Characterization of CDOM in saline and freshwater lakes across China using spectroscopic analysis

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Abstract: Colored dissolved organic matter (CDOM) is a major component of DOM in waters, and plays a vital role in carbon cycling in inland waters. In this study, the light absorption and three-dimensional excitation-emission matrix spectra (EEMs) of CDOM of 936 water samples collected in 2014–2017 from 234 lakes in five regions across China.
China were examined to determine relationships between lake water sources (fresh versus saline) and their fluorescence/absorption characteristics. Results indicated significant differences regarding DOC concentration and $a_{CDOM}(254)$ between freshwater ($6.68 \text{ mg C L}^{-1}, 19.55 \text{ m}^{-1}$) and saline lakes ($27.4 \text{ mg C L}^{-1}, 41.17 \text{ m}^{-1}$). While humic-like ($F_5$) and fulvic-like ($F_3$) compounds contributed to CDOM fluorescence in all lake waters significantly, their contribution to total fluorescence intensity ($F_T$) differed between saline and freshwater lakes. Significant negative relationships were also observed between lake altitude with either $F_5$ ($R^2=0.63, N=306$) or $F_T$ ($R^2=0.64, N=306$), suggesting that the abundance of humic-like materials in CDOM tends to decrease with increased in lakes altitude. In high-altitude lakes, strong solar irradiance and UV exposure may have induced photo-oxidation reactions resulting in decreased abundance of humic-like substances and the formation of low molecular weight compounds. These findings have important implications regarding our understanding of C dynamics in lacustrine systems and the contribution of these ecosystems to the global C cycle.

**Keywords:** Absorption, CDOM, EEMs, freshwater, saline water
1. Introduction

Dissolved organic matter (DOM) is considered the largest pool of organic matter in natural waters, accounting for >90% of the total organic matter (Kececioglu et al., 1997; Cole et al., 2007; Para et al., 2010). Dissolved organic carbon (DOC) regulates metabolic and biogeochemical processes in water bodies, and ultimately determines the contribution of aquatic ecosystems to the global carbon cycle (Borge et al., 2015; Catalan et al., 2016). Part of the DOM is termed chromophoric dissolved organic matter (CDOM) based on the absorption of ultraviolet (UV) and photo-synthetically available radiation (PAR), whereas other DOM fractions are referred to as fluorescent DOM (FDOM) based on the emission of fluorescence photons after radiation absorption; both fractions are responsible for the optical properties of DOM (Effler et al., 2010). CDOM is a complex mixture of organic compounds of both allochthonous and autochthonous origin (Coble, 2007; Zhang et al., 2009). As an optically-active substance, CDOM absorption properties are significantly affected by several factors, including DOM concentration and its chemical composition (Minero et al., 2007), photo-induced and microbial processes in aquatic environments (Shank et al., 2010), and salinity (Singh et al., 2010).

Inland waters account for a significant portion of the global freshwater storage (Oki
& Kanae, 2006), and in China saline lakes comprise a large share of all inland waters (Ma et al., 2011). Although several studies and reviews have been carried out to examine the optical and chemical properties of CDOM, and evaluate relationships between salinity and CDOM fluorescence (Coble, 2007; Zhang et al., 2010; Moore et al., 2011; Song et al., 2013), little is known about the composition, sources, and the factors regulating the dynamics of CDOM in saline inland waters, especially across large national-scale regions. Previous studies have shown a linear inverse relationship between CDOM absorption and salinity (Singh et al., 2010), and elevated dissolved carbon concentration in inland waters from semi-arid and arid climates (Brooks and Lemon, 2007; Song et al., 2017). Increased water retention time in saline lakes could alter the absorption and fluorescence properties of CDOM through microbial degradation and strong photo-induced radiation reactions (Catalan et al., 2016). These phenomena are likely of lesser significance in freshwaters. Thus, studies are needed for characterizing CDOM absorption and fluorescence characteristics in inland saline waters (Duarte et al., 2008; Tranvik et al., 2009), and this is crucial to our ability to quantify the role of inland saline waters to the global carbon cycle (Cole et al., 2007; Tranvik et al., 2009). Moreover, the dynamics and characteristics of CDOM in saline waters may differ depending on their relative altitude (above sea level) and the amount
of solar radiation received. In high elevation saline lakes, exposure to UV-B radiation is expected to be significant and that could in turn increase the photo-chemical degradation of CDOM (Jansson et al., 2008). In addition, the terrestrial input of CDOM to aquatic systems is expected to be insignificant given the generally low terrestrial productivity and limited human activity in high altitude regions.

CDOM is a complex mixture of organic constituents, the composition of which is difficult to elucidate. Spectrophotometry and three-dimensional excitation-emission matrix spectra (EEMs) have provided useful information about CDOM composition, sources, and molecular size (Liu et al., 2007; Wang et al., 2007; Wen et al., 2018). Specifically, the spectral slope (S) provides important information on CDOM origin, chemical composition and sources (Fichot and Ronald, 2012), and the S value is a good proxy for indicating the proportion of terrestrial DOC in waters (Gonnelli et al., 2013).

The SUVA$_{254}$ has been adopted to estimate the aromatic carbon content and to understand the chemical characteristics of DOM (Weishaar et al., 2003; Świetlik and Sikorska, 2004). In recent years, the EEMs fluorescence spectroscopy has been widely used to differentiate FDOM from fresh, riverine, coastal marine sources (Stedmon et al., 2003; Henderson et al., 2009; Zhao et al., 2016). Three popular methods have been used for extraction of useful information regarding FDOM, including the traditional
“peak-picking” method (Coble, 1996), EEM coupled with parallel factor analysis (PARAFAC) (Stedmon et al., 2003), and EEM coupled with fluorescence regional integration (FRI) (Chen et al., 2003; Yan et al., 2018). Compared to the PARAFAC techniques, FRI is a quantitative approach that integrates all the wavelength-dependent fluorescence intensity data and has been proven as an effective method to represent the FDOM components. With the FRI method, five FDOM components (including tyrosine-like component, tryptophan-like component, fulvic-like component, microbial protein-like component, humic-like component) (Chen et al., 2003) have been identified, and these components vary with the hydrologic conditions (such as water retention time), geographical settings, climatic zones and regional landscape characteristics. Therefore, FRI can be applied to large continental-scale examinations of CDOM sources in different types of inland lakes (freshwater and saline) in an effort to identify drivers of carbon cycling in these aquatic ecosystems.

In the present study, the FRI technique was used to identify the FDOM components in 936 water samples from 234 lakes across five lake regions in China. The lakes differed in salinity and elevation (relative to sea level, msl). We also determined the absorption, source characteristics, and fluorescence of CDOM. The main objectives of this study were to: (1) determine CDOM absorption coefficients in saline and freshwater
inland lakes; (2) characterize CDOM components with FRI and EEM techniques, and examine their potential for source tracking; (3) examine correlations between FRI-EEM parameters and CDOM absorption coefficients; and (4) examine the relationship between FDOM and elevation of saline lakes.

2 Materials and Methods

2.1 Five lake regions in China

Based on broad regional variations of landforms and climate characteristics, the lakes in China have been grouped into five regions (Ma et al., 2011), namely: Northeastern lake region (NLR), Inner Mongolia-Xinjiang lake region (MXR), Tibetan-Qinghai Plateau lake region (TQR), Yungui Plateau lake region (YGR) and Eastern lake region (ELR). The NLR is located in the humid and semi-humid monsoon climate zone, and the lakes (3.3% of all lakes in China) are mainly distributed in plain areas (Fig. 1). The formation of lakes is related to volcanic calderas, or to the formation of swamps on low-permeability geological formations. Due to the specific local geographical and climatic conditions, many saline soda lakes are formed in NLR (Song et al., 2013). The MXR lakes region is characterized by arid and semi-arid climate with low annual precipitation (175 mm) and high evaporation level. Many lakes in the MXR region are
brackish to salty. The TQR is located in an area with an average elevation over 4,000 m
(above msl). The high altitude and strong solar radiation contribute to the unique
ecological characteristics of that region. Largely associated with endorheic drainage
basins, most of the TQR lakes are saline. The TQR region includes some of the highest
alpine lakes on the Earth, and represents an area with the highest density of lakes in the
world. The YGR is located in a subtropical monsoon climate zone. The lakes in this
region are formed along fault zone or steep valleys, and are mostly tectonic lakes. The
ELR region, situated in the middle/lower reaches of the Yangtze River, Yellow River,
Hai River and Huai River, is the most developed region in China. About 30% of the
total lake areas in China are centered in the ELR, including the largest five freshwater
lakes in the country (Fig. 1).

2.2 Field sampling and measurements

Both freshwater lakes and saline water lakes (Electrical conductivity (EC) threshold:
1,000 $\mu$S cm$^{-1}$) were selected in the five lake regions across China in order to
characterize the features of FDOM among freshwater or saline water lakes. Lakes were
selected with respect to both salinity and watershed characteristics. In total, 936 water
samples (630 freshwater, 306 saline) were collected from 234 lakes between 2014 and
For each sampling point, geographical coordinates (latitude, longitude) and elevation were recorded in situ with a GPS receiver. Some water chemical parameters including EC, turbidity, temperature were measured in situ with an YSI EXO1 portable multi-parameter water quality probes. At the time of sampling, water clarity was also determined with a Secchi disk depth (SDD). All lake water samples were collected in 1-L acid-cleaned plastic bottles, held on ice packs, and transported to the laboratory as soon as possible. In the laboratory, samples were stored at 4°C in a refrigerator, and analyzed within 2 days.

### 2.3 Water quality measurements

In the laboratory, water samples were analyzed for DOC concentration on a Shimadzu TOC-5000 Analyzer (680 °C). Total nitrogen (TN) and total phosphorus (TP) were analyzed according to APHA/AWWA/WEF (1998). Chlorophyll-a concentration was obtained through extraction of a filtered sample (0.45 µm Whatman GF/F) with 90% acetone solution, and determination of the chlorophyll-a concentration (Chl-a) with a Shimazu UV-2600PC spectrophotometer. Total suspended matter (TSM) was obtained gravimetrically as described in Song et al. (2013).
2.4 CDOM absorption analysis

The water samples in the laboratory were filtered through a 0.7 µm pre-combusted Whatman GF/F filter, and then through a 0.22 µm Millipore membrane cellulose filter. CDOM absorption spectra were determined using a Shimadzu UV-2600PC UV–Vis spectrophotometer, fitted with a 1-cm quartz cuvette, in the spectral region between 200 and 800 nm at 1 nm intervals. CDOM spectra for Milli-Q water was used as reference. The CDOM absorption coefficient \(a_{(\lambda)}\) was computed according to Eq.1:

\[
a_{(\lambda)} = \frac{2.303A_{(\lambda)}}{L} \tag{1}
\]

where \(L\) is the cuvette length (m), and \(A_{(\lambda)}\) the measured optical density.

The spectral absorbance can be modeled with Eq. 2:

\[
a_{\text{CDOM}}(\lambda) = a_{\text{CDOM}}(\lambda_0) \exp(-S(\lambda_0 - \lambda)) \tag{2}
\]

where \(\lambda_0\) is a reference wavelength of 700 nm and \(S\) is the spectral slope.

In this study, the absorption coefficient of CDOM at 254 nm \(a_{254}\) was used as a proxy for CDOM concentration. The spectral slope \((S)\) between 275-295 nm \(S_{275-295}\) was calculated using Eq. 2, and used as a proxy for DOM molecular weight which is linked to CDOM sources (Helms et al., 2008; Zhang et al., 2011). The specific UV absorbance at 254 nm \(SUVA_{254}\) is defined as the absorbance at 254 nm \((m^{-1})\) divided by the DOC concentration \((mg C L^{-1})\) (Weishaar et al., 2003). Unlike \(S_{275-295}\), \(SUVA_{254}\)
increases with decreasing CDOM molecular size (Song et al., 2017).

2.5 CDOM fluorescence measurement and FRI analysis

CDOM fluorescence excitation-emission matrices (EEMs) were measured using a Hitachi F-7000 fluorescence spectrometer equipped with a 700-V xenon lamp. The excitation (Ex) and emission (Em) scanning ranges were 200-450 nm (5 nm intervals) and 250-500 nm (1 nm intervals), respectively. The spectra were recorded at a scan rate of 2400 nm min\(^{-1}\) using excitation and emission slit bandwidths of 5 nm. EEMs of Milli-Q water blanks were subtracted to eliminate the water Raman scatter. The elimination of the inner-filter effect was performed by adjusting for CDOM absorbance at the corresponding wavelengths according to Eq. 3 (McKnight et al., 2001; Kothawala et al., 2013). Interpolation was used to remove the effect of Rayleigh scattering (Stedmon and Bro, 2008).

\[
F_{\text{corr}} = F_{\text{obs}} \times 10^{(A_{\text{Ex}} + A_{\text{Em}})/2}
\]  

(3)

where \(F_{\text{obs}}\) and \(F_{\text{corr}}\) represent fluorescence intensity of EEMs before and after calibration, respectively. The \(A_{\text{Ex}}\) and \(A_{\text{Em}}\) represent corrected absorbance at the corresponding excitation and emission wavelengths, respectively. The fluorescence was normalized to the integral of Raman signal to eliminate the effect daily variation in lamp intensity (Lawaetz and Stedmon, 2009).
Fluorescence regional integration (FRI) is a new quantitative approach to analyze the total wavelength-independent fluorescence intensity data based on EEM spectra (Chen et al., 2003; Sun et al., 2016). EEM maps were divided into five regions, and a description of each region is provided in Table 1. The integral volume ($F_i$) can be expressed as follows:

$$F_i = \sum_{E_x} \sum_{E_m} l(\lambda_{EX}, \lambda_{EM}) \Delta\lambda_{EX} \Delta\lambda_{EM}$$

(4)

where $l(\lambda_{EX}, \lambda_{EM})$ is the fluorescence intensity at each excitation–emission wavelength pair. $\Delta\lambda_{EX}$ is the internal excitation wavelength (taken as 5 nm), $\Delta\lambda_{EM}$ is the internal emission wavelength (taken as 5 nm). The sum of the fluorescence intensities of FRI-divided FDOM components were presented by $F_T$ (unit: nm). The percent fluorescence response in a specific region ($P_i = 1, 2, 3, 4, 5$) was calculated as following:

$$P_i = \frac{F_i}{F_T} \times 100\%$$

(5)

The humification index (HIX) represents the ratio of allochthonous fluorescence intensity $F_{3&5}$ to that of the autochthonous fluorescence intensity $F_{1&2&4}$ (Bilal et al., 2010). Humic-like fluorescence indicated as $F_n(355)$ was excited at 355 nm and its emission was measured at 445-455nm (Vignudelli et al., 2004).
2.6 Statistical analyses

Statistical analyses, including mean values, standard deviations, linear or non-linear regressions, and t-tests were performed using SPSS 16.0 software package (Statistical Program for Social Sciences, Chicago, IL). Difference is considered statistically significant when p < 0.05. Spatial mapping of sampling sites and land cover types were conducted using ArcGIS 10.1 (Environmental Systems Research Institute, Redlands, CA). Principal components analysis (PCA) was conducted using Origin 9.0 (Microcal Software, Inc., Northampton, MA) by using the five FRI fluorescent components according to different trophic status and lake regions.

3. Results

3.1 Biogeochemical characteristics

Analysis results of the 936 lake water samples collected in the present study indicated that the lakes studied in the five lake regions of China are diverse, not only in terms of their geomorphological and climatic settings, but also in regards to water chemistry as exemplified by the difference in DOC, TN, TP, Chl-a, TSM and EC values for lakes in the different regions (Table 2). The differences in water quality parameters among lakes from the five regions were all statistically significant (p<0.001).

The average DOC and TN, TP concentration in the saline waters was noticeably
higher than that of the freshwater lakes (Table 2). The difference in DOC concentration (and other water chemical parameters) between saline water and freshwater lakes was statistically significant (p<0.001). Among the five lake regions of China, DOC concentration ranged from 5.25 to 35 mg C L\(^{-1}\) (Fig.2a), with a mean DOC concentration of 27.4 and 6.68 mg C L\(^{-1}\) for saline and freshwater lakes, respectively. The DOC concentration in NLR, MXR and TQR region lakes were higher than that of lakes from the ELR and YGR regions. The mean TN concentration in the five lake regions was, from highest to lowest, in the order: NLR (5.54 mg N L\(^{-1}\)), TQR (3.82 mg N L\(^{-1}\)), MXR (3.23 mg N L\(^{-1}\)), ELR (0.98 mg N L\(^{-1}\)) and YGR (0.90 mg N L\(^{-1}\)). Similarly, the TP concentration exhibited significant regional variability, ranging from 0.02 to 1.28 mg P L\(^{-1}\). The highest TN and TP were measured in the NLR lakes, and the lowest TN was observed in lakes from the YGR region, while the lowest TP concentration was observed in the ELR region lakes. The EC values and TSM concentration in the saline lakes was higher than in the freshwater lakes. The mean EC of lake waters ranged from 345.3 to 12,722.9 µS cm\(^{-1}\). The range for Chl-a concentration was 1.94 to 55.21 µg L\(^{-1}\) for different regions. The highest EC (12,722.98 µS cm\(^{-1}\)) was observed in the TQR while the highest Chl-a concentration was found in the ELR (55.21 µg L\(^{-1}\)). All of the water chemical parameters in saline waters were
significantly different for each lake region (p<0.01).

[Insert Table 2 about here]

[Insert Fig. 2 about here]

3.2 CDOM absorption characteristics

Among the 936 lake water samples, $a_{CDOM}(254)$ ranged from 1.39 m$^{-1}$ to 530.03 m$^{-1}$, with a mean of 41.17 m$^{-1}$ for saline lakes and 19.55 m$^{-1}$ for freshwater lakes. The $a_{CDOM}(254)$ of saline lakes were significantly different from that of fresh lakes (p<0.01). When all five lake regions were considered together, the $a_{CDOM}(254)$ in saline waters were much higher than that of the freshwaters in the NLR, MXR and TQR (Fig.2b). Another interesting result of this study (Fig.2b) is that, for the saline lakes in the NLR, MXR and TQR region, the CDOM absorption at 254 nm decreased significantly with increased lakes elevation.

The $S_{275-295}$ in this study ranged from 10 µm$^{-1}$ to 61.15 µm$^{-1}$, and the mean values were 0.031nm$^{-1}$ for saline lakes and 0.024 nm$^{-1}$ for freshwater lakes (Fig.3a). The slope for both saline lakes and freshwater lakes in the five regions were significantly different (p<0.01). The CDOM slopes for saline lakes in each individual lake region were higher than that of the freshwater lakes in that region. The $S_{275-295}$ increased while the SUVA$_{254}$ decreased inversely. The SUVA$_{254}$ in this study ranged from 0.04 to 5.62 L mg$^{-1}$ m$^{-1}$ for
saline waters, and from 0.35 to 6.99 L mg\(^{-1}\)m\(^{-1}\) for freshwaters (Fig.3b). The SUVA\(_{254}\) for both saline and freshwater lakes in the five regions were significantly different (p<0.01). The SUVA\(_{254}\) for saline lakes in a given region was significantly lower than that of freshwater lakes in the corresponding region. For both freshwater and saline lakes, we observed a significant (p<0.01) increase in the S\(_{275-295}\) values with increased lakes elevation in the NLR, MXR and TQR regions. In contrast, a corresponding decrease in SUVA\(_{254}\) values with increased lakes elevation was noted. We had also organized our dataset with seasons (spring, summer and autumn), it was found that S\(_{275-295}\) of saline waters in all three seasons was higher than that of fresh waters, the inverse trends of SUVA\(_{254}\) was also observed in the corresponding seasons (Fig. S3a-b). For all water types and seasons, the a\(_{CDOM}(254)\), SUVA\(_{254}\) were all negatively correlated to elevation (p<0.01) (Table S1). Details of these intriguing phenomena will be discussed in later sections.

3.3 FRI-Based CDOM Fluorescent Components

In this study, the FRI-based EEMs in saline and freshwater lakes of China were analyzed to document trends in CDOM fluorescence characteristics in relation to lake types. The excitation-emission area volumes F\(_i\) and P\(_i\) (i = 1, 2, 3, 4, and 5) were
proportional to the total fluorescence intensity and the relative contribution of the five different components to the total fluorescence intensity. Examples of EEM fluorescence spectra from Lake Seli Co (TQR), Lake Aibi (MXR) and Lake Khanka (NLR) were selected as representative of lakes in these regions (Fig. 4). The total fluorescence intensity \( F_T \) ranged from \( 3.78 \times 10^{10} \) nm to \( 4.55 \times 10^{12} \) nm for all water samples, and the mean \( F_T \) for saline waters and freshwaters was \( 4.91 \times 10^{11} \) nm and \( 3.39 \times 10^{11} \) nm respectively (Fig. 5). The \( F_T \) in saline waters was higher than that of the freshwaters in lakes from the NLR, MXR and TQR. The highest \( F_T \) (\( 1.05 \times 10^{12} \) nm) was observed in the saline waters from the NLR, while the lowest \( F_T \) (\( 1.99 \times 10^{11} \) nm) was obtained in the freshwaters from the TQR. For different seasons, the corresponding FRI-EEM component in the fresh waters was lower than that of saline waters for various seasons (Fig. S3c-d).

The relative contribution of individual components \( F_i \) (with \( i = 1, 2, 3, 4, 5 \)) to total fluorescence intensity differed among lake types and regions (Fig. 6). It was found that, for both saline and freshwater lakes in different regions, the \( F_5 \) (humic-like) and \( F_3 \) (fulvic-like) compounds were predominant in FDOM. Furthermore, by comparing the fluorescence intensity of different components \( P_i \) (with \( i = 1, 2, 3, 4, 5 \)) across lake regions (Fig. 7), it was found that the percent of \( F_5 \) to total fluorescence intensity in
saline lakes was higher than that of freshwater lakes in the NLR, MXR and TQR regions. The inverse was observed in regard to the F₃ fluorescent component. These results indicated that the fluorescence intensity of the five components and their relative contribution to the total fluorescence intensity differed between saline and freshwater lakes across the five lake regions of China. The Fn(355) ranged from 3.28 to 1,018 nm, with a mean Fn(355) of 108.50 and 88.75 nm for freshwater and saline lakes, respectively. The relative humic-like fluorescence content of saline waters was higher than that of the freshwaters. However, the mean humification index (HIX) for freshwater lakes (8.45) was nearly 2-fold higher than for saline waters (4.39).

[Insert Fig. 4-7 about here]

3.4 PCA of FRI Fluorescent Components

PCA was conducted, using the relative scores of the five FRI fluorescent components (F₁, F₂, F₃, F₄ and F₅) for all 936 lake water samples, to determine the degree of separation between the saline lakes and freshwater lakes investigated. For the saline lakes, the first two PCA axes (i.e., components 1 and 2) explained 91.2% of the total variance in the dataset, with component 1 and component 2 accounting for 68.1% and 23.1%, respectively (Fig.8a). For the freshwater lakes, the first two PCA axes (i.e., components 1 and 2) explained 96.4% of the total variance in the dataset, with
component 1 and component 2 accounting for 82.5% and 14.9%, respectively (Fig. 8c).

Each PCA axis is a linear combination of the five FRI fluorescent components (Table 3).

The five FRI fluorescent components showed positive component 1 loadings (Fig. 8a and 8c). The fulvic-like (F3) and humic-like (F5) compounds showed positive loadings for component 2. The FRI-PCA in this study could differentiate, on the basis of fluorescent characteristics, between the allochthonous substances [fulvic-like (F3) and humic-like (F5) compounds] and the autochthonous substances [tyrosine-like (F1), tryptophan-like (F2), and microbial protein-like (F4) compounds]. Further, a plot of the PCA component 1 and component 2 scores for all 306 saline water samples (Fig. 8b) showed a general clustering of most saline water samples, with component 1 scores in the range of -3 to 8 and component 2 scores ranging from -3 to 4. The water samples from the NLR region lakes were generally scattered, with high component 1 and component 2 scores. However, the saline water samples from TQR lakes were generally clustered along the negative axis of component 1 and component 2. With increased in altitude from NLR and MXR to the TQR region lakes, the PCA score distribution for the saline samples decreased along the vertical axis of component 2. In contrast, for the...
freshwater lake samples (scores ranging from -2 to 4 for PCA component 1 and from -1 to 3 for PCA component 2), the scores distribution (Fig. 8d) was random and did not follow patterns that could be associated with specific lake regions (although a weak cluster was noted for samples from YGR and TQR along the negative axis of component 2).

4. Discussion

4.1 DOC and CDOM absorption of saline and freshwaters

Variability in DOC concentration among the inland lakes investigated reflects the diversity of the geological materials, land use, climatic conditions and human activity within the lakes drainage basins (Tranvik et al., 2009; Webster et al. 2008). The range of DOC concentration (5.25-35.07 mg C L\(^{-1}\); Fig. 2a) measured in the present study overlaps nicely the results of an earlier study by Song et al. (2013) in which DOC concentration in the range of 3.61-32.60 and 1.01-14.23 mg C L\(^{-1}\) was measured in saline and freshwater lakes, respectively. Similar results have also been reported by Curtis and Adams (1995) for lakes in the semi-arid region of Alberta, Canada. The high DOC content in saline water indicated that DOC tends to accumulate with long water retention time (Catalan et al., 2016), and may also reflect the input of carbonaceous
A key finding of the present study is the observation that, in both freshwater and saline lakes, DOC concentration tends to decrease gradually with increased lakes altitude in the NLR and MXR to the TQR region. Conversely, in YGR and ELR regions, DOC concentration tends to rise consistently with decreased lake elevation. A similar trend was previously reported in the YGR region by Zhang et al. (2009). Indeed, different land use types (Fig. S1) and climatic conditions may have contributed to the variability in DOC input and DOC optical characteristics. For example, the much higher DOC concentration and predominance of humic substances in lake waters from the NLR region could be ascribed to the forestland and unique soils in that region (Zhao et al., 2016). But above all, our research findings revealed that landscape elevation plays an important role in driving the DOC variability.

The $a_{CDOM}(254)$ for saline waters was significantly different ($p<0.01$) from that of freshwaters across the five regions (Fig. 2b). Compared with other previous studies, our $a_{CDOM}(254)$ values were comparable to the results of Zhang et al. (2018) who reported $a_{CDOM}(254)$ between 1.68 and 92.65 $m^{-1}$ for 22 lakes along a trophic gradient in China. The diversity of CDOM absorption at 254 nm may be due to the following factors.
Firstly, the CDOM absorption is dependent on the total DOM concentration in the lake waters. The regional hydrogeological conditions and the variety of climatic situations additionally affect the variability of DOM in lake waters. Because of the prevailing dry climate in the areas surrounding most of the saline lakes, high rates of evaporation likely occur and that may have contributed to the high DOM concentrations measured in these lakes. Secondly, the saline waters are generally terminal lakes in semi-arid or arid regions, and consequently DOM from terrestrial sources accumulates in these lakes (and is not exported downstream).

4.2 CDOM sources for saline and freshwaters

The slopes for both saline lakes and freshwater lakes in the five regions were significantly different (p<0.01). The CDOM slopes for saline lakes in the five regions were all lower than those of the freshwater lakes in the corresponding regions (Fig. 3a). This observation is similar to those of Wen et al. (2016) who noted that the slope of CDOM absorption for Mongolian plateau saline lakes dominated by autochthonous sources of CDOM was higher than that of freshwater rivers in the region. In contrast, in many fresh water lakes, significant input of CDOM from surrounding landscapes can occur, especially during extreme hydrological events. This interpretation is in line with the work of Zhou et al. (2016) who reported a parallel increase in the concentration and
relative molecular size of terrestrial humic-like CDOM molecules in Qiaodao Lake waters during periods of high inflow.

In the saline lakes across different regions, we observed a significant increase in $S_{275-295}$ with increase in the altitude of the lakes, from NLR and MXR to TQR region, while the SUVA$_{254}$ values significantly decreased (Fig. 3b). Our SUVA$_{254}$ results (Fig.3b) were similar to those reported for terminal lakes of the Inner Mongolia Plateau (saline waters, SUVA$_{254}$=1.90 mg C$^{-1}$ m$^{-1}$; freshwaters, SUVA$_{254}$=2.74 mg C$^{-1}$ m$^{-1}$), and for saline lakes of Northeast China (2.8-5.7 mg C$^{-1}$ m$^{-1}$) (Wen et al., 2016; Zhao et al., 2017). The higher S and lower SUVA$_{254}$ may be due to the effect of salinity on photosynthesis in terrestrial higher plants, and to microbial degradation and the stronger photo-degradation processes resulting from prolonged water residence time and greater UV radiation exposure. Wu et al., 2005; Brooks et al., 2007; Madsen-Østerbye et al., 2018). Moreover, our results suggest that the proportion of terrigenous DOC in saline waters was lower than that of the freshwater lakes. The diversity of hydrogeological conditions, longer residence time of DOM and the photo-degradation of DOM in saline waters could significantly contribute to the variability of CDOM absorption and composition (Spencer et al., 2012; Song et al, 2013; Wen et al., 2018).

This result was consistent with Kellerman et al. (2015) who concluded that degradation
processes preferentially remove oxidized, aromatic compounds in aquatic systems.

[Insert Fig.9 about here]

The differences in the correlation between DOC and CDOM for saline waters and freshwaters suggest that salinity may have modulated the relationships between these variables in the lakes investigated. Our results were similar to those from previous studies that have reported good correlations between CDOM absorption and DOC in coastal areas and inland waters (Chen et al., 2002; Rochelle-Newall and Fisher, 2002; Song et al., 2018). But, the variability in the strength of these correlations for different regions was ascribed to differences in regional geological characteristics and salinity.

The presence of CDOM components such as allochthonous fulvic and humic acids can also be responsible for the good correlation that is usually observed between CDOM absorbance and DOM content in a variety of waters (Vione et al., 2010). Therefore, the results indicated that human disturbances, non-point agricultural pollutant and climatic conditions may have contributed to the accumulation of terrestrial humic acids in the freshwater lakes investigated. The general slope gradient was in the order (from high to low): TQR, MXR and NLR. The high slopes DOC/CDOM (Fig.9) indicate that the higher slopes in saline lakes were associated with the high autochthonous production CDOM and photo-induced degradation of CDOM. The photo-chemical degradation of
CDOM changed its optical and chemical properties; it involves the decomposition of CDOM chromophores and results in reduced CDOM absorptivity of UV and visible radiations (Fichot and Miller, 2010). Finally, due to long water retention time of saline lakes, more aromatic compounds for allochthonous matters are preferred to degradation to form low molecular CDOM components that may have also influenced CDOM composition and ultimately the correlation between CDOM and DOC.

4.3 Correlation between CDOM and Fluorescent Components

The PCA results for saline waters indicated that the fulvic-like ($F_3$) and humic-like ($F_5$) components were predominant in FDOM for saline waters in the NLR region. With increased lake altitude, the contribution of autochthonous substances increases in saline waters. The PCA score of the freshwaters indicated that the fluorescent CDOM varied with different hydrogeological conditions within individual lake regions, and that the allochthonous substances ($F_3$ and $F_5$) were predominant in the terminal lakes of NLR and MXR regions while the more autochthonous substances ($F_1$, $F_2$ and $F_4$) were produced in lakes of the YGR and TQR regions. Conversely, the high percentage of $F_1$, $F_2$ and $F_4$ in the ELR lakes indicated that the low-altitude freshwater lakes in that region are highly polluted, exhibiting high content of the tyrosine-like, tryptophan-like, and
microbial protein-like materials. This interpretation is consistent with previous studies (Baker, 2001; Lu et al., 2014; Zhao et al., 2017). Baker (2001) related the high fluorescence intensity of the tryptophan-like component of FDOM to wastewater discharge. Likewise, Zhao et al. (2017) detected the highest concentration of the tryptophan-like and the microbial protein-like components in highly polluted river basins. Lu et al. (2014) concluded that anthropogenic activities (cropland, pasture and urban) increased microbial activity and enhanced the relative abundance of protein-like fluorescence of DOM.

Correlations among the five fluorescent components and with other optical parameters \(a_{\text{CDOM}(254)}, \text{HIX and Fn(355)}) showed, for both saline and freshwater lakes, strong positive linear relationships between \(F_1\) and \(F_4\) (Fig.10a) (saline waters: \(R^2=0.71, p<0.01\); freshwaters: \(R^2=0.93, p<0.01\)), suggesting that parts of the tyrosine-like component \(F_1\) and microbial protein-like component \(F_4\) are likely from the same autochthonous sources. Similar results were also found between \(F_3\) and \(F_5\) (Fig.10b) which indicated that the fulvic-like components \(F_3\) and the humic-like components \(F_5\) may originate from similar allochthonous sources. The saline lakes are largely distributed in the endorheic region of China, and are characterized by long water
residence times and strong water evaporation. Consequently, organic materials (autochthonous and allochthonous) progressively accumulate in these lakes along with salinity, and that may have contributed to the greater concentration of CDOM and FDOM measured in the saline lakes (Catalan et al., 2016; Song et al., 2018). Strong positive correlations between the tryptophan-like F2 and the fulvic-like components F3 were observed in saline waters (Fig.10c) (slope=0.03, $R^2=0.67$) and in freshwater lakes (slope=0.09, $R^2=0.54$), indicating that part of the tryptophan-like fluorophore may have originated from the fulvic-like components, and that the ratio of tryptophan-like component to fulvic-like component was higher in freshwaters than in saline waters. There was also a weak correlation between the total allochthonous substance F$_{3&5}$ and the a$_{CDOM}(254)$ for saline waters (slope=0.07, $R^2=0.39$) and freshwaters (slope=0.03, $R^2=0.39$) (Fig.10d). Moreover, correlation between a$_{CDOM}(254)$ and Fn(355) were also found for saline waters (slope=0.27, $R^2=0.54$) and freshwaters (slope=0.18, $R^2=0.61$) individually. These results suggested that the CDOM was dominated with allochthonous substances in both saline waters and freshwaters. There were also moderate positive linear relationships between a$_{CDOM}(254)$ and HIX for saline waters (slope=5.71, $R^2=0.79$) and freshwaters (slope=0.99, $R^2=0.35$) respectively. These results indicated that the relative humic-like components were more abundant in freshwaters, in accord with
higher degree of humification of CDOM in freshwater than in saline waters. Long periods of exposure to sunlight have likely increased the extent of photochemical oxidation processes in saline waters. The photo-production process significantly increases with salinity in natural waters (Nieto-Cid et al., 2006; Mostofa et al., 2009; Osburn et al. 2009). Our result indicated that the linkage of the fluorescent CDOM components to CDOM in saline waters and freshwaters can be very complex due to the effect of various hydro-geographical and climatic factors, including precipitation, salinity, wastewater discharge and the trophic status of the lakes.

4.4 Elevation versus FDOM components for saline water

Examination of the correlations between lake elevation and fluorescent CDOM characteristics showed weak relationships between these variables in the freshwater lakes (Fig. S2). However, as shown in the results, for all water types (fresh waters and saline waters) and seasons, the $a_{CDOM}(254)$, SUVA$_{254}$, $F_5$ and $F_T$ were all negatively correlated to elevation ($p<0.01$) (Table S1). This result indicated that with the increasing of elevation, less human activity decreased CDOM input, and stronger photochemical degradation of allochthonous CDOM, and seasonal changes and elevations would affect the CDOM compositional changes in fresh waters mutually significantly. Therefore, the sole effect of elevation on fluorescent characteristics of CDOM was not as pronounced
as in saline waters. Future studies could investigate the seasonality of CDOM composition in lakes of different altitudes especially for fresh waters. Meanwhile, these relationships are likely driven by variations in geological settings, climate characteristics, irradiance conditions, and anthropogenic factors and their effect on water residence time and DOC dynamics in lacustrine systems. The short water retention time as well as differences in the origin of CDOM may account for the weak relationships observed in the freshwater lakes (Moran et al., 2000; Winter et al., 2007; Catalan et al., 2016).

[Insert Fig.11 about here]

The correlation between elevation and CDOM absorption at 254nm ($R^2=0.51$, $N=306$), and between elevation and SUVA$_{254}$ in the saline lakes ($R^2=0.41$, $N=306$) was moderate (Fig. 11a and 11f). These trends indicated that the CDOM absorption was higher in the high altitude than in the lower altitude lakes, and that the molecular weight and aromaticity of CDOM trends to decrease with increased lakes elevation. No strong correlations between the tyrosine-like F$_1$, the tryptophan-like F$_2$, and the microbial protein-like F$_4$ components were observed. There was a negative linear correlation between the humic-like F$_5$ and elevation ($R^2=0.63$, $N=306$). However, a strong correlation was found between the total fluorescent intensity F$_T$ and elevation ($R^2=0.64$, $N=306$).
suggested that humic-like materials were predominant of the FDOM in the saline waters and tended to decrease with increasing altitude. That interpretation would be consistent with the moderate negative correlation between elevation and humification index (HIX) \( (R^2=0.56, N=306), \) and the strong correlation between elevation and \( \text{Fn}(355) \) \( (R^2=0.70, N=306), \) indicating that the concentration of humic-like substances and the contribution of allochthonous sources of CDOM tended to decline in the high altitude lakes due to the preferred degradation processes.

The linkages between fluorescent CDOM characteristics and elevation in the saline lakes can be difficult to explain based solely on the data collected during the present study. However, in light of the information presented in past studies, several mechanisms can be invoked to explain the observed trends regarding lakes elevation and fluorescence characteristics of CDOM in these lakes. Solar radiation is a key factor for photo-induced degradation of DOM and organic contaminants in waters (Dobrovic´ et al., 2007; Mostofa et al., 2009). Previous studies have shown that increase in the fluxes of UV radiation can substantially increase the quantity of reactive free radicals such as \( \text{HO}^\cdot \) and \( \text{H}_2\text{O}_2 \) in waters (Qian et al., 2001; Yocis et al., 2000). The effect of irradiance on the degradation processes and fluorescence properties of CDOM are generally highest in surface waters, but tends to be less significant in regard to
deep-water DOM because of the lower sunlight irradiance in the deeper water layers (Laurion et al., 2000). Analysis of the relationships between lakes elevation and meteorological factors (100 stations), showed a moderate positive correlation with solar irradiance \( R^2 = 0.41, N=100, p<0.01 \) and a weak positive correlation with duration of daylight \( R^2 = 0.18, N=100, p<0.01 \) (Fig. S4). This result indicated that with increased lakes elevation, solar irradiance is a determining factor controlling the decrease in the humic-like fluorescent component of CDOM. Solar irradiation can trigger photo-induced degradation of CDOM and the release of small molecular weight CDOM moieties. The intensity of these processes and their impact on the concentration, absorption spectrum and molecular attributes of CDOM are expected to vary depending on lakes altitude. It demonstrated that the humic materials in CDOM undergo the photo-induced degradation in natural waters and would decrease significantly with increased elevation.

The relationships between FDOM and elevation can therefore be complex, and depend on several factors related to variable local conditions including, solar radiation, molecular nature of DOM, salinity and even global warming. Further investigations of these factors are needed to further elucidate the transformation of CDOM components, and the contribution of photo-induced reactions and microbial processes on these
transformations. These future studies would inform our understanding of photo-induced and microbial-mediated alterations in CDOM absorption characteristics in inland waters, and ultimately contribute to our ability to assess the significance of lacustrine ecosystems to the global C cycle.

5. Conclusions

Information about the optical properties and fluorescence characteristics of CDOM in saline inland waters for large geographical regions is rare and generally incomplete. For a better understanding of carbon cycling in inland lakes, a large-scale study was conducted across five lake regions in China to explore the characteristics of CDOM absorption and CDOM fluorescence. Compared to freshwater lakes, higher DOC concentration, $a_{CDOM}(254)$ and SUVA$_{254}$ were measured in the saline inland lakes. Analysis of CDOM fluorescence characteristics provided important insights regarding the sources (allochthonous vs authochnous, natural vs anthropogenic), fates and transformation of DOM in inland lakes. Specifically, the analysis showed that low molecular CDOM fractions were relatively more abundant (hence, low humification index of CDOM) in saline than in freshwater lakes. Since the saline lakes are largely located in endorheic drainage basins and experience long periods of exposure to solar
radiation, these results were interpreted as the consequence of UV-induced photochemical oxidation reactions in saline lakes whereby complex humic molecules are decomposed into low molecular weight fulvic-like substances. Our results further indicate that this photo-oxidation process is much stronger in high-altitude lakes. These findings have important implications regarding our understanding of C dynamics in inland lacustrine systems and the contribution of these ecosystems to the global C cycle.

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Tables

Table 1 The Excitation and Emission wavelength ranges of the five integrated regions identified by Fluorescence Regional Integration (FRI) *

<table>
<thead>
<tr>
<th>FRI Region</th>
<th>Excitation (nm)</th>
<th>Emission (nm)</th>
<th>Description and Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>F₁</td>
<td>200-250</td>
<td>250-330</td>
<td>Tyrosine-like protein</td>
</tr>
<tr>
<td>F₂</td>
<td>200-250</td>
<td>330-350</td>
<td>Tryptophan-like protein</td>
</tr>
<tr>
<td>F₃</td>
<td>200-250</td>
<td>350-500</td>
<td>Fulvic acid-like</td>
</tr>
<tr>
<td>F₄</td>
<td>250-280</td>
<td>250-380</td>
<td>Microbial-like</td>
</tr>
<tr>
<td>F₅</td>
<td>280-400</td>
<td>380-500</td>
<td>Humic-like</td>
</tr>
</tbody>
</table>

*Five regions identified by FRI method according to Chen et al. (2003).
Table 2 The water chemical parameters of various lake regions

<table>
<thead>
<tr>
<th>Region</th>
<th>DOC (mg/L)</th>
<th>EC (µs/cm)</th>
<th>TN (mg/L)</th>
<th>TP (mg/L)</th>
<th>Chl-a (mg/L)</th>
<th>TSM (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLR</td>
<td>27.22</td>
<td>3855.21</td>
<td>5.54</td>
<td>1.28</td>
<td>24.10</td>
<td>127.00</td>
</tr>
<tr>
<td>MXR</td>
<td>25.78</td>
<td>9388.91</td>
<td>3.23</td>
<td>0.17</td>
<td>6.99</td>
<td>24.88</td>
</tr>
<tr>
<td>TQR</td>
<td>34.96</td>
<td>9671.30</td>
<td>3.82</td>
<td>0.49</td>
<td>1.94</td>
<td>29.64</td>
</tr>
<tr>
<td>ELR</td>
<td>5.92</td>
<td>345.32</td>
<td>0.98</td>
<td>0.02</td>
<td>55.21</td>
<td>13.33</td>
</tr>
<tr>
<td>YGR</td>
<td>5.25</td>
<td>388.12</td>
<td>0.90</td>
<td>0.05</td>
<td>24.41</td>
<td>41.99</td>
</tr>
<tr>
<td>All saline</td>
<td>27.40</td>
<td>12722.98</td>
<td>3.55</td>
<td>0.40</td>
<td>7.01</td>
<td>42.97</td>
</tr>
<tr>
<td>All Fresh</td>
<td>6.68</td>
<td>970.75</td>
<td>2.38</td>
<td>0.13</td>
<td>47.99</td>
<td>19.91</td>
</tr>
</tbody>
</table>
Table 3 The PCA component 1 and 2 as the dependent variables expressed with FRI fluorescent components as independent variables

<table>
<thead>
<tr>
<th>Types</th>
<th>Equations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline waters</td>
<td>Component 1 = 0.417F₁ + 0.501F₂ + 0.484F₃ + 0.446F₄ + 0.377F₅</td>
</tr>
<tr>
<td></td>
<td>Component 2 = -0.564F₁ - 0.014F₂ + 0.373F₃ - 0.390F₄ + 0.625F₅</td>
</tr>
<tr>
<td>Fresh waters</td>
<td>Component 1 = 0.457F₁ + 0.473F₂ + 0.430F₃ + 0.468F₄ + 0.404F₅</td>
</tr>
<tr>
<td></td>
<td>Component 2 = -0.427F₁ - 0.266F₂ + 0.506F₃ - 0.319F₄ + 0.624F₅</td>
</tr>
</tbody>
</table>
Figures

Fig. 1 The distribution of sampled saline and freshwater lakes in different lake regions with respecting to elevation across China.
Fig. 2 (a) the concentration variations of dissolved organic carbon (DOC), and (b) the variation of α_{CDOM}(254) and comparison for saline (S) and fresh waters (F) in five lake regions across China.
Fig. 3(a) The variation and comparison of CDOM absorption spectral slope ($S_{275-295}$), and (b) SUVA$_{254}$ values for saline (S) and freshwater (F) lakes across five lake regions of China. The line and circle within each box represent the median and mean, respectively. The horizontal edges of each box denote the 25th and 75th percentiles, the whiskers denote the 10th and 90th percentiles, and × represent outliers.
Fig. 4 The EEM fluorescence spectra and FRI distribution of Lake Selin Co from the TQR, Lake Aibi from the MXR and Lake Khanka from the NLR.
Fig. 5 The comparison of total fluorescent intensity of saline (S) and fresh water (F) lakes in different lake regions across China.
Fig. 6 The comparison of individual fluorescent intensities of saline (S) and fresh waters (F) in different lake regions. F1 represents tyrosine-like protein, F2 represents tryptophan-like protein, F3 represents fulvic acid-like organics, F4 represents soluble microbial by-product-like materials, and F5 represents humic acid-like organics.
Fig. 7 The proportions of individual fluorescent intensities of saline (S) and fresh waters (F) in different lake regions. \( P_1 \) represents the percentages of EEM-FRI extracted FDOM related to tyrosine-like protein. \( P_2 \) represents the percentages of EEM-FRI extracted FDOM related to tryptophan-like protein. \( P_3 \) represents the percentages of EEM-FRI extracted FDOM related to fulvic acid-like organics. \( P_4 \) represents the percentages of EEM-FRI extracted FDOM related to soluble microbial by-product-like materials. \( P_5 \) represents the percentages of EEM-FRI extracted FDOM related to humic acid-like organics.
Fig. 8 The principal component analysis (PCA) results of FRI-based EEMs (a) PCA components and (b) PCA factor scores for saline waters; (c) PCA components and (d) PCA factor scores for freshwaters.
Fig. 9 The correlation between $a_{CDOM}(254)$ and DOC concentration for various lake regions in China. (a). TQR (The high saline waters are with the mean EC of 25000 us/cm, the low saline water are with the mean EC of 9500 us/cm); (b). ELR; (c). YGR; (d). NLR; (e). MXR.
Fig. 10 The correlations between $a_{\text{CDOM}}(254)$ and FRI-EEMs characteristics, (a) cumulative volume $F_1$ and $F_4$ by EEM-FRI for water samples in saline lakes, (b) $a_{\text{CDOM}}(254)$ and Humic-like Fluorescence (Fn355), (c) $a_{\text{CDOM}}(254)$ and humification index, (d) cumulative volume $F_2$ and $F_3$ by EEM-FRI for water samples in saline lakes, (e) $a_{\text{CDOM}}(254)$ and cumulative volume $F_3+F_5$ by EEM-FRI for water samples in saline lakes, (f) cumulative volume $F_3$ and $F_5$ by EEM-FRI for water samples in saline lakes.
Fig. 11 The correlation between elevation and CDOM absorption and FRI-EEM extracted indices for saline waters, (a) $a_{\text{CDOM}}(254)$ versus elevation, (b) $F_5$ versus elevation, (c) $F_n355$ versus elevation, (d) $F_T$ versus elevation, (e) HIX and elevation, and (f) SUVA$_{254}$ versus elevation.
Highlights

1. The 936 lake samples in China were examined to explore CDOM optical properties
2. FRI was used to characterize CDOM sources of saline waters and fresh waters
3. The relationships between CDOM absorption and FDOM components were analyzed
4. Close relations of elevation and saline waters FDOM components were identified
Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: