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Optical properties of chromophoric dissolved organic matter (CDOM) of water body in Sanyang wetland, China

Qi Wang[a,b,*], Ying Liu[a,b], Hengguo Yu[b], Qiang Ke[a,b] , Chuanhua Wang[a,b], Chuanjun Dai[a,b], Min Zhao[a,b,*]

[a]*College of Life and Environmental Science, Wenzhou University , Wenzhou, China, email: victor527@126.com (Q. Wang), 474160854@qq.com (Y. Liu), 851059575@qq.com (Q. Ke), 460844396@qq.com (C. Wang), 1121639138@qq.com (C. Dai), zmcnzj@sina.com (M. Zhao)*
[b]*Key Laboratory for Subtropical Oceans & Lakes Environment and Biological Resources Utilization Technology of Zhejiang, Wenzhou University, Wenzhou, China, email: yuhengguo5340@163.com (H. Yu)*

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ABSTRACT

Sanyang wetland in Wenzhou plays an important role in urban ecological system. However, Water body in Sanyang wetland is facing many threats of industrial pollution, agricultural non-point source pollution and domestic living pollution. In order to further understand specific pollution situation, three-dimensional fluorescence fingerprinting technology and ultraviolet spectrophotometry were used to evaluate spatial distribution and temporal trends of optical properties of colored dissolved organic matter (CDOM) in Sanyang wetland. One hundred and sixty eight experimental data in seven sampling sites of Sanyang wetland were monitored during years 2015–2016. The results showed that the degree of organic pollution in Sanyang wetland was different in time and space. In summer, the organic pollution could reach a high level, while the difference in spatial distribution was small, which was related to the intertwined rivers in the wetland. Moreover, It should be emphasized that the characteristics of biological CDOM fluorescence indices in Sanyang wetland were mainly owing to more microorganisms and phytoplankton. Finally, all these results would be expected to be of use in the study of pollution problems of wetland water quality.

Keywords: Optical properties; Chromophoric dissolved organic matter (CDOM); Humification index (HIX); Biological source index (BIX)

1. Introduction

Colored dissolved organic matter (CDOM), also known as soluble organic chromophores, or mucolytic solutes. Earliest, it is called a yellow substance, an optically measurable component of dissolved organic matter in water [1,2]. CDOM is widely found in natural water. Its main components are humic and protein-like, including humic acid, fulvic acid, humic, and peptidoglycan, which can play an important role in the biological activities of water environment, the migration and transformation of pollutants [3,4]. It can interact with many inorganic or organic substances and directly affects its stability and bioavailability [5]. Sources of CDOM in water include exogenous inputs and biological activities [6]: Exogenous inputs are emissions from river inputs and human activities. Sewage may contain high concentrations of organic matter, faecal bacteria, viruses and chemicals, such as heavy metals, hydrocarbons, etc. On the one hand, many of the main sources of freshwater are terrestrial plant degradation, soil erosion and plankton activity [7]. The characteristics of dissolved organic matter (DOM) will affect the acid-base properties of water in water environment, nutrient availability and pollution of environment behavior, such as pollutant toxicity, migration and transformation characteristics and biodegradability.

*Corresponding author.

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At the same time, the migration behavior of heavy metals and organic pollutants in the water environment also has an important impact. Moreover, DOM properties in source water will significantly affect the operation of drinking water treatment process (e.g., disinfection by-products) [8,9]. CDOM is some macromolecular polymer with aromatic ring structure, accounting for DOM of total water absorption in 50%~70%, an important component of the DOM library, the main absorption of ultraviolet light and blue violet, it is not only to protect the marine biological level from ultraviolet radiation B damage, but also control the sun light in the water transmission depth, and then affects the primary productivity of the water body [10,11].

CDOM has obvious optical properties, and has light absorption properties in the ultraviolet and visible light regions, and then the fluorescent material therein can emit fluorescence under certain conditions [12]. In particular, the absorption of ultraviolet light is particularly strong. Different pollutants have different fluorophores, and can emit light with a characteristic wavelength under a certain range of excitation light. Spectral analysis by Ex/Em (excitation wavelength/emission wavelength) determines the type of dissolved organic matter [13]. As a fast and easy method to study the composition and origin of CDOM, three-dimensional fluorescence spectroscopy has the advantages of high sensitivity, small sample amount, fast processing speed and large measuring range [14,15]. In recent years, three-dimensional fluorescence method for lake, river and other water CDOM analysis has been more extensive [16–18].

In this paper, we studied the spatial and temporal distribution characteristics of CDOM and its fluorescence components in rivers and streams from 2015 to 2016 and their sources by using three-dimensional fluorescent fingerprint spectroscopy. On this basis, the relationship between CDOM and total nitrogen (TN), total phosphorus (TP), chlorophyll a (Chl a) and dissolved organic carbon (DOC) were discussed in order to provide basic information for further revealing the dynamic mechanism of CDOM source and its environmental behavior.

2. Material and methods

2.1. Sampling and pretreatment

The Sanyang wetland is located in the East China coastal zone, close to the center of Wenzhou city, a location that is well-known in China for its fast economic development over the last two decades, which is shown in Fig. 1. We will set 7 sampling sites (1–7), which are chosen in the Sanyang Wetland of Wenzhou (27º03′~28º36′N, 119º37′~121º18′), Southeast of Zhejiang Province in China. All the grab water samples are collected at a depth of 0.5 m and a global position system (GPS) is used to locate the sampling sites. Samples are obtained on Jan. 2015 to Dec. 2016. Sampling and analysis information for water quality parameters would be acquired, including temperature, dissolved oxygen (DO), pH, dissolved organic carbon (DOC), total nitrogen (TN), total phosphorus (TP), chlorophyll a (Chl a) and total dissolved solids (TDS). For analysis, each sample is collected in a pre-cleaned amber glass bottle and stored at 4°C, processed within 24 h. All the water samples are filtered through a 0.45 μm filter after the samples are shipped to the laboratory. The filtered-samples should be refrigerated at 4°C in the dark and completed the analysis within one week.

2.2. Absorption measurements and fluorescence spectroscopy

In this study, the CDOM concentrations are represented using a355 (absorption coefficient a at a wavelength of 355 nm), and to facilitate comparison with other studies. Absorption spectra are obtained between 200 and 800 nm at 1 nm intervals using a Perkin Elmer Lambda 750 UV/Vis spectrophotometer equipped with matching 10 cm quartz cells. Milli-Q water is used as baseline for absorbance spectrum (resistivity > 18 MΩ·cm), and at the medium scanning speed. Fluorescence excitation-emission matrices (EEM) of all water samples are measured using a fluorescence spectrometer (Hitachi F-4600, Japan) with excitation light source of xenon lamp. Excitation scan is conducted from a wavelength range of 200–500 nm in 5 nm increments at a stepwise increase of 2 nm with an emission wavelength range of 250–600 nm, with a scanning speed of 12000 nm·min^{-1}. Water Raman scatter peaks are calibrated by subtracting EEM of Milli-Q water blanks on the same day.

2.3. Statistical analysis

The statistical analysis, including mean values, Boxplots, standard deviations, calculation of descriptive statistics, and correlation analysis are performed with Statistical Product and Service Solutions software (SPSS 22.0). In addition, contour excitation-emission matrix (EEM) plots are conducted in MATLAB R2016b.

3. Results and discussion

3.1. Regular water quality parameters

Water quality is typically characterized by parameters, such as temperature, DO, pH, DOC, TN, TP, Chl a and TDS, heir average values are 21.96°C, 3.15 mg/L, 7.52, 13.67 mg/L, 4.78 mg/L, 0.31 mg/L, 31.50 μg/L and 219.62 mg/L, respectively. Higher values are concentrated in the northern regions AY, LG, YX, but the concentration of Chl a is just the opposite trend. In particular, ZH except TDS this indicator has been larger, the remaining parameter values are kept to a minimum. Due to the important role of nutrients in primary productivity, concentration and CDOM breakdown [19], high levels result in poor water quality. The sampling sites Chl a concentrations are greater than 10 μg/L, only ZH bridge values are significantly lower, the other sampling points were at similar levels. In season, winter is the lowest season for all parameters. Chl a in summer is significantly higher than in other seasons, and the cooler the colder, the lower the weather, which should be related to the growth of aquatic plants. Chl a concentration and fluorescence intensity distribution of each sampling point basically agree with, showing the higher the concentration, the stronger the fluorescence intensity. The trend of TN and TP are very similar. Spring and summer are higher than autumn and winter, and peak in May and June. To the best of our knowledge, DO is significantly lower in summer

Fig. 1. Distribution of sampling sites in Sanyang Wetland; Sampling points 1 to 7, represent Anyang Bridge(AY), Ligong Bridge(LG), Yixian Bridge(YX), Shuixian Bridge(SX), Qixian Bridge(QX), Zhihe Bridge(ZH) and Nanxian Bridge(NX), respectively.

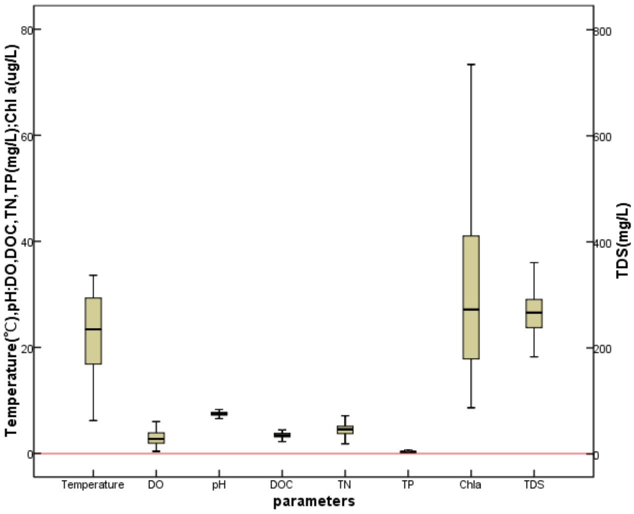

Fig. 2. Box-plots of temperature, DO, pH, DOC, TN, TP, Chl a, TDS in Sanyang Wetland.

and winter, on the one hand, related to hot weather, and on the other hand, as caloric evaporation increases the amount of dissolved salts, on the other hand, as algal growth and decomposition of organic matter are accelerated, dissolved oxygen decline. In addition, Chl a is thought to degrade more bacteria by hydrolyzing phytoplankton rather than by land inputs and sunlight [1,8,20]. However, TN and TP are mainly affected by terrestrial sources, DOC in the urban water samples mainly originated from terrestrial sources and a small portion from sewage [9,21], and these are also of great significance for exploring the optical properties of CDOM.

3.2. Fluorescent component characteristics of CDOM

Different sources of dissolved organic matter have different fluorescence groups, and the position and intensity of fluorescence peaks can vary from 2015 to 2016. The three dimensional fluorescence spectra of CDOM in Sanyang Wetland of Wenzhou City has two protein-like fluorescence peaks (Fig. 3). One is tryptophan fluorescence (Peak A) [22], the maximum excitation wavelength and emission wavelength are 225 nm and 340 nm, respectively; another protein-like peaks is considered to be a tyrosine substance tyrosine (Peak B) [23], the maximum excitation and emission wavelengths are 275 nm and 330 nm, respectively. Such fluorescence peaks are mainly produced by the action of microorganisms and phytoplankton, reflecting biological sources. However, their production is also affected by the microbes carried by direct discharge of treated or untreated wastewater, including domestic sewage and industrial wastewater [24]. The composition of the groundwater CDOM is also found in the composition, the main source for human activities, microbial production and terrestrial input.

By comparing the seasonal variation characteristics of fluorescence intensity, it is found that the fluorescence intensity in summer is significantly higher than that in other seasons, and the fluorescence intensity in spring and winter is relatively weak. The fluorescence intensity of spring 2015 is weaker than that of 2016, but the contrary is true in winter. Due to the abundant precipitation in summer, Rain Water scour the surface plants, has a large river runoff, high input intensity of land source [25], and the death of phytoplankton. Degradation also produces CDOM, especially microbes and phytoplankton in summer waters, which are important sources of CDOM in water [13]. The reason is that terrestrial input may directly lead to the increase of protein-like

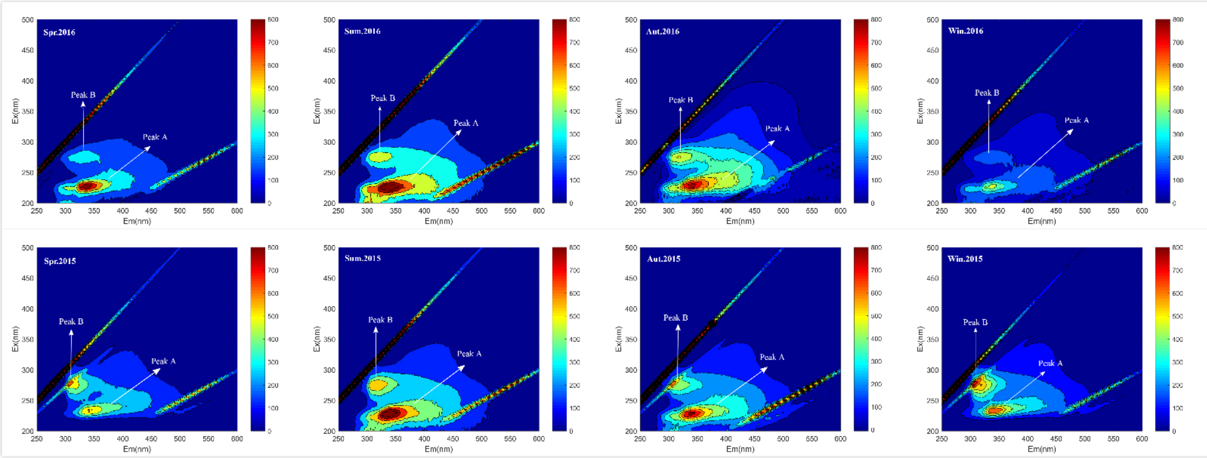

Fig. 3. Contour plots of EEM fluorescence spectra of different seasons (2015–2016).

components, and the input of surface plant residues may induce a large number of algae growth in water, which in turn can promote the increase of protein-like components. In addition, the life sewage of the surrounding residents and the pollution of the industrial and agricultural production is also a factor. In the autumn and winter, the intensity of the CDOM absorption spectrum in the water body will start to weaken. All the things in spring are in slow growth and long-term, and the biological activity is not violent, which is an important reason for the low fluorescence intensity of CDOM .

3.3. CDOM distribution characteristics and seasonal changes

The fluorescent material can contain different concentrations of different substances in the water, so its fluorescence spectrum will change [26]. Fluorescence spectra are used to visually reflect the different substances in the water of Sanyang wetland, and the fluorescence fingerprint characteristics index (B/A), fluorescence index (f450/500), humic index (HIX), biological source index (BIX) indicators to reflect the fluorescence characteristics of CDOM in Sanyang wetland water, and the organic pollution degree and CDOM sources.

1) The fluorescence fingerprint characteristic index is used to reflect the structure of the protein in the water body, which can indicate that the dissolved organic matter pollution of the water body is B/A. The lower the B/A value, the higher the proportion of refractory substances in the water body, the higher the degree of refractory water [14,15]. A and B are two fluorescence peaks, respectively, in the latter analysis. In general, the B/A value can change differently in two years. It can reach its highest value in winter, but there are more low values, and then it will go down. According to the climatic characteristics of Wenzhou, we can infer that there is a correlation between the B/A value and the temperature. Too high or too low temperature is not conducive to the degradation of organic matter in water body. In 2015, the B/A value of Sanyang Wetland has maintained a high value in spring. It can be concluded that the B/A value of Sanyang Wetland has a certain relationship with the season, which may be due to the strong activity of microorganisms in spring. Thus, these results show that the activity is frequent and the degradation ability of organic matter is stronger than that of the other three seasons.
2) McKnight [12] proposed f450/f500 fluorescence index (FI), namely in the excitation wavelength of Ex = 370 nm, the ratio of fluorescence intensity of emission wavelength of Em = 450 nm and 500 nm, to indicate the sources of water dissolved organic matter. And some research pointed out that when the FI value was greater than 1.9, dissolved organic matter derived mainly from aquatic organisms and microorganisms, when the less than 1.3, dissolved organic matter originated from land and soil [27]. However, it should be stressed from our researched results that the FI is close to 1.9 and part of the site is larger than 1.9 in part of time. Therefore, it can be successfully inferred that the dissolved organic matter of Sanyang Wetland mainly comes from aquatic organisms and microorganisms.
3) Zsolnay [28] pointed out that the excitation wavelength, emission wavelength of Em between 300 and 480 nm, and the Humidity Index, HIX254, can show the sum of the fluorescence intensities at em from 435 to 480 nm, 345 nm, which can be used to characterize the degree of humification of dissolved organic matter. He suggested that the humification index can also be used to estimate the maturity of soil dissolved organic matter. Humidification index can reflect the source of DOM and the degree of pollution. The higher the humification index, the higher the degree of humification, and the main source of terrestrial sources, while the smaller the humification index indicates a low degree of humification [9, 29], when Humic Index < 4, local microorganisms

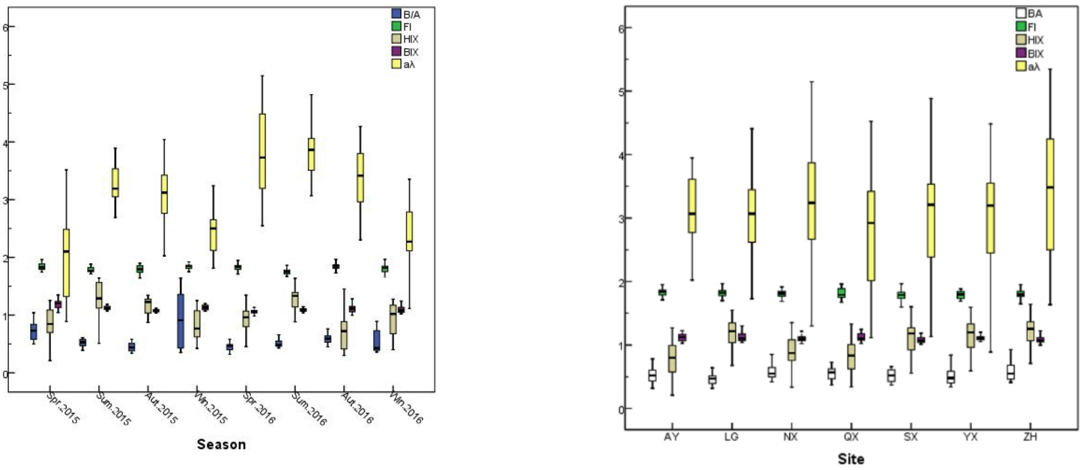

Fig. 4. Variations of fluorescence characteristic parameters (B/A, FI, HIX, BIX, aλ) in different seasons and sites.

and algae was the main source . However, the HIX value of Sanyang Wetland is significantly lower than 4, indicating that dissolved organic matter is mainly derived from local microbes, algae and so on, and the degree of humification is relatively low. The spatial differences are not obvious and the values of LG and SX are slightly higher. From spring to winter, the values of HIX index of each sampling point is similar, which can show a trend of increasing and decreasing gradually. All these results can further further illustrate that the summer has more obvious characteristics of exogenous humus input.

4) Biogenic index(BIX) [30] was the fluorescence intensity ratio at excitation wavelength of Ex = 310 nm and emission wavelength Em at 380 nm and 430 nm, respectively. The biogenic index can be used to evaluate the relative contribution of dissolved organic matter from microorganisms in the sample being measured. Biogenic index >0.6 means that the fresh dissolved organic matter produced by the microorganism is more, and <0.6 means that the dissolved organic matter contains less dissolved organic matter from the microorganism source. Obviously, the BIX value of Sanyang wetland is significantly higher than 0.6, and it could be preliminarily judged that the fresh DOM produced by microorganisms in Sanyang wetland is more than that in Sanyang wetland.
5) The calculation formula for the coefficient of light absorption of [19] :

$$a_{\lambda'} = \frac{2.303 D_{\lambda}}{r} \tag{1}$$

Type (1), aλ′ absorption coefficient for the wavelength lambda uncorrected, M^{-1}; Dλ is the absorbance; *r* optical path, M.

In order to remove the noise, caused by the remaining particles in liquid filtration or scattering caused by the refractive index of the sample and blank, hereby 750 nm zero correction:

$$a_{\lambda} = \frac{a_{\lambda'} - a750'\cdot\lambda}{750} \tag{2}$$

In Eq. (2), aλ is the absorption coefficient of λ; λ is wavelength, nm .

In this paper, the absorption coefficient a355 of corrected CDOM at 355 nm is used to characterize the CDOM content. The median range of absorption coefficient a355 of Sanyang Wetland is between 2.1~0.9. There is no significant difference among different sampling points. However, Zhihe Bridge is generally higher than the other 6 points in 12 months, so it is preliminarily inferred that the organic pollution degree of Zhihe Bridge is relatively high in 7 sampling sites. There is a great difference in different seasons, and the trend of rising first and then decreasing in the order of spring, summer, autumn and winter, which may be related to the activity of microorganism. Especially, the degree of organic pollution is the highest in summer.

It can be seen from the above three-dimensional fluorescence spectrum that the central position of the fluorescence peak is not consistent. According to fluorescence spectrum analysis, the organic pollution degree of seven sampling sites in Sanyang Wetland is different in time and space. There are differences between upstream sampling sites and downstream sampling sites, no matter whether it is B/A, FI, HIX or BIX. It is preliminarily concluded that the DOM of Sanyang Wetland is mainly derived from microorganisms and algae in the water, and the fresh DOM produced by microbes is more. This is due to the Sanyang Wetland as a city of Wenzhou. The largest wetlands, rivers intertwined vertically and horizontally in wetlands, dense as cobwebs, with a water area to land ratio of 1.1: 1. The Sanyang Wetland is rich in resources, and this spatial advantage has brought benefits to microbes and algae growth. And in recent years, The industrial development of Wenzhou makes Sanyang Wetland face the dilemma of industrial pollution, so some of the organic matter in the water will be brought in by the outside world due to the flow of rivers and so on. The seasonal trend of FI and BIX between 2015 and 2016 is approximately the same, and the degree of organic pollution and the degradation ability of CDOM can decrease in low temperature months, which is due to the unfavorable living conditions of the microbes that can degrade CDOM and decrease microbial activity. In addition, it is related to the amount of rainfall. In the rainy season, more land-based substances are washed into the water, which can lead to the increase of protein-like substances.

3.4. Relationship between fluorescence indices and water quality parameters

The correlation analysis between the conventional water quality parameters and the fluorescence characteristic parameters can show that the correlation between the fluorescence components in Sanyang wetland can basically reach a significant level. Among them, B/A is extremely significantly correlated with FI, HIX and aλ, which may show the common features of biological origin of CDOM in Sanyang wetland. HIX is negatively correlated with BIX and positive with aλ; FI is negatively correlated with HIX and positively correlated with BIX; BIX is positively correlated with aλ. However, the correlation between DOC concentration and other parameters is significant, which may be due to the existence of a large number of non-chromophores dissolved organic matter in water, which can not fully reflect the characteristics of DOM, this is consistent with the research by Liu Yang et al. [31]. DOC values can show the amount of organic matter produced by local microorganisms, mainly plant residues and microbial metabolites. It can indicate that BIX is a valuable proxy for distinguishing DOC sources, and this can be comparable to other environmental samples or sample treatments [32]. TN shows extremely significant correlation with most of the parameters except for pH and DOC, indicating that organic pollution caused by surface input also has a great impact due to human intervention. The increase of nutrients in water can cause the proliferation of phytoplankton and microbes to increase, so that the water is eutrophic state with increasing pollution. Moreover, there is a correlation between Chl a and pH, BIX, DO, pH, TN, Chl a is associated with phytoplankton and microbes in water, which can be confirmed by the protein-like peaks appearing in the fluorescence spectra. At the same time, the above analysis of the fluorescence index shows that Sanyang wetland DOM comes from micro-organisms and algae (such as green algae, cyano bacteria, diatom), and the DOM produced by microorganisms are more. Moreover, phytoplankton decomposition through photo degradation and microbial activity can also contribute to the elevated CDOM at the surface [19,20]. Therefore, the concentration of Chl a can reveal the fluorescence intensity at a certain level, but the single use can not effectively analyze the pollution of organic matter.

4. Conclusion

Our findings suggest that: i) Two kinds of fluorescent proteins of three types of Sanyang wetlands are obtained by three-dimensional fluorescence spectroscopy: Tryptophan A (225/340) and Tyrosine B (275/330); ii) The correlation analysis between conventional water quality parameters and five fluorescence parameters can show that CDOM is a common feature of biological sources in origin; iii) The fluorescence intensities of the two fluorescent components can illustrate the characteristics of summer high in the seasons, mainly depends on the influence of terrestrial input. But, the spatial distribution of the difference is not obvious, ZH organic pollution is slightly serious, which can initially concluded that the flow of rivers can cause ZH organic pollution.

Consequently, as an ecosystem of wetland, the organic pollution of Sanyang wetland can affect the biodiversity of the wetland and lead to the loss of habitats for some of them. It is imperative to treat organic pollutants in Sanyang wetland. For places and time periods with high organic pollution, efforts should be made to intensify their efforts. For those periods with low levels of organic pollution, prevention can be the main part, and artificial control can be given in combination with the purification capacity of wetland ecosystem.

Table 1
Correlations CDOM fluorescent index and water quality parameters; **indicates significant correlation at level 0.01 (bilateral); *indicates significant correlation at 0.05 level (bilateral); Pearson, n = 169

	B/A	FI	HIX	BIX	aλ	T	TDS	DO	pH	DOC	TN	TP	Chl a
B/A	1												
FI	0.216**	1											
HIX	–0.531**	–0.240**	1										
BIX	0.184*	0.280**	–0.256**	1									
aλ	–0.330**	–0.084	0.246**	–0.254**	1								
T	–0.344**	–0.267**	0.261**	–0.065	0.414**	1							
TDS	0.534**	0.112	–0.192*	0.148	–0.299**	–0.254**	1						
DO	0.222**	–0.008	–0.089	0.121	–0.116	–0.15	0.148	1					
pH	0.033	0.084	0.02	0.091	0.145	–0.068	–0.118	0.356**	1				
DOC	–0.044	–0.018	0.077	–0.002	0.064	0.113	–0.026	0.025	0.049	1			
TN	0.477**	0.294**	–0.351**	0.290**	–0.314**	–0.540**	0.426**	0.269**	0.121	–0.073	1		
TP	0.051	0.196*	–0.163*	0.160*	–0.043	–0.037	0.04	–0.353**	–0.137	–0.046	0.465**	1	
Chl a	0.005	–0.113	0.102	0.197*	–0.145	0.087	0.055	0.459**	0.210**	0.038	0.218**	0.09	1

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