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Fluorescence characteristics of chromophoric dissolved organic matter in shallow water along the Zhejiang coasts, southeast China

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Abstract: Twenty-eight surface water samples from rivers, muddy intertidal flats, sand shores, and bedrock coasts were collected along the Zhejiang coastline in southeastern China. In addition, three samples from the Changjiang (Yangtze River) were collected for comparison. CDOM (chromophoric dissolved organic matter) absorption and fluorescence excitation-emission matrix (EEM) spectroscopy, as well as nutrients and DOC were measured in these samples. According to salinity, nutrient, and DOC constituents, the 28 Zhejiang samples were categorized into four groups, i.e. highly-polluted, river derived, muddy-flat derived, and saltwater dominated ones. Among the six parameters (two humic-like and two protein-like peak intensities in fluorescence EEM contours, absorption at 300 nm, and DOC concentration) for the Zhejiang samples, any two of them were positively correlated. The submarine groundwater discharge, rather than local rivers, might have provided most of the freshwater that interacted with the saltwater during the mixing process. The high protein-like EEM peaks in samples from muddy salt marshes and rivers were probably caused by terrestrial inputs, land-based pollution, and local biological activities in combination.

Keywords: fluorescence spectroscopy; biogeochemical cycle; pollution effects; submarine groundwater discharge; salt marshes; coastal waters; East China Sea

1. Introduction:

Chromophoric dissolved organic matter (CDOM), absorbing light in the UV-visible range, represents one important fraction of dissolved organic matter (DOM) pool (Coble, 2007). Excitation-emission matrix (EEM) spectroscopy of CDOM has been applied for characterizing fluorescence properties of DOM in recent decades (Coble et al., 1990; Mopper and Schultz, 1993). This technique yields important information on the dynamics and chemical nature of bulk DOM as a function of its fluorescent functional groups and has been used to distinguish and track water masses (e.g. Del Castillo et al., 1999; Boyd and Osburn, 2004; Guéguen et al., 2007). Construction of an EEM contour allows the detection of the position of maximum emission at maximum excitation, i.e. $(Ex/Em)_{max}$, and it is a simple, rapid and sensitive method for CDOM concentration and composition requiring a small volume of sample.

The $(Ex/Em)_{max}$ peaks in EEM contours have generally been associated with what have been termed humic-like fluorescence (defined as peaks A, C, and M) and protein-like fluorescence (defined as peaks B and T) (Coble, 1996). Protein-like peaks result from the fluorescence of the aromatic amino acids tyrosine and tryptophan either in their monomeric forms or, more likely, incorporated into reactive protein/peptides or more refractory humic-type materials (Determann et al., 1998; Mayer et al., 1999; Yamashita and Tanoue, 2003). Due to its ability to identify protein-like materials, EEM spectroscopy has been used studying water quality evaluation (Baker, 2001; Baker and Inverarity, 2004) and marine algal and bacteria dynamics (Determann et al., 1998). Furthermore, since both CDOM and chlorophyll strongly absorb blue light, it is essential to understand how changes in concentration and optical properties of CDOM can affect the interpretation of remote sensing data (Siegel et al., 2002; Magnuson et al., 2004).

Estuarine, coastal, and marsh ecosystems are hot spots of DOM cycling because their DOM composition is controlled by the relative abundance of many different DOM sources as well as the complex interactions of physical, photochemical and microbial processes. The highest concentrations of CDOM are always found in coastal margins and semi-enclosed seas, where direct sources of terrestrial DOM are present (e.g. Mayer et al., 1999; Del Castillo et al., 1999; Kowalczyk et al., 2003). The geographical extent of the terrestrially dominated regions varies seasonally, depending on the magnitude of freshwater inputs.

Zhejiang is one of the most developed provinces in China, and its adjacent coastal areas are greatly affected by the rapid increasing population and economic development. The terrestrial

substances are transported to the seas by local rivers, and also probably by underground water discharge. At the same time, the Zhejiang coasts are surrounded by the sub-aqueous mud wedge originating from the Changjiang Estuary (Liu et al., 2007), which has been suggested in other regions to be a potential CDOM source to the overlying water (Clark et al., 2008; Tzortziou et al., 2008). Therefore, measurements of CDOM properties in different types of water samples along the Zhejiang coasts can help explain the varying effects of a series of controlling factors, such as human effects, terrestrial inputs, muddy salt marsh releases, and pelagic saltwater upwelling and intrusion, on the CDOM dynamics.

In this study, we present results of CDOM absorption and fluorescence in water samples from various environments along the Zhejiang coasts, in the inner shelf of East China Sea. Little is known about the optical quality, composition, and transportation of CDOM within this marsh-estuarine ecosystem. Our objectives are (1) to illustrate the distribution and variability of CDOM in water samples along the Zhejiang coasts, and (2) to understand the processes that contribute to the CDOM source and dynamics during the mixing process between fresh- and salt- waters.

2. Study Area and Methods

2.1 Study area

Muddy banks are widely distributed along the Zhejiang coastline. The seismic profiling and coring show that the sub-aqueous mud wedge extends from the Changjiang mouth southward off the Zhejiang and Fujian coasts into the Taiwan Strait (Liu et al., 2007). There are numerous small islands and semi-enclosed bays along the Zhejiang coasts, favoring the development of muddy tidal flats (Wang and Jin, 1983). Most of the muddy sediments come from the Changjiang, with a few sources from small local rivers (Liu et al., 2007). From north to south along the study areas (Fig. 1), there are four mountainous rivers flowing in, i.e. Jiaojiang, Oujiang, Feiyunjiang, and Aojiang, which rank the 3rd, 2nd, 7th, and 8th, respectively, in terms of the catchment area among all the rivers in Zhejiang (Liu G.Y., unpublished data, http://www.zjwater.gov.cn/pages/document/10/document_313.htm). The detailed information about the four rivers is shown in Table 1.

Due to the rapid economic development and population growth, enormous quantities of nutrients and other pollutants are transported from land to sea in Zhejiang (Hua et al., 2008), which might have largely resulted in the increasing number and scale of harmful algal bloom events in the adjacent sea

(Zhou et al., 2008). In recent decades, to meet the demand of social and economic development, more and more intertidal flats have been reclaimed for arable land and aquatic farms. Rapid land use changes have occurred along the Zhejiang coasts, in which urban areas have increased dramatically (Ding et al., 2007).

2.2 Sampling

Twenty-eight surface water samples from rivers, muddy intertidal flats, sand shores, and bedrock coasts were collected along the Zhejiang coastline (Fig. 1). In the north part of the study area, the coastline is characterized by distribution of broad muddy intertidal flats that are bordered landwards by the man-made seawalls (generally against 20–50 year floods), and bedrock coasts, gravel and sand shores were only present around some coastal headlands. To the south, the proportion of the muddy coasts decreases and they only appear in some semi-enclosed bays. On the other hand, the bedrock coasts begin to dominate the coastline, so that few seawalls are necessary. At the stations where samples 20–24 were collected, the dotted-distributed muddy flats were generally tied to the coastal bedrocks with a narrow sand shore transition or absent. At the two southernmost stations for sample 25 and 26 (which were actually located in the Fujian Province), the seawater fronts directly to the coastal bedrocks without any transition of intertidal muddy, sand, or gravel shores. Meanwhile, the water color in the two samples changed from brown to blue, presumably related to the reduced resuspended muddy particulates and the inherent CDOM property change. Besides the above Zhejiang samples, three additional ones 29–31 were also collected from the Changjiang (near Xuliujing) for comparison (Fig. 1), and the detailed information about all samples can be found in Table 2.

At each station, water samples were collected and then stored in dark immediately in an ice-box. Then, the samples obtained on that day were filtered into HDPE bottles using a pre-combusted GF/F filter (0.7 μm in pore-size, Whatman Inc.) and then frozen in dark under -20°C condition and defrosted overnight before being analyzed (within 12 days). The above sample storage protocol is commonly used for CDOM studies (e.g. Yamashita and Tanoue, 2003; Burdige et al., 2004; Jiang et al., 2008), when sample sites are remote from laboratories.

2.3 Measurements

2.3.1 CDOM absorption

Measurements of CDOM absorption were performed using a UV-visible spectrophotometer (Model: TU1810DPC, Beijing Purkinje Inc.) with a 5-cm path-length quartz cuvette. Measurements

were baseline-corrected using Milli-Q water and by running a new blank before each sample. Measurements covered the wavelength range of 300 to 800 nm (0.5-nm interval). The data were corrected for scattering and baseline fluctuations by subtracting from each spectrum the value of absorption at 700 nm (Bricaud et al., 1981). The absorptions at 300 nm, $a_{\text{CDOM}}(300)$, were chosen as an index of CDOM abundance. The spectral slope $S_{\text{CDOM}}(300-500)$ that determined the rate of the exponential decline of CDOM absorption with increasing wavelengths, was estimated after applying nonlinear exponential regression to $a(\lambda)$ measured in the wavelength range of 300–500 nm. R^2 values of the nonlinear fits were in almost all cases higher than 0.99.

2.3.2 CDOM fluorescence

CDOM fluorescence EEM contours were obtained with a Hitachi F-2500 spectrofluorometer similar to some previous studies (Coble et al., 1993; Mayer et al., 1999; Jiang et al., 2008), using a 1-cm quartz cell (Hitachi). Briefly, the excitation and emission bandwidths were both set at 5 nm. The spectrofluorometer was set at a scan speed of 300 nm min⁻¹, a PMT voltage of 700 V, and an auto response time. If necessary, samples were diluted before being measured to make the absorption at 370 nm lower than 0.1 using the 5-cm path length cuvette; and under such condition both re-absorption and inner filter effects were minimized (Guéguen et al., 2007). EEMs were recorded by scanning emission wavelengths from 230 to 600 nm repeatedly (by 0.5-nm intervals) at excitation wavelengths from 220 to 450 nm (by 5-nm increments). Final EEMs were interpolated (by 2.5-nm interval) and smoothed (by 5 × 5 moving average method) using the Surfer software (version 8, Golden Software Inc.). Daily-determined Milli-Q water was used as a blank and subtracted from sample spectra to remove Raman signals. Rayleigh scatter effects were removed by only including certain emission measurements shown in all EEM contours in this paper. The (Ex/Em)_{max} positions and intensities of the four fluorescence peaks (i.e. $F(\text{SR})$, $F(\text{BT})$, $F(\text{A})$, and $F(\text{C})$) were determined by examining the smoothed EEM contours. Fluorescence intensities are reported using the Raman Units (nm⁻¹) according to some previous studies (Nieke et al., 1997; Huguet et al., 2009).

Just in recent years, some studies (e.g. Spencer et al., 2007) suggested that freezing is not a suitable sample storage protocol for absorption and fluorescence measurements; and ideally, samples have to be stored in the cold (4°C) and dark for less than 48 h to be representative of in situ conditions. To test the freeze/thaw procedure on the optical results of our samples, a water sample was collected from Huangpujiang (31°01'02"N, 121°28'39"E; a river flowing through the city of Shanghai) and

measured after using three storage protocols, i.e. stored in 4°C for less than 48 h, stored in 4°C for 12 days, and stored in freezing (-20°C) for 12 days. Similar to most of our samples, the Huangpujiang sample also suffered from a great anthropogenic influence (Yang et al., 2007). Our results show that although there are obvious modifications of measurements after 12 days (in freezing or in 4°C) for the five quantitative indexes (i.e. $a_{CDOM}(300)$, $F(SR)$, $F(BT)$, $F(A)$, and $F(C)$), the percent differences are all less than 15% (Table 3), much smaller than the variation ranges of the Zhejiang samples over one or even two orders of magnitude for the five indexes (mentioned later). For the two qualitative indexes of S_{CDOM} (300–500) and $F(A)/F(BT)$, the maximal differences among the nine measurements (three for each protocol) are 0.0009 nm⁻¹ and 0.04 nm⁻¹/nm⁻¹, respectively, still much smaller than those Zhejiang samples (0.0067 nm⁻¹ and 3.03 nm⁻¹/nm⁻¹). In terms of (Ex/Em)_{max} positions for the four fluorescence peaks, no significant modification has been introduced by freezing (Table 3). Therefore, we believe that the main conclusions in this study should not have been changed by the measurement biases induced from the freeze/thaw procedure.

2.3.3 Additional measurements

Nutrients were determined using a Segmented Flow Analyzer (Model: San^{plus} System, SKALAR Inc.) (Grasshoff et al., 1999). DOC concentrations were determined by thermal catalysis at 680°C in a Shimadzu TOC-VCPN instrument, and samples were acidified to about pH=2 with 85% phosphoric acid and then sparged for 10 min before being injected. Salinity and pH were measured directly by YSI 650MDS multi-parameter sonde. Estimation of suspended particulate matter (SPM) was based on 200–400 ml water samples filtered through pre-weighed GF/F filters that were then dried at 50°C for 48 h and weighed again; the difference of filter weights with and without particulates, together with the volume of filtered water, was used to calculate the SPM values.

3. Results

3.1 Nutrients and DOC

Nutrients, DOC, and salinity data (shown in Fig. 2) were statistically analyzed by principal component analysis (PCA) (SPSS version 11.5, SPSS Inc.), which is a variable-reduction procedure that can be defined as a linear combination of optimally weighted variables. The optimal weights are created by the principle of least squares similar to the linear regression. In our case, PCA was done with data from the seven variables (i.e. salinity, five nutrients, and DOC) for the 28 cases (i.e. the 28

Zhejiang samples). The 28 samples were then artificially classified into four types (Type A, B, C, and D) mainly according to the PCA results (Fig. 3).

The two samples 16 and 19 are categorized into Type A, with extremely high NH_4^+ and near-zero NO_3^- values (Fig. 2b and d); in addition, they show the highest DOC levels (Fig. 2f). During the field investigation, sample 16 was collected from a tidal pond on a higher muddy flat, which seemed to have not been refreshed by the upcoming tidal currents for several semidiurnal cycles. Sample 19 was collected in a ferry port in Aojiang that had been greatly polluted by municipal wastewater. The three samples 1, 27, and 28 are classified as Type B, which were all collected from local rivers and with salinity less than 0.3. In the nutrient-salinity relationships, the two samples from Oujiang (27 and 28) show relatively higher NO_3^- and NO_2^- and lower NH_4^+ and DOC concentrations (Fig. 2b–d, f). Most (19 out of 28) of the samples (2–15, 17, 18, and 20–22) are Type C, because they were all collected from the muddy flats along the Zhejiang coasts. The last four samples (23–26), obtained in the southern part of the study area, are grouped into Type D since they show different fluorescence features (discussed later). Among the above four samples, two of them (25 and 26) were collected from bedrock coasts with blue water color, partly because they contained lower SPM values (Table 2). The NO_2^- and PO_4^{3-} concentrations for the four samples in Type D are relatively higher than others, whereas their DOC values are somewhat lower (Fig. 2c, e, and f). The negative relationships between concentration and salinity (Fig. 2a, b, and f) suggest that distributions of SiO_3^{2-} , NO_3^- , and DOC in the Zhejiang samples were in part controlled by the mixing process between fresh- and salt- waters.

3.2 CDOM absorption

The CDOM abundance index $a_{\text{CDOM}}(300)$ is highly linear correlated with DOC (Fig. 4), suggesting CDOM signatures can be used to represent DOC concentration in the study area. The positive intercept in the y-axis (48.1 μM of DOC when $a_{\text{CDOM}}(300)$ equals zero) implies the presence of DOC compounds unrelated to the CDOM dynamics.

The CDOM spectral slope, $S_{\text{CDOM}}(300\text{--}500)$, is believed to reflect the CDOM composition change of humic to fulvic or high-molecular-weight to low-molecular-weight proportions (Carder et al., 1989; Twardowski and Donaghay, 2002). In the present study, the $S_{\text{CDOM}}(300\text{--}500)$ results range between 0.0082 to 0.0149 nm^{-1} , in which sample 16 and 13 contribute to the highest and lowest values, respectively. In previous studies, Kowalczyk et al. (2005) found the increasing spectral slopes with decreasing absorptions in the Baltic Sea. Green and Blough (1994) also observed that the lowest

spectral slopes and highest absorptions always correspond to inland and coastal samples, while the offshore blue waters generally have the opposite values. In contrast with the two studies, the two parameters among the 28 Zhejiang samples are slightly positively correlated (the solid line in Fig. 5). However, when only the two groups of samples in Type C and Type D are compared, a slightly negative regression, rather than positive, between $S_{CDOM}(300-500)$ and $a_{CDOM}(300)$ occurs (the dotted line in Fig. 5).

3.3 CDOM fluorescence

Fluorescence EEM contours we obtained (Fig. 6) contain a number of distinct peaks that are generally ascribed to either humic-like or protein-like fluorescence. Coble (1996) reported the two protein-like fluorescence peaks: peak B at $(Ex/Em)_{max}=275/310$ nm due to tyrosine, and peak T at $(Ex/Em)_{max}=275/340$ nm due to tryptophan. Later, Mayer et al. (1999) found the additional two protein-like fluorescence peaks at lower excitation wavelengths ($Ex_{max}=220-230$ nm) and similar emission wavelengths, as had not been found by Coble (1996) because the author did not use excitation wavelengths lower than 260 nm. Burdige et al. (2004) named these additional peaks as peak S for tyrosine and peak R for tryptophan. In this study, similar to Burdige et al. (2004), we also quantify protein-like fluorescence simply in terms of high and low excitation wavelengths, i.e. combined peaks of BT and SR, without further discrimination of the two amino acid sources.

In samples belonging to Type A, B, and C (Fig. 6a–f), in addition to the two protein-like peaks (i.e. SR and BT), the EEM contours show clearly a third peak that has been linked to the humic-like peak A (Coble, 1996). In the other four samples of Type D (Fig. 6g and h), the peak A becomes the only distinct one in the EEM contours. In all the 28 EEM contours, the humic-like peaks C and M (Coble, 1996; Parlanti, et al., 2000) generally only appear as shoulders on the tail of the adjacent prominent peaks (Fig. 6), and it is not easy to precisely distinguish the $(Ex/Em)_{max}$ positions of the two peaks. Hence, in this study we only quantify the heights of peak C at the fixed Ex/Em wavelength of 350/450 nm recommended by Coble (1996).

The EEM contour of sample 16 (Fig. 6a) shows much higher fluorescence values than the other 27 samples throughout the spectrum; hence this sample is removed in the following correlation calculations. Table 4 shows that any two of the four EEM peak intensities (i.e. $F(SR)$, $F(BT)$, $F(A)$, and $F(C)$) among the remaining 27 samples are significantly correlated; in addition, the four peak values are also highly correlated with DOC or $a_{CDOM}(300)$.

PCA is employed for the second time on the 13 variables (i.e. the seven variables in the first PCA in Fig. 3 plus $a_{CDOM}(300)$, $S_{CDOM}(300-500)$, and the four fluorescence EEM peak heights) for the 28 samples. Compared to those in Fig. 3, the relative positions for the 28 samples in the new PCA plot are not largely changed after introducing the six new parameters (plot not shown); and the previous classification of the four types for the 28 samples is still valid.

4. Discussion

4.1 Comparison with the Changjiang freshwater

Three Changjiang water samples (29–31, shown in Fig. 1) were collected in March and April 2008 with salinity all less than 0.3, and their EEM contours are shown in Fig. 7. In summer under the prevailing southeast monsoon, the northward Taiwan Warm Current intensifies, and correspondingly the southward Zhejiang-Fujian Coastal Current weakens (Beardsley et al., 1985; Lee and Chao, 2003). Hence during the investigation period of July 2007, the influence of the Changjiang Diluted Water on Zhejiang coastal seawater is believed to be minimized. As has been mentioned, the EEM contours of the 28 Zhejiang samples can be separated into two categories. Samples 1–22 and 27–28 (Type A, B, and C; Fig. 6a–f) belong to the first category, whose EEM contours show distinct SR and BT peaks with heights comparable to or even higher than peak A. However, in the second category, EEM contours of samples 23–26 (Type D, Fig. 6g and h) show SR or BT signatures generally only as the shoulders of peak A. Concerning the three Changjiang freshwater samples (Fig. 7), their EEM contours seem to lie between those two categories of Zhejiang samples, because the fluorescence ratio of peak A to peak BT, $F(A)/F(BT)$, for the three Changjiang waters ranges between 2.0–2.2, comparing to 0.4–1.6 for Zhejiang samples in the first category and 2.4–3.4 in the second.

Relationships between salinity and the four EEM peak heights (i.e. $F(SR)$, $F(BT)$, $F(A)$, and $F(C)$) for all the 31 samples (the 28 Zhejiang samples plus the three Changjiang samples) are shown in Fig. 8. The two samples from Oujiang (sample 27 and 28) and the three ones from Changjiang (sample 29–31) are always clustered together at the bottom left area of the diagrams. If the five data points and that of sample 16 are removed, negative linear correlations can generally be established (Fig. 8). Different from Jiaojiao, Feiyunjiang, and Aojiao (sample 1, 18, and 19), the five river samples from Oujiang and Changjiang with much higher freshwater runoff (Table 1) provide fluorescence values much lower than those the calculated regression lines suggest (Fig. 8), implying Oujiang is not a main source of

fluorophores measured in other Zhejiang samples. The above results also suggest that the concentrations of fluorescent compounds in large rivers (e.g. Changjiang and Oujiang) may be determined by different mechanisms from those of smaller ones (e.g. Jiaojiang, Feiyunjiang, and Aojiang).

4.2 Identification of the freshwater end-member

According to the fluorescence-salinity relationships (Fig. 8), the fluorophores from Oujiang should not be the main origin of CDOM measured in other samples. Also, due to the much lower freshwater outputs (Table 1), CDOM compounds in most Zhejiang samples unlikely originated from Jiaojiang, Feiyunjiang, and Aojiang. In fact, from the salinity results in the 28 samples along the Zhejiang coasts (Fig. 9) we can hardly find their specific variations in response to the freshwater outputs from nearby local rivers. It is consequently important to determine the freshwater source, which is also closely associated with the origins of nutrients, DOC, and CDOM molecules since their concentrations are primarily or partly controlled by salinity in most samples (Figs. 2, 4, and 8).

We notice that a surprisingly low salinity 1.9 is shown in sample 16, which was collected in a high-elevation tidal pond having not been recharged by upcoming tidal currents for several semidiurnal cycles. Firstly, this sample unlikely originated from the adjacent local rivers because (1) there are no small rivers nearby, and (2) its salinity is much lower than those adjacent samples (14, 15, 17, and 18) that are closer to Oujiang and Feiyunjiang (Fig. 1). Secondly, sample 16 was not influenced by rainwater because (1) there was no rain record during the investigation days, and (2) its nutrient results (Fig. 2) are dramatically different from those rainwater samples obtained in the nearby regions (Zhang et al., 2005). In fact, the first author (Gao L.) had also collected a rainwater sample in the Changjiang Estuary in August 2005, whose nutrient concentrations were 4.5 μM for SiO_3^{2-} , 1.4 μM for NO_2^- , 113.4 μM for NH_4^+ , 95.0 μM for NO_3^- , and 0.5 μM for PO_4^{3-} (unpublished data). Compared to sample 16, the rainwater contains much lower SiO_3^{2-} (4.5 vs. 223.7 μM) and NH_4^+ (113.4 vs. 1049 μM), and higher NO_3^- (95.0 vs. 2.0 μM) concentrations.

Submarine groundwater discharge (SGD) in coastal areas was neglected scientifically for many years because of the difficulty in its quantification. In recent years, according to radiochemical and stable isotope measurements, more and more studies showed that SGD outputs are chemically and ecologically important to coastal waters. For example, Dulaiova et al. (2006) found the SGD runoff can represent about 20% of the river flow during low-flow periods and 4% during high-flow conditions in

the estuarine region of the Chao Phraya River (discharging into the Gulf of Thailand). Paytan et al. (2006) estimated that nitrogen inputs by SGD range between 3 to 800 mmol h⁻¹ per meter of shoreline in the coral reef ecosystems around the world. Other studies also showed that terrestrially derived inputs from SGD play an important role in the discharge of nutrients and other compounds, especially in areas where the terrestrial outputs by rivers are not significant (Burnett et al., 2003; Charette and Buesseler, 2004; Hays and Ullman, 2007).

During the investigation for the present study, 11 sediment cores (with lengths of about 1 m) were also obtained by manual digging at low tides in different muddy intertidal flats. During the digging process, rapid porewater advection filling into the excavation hole was always observed, which directly verified the considerable underground water discharge in the study area.

Therefore, we may consider that the sample 16 is actually from SGD outputs. This sample had hardly been affected by the recycled seawater due to its very low salinity, although it might have been greatly modified by inherent chemical processes. The tidal pond where sample 16 was collected is in fact acting as the ‘monitoring well’, by which SGD samples in coastal areas were collected in many previous studies (Dulaiova et al., 2006; Hays and Ullman, 2007; McCoy et al., 2007). The salinity variation among the Zhejiang samples (Fig. 9) presumably largely reflects the varying magnitude of the SGD fluxes from local permeable sediments. Further, the variation among the three samples 2–4 (with salinity ranging from 25.6 to 33.7), which were collected at the same station and on the same day but at different tidal phases, presumably reveals the reciprocating motion of the subterranean freshwater-saltwater interface within a tidal cycle (Robinson et al., 2007).

In the DOC-salinity relationship (Fig. 2f), the value of sample 16 is in the same order of magnitude when compared with the freshwater end-member value suggested by the regression line. In the *F*(A)-salinity and *F*(C)-salinity diagrams (Fig. 8c and d), similar distributions of data points are also observed. The above results imply that the SGD might provide a part of DOC (Fig. 2f), CDOM absorbents (deduced from Figs. 2f and 4) and humic-like fluorophores (Fig. 8c and d) measured in the most Zhejiang samples, whose concentrations decrease near-conservatively during the freshwater-saltwater mixing process. When, however, considering the protein-like peaks SR and BT, their levels in sample 16 are unusually high (Fig. 8a and b, note the breaks in the y-axes). If the sample 16 was considered as the freshwater end-member, the protein-like fluorophores would have been largely removed/assimilated at intermediate salinities, presumably by photobleaching, adsorption to

particles, and/or flocculation (Miller, 1999; Abril et al., 2002; Stedmon and Markager, 2003). Another possibility is that $F(\text{SR})$ or $F(\text{BT})$ values in sample 16 had been greatly elevated by in situ processes. The various processes changing the fluorescence values of the two protein-like peaks are discussed in the next section.

4.3 The high protein-like fluorescence peaks

The two protein-like peaks can be observed in most EEM contours of samples in the present study, consistent with other Chinese coastline surface seawaters (Hong et al., 2005; Jiang et al., 2008). The high contribution of protein-like materials to the fluorescence EEM contours was also found in a large number of coastal oceans worldwide (e.g. Mayer et al., 1999; Kowalczyk et al., 2003; Burdige et al., 2004). All of the above studies highlight the importance of elucidating the origin of these protein-like fluorophores.

Baker et al. (2001) studied the EEM contours in the sewage-impacted waters and found that these samples always displayed high protein-like fluorescence peaks; they concluded that the sewage (even after treatment) and other agricultural waste and industrial discharges can behave as a high-protein source to adjacent coastal seawaters. Similar conclusion was also reached by Hudson et al. (2007). Our study area is surrounded by two big cities of Wenzhou and Taizhou (shown in Fig. 1), which are under the influence of heavy urbanization and industrialization. Also, the sampling stations were always close to the aquatic farms for fisheries and agriculture. Therefore, it is reasonable that the distinct protein-like peaks in most samples are caused at least partly by the land-based protein pollution.

The contribution of anthropogenic activities to the protein-like peaks can be validated by the results of sample 19, which was collected from a ferry port in Aojiang. This sample contains the second, third, and second highest SR and BT fluorescence heights and NH_4^+ concentration, respectively (Figs. 2d, 8a and b), and it also exhibits near-zero NO_3^- level (Fig. 2b).

It is still possible that some protein-like fluorophores originate from inherent aquatic processes, such as primary productivity and respiration consumption by phytoplankton, and degradation of bacteria. The co-occurrences of the high NH_4^+ concentration and the two protein-like peaks in sample 16 strongly suggest the biological degradation processes in the field. Also, although the near-zero NO_3^- concentration in sample 16 is similar to that in Aojiang (sample 19), it is obviously lower than those in other local rivers (sample 1, 18, 27, and 28; Fig. 2b) and most of the rivers in other regions suffering from a great influence of human activities (e.g. the Huangpujiang river, see Yang et al., 2007). Several

360 studies have observed high benthic denitrification rates in the Changjiang Estuary intertidal flats, which
 361 remarkably decreased the NO_3^- concentrations in the overlying water (Wang et al., 2007; Gao et al.,
 362 2008). Therefore, sample 16 might have also been experiencing, until being sampled, considerable
 363 denitrification that not only reduced NO_3^- levels but also might have altered the concentrations and
 364 properties of DOC and CDOM substances, including the protein-like fluorophores.

365

366 5. Conclusions

367 The Zhejiang coasts are a representative area in which great physical, chemical, geological and
 368 biological processes, as well as human activities all exert their complex influences on the CDOM
 369 distribution and biogeochemistry. In the present study, we classify the 28 surface water samples
 370 obtained along the Zhejiang coasts into four types, according to their nutrient, DOC, and optical
 371 measurements. The two samples in Type A (16 and 19) are highly polluted and/or greatly in situ
 372 modified, because they contain extremely high NH_4^+ and protein-like fluorescence values as well as
 373 near-zero NO_3^- concentrations. The three samples in Type B (1, 27, and 28) were all collected from
 374 local rivers; in which the two samples from Oujiang (27 and 28) show similar properties with the
 375 Changjiang waters, in terms of the relationships between salinity and fluorescence values of the four
 376 EEM peaks. Most of the samples (19 out of 28) belong to Type C, since they were generally obtained
 377 from the northern study areas that were under the influence of the ‘mud belt’ depositing on the inner
 378 shelf of East China Sea. Concerning the fluorescence EEM contours, the two protein-like peaks (SR
 379 and BT) in samples of Type A, B, and C can be easily discerned with magnitudes comparable to or
 380 even higher than those of the humic-like peak A. However, in the EEM contours of Type D samples
 381 (23–26), the protein-like signals only exhibit as shoulders of the dominant peak A. The underground
 382 water discharge, rather than local river inputs, might have actually provided the most CDOM
 383 substances measured in the Zhejiang samples. The protein-like fluorophores in samples of Type A, B,
 384 and C should have multiple sources, such as terrestrial inputs, land-based pollution, and local biological
 385 activities.

386

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530

531

Table 1. Characteristics of the four local rivers in the study area

River	Water Flux ($10^9 \text{ m}^3 \text{ yr}^{-1}$)	Sediment Flux (10^6 t yr^{-1})	Length (km)	Catchment Area (10^3 km^2)
Jiaojiang	3.9 ^a	0.8 ^a	209 ^b	6.6 ^b
Oujiang	16.7 ^a	2.2 ^a	384 ^b	18.1 ^b
Feiyunjiang	2.4 ^a	0.4 ^a	193 ^b	3.7 ^b
Aojiang	0.5 ^a	0.08 ^a	81 ^b	1.5 ^b

532 ^a cited from Sun and Huang (1984).533 ^b cited from Liu G.Y., unpublished data.

534

535

536 Table 2. Characteristics of the 31 surface samples collected along the Zhejiang coasts (in 2007) as well

537

as in the Changjiang river (in 2008)

Sample #	Sampling Date	Lat (N) & Long (E)	Salinity	pH	SPM (mg L ⁻¹)	DOC (μM)	<i>a</i> _{CDOM(300)} (m ⁻¹)
1	Jul-14	28°41'12", 121°25'44"	0.13	7.88	520	234.3	15.43
2	Jul-15	28°32'27", 121°33'29"	25.55	7.58	455	101.0	3.27
3	Jul-15	28°32'27", 121°33'29"	33.68	7.97	305	176.9	7.23
4	Jul-15	28°32'27", 121°33'29"	33.40	7.75	495	162.0	5.48
5	Jul-16	28°17'54", 121°34'40"	31.24	7.88	360	95.3	2.03
6	Jul-16	28°18'34", 121°33'49"	22.51	7.57	245	85.1	2.21
7	Jul-16	28°19'23", 121°32'00"	33.52	7.77	495	114.5	2.44
8	Jul-17	28°12'48", 121°23'53"	32.13	8.06	350	118.5	4.10
9	Jul-17	28°11'38", 121°22'09"	32.17	7.89	118	101.4	3.13
10	Jul-17	28°10'14", 121°19'25"	30.19	8.04	905	105.3	4.01
11	Jul-18	28°21'10", 121°10'21"	21.17	7.81	420	132.9	6.77
12	Jul-18	28°16'48", 121°06'33"	26.09	7.88	340	135.4	5.11
13	Jul-18	28°07'28", 121°02'30"	27.98	7.81	905	114.3	4.65
14	Jul-19	27°56'28", 120°56'16"	18.48	7.73	1,765	168.5	9.30
15	Jul-19	27°55'14", 120°59'50"	28.46	7.72	885	126.3	3.96
16	Jul-19	27°47'38", 120°46'58"	1.87	8.43	330	364.7	14.42
17	Jul-20	27°43'34", 120°44'10"	29.27	7.91	4,475	197.2	9.03
18	Jul-20	27°45'51", 120°37'11"	14.37	7.89	650	97.3	4.33
19	Jul-21	27°35'33", 120°33'01"	7.98	7.87	845	236.9	10.55
20	Jul-21	27°30'24", 120°37'33"	32.00	7.90	1,420	142.7	5.85
21	Jul-22	27°23'25", 120°36'30"	32.91	8.17	1,805	86.2	2.30
22	Jul-22	27°21'35", 120°32'06"	32.64	8.14	325	82.7	1.61
23	Jul-22	27°23'05", 120°33'10"	33.89	8.01	1,120	63.1	1.47
24	Jul-22	27°12'37", 120°27'30"	33.83	7.87	370	74.0	2.16
25	Jul-23	27°09'48", 120°25'19"	32.65	7.88	140	54.8	0.83
26	Jul-23	27°13'44", 120°23'56"	31.19	7.95	137	60.5	1.06
27	Jul-24	28°08'22", 120°30'05"	0.22	7.07	335	142.6	5.67
28	Jul-24	28°01'42", 120°37'23"	0.12	7.54	605	118.7	5.30
29	Apr-1	31°47'27", 121°03'50"	0.18	7.84	95	107.3	4.19
30	Apr-1	31°57'38", 120°51'59"	0.15	7.86	50	92.4	4.10
31	Mar-8	31°46'13", 120°55'40"	0.30	7.85	39	96.4	4.38

538

539

540 Table 3. Comparison of the optical measurements using three different storage protocols for a river

541 sample collected from Huangpujiang

Index	$a_{\text{CDOM}}(300)$ [m ⁻¹]	$S_{\text{CDOM}}(300-500)$ [nm ⁻¹]	$F(\text{SR})$	$F(\text{BT})$	$F(\text{A})$	$F(\text{C})^a$	$F(\text{A})/F(\text{BT})$ [nm ⁻¹ /nm ⁻¹]
stored in cold for less than 48 h (ave.±std., $n=3$)							
	5.85±0.05	0.0167±0.0001	5.2±0.1	6.8±0.3	5.8±0.2	1.49±0.05	0.86±0.01
(Ex/Em)max. [nm/nm]			237.5/355	277.5/317.5	(255–257.5)/ (440–445)		
stored in cold (4°C) for 12 days (ave.±std., $n=3$)							
	6.09±0.08	0.0162±0.0002	4.4±0.1	6.0±0.1	5.1±0.1	1.41±0.02	0.86±0.01
(Ex/Em)max. [nm/nm]			237.5/ (355–357.5)	277.5/317.5	(255–257.5)/ (437.5–445)		
Recovery	104%		85%	88%	88%	95%	
stored by freezing (-20°C) for 12 days (ave.±std., $n=3$)							
	6.31±0.06	0.0167±0.0002	4.6±0.1	6.0±0.2	5.1±0.2	1.36±0.02	0.84±0.01
(Ex/Em)max. [nm/nm]			237.5/ (355–357.5)	277.5/ (315–317.5)	(260–265)/ (435–442.5)		
Recovery	108%		88%	88%	88%	91%	

542 ^a at the fixed Ex/Em wavelength of 350/450 nm.

543

544

545 Table 4. Positive correlation coefficients (R^2) between the six parameters of the four fluorescence peak
 546 heights of $F(\text{SR})$, $F(\text{BT})$, $F(\text{A})$, and $F(\text{C})$ in EEM contours, and $a_{\text{CDOM}}(300)$ and DOC concentration,
 547 for the samples obtained along the Zhejiang coasts (sample 16 is not included in the correlation
 548 calculations due to its extremely high values)

R^2	$F(\text{SR})$	$F(\text{BT})$	$F(\text{A})$	$F(\text{C})$	$a_{\text{CDOM}}(300)$	DOC
$F(\text{SR})$	—	0.92**	0.93**	0.94**	0.85**	0.79**
$F(\text{BT})$	0.92**	—	0.86**	0.89**	0.92**	0.85**
$F(\text{A})$	0.93**	0.86**	—	0.97**	0.88**	0.77**
$F(\text{C})$	0.94**	0.89**	0.97**	—	0.90**	0.81**
$a_{\text{CDOM}}(300)$	0.85**	0.92**	0.88**	0.90**	—	0.88**, ^a
DOC	0.79**	0.85**	0.77**	0.81**	0.88**, ^a	—

549 ** . Correction is significant at the 0.01 level.

550 ^a. also shown in Fig. 4 but only for 27 samples here.

551

Figure Captions:

Fig. 1. Map of the study area and the sampling stations, including the 28 samples (1–28) along the Zhejiang coasts and the three (29–31) in the Changjiang river.

Fig. 2. Variation of nutrients and DOC against salinity for the 28 samples along the Zhejiang coasts. The samples are classified into four types denoted by different symbols (see details in Section 3.1). Note the $\log_{10}$ scale in the y-axis of Fig. 2d.

Fig. 3. PCA results with data from salinity, DOC, and five nutrients (i.e. SiO_3^{2-} , NO_3^- , NH_4^+ , NO_2^- , and PO_4^{3-}) (the seven variables) for the 28 samples along the Zhejiang coasts. These samples are then separated into four groups of Type A, B, C, and D. Symbols are as in Fig. 2.

Fig. 4. Correlation between DOC concentration and the CDOM absorption $a_{\text{CDOM}}(300)$ among the 28 samples along the Zhejiang coasts. Symbols are as in Fig. 2.

Fig. 5. Relationship between the CDOM absorption $a_{\text{CDOM}}(300)$ and the spectral slope $S_{\text{CDOM}}(300-500)$ for the 28 samples along the Zhejiang coasts. The solid line shows the correlation for all the 28 data points; whereas the dotted line, only for those of Type C and D. Symbols are as in Fig. 2.

Fig. 6. Representative fluorescence EEM contours for samples of Type A (Fig. 6a and b), B (Fig. 6c and d), C (Fig. 6e and f), and D (Fig. 6g and h) selected from the 28 samples along the Zhejiang coasts. The fluorescence intensities are quantified using Raman Units (nm^{-1}). The $(\text{Ex/Em})_{\text{max}}$ positions for peaks SR, BT, A, and C are denoted in Fig. 6a and b.

Fig. 7. Fluorescence EEM contours for the three samples collected in the Changjiang river. The fluorescence intensities are quantified using Raman Units (nm^{-1}). The $(\text{Ex/Em})_{\text{max}}$ positions for peaks SR, BT, A, and C are denoted in Fig. 7a.

Fig. 8. Variations of the four fluorescence EEM peak heights (i.e. $F(\text{SR})$, $F(\text{BT})$, $F(\text{A})$, and $F(\text{C})$) against salinity among the 31 samples (including the 28 samples along the Zhejiang coasts and the three from Changjiang). In each diagram, the data points enclosed by the ellipses are not incorporated into the correlation calculations. Note the breaks in the y-axes of Fig. 8a and b.

Fig. 9. Salinity variation in the 28 Zhejiang samples arrayed roughly from north to south along the coastline. Note that samples 2–4 were collected at the same station and on the same day but at different tidal phases.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

ACCEPTED MANUSCRIPT

Figure 6

Figure 7

Figure 8

Figure 9