples were filtered using a vacuum pump through GF/F filters (Whatman, U.K, φ=25 mm, pre-combusted at 450°C for 4 h). The filtrate was stored in 60 ml brown glass bottle (pre-combusted at 450°C for 4 h) frozen at -20°C in the dark for later organic matter analysis.

Measurement of dissolved organic matter fluorescence, DOC and salinity

The EEMs fluorescence of the DOM was measured using a Hitachi F-4500 fluorescence spectrophotometer, with normal operating conditions of 5 nm slit widths of excitation and 10 nm slit widths of emission, a PMT voltage of 700 V. Fluorescence measurements were made by scanning emission spectra from 250 to 650 nm with 5 nm intervals, excitation wavelengths from 200 to 500 nm with 5 nm.The EEMs were normalized to quinine sulfate units using 0.01 mgL^{-1} quinine sulfate monohydrate in a solution of 0.05 mol L^{-1} H_2SO_4. The EEMs of each sample was Raman calibrated and subtracted from a Raman normalized Mill-Q water EEMs. Set the data which influenced by Rayleigh scattering to zero. EEMs were converted to R.U. by correcting with the area under the water Raman Peak at 350 nm excitation [20]. DOC was measured using a Shimadzu TOC-V total organic carbon analyser. Salinity was determined by CTD.

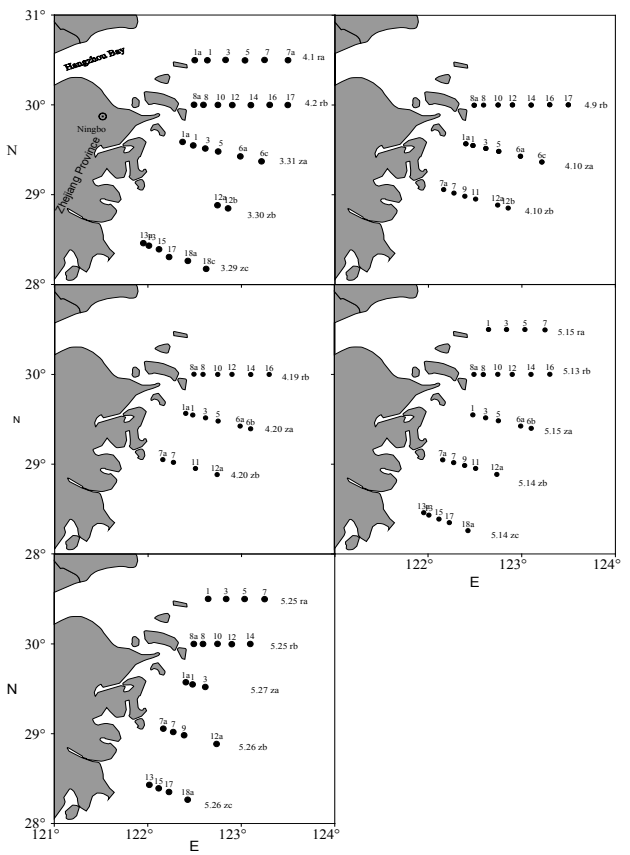

Figure 1: Sample Stations in the East China Sea.

PARAFAC modeling

A total of 552 EEMs were subject to the multivariate modeling technique PARAFAC [21] using MAT-LAB 2010a with "the N-way toolbox for MATLAB'. The number of components was validated by split-half analysis.

HIX, FI and BIX calculation

The degree of DOM humification is an indicator of a material's age and recalcitrance within a natural system [22,23]. The humification index (HIX) determined from the ratio of two integrated regions of an emission scan (sum from λ_{Em} 435–480 nm divided by the sum from λ_{Em} 300–345 nm) collected with excitation at 255 nm as a method for comparing the relative humification of DOM samples [22]. Fluorescence intensity ratios can be used to infer the relative contributions from autochthonous and allochthonous OM in natural waters. Fluorescence index (FI) was the ratio of the fluorescence intensity at emission 450 and 500 nm at afixedexcitation wavelength of 370 nm [24]. The biological/autochthonous index or BIX was used to assess the relative contribution of autochthonous DOM and is calculated from the ratio of emission intensities at λ_{Em} 380 nm and λ_{Em} 430 nm wavelength using a fixed excitation (λ_{Ex}310 nm) [24].

Results and Discussion

The fluorescence characteristic of DOM

Three fluorescent components were identified using PARAFAC (Figure 2 and Table 1): C1 (230 nm, 280/320 nm), C2 (240 nm, 305/355 nm) and C3 (270 nm, 340/480 nm).

The EEMs spectral characteristics of C1 was composed of two peaks with 320 nm emission maxima wavelength at excitation maxima wavelength 230 nm and 280 nm which was similar to a tyrosine-like fluorescent compound previously observed [1,25,26]. The spectral features were also similar to the high fluorescence intensities tyrosine-like fluorescent component observed in red tide water in the East China Sea [12].

The C2 component with emission maxima wavelength at 355nm and two excitation maxima wavelength at 240 nm and 305 nm were categorized as the previously defined autochthon-nous tryptophan-like fluorescence peak. This component was also similar to the previously reported PARAFAC components of amino acids that are free or bound in proteins [27,29,30].

The C3 component was also composed of two peaks with excitation maxima wavelength at 270 nm and 340 nm at 480 nm emission maxima wavelength that were similar to the terrestrial humic-like fluorescence peak A and peak C. The spectral features were also similar to those reported for terrestrial-derived humic-like PARAFAC components [31].

Homology analysis of components

To investigate whether the three components had the same sources, we calculated the correlation coefficient between three components in different red tide period, as listed in Table 2. It showed that they

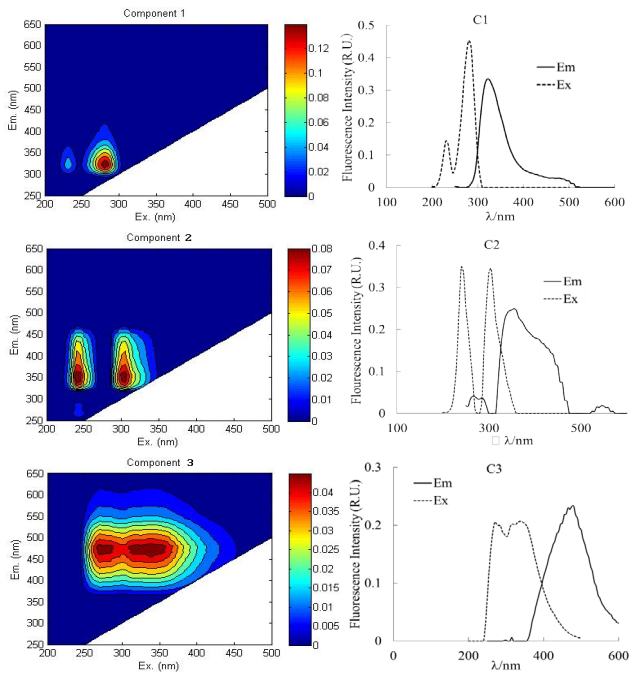

Figure 2: Fluorescent components of DOM identified by the PARAFAC model.

Fluorescence Components	Peak position λEx/Em(nm)	Literature reported		
		Description	Ex/Em (nm)	Reference
C1	230,280/320	tyrosine-like	230/325-350 280/320-350; 270-280/320-350;	[1] [25] [26]
C2	240,305/355	tryptophan-like	<250(290)/360 <250(290)/360	[27] [28]
C3	270,340/480	Terrestrial humic-like	230-260/380-480 320-360/420-480	[29]

Table 1: Positions of the fluorescence maxima of the three components.

were positive related significantly between three components (R>0.72, P<0.01). Particularly, tryptophan-like component C2 had higher correlation (R>0.8) with tyrosine-like C1 and humic-like component C3, which illustrated that they had some connection or difference in source or structure.

The vertical distribution of the three components fluorescence intensities

Table 3 showed that the fluorescence intensities of three components in surface, middle and bottom layers in different red tide period. Chen and Bada [32] reported that fluorescence intensity of protein-like fluorescence was the highest in the surface water, decreasing with the increase of depth. But the result of our study was different. No matter in surface water or in middle water, fluorescence intensity of protein-like components C1 and C2 were almost the same, sometimes it was higher in the middle water than the surface, but both were higher than the bottom which was in accordance with biological activity. Similarly, humic-like component C3 showed the same distribution that the fluorescence intensity was similar in surface and middle, but higher than the bottom. It was not consistent with previously views that fluorescence intensities of humic-like increased with the depth [1,33].

The horizontal distribution of PARAFAC components

Owing to the high relationship between the three components and the fluorescence intensity of component C1 was the highest, we chose component C1 as characteristic component. Figure 3 displayed the horizontal distribution variation of component C1 with the red tide succession. On the whole, fluorescence intensity of DOM inshore was higher than that in the open sea. Diatom bloom erupted far offshore during March 31 to April 2 and the highest fluorescence intensity appeared in offshore. Then diatom bloom began to dispersion in April 9 to 10, the highest fluorescence intensity transfer toward north area. Following it appeared in southern area of surveyed waters in April 19 to 20, the distribution patterns kept until May 13 to15, which accord with relatively high dinoflagellate density in this area. During May 25 to 27, dinoflagellate bloom broke full-scale, fluorescence intensity were relatively high in the southern coastal area, conform to the result that dinoflagellates finally outbreak in the near shore, which demonstrated that phytoplankton activity had certain contribution to fluorescent components.

Spectroscopic indication of the sources of DOM in the East China Sea

To analysis the influence of terrestrial input on DOM in different red tide period, Table 4 showed the relationships between the three components and salinity. Although tyrosine-like C1 component had significant correlation with salinity, the correlation coefficient can reach -0.6749 in April 9 and April 10. Combining with the process of algal bloom showed that the C1 component was affected by algal succession in other periods. Tryptophan-like C2 component and humic-like C3 component had different correlation with salinity in different periods of red tide. C2 and C3 had better correlation with salinity during the diatom erupted until it dispersed, which showed that terrestrial input was a major contributor to them. The result was consisting with the conclusion that terrestrial input contributed a lot of tryptophan-like matter to the East China Sea. However, correlation had been reduced during the outbreak of dinoflagellate bloom, which indicated that the biological effects bad been increased.

FI values can infer the relative contributions from autochthonous and allochthonous organic matter in natural waters. It suggested that FI values of 1.4 or less indicated humic DOM of terrestrial origin and values of 1.9 or higher correspond to microbially-derived material [24]. In this study, the FI in the East China Sea is in the range of 0.40~2.11, average 1.08. Most of the samples are less than 1.4, only one of them is larger than 1.9, indicating that terrestrial origin is the mainly sources of DOM in this area, which is consistent with above the relationship between DOM fluorescence components and salinity.

BIX Values between 0.8 and 1.0 correspond to freshly produced DOM of biological or microbial origin, whereas values below 0.6 are considered to contain little autochthonous OM [24]. In this area,

Time	$R_{C1/C2}$	$R_{C1/C3}$	$R_{C2/C3}$	N
3.31-4.2	0.8019	0.7456	0.9526	77
4.9-4.10	0.9213	0.9161	0.9533	50
4.19-4.20	0.9599	0.7265	0.8345	45
5.13-5.15	0.8764	0.7365	0.8652	72
5.25-5.27	0.8731	0.7941	0.9722	57
All above	0.8578	0.7446	0.9085	448

Table 2: Correlation coefficient between three components C1, C2, C3, (P <0.01).

Component	water layer	$FI_{3.31-4.2}$	$FI_{4.9-4.10}$	$FI_{4.19-4.20}$	$FI_{5.13-5.15}$	$FI_{5.25-5.27}$
C1	surface	0.0965 ± 0.0602	0.0835 ± 0.054	0.1132 ± 0.0857	0.0833 ± 0.0314	0.0973 ± 0.0415
	middle	0.0753 ± 0.0337	0.0834 ± 0.0393	0.1467 ± 0.1289	0.0884 ± 0.0355	0.0978 ± 0.0409
	bottom	0.0577 ± 0.0522	0.0747 ± 0.0482	0.0911 ± 0.1004	0.0479 ± 0.0367	0.0981 ± 0.055
	mean	0.0762 ± 0.0459	0.0807 ± 0.0465	0.1199 ± 0.1076	0.0714 ± 0.0389	0.0977 ± 0.0455
C2	surface	0.0303 ± 0.0187	0.0246 ± 0.0116	0.0315 ± 0.0154	0.0241 ± 0.008	0.0275 ± 0.0117
	middle	0.0249 ± 0.0131	0.0262 ± 0.0131	0.0368 ± 0.0251	0.0265 ± 0.0105	0.0268 ± 0.0114
	bottom	0.0179 ± 0.0147	0.0242 ± 0.015	0.0238 ± 0.0163	0.0146 ± 0.008	0.0246 ± 0.0139
	mean	0.0242 ± 0.0164	0.0251 ± 0.0129	0.0315 ± 0.0201	0.0211 ± 0.0102	0.0262 ± 0.0122
C3	surface	0.0283 ± 0.0149	0.0261 ± 0.0106	0.0286 ± 0.0122	0.0236 ± 0.008	0.0306 ± 0.0105
	middle	0.0261 ± 0.0105	0.0265 ± 0.0114	0.0324 ± 0.0195	0.0239 ± 0.0064	0.0298 ± 0.0094
	bottom	0.0197 ± 0.0144	0.0258 ± 0.0138	0.024 ± 0.0127	0.0159 ± 0.0064	0.0269 ± 0.0141
	mean	0.0244 ± 0.013	0.0261 ± 0.0117	0.0288 ± 0.0155	0.0209 ± 0.0079	0.0289 ± 0.0115

Table 3: The fluorescence intensities of three components in surface, middle and bottom layers in different red tide period (R.U.).

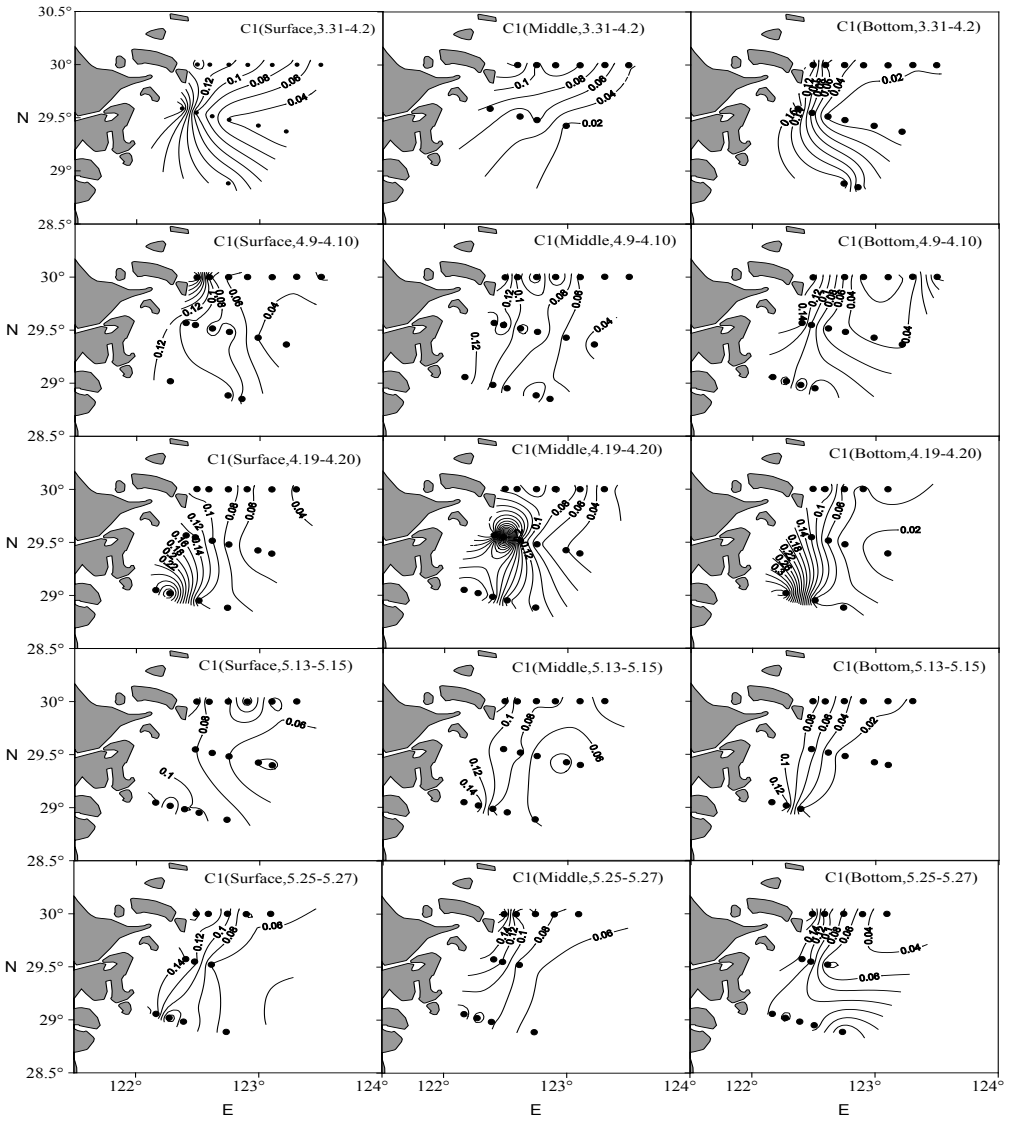

Figure 3: Horizontal distribution of component C1 in different red tide period.

Component	Mar.31 to April 2	April 9 to 10	April 19 to 20	May 13 to 15	May 25 to 27
C1	R=-0.4267 P<0.01 N=77	R=-0.6749 P<0.01 N=50	R=-0.4489 P<0.01 N=45	R=-0.3672 P<0.01 N=72	R=-0.4853 P<0.01 N=57
C2	R=-0.7253 P<0.01 N=77	R=-0.7226 P<0.01 N=50	R=-0. 6021 P<0.01 N=45	R=-0.5612 P<0.01 N=72	R=-0.5863 P<0.01 N=57
C3	R=-0.6908 P<0.01 N=77	R=-0.6682 P<0.01 N=50	R=-0.6942 P<0.01 N=45	R=-0.6424 P<0.01 N=72	R=-0.5583 P<0.01 N=57

Table 4: Positions of the fluorescence maxima of the three components.

Time	$R_{C1/DOC}$	$R_{C2/DOC}$	$R_{C3/DOC}$	N
3.31-4.2	0.1893	0.309*	0.3069*	77
4.9-4.10	0.121	0.2089	0.1068	50
4.19-4.20	-0.3045*	-0.1982	0.0635	45
5.13-5.15	0.1422	0.301**	0.4483**	72
5.25-5.27	0.1488	0.3209*	0.3385*	57
All above	0.8578	0.7446	0.9085	448

*significant level P<0.05 **significant level P<0.01

Table 5: Relationship between fluorescence intensity of three components and DOC.

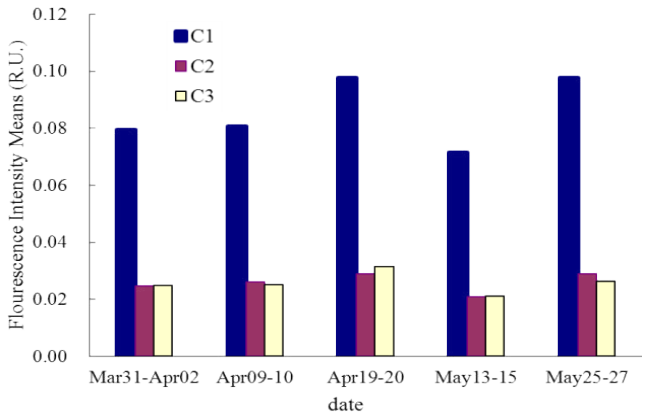

Figure 4: Change of the fluorescence intensity means of the three components on sections rb, za and zb.

BIX values were in the range from 0.39~1.48, average 0.9, about 81% samples were larger than 0.8, only 7 samples were less than 0.6. Red tide process is an important organic synthetic process; freshly produced DOM undoubtedly became an important distributor to the CDOM in the investigated water area.

The humification index (HIX) is used to investigate the degree of humification of CDOM [22,23]. Huguet et al. [33] measured HIX for samples from the Gironde Estuary (France). The high values between 10 and 16 were of terrestrial origin, but the low values (<4) were related to autochthonous organic matter. Yang et al. [34] got HIX values 0.53 and 2.11 sampled from the submarine hydrothermal vents off NE Taiwana indicating a biological or aquatic bacterial source. We calculated HIX values ranged from 0.45~7.53, averaged 1.37. Only several near shore or bottom samples have higher HX values (>2), especially near the Changjiang Estuary, the HIX value was 7.53. Most of samples had relatively lower HIX values (<2), indicating the DOM had the low humification degree and was the freshly produced by biological activity.

The fluorescence intensity means variation of the three components on the typical sections in the red tide succession process

Figure 4 showed the change of the fluorescence intensity means of the three components on sectionsrb, za and zb. Combining with Table 3, we knew that fluorescence intensities of the three components reached maximum in April 19 and April 20. Moreover, fluorescence intensities of tyrosine-like C1 component changed distinctly with red tide succession, while C2 component and C3 component varied not obviously.

Tryptophan fluorescence peak can only be tested if a protein molecule containing both tyrosine and tryptophan. So tyrosine-like fluorescence peak only be detected from tryptophan monomer or protein molecules only containing tyrosine without tryptophan [35,36]. Determann [37] effectively explored the fluorescence properties of protein from phytoplankton and bacteria through laboratory culture experiment and identified distinct tryptophan-like fluorescence peak from algae cells and bacteria, while tyrosine-like fluorescence peak only existed in phytoplankton and fluorescence intensities of tyrosine only a third of tryptophan-like. So the tyrosine-like fluorescence can be used to judge the composition and source of protein in water. Figure 4 showed that fluorescent intensities of tyrosine-like component C1 is three times more than that of tryptophan-like component C2, speculating that phytoplankton is the main source of protein in water.

Basing on the above characteristics of component C1 and its correlation with salinity, we conjectured that phytoplankton activity is the significant cause of the variation of C1 fluorescence intensities, diatom decomposition released plenty of tyrosine-like matter into water causing C1 fluorescence intensities rising in late March and early April; 8μm unknown algae took advantage of organic matter making C1fluorescence intensities reduce since April 20; then the outbreak of dinoflagellates enabled it rise up until the middle of May.

Relationship between fluorescence intensity of three components and DOC

Many scholars attempted to establish the correlation between fluorescence and dissolved organic carbon (DOC) and tried to use fluorescence to invert the content of DOM [38]. Ferrari [39] found that humic-like was positively correlated with DOC (R>0.5) in the Mediterranean and the Atlantic coast, and Ji et al. [38] discovered that protein-like substance had good linear relation with DOC and humic-like substance had bad liner relation with DOC during red tide occurrence in Jiaozhou bay, Qingdao in China. In this study only part of the five curises showed somecorrelations between the three components and DOC listed in Table 4, which suggested the source complexity of the CDOM in the investigated water area.

Conclusions

Dissolved fluorescent organic matter was measured using EEMs combined with PARAFAC. The fluorescence intensity of tyrosine-like C1 was relatively high and changed along with the succession of red tides. Phytoplankton activity was the important factor effecting on the fluorescence intensity change of tyrosine-like C1 components. The fluorescence intensities of component C2 and C3 were low relatively and changed not very noticeable. All of the relationships between the three fluorescence components and salinity, low FI, higher BIX and

low HIX indicated that terrestrial input and biological activity were the major sources of the CDOM components in different red tide periods. Our studies showed that the application of EEMs-PARAFAC modeling can characterize the qualities and sources of the dissolved organic matter in the coastal red tide-frequent-occurrence area.

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