Quantitative study on the structure-bioavailability relationship of dissolved organic nitrogen in wastewater treatment plant effluent

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Abstract
This study systematically investigated the relationship between the structure properties and biological characteristics of dissolved organic nitrogen (DON) in the effluents from municipal wastewater treatment plants. Ultrafiltration, Fourier transform infrared (FTIR) spectroscopy, ultraviolet (UV) spectroscopy, and excitation-emission matrix (EEM) fluorescence spectroscopy were used to characterize the structure of organic matters in the effluent samples, and the bioavailability of DON was determined by algal/bacterial-based bioassay. The quantitative analysis of EEM spectra conducted by fluorescence regional integration method showed that the organic portion of all samples was mainly consistent with fulvic acid and protein. Combined with the bioassay results, a positive correlation between the DON bioavailability and the protein content (sum of region I and region II) \( r = 0.80, P < 0.02 \) and soluble microbial byproduct-like materials (region IV) \( r = 0.76, P < 0.03 \) were observed. Nevertheless, the humic substances content represented by the region III and V would negatively affect the DON bioavailability. High humification degree (high HIX value) \( r = -0.77, P < 0.03 \) was related to low bioavailability. Furthermore, according to UV spectroscopy results, strong aromaticity (high UV \(_{254}\) values) \( r = -0.78, P < 0.03 \) suggested low DON bioavailability. One protein-like component (C3) and two humic-like components (C1 and C2) were identified via fluorescence excitation-emission matrices-parallel factor analysis (EEM-PARAFAC), and component C3 values were positively correlated to the BAN/DON ratio \( r = 0.74, P < 0.03 \). The ultrafiltration showed that the low molecular weight DON (<3 kDa) accounted for 30–73% of the total DON, and no notable relationship was observed for DON molecular weight and its bioavailability.

Keywords Eutrophication potential · Characterization · Spectroscopy techniques · Algal bioassay · EEM-PARAFAC · Correlation

Introduction
Municipal wastewater effluent discharged from urbanized areas is a crucial source of anthropogenic nitrogen load in surface water (Liu et al. 2011; Huang et al. 2020). It is estimated that the wastewater-derived nitrogen accounts for 12–33% of nitrogen input in global river (Howarth 2004). With the development of advanced nutrient removal system, high inorganic nitrogen removal could be achieved during wastewater treatment process (Yan et al. 2022). This led to the dissolved organic nitrogen (DON) became a major nitrogen form of the total dissolved nitrogen (TDN) in wastewater effluent (Yan et al. 2022).

Previous research suggested that inorganic nitrogen can be readily used by phytoplankton, while the bioavailability of DON varies greatly due to its complicate composition (Fan et al. 2018). For instance, urea nucleic acids were taken up readily by planktonic microbiota and stimulated algae growth, while humic bound nitrogen substances were often resistant to biodegradation (Berman and Bronk 2003; Fiedler et al. 2015; Liu et al. 2011). However, it is difficult to evaluate the contribution of different DON compounds in...
effluent to eutrophication as current methods are only able to identify about 10% of the DON in wastewater treatment plant (WWTP) effluent (Fan et al. 2018). On the other hand, the quantitative study on the relationship between DON bioavailability and its structure properties can be an alternative since certain researches reported the DON bioavailability is somewhat related to its molecular structure. For example, the study conducted by Eom et al. (2017) suggested that the DON bioavailability was related to its molecular weight, and DON with high molecular weight tended to recalcitrant. Feng et al. (2018) pointed out that the hydrophilic DON was readily assimilated by algal, while the hydrophobic DON was less likely to support algae growth. It is known that the hydrophilicity/hydrophobicity of DON characterizes the molecular polarity and the hydrogen bond between molecules. Besides, Yan et al. (2021) suggested that the DON bioavailability was also related to its C/N ratio, as it was reported that low C/N ratio contained DON compounds, such as amino acid and protein, had high bioavailability toward algae.

Above studies suggested that the structure of DON was closely related to its bioavailability, and the variation of DON bioavailability was probably due to the different structures of different DON. Therefore, proper DON structure characterization is the key to understand the relationship between the DON structure and its bioavailability. With the development of spectroscopy technology, some advanced spectroscopy techniques have been widely used in DON structural characterization, including the characterization of functional group composition, aromaticity, and degree of humification of DON. For example, excitation-emission matrix (EEM) fluorescence was used to analyze the humic-like and protein-like materials in eutrophic lakes (Yan et al. 2018). Ultraviolet (UV) spectroscopy could reflect the aromaticity and the degree of substitution on benzene rings of dissolved organic matter (DOM) through the detection of absorbance UV254 and the absorbance ratio of UV240/UV203 and UV235/UV203 (Huoivinen et al. 2003; Hua et al. 2017; Sun et al. 2018). Yang et al. (2017) used Fourier transform infrared (FTIR) spectroscopy to characterize the functional groups of DON in Da Qaidam Lake. However, the relationship between the bioavailability of DON and its structure was not systematically studied, and whether there is mathematical correlation between spectral characterization parameters and DON bioavailability is still unclear.

Therefore, in this paper, the structure properties of DOM in wastewater effluent, including the molecular weight, functional groups, aromaticity, humification degree, and humic substances category, were characterized by ultrafiltration, FTIR spectroscopy, UV spectroscopy, and EEM fluorescence spectroscopy, respectively. Combined with the DON bioavailability results obtained by 14-day nitrogen uptake bioassay, the relationship between the bioavailability and structure properties of DON was systematically and quantitatively investigated. This finding could provide a mathematical correlation to express the relationship between the bioavailability of DON and its structure.

Materials and methods

Sampling

Twenty-four effluent samples collected from 8 WWTPs in Chengdu, China, were used in this study (Table S1 and Fig. S1 of the supporting information, SI). After collection, all samples were filtered through 1.0-μm and 0.45-μm Milipore filters to remove suspended particulate and then stored at 4 °C prior to use. The effluent samples were labeled E1–E8 in this paper.

Chemical analysis

The concentration of total nitrogen (TN) and total dissolved nitrogen (TDN) were analyzed using the persulfate digestion-ultraviolet spectrophotometric method. This method converts all nitrogenous compounds to nitrates (Clescerl et al. 1998). The concentration of nitrate nitrogen (NO3-N) and nitrite nitrogen (NO2-N) was determined using UV spectrophotometry. The ammonia nitrogen (NH4-N) in water was determined by Nessler’s Reagent Spectrophotometry (Table S2 of SI). DON was calculated as the difference between measured TDN and sum of measured dissolved inorganic nitrogen (DIN) species using the following equation (Tang et al. 2021).

\[ \text{DON} = \text{TDN} - (\text{NO}_3^- + \text{NH}_4^+ + \text{NO}_2^- + \text{N} + \text{NO}_4^+ - \text{N}) \]

Resin separation

High inorganic nitrogen concentration might negatively affect the calculation accuracy of the DON concentration. Thus, resin separation was used as pretreatment to reduce the DIN concentration. Prior to use, the 100 g ECA90 anion exchange resin (Yong Zhou Water Treatment Tech Co, Ltd) packed in a borosilicate glass column was cleaned with 10% sodium chloride (Sigma-Aldrich, Inc. ≥ 99.0%) and then twice with ultrapure water. After cleaning, the effluent was slowly passed through the resin with 5 mL/min for inorganic nitrogen extraction. After treating the samples with ion exchange, the removal rate of DIN ranged from 87.9 to 97.8%, and the average removal rate of DIN was 93.1% (Table S3). This operational condition ensured minimal DON adsorption on ion exchange resin.
The detailed information about the DON concentration before and after ion exchange was provided in Table S4.

Algal bioassay

*Selenastrum capricornutum* was used as the test algae, which can be easily cultured in the laboratory and has been used as a standard test organism for eutrophication studies for over 45 years (Yan et al. 2022; Fan et al. 2017a, b). The freshwater algae culture was maintained using the nutrient medium suggested by Miller et al. (1978). Nutrient stress was induced by incubating algae cultures with nitrogen free medium 7–10 days prior to the bioassay (Fan et al. 2017a, b).

The experiment was initiated by adding nitrogen starved algae (initial chlorophyll-a concentration 30 μg/L of) to the samples (Fan et al. 2018, 2020). Control samples with incubated with algae and bacteria were also prepared to understand the influence of bacteria during DON uptake. Specifically, 3 L of mixed sample from each treatment plants were filtered through 1-μm filter to remove large particulate pollutants and then through a 0.2-μm membrane filter. The retentate on the 0.2-μm filter was resuspended in 100 mL of 0.2 μm filtered wastewater effluent and used as the bacterial inoculum (Liu et al. 2011). Triplicates of each sample were incubated and the results were averaged for the final calculations.

During the algal bioassay experiments, all samples were incubated at the temperature of 25 ± 1 °C under continuous fluorescent lighting of 1300 Lm in a horizontal shaker at 110 rpm for 14 days. During the bioassay, all nutrients (except N) were added twice a week to avoid nutrient co-limitation. To ensure the maximum algae potential growth rate, the optimum incubation environment was maintained for all samples (Fan et al. 2017a, b). The freshwater algae culture was maintained using the nitrogen free medium 7–10 days prior to the bioassay (Fan et al. 2017a, b). The freshwater algae culture was maintained using the nutrient medium suggested by Miller et al. (1978). Nutrient stress was induced by incubating algae cultures with nitrogen free medium 7–10 days prior to the bioassay (Fan et al. 2017a, b).

Fluorescence spectra of effluents samples were recorded using a HORIBA/FM4PC fluorescence spectrophotometer (PerkinElmer, USA), which was equipped with double monochromators both at the excitation and the emission sides. The fluorescence EEM spectra were collected by subsequent scanning emission from 240 to 800 nm at sequential 2 nm increments; for each excitation wavelength, the emission wavelength from 240 to 800 nm was detected at sequential 3-nm increments. The water Raman scattering and second order Rayleigh scattering are eliminated by using Milli-Q water as blank.

Based on the fluorescence regional integration (FRI) method, the excitation and emission boundaries could be classified into five regions, and each region represented materials like aromatic protein, fulvic acid, soluble microbial byproduct, and humic acid. The volume (Ω) beneath region *i* of an EEM can be calculated with:

\[
\Phi_i = \int_{\lambda_{ex}}^{\lambda_{em}} \int_{\lambda_{em}}^{\lambda_{ex}} I(\lambda_{ex}, \lambda_{em}) d\lambda_{ex} d\lambda_{em}
\]

where \(I(\lambda_{ex}, \lambda_{em})\) is the fluorescence intensity at each excitation–emission wavelength pair, \(d\lambda_{ex}\) is the differential of the excitation wavelength, and \(d\lambda_{em}\) is the differential of the emission wavelength. For discrete data, the volume (\(\Phi_i\)) is expressed by:

\[
\Phi_i = \sum_{\lambda_{ex}} \sum_{\lambda_{em}} I(\lambda_{ex}, \lambda_{em}) \Delta\lambda_{ex} \Delta\lambda_{em}
\]

where \(\Delta\lambda_{ex}\) is the excitation wavelength increment, \(\Delta\lambda_{em}\) is the emission wavelength increment.

The normalized excitation-emission area volumes (\(\Phi_{in}\)) and normalized percentage (\(P_{in}\)) were calculated according to following equation:

\[
\Phi_{in} = MF_i \Phi_i
\]

\[
P_{in} = \frac{\Phi_{in}}{\Phi_{Tn}} \times 100\%
\]

Meanwhile, PARAFAC analysis was carried out on the 8 effluent samples using the DOMFluor toolbox in MATLAB R2018a following the procedure provided by Stedmon (2008).

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UV

UV absorbance measurements were conducted on a Lambda950 UV spectrophotometer (PerkinElmer, USA) between 200 and 800 nm with 1 nm as the scanning interval. One-centimeter light path was provided with a quartz and the Milli-Q water was used as blank sample during UV absorbance measurements. The UV254 value was defined as UV absorbance measured at the wavelengths of 254 nm. UV240/UV420 and UV253 /UV203 were the absorbance ratio at the wavelengths of 240 nm, 420 nm, 253 nm, and 203 nm, respectively.
**FTIR**

FTIR spectra of the dried DON samples were obtained on a VERTEX 70 Fourier-transform infrared (SHIMADZU, Japan). The pellets were made from the mixture of dried DON samples and KBr in a 1:100 ratio. The instrument was set up to scan from 4000 to 400 cm\(^{-1}\) averaging 10 scans at 1.0-cm\(^{-1}\) intervals with a resolution of 4.0 cm\(^{-1}\).

**Molecular weight distribution**

For ultrafiltration, pre-treated effluent samples were fractionated using 400 mL commercial stirred cell units. The samples were fractionated into five groups using a series of cellulose-derivative ultrafiltration membranes with MW cutoffs of 100 kDa, 30 kDa, 10 kDa, and 3 kDa, respectively, under high purity nitrogen gas at 0–0.15 MPa. The membrane was soaked overnight in advance and filtered with Milli-Q water for half an hour before each filtration to remove any organic matter attached to the membrane and obtain a stable permeate flux (Tan et al. 2017). Prior to the experiment, the 400-mL commercial stirred cell units was rinsed with a small amount of pre-treated effluent samples. During filtration process, the first 20 mL of effluent sample was discarded and the remaining effluent samples were collected as the retentate. Repeat the above operation for each replacement of the ultrafiltration membrane. The percentages of DON in each size range were calculated as follows (Lee and Westerhoff 2006).

\[
\% < 3kDa = \frac{C_{3kpermeate}}{C_{raw}} \times 100(\%)
\]

\[
\% 3kDa - 10kDa = \frac{C_{10kpermeate} - C_{3kpermeate}}{C_{raw}} \times 100(\%)
\]

\[
\% 10kDa - 30kDa = \frac{C_{30kpermeate} - C_{10kpermeate}}{C_{raw}} \times 100(\%)
\]

\[
\% 30kDa - 100kDa = \frac{C_{100kpermeate} - C_{30kpermeate}}{C_{raw}} \times 100(\%)
\]

\[
\% > = \frac{C_{raw} - C_{100kpermeate}}{C_{raw}} \times 100(\%)
\]

**Results and discussion**

**DON bioavailability**

In order to effectively remove DIN and improve the calculation accuracy of DON, the effluent samples were pretreated with ion exchange before the N composition analysis. The obtained results showed that the DON concentration of the effluent samples was ranged from 0.78 to 1.52 mg/L and the DIN concentration was less than 0.5 mg/L. The DON bioavailability of effluents was assessed using algal/bacterial based bioassay, and the obtained results showed that the bioavailable nitrogen (BAN) concentration of \(E_1-E_8\) samples of algae group accounted for 35.01%, 20.24%, 35.80%, 27.18%, 24.85%, 29.29%, 29.36%, and 32.83% of the DON, respectively (Fig. 1). The chlorophyll-a concentration increased by 112.48–760.60 μg/L after 14 days of incubation. On the other hand, for control sample, the added \(\text{NO}_3^-/\text{NH}_4^+\) (TN concentration of 3 mg L\(^{-1}\)) was completely used up during the 14 days bioassay and it showed much higher Chl-a value by the end of the incubation. This indicated that the recalcitrant DON accounted for majority fraction (> 60%) of the TDN in the WWTP effluent, and their support to algal growth was much lower than that of the DIN. This was consistent with the results reported by Fan et al. (2017a, b) when

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**Fig. 1** (a) The DON bioavailability of all effluent samples; (2) Chlorophyll-a concentration for all samples after 14 days of incubation.
the DON bioavailability in the effluent of 9 WWTPs in unite state was studied. The concentration of BAN increased by 6.04–17.43% and the chlorophyll-a concentration increased by 13.10–85.18% with the presence of bacteria, similar to previous study, which suggested that the addition of bacteria could convert the high molecular weight DON into low molecular weight DON, which favored the algal growth stimulation (Yan et al. 2022).

UV spectroscopy

The UV spectrum characteristic parameters of DON in the effluent were shown in Table 1. The UV_{254} value (ranged from 0.052 to 0.179) that was often used to characterize the aromaticity of DOM, and according to the bioassay, the DON bioavailability was showed to be negatively correlated with the UV_{254} value ($r = -0.78, P < 0.03$). This result was probably due to aromatic and hydrophobic fraction of humic substances usually exhibited higher absorption at 254 nm than aliphatic and non-humic substances (Kumari and Gupta 2019). The aromatic and hydrophobic fraction of humic substances tended to be recalcitrant (Chao et al. 2015). The UV_{240}/UV_{420} values for all effluent samples ranged from 9.82 to 16.11, with an average value of 12.90, suggesting that the humus in the samples was mainly fulvic acid (Sun et al. 2018). High UV_{253}/UV_{203} value result indicated that there were more substituents with carboxyl, carbonyl, hydroxyl, and ester content on the aromatic ring. These functional groups could easily provide sites for heavy metal coordination complexation and adsorb heavy metal ions in water to form macromolecular complexes, which would greatly reduce the DON bioavailability (Velazquez-Jimenez et al. 2013). This was in agreement of the bioassay results, which showed that the DON in sample E$_2$ and E$_7$ with the highest UV_{253}/UV_{203} value was highly recalcitrant compared to the rest of the samples.

EEM fluorescence spectroscopy

The EEM spectrum of all effluent samples is shown in Fig. 2. Three fluorescence indices (FI), humification index (HIX), and biological index (BIX) were calculated to characterize the DOM in samples (Table 1). The FI value was used to estimate the source of DOM. The DOM was considered to be originated from microbial sources when the FI value was $>1.90$, while it might be from terrestrial and soil sources when the FI value was $<1.40$ (Huguet et al. 2008). The HIX value often characterize the humification degree of DOM. HIX higher than 10 suggested high humification degree of the fluorescent component, while HIX lower than 4 indicated low humification degree (Yan et al. 2018; Guang et al. 2018). For BIX value, when it was observed to be greater than 1, the DOM was believed to be newly released into the water. When the BIX was 0.60–0.70, the DOM was considered to be composed with less autogenic matters (Yan et al. 2018). As shown in Table 1, the FI values for tested effluent samples were ranged from 1.41 to 1.83. For samples E$_1$, E$_3$, E$_4$, E$_5$, and E$_8$, the FI values were close to 1.90, indicating that the DON from these samples was originated from microbial sources (Maie et al. 2006). Similar phenomenon was observed by Yang et al. (2017), who reported that the DOM in secondary effluent was mainly from a biological source. The HIX values less than 4 were detected for all effluent samples, indicating that the humification degree of DON was relatively low. The HIX and the BAN/DON ratios were negatively correlated ($r = -0.77, P < 0.03$), which indicated that the lower the humification degree, the higher the bioavailability of DON. The BIX value was observed between 0.88 and 0.97, implying that the DON in water was mainly from autogenous sources. This result was in consistent with the conclusion obtained from the FI value, and similar results were also proposed by other researchers (Cory and McKnight 2005; Yang et al. 2018).

According to Chen’s method, the EEM spectra could be divided into five consecutive regions (Chen et al. 2015). As shown in Table 2, the proportion of the sum of fluorescent protein region I and region II ranged from 33.53 to 55.77%. The proportion range of fulvic acids in the region III and humic acids in the region V was 36.94–61.37% and 1.72–2.06%, respectively. In region IV, the proportion of soluble microbial byproduct-like materials in the fluorescence region ranged from 3.08 to 5.64%. These results showed that

| Table 1 Fluorescence parameters and UV parameters of DON in the effluent samples |
| --- |
| **FI** | **HIX** | **BIX** | **UV$_{254}$** | **UV$_{240}$/UV$_{420}$** | **UV$_{253}$/UV$_{203}$** |
| E$_1$ | 1.833 | 1.370 | 0.882 | 0.052 | 12.439 | 0.010 |
| E$_2$ | 1.413 | 2.564 | 0.947 | 0.179 | 16.106 | 0.029 |
| E$_3$ | 1.805 | 1.532 | 0.967 | 0.059 | 10.820 | 0.012 |
| E$_4$ | 1.525 | 1.669 | 0.910 | 0.086 | 13.323 | 0.026 |
| E$_5$ | 1.600 | 2.798 | 0.834 | 0.079 | 13.439 | 0.022 |
| E$_6$ | 1.465 | 2.308 | 0.930 | 0.065 | 13.232 | 0.016 |
| E$_7$ | 1.431 | 1.832 | 0.943 | 0.145 | 14.398 | 0.027 |
| E$_8$ | 1.733 | 1.705 | 0.936 | 0.063 | 12.424 | 0.020 |
the effluent samples were mainly contained with humic-like substances and protein-like substances. This is similar to the study by Wei et al. (2019), which showed that the urban streams were enriched with anthropogenic and aquagenic fulvic acid and protein-like DOM. Besides, this was also in consistent with the result of the UV_{250}/UV_{420} values. Combined with the bioassay results, it can be concluded that the protein-like content was positively correlated with the BAN/DON ratios (r = 0.80, P < 0.02), while the humic substance content was negatively correlated to the BAN/DON ratio (r = −0.86, P < 0.01). This was in agreement with the previously reported results that protein compounds were highly bioavailable due to its low C/N ratios and the high presence of nitrogen-rich hydrophilic components, while humic substances tended to be resistant to biodegradation (Yan et al. 2021; Huo et al. 2013). Thus, high value of region I + region II indicated high DON bioavailability, while high value of region III + region V reflected low DON bioavailability. Moreover, the generated results also exhibited a positive correlation between the content of soluble microbial byproduct-like materials (proportion of region IV) and BAN/DON ratios (r = 0.76, P < 0.03), because microorganism-derived DON was a nitrogen source to algae and bacteria (Liao et al. 2020). Protein compounds with a low C/N ratio have more nitrogen-rich hydrophilic components, which have high bioavailability.

The spectral shapes of the four components were derived by PARAFAC, in which C1, C2, and C4 components were classified as humic-like, and C3 was categorized as protein-like. Component C1 displayed an emission maximum at 250 nm, and an excitation maximum at 418 nm, which was attributed to humic-like peak (Zhang et al. 2015). Component C2 exhibited an excitation maximum at <250 nm, and an excitation maximum at 400 nm (Jamieson et al. 2014; Cui et al. 2019). This could also be categorized as a humic-like peak. Component C4 (Ex/Em = 360/500 nm) was categorized as fulvic acid substance (Stedmon and Markager 2005; Zhang et al. 2015). Component C3 was the only protein-like component observed and showed an excitation/emission maximum of 270/320 nm (Jamieson et al. 2014; Cui et al. 2019; Cuss et al. 2019). Figure 3 indicates that the maximum fluorescence intensity (F_{max}) value of C3 varied greatly among different WWTPs; the F_{max} value for component C3 for sample E1 was higher than those from other samples, indicating that the E1 sample had the highest protein content, and the component C3 values were positively correlated to the BAN/DON ratios (r = 0.74, P < 0.03). The dominance of the component C2 in all WWTP effluent samples indicated that humic substances in the secondary effluent were difficult to remove via biological treatment (Yan et al. 2022). The E2 sample with the largest proportion of humic and fulvic acid presented the lowest bioavailability, which was similar to the results obtained by FRI method.

**FTIR spectroscopy**

FTIR was used to characterize the DOM functional groups of all samples, and the obtained spectra are shown in Fig. 4. Due to the overlap of functional groups, the spectra of all effluent samples showed similar patterns in the position of principal absorption bands (Abdulla et al. 2010). All effluent samples formed a broad peak around 3420 cm^{-1}, which can be assigned to the of O–H stretching of carbohydrate,
carboxylic acid, and phenol compounds. The relative wide breadth of this peak was probably due to the intra-molecular and inter-molecular hydrogen bond interactions of carboxylic acids (Pavia et al. 2009; Abdulla et al. 2010; Yang et al. 2017). The sharp peaks around 2970–2930 cm⁻¹ and 2870–2850 cm⁻¹ were attributed to asymmetric stretching of aliphatic C-H and related to aliphatic compound methyl (-CH₂) groups and methyl (-CH₃) groups (Gandois et al. 2013). These two peaks were more pronounced for sample E₂, illustrating the presence of more aliphatic compounds in sample E₂ compared to other samples. The absorption peak near 1720 cm⁻¹ was attributed to the C=O stretching of the conjugated carbonyl groups in carboxylic acids, ketones, and aldehydes. The absorption peak observed at around 1640 cm⁻¹ was related to the C=O stretching of the amide groups in the protein-like component (Giovanela et al. 2004). Sample E₁ showed the highest peak area of 206 at 1640 cm⁻¹, while the peak area at this peak for sample E₂ was only 105. The bioassay also indicated that the bioavailability of DON in sample E₁ was much higher than that of sample E₂, since previous studies showed that protein could be taken up readily by algae (Liu et al. 2011; Fan et al. 2018). In addition, the peak of hydrophilic component at 1640 cm⁻¹ was significantly larger than that of hydrophobic component, and the bioavailability of hydrophilic DON was greater than that of hydrophobic DON (Sharma et al. 2017), similar to UV results, with the E₁ plant having a lower UV₂₅₄ value for poor aromaticity, which tends to be negatively correlated with hydrophilicity (Kumari and Gupta, 2019). The two absorption peaks around 1170 cm⁻¹ and 1070 cm⁻¹ were ascribed to the C-O stretching of polysaccharides (Gandois et al. 2013).

### Molecular weight distribution

Figure 5 presented the DON molecular weight distribution of all effluent samples. The results showed that the low molecular weight DON accounted for 30–73% of the total DON concentration, which indicated that DON in the effluent was mainly contained with low molecular weight DON. However, unlike conclusions proposed by other researchers, in this study, the low molecular weight DON content was not evidently correlated with its bioavailability. This was probably because that some high molecular weight DON compounds, such as certain type of humic acid can also be decomposed by algae. While some low molecular weight DON compounds, such as Uracil and Aminobenzoic acid, tend to be recalcitrant to biodegradation (Fan et al. 2018). Besides, it was reported that some low molecular weight nitrogenous compounds, such as amino acids, purines, and pyrimidine might be adsorbed onto the humic core structure, and these adsorbed compounds could not be identified as low molecular DON using ultrafiltration (Antia et al. 1991; Zhai et al. 2016; Zhang et al. 2016).

| Sample | Region I | Region II | Region III | Region IV | Region V | Region I | Region II | Region III | Region IV | Region V |
|--------|----------|-----------|------------|-----------|----------|----------|-----------|------------|-----------|----------|
| E₁     | 1.036    | 41.7      | 43.201     | 17.160    |
|        | 2.331    | 41.7      | 97.203     | 38.609    |
|        | 4.366    | 21.3      | 92.996     | 36.938    |
|        | 1.776    | 21.3      | 14.030     | 5.573     |
|        | 3.330    | 1.1       | 4.329      | 1.720     |
| E₂     | 0.530    | 41.7      | 22.101     | 11.148    |
|        | 1.064    | 41.7      | 44.369     | 22.381    |
|        | 5.712    | 21.3      | 121.666    | 61.371    |
|        | 0.773    | 21.3      | 6.105      | 3.080     |
|        | 3.080    | 1.1       | 4.004      | 2.020     |
| E₃     | 0.851    | 41.7      | 35.487     | 15.009    |
|        | 2.014    | 41.7      | 83.973     | 35.517    |
|        | 4.650    | 21.3      | 99.045     | 41.892    |
|        | 1.688    | 21.3      | 13.331     | 5.639     |
|        | 3.533    | 1.1       | 4.592      | 1.942     |
| E₄     | 0.747    | 41.7      | 31.150     | 15.304    |
|        | 1.593    | 41.7      | 66.428     | 32.635    |
|        | 4.368    | 21.3      | 93.038     | 45.709    |
|        | 1.176    | 21.3      | 9.290      | 4.564     |
|        | 2.800    | 1.3       | 3.640      | 1.788     |
| E₅     | 0.634    | 41.7      | 26.438     | 12.452    |
|        | 1.370    | 41.7      | 57.108     | 26.897    |
|        | 5.416    | 21.3      | 115.361    | 54.332    |
|        | 1.188    | 21.3      | 9.385      | 4.420     |
|        | 3.102    | 1.3       | 4.033      | 1.899     |
| E₆     | 0.693    | 41.7      | 28.898     | 13.244    |
|        | 1.641    | 41.7      | 68.439     | 31.566    |
|        | 5.135    | 21.3      | 109.376    | 50.127    |
|        | 0.923    | 21.3      | 7.292      | 3.342     |
|        | 3.224    | 1.3       | 4.191      | 1.921     |
| E₇     | 0.568    | 41.7      | 23.686     | 12.121    |
|        | 1.777    | 41.7      | 49.060     | 25.106    |
|        | 5.629    | 21.3      | 111.378    | 56.997    |
|        | 0.897    | 21.3      | 7.086      | 3.626     |
|        | 3.231    | 1.3       | 4.200      | 2.149     |
| E₈     | 0.780    | 41.7      | 32.526     | 14.243    |
|        | 1.853    | 41.7      | 77.249     | 33.827    |
|        | 4.875    | 21.3      | 103.838    | 45.469    |
|        | 1.365    | 21.3      | 10.784     | 4.722     |
|        | 3.055    | 1.3       | 3.972      | 1.739     |
Conclusion

The present study investigated the relationship between the structure and bioavailability of DON in the effluent of WWTPs using spectroscopy characterization techniques and algae/bacterial based bioassay. The EEM spectrum and bioassay results showed that the protein content (sum of region I and region II) and soluble microbial byproduct-like material content (region IV) in DON were positively correlated with bioavailability, while the humic substance content (region III and region V) was negatively correlated. The high humification degree (high HIX values) corresponded to low DON bioavailability. According to the DON fluorescence excitation emission spectra, there were one protein-like component (C3), and two humic-like components (C1 and C2), and component C3 values were positively correlated to the DON bioavailability. The UV spectroscopy analysis results showed that low bioavailability DON had stronger aromaticity with higher UV\textsubscript{254} values. Molecular weight distribution experiments showed that there was no significant correlation between molecular weight and bioavailability.
Supplementary Information  The online version contains supplementary material available at https://doi.org/10.1007/s11356-022-21567-2.

Author contribution  Sampling, investigation, and interpretation were performed by CY, Z, JL, and L. Review and editing were performed by CY, JC, and LF. Conceptualization and writing were performed by CY and LF. Initial idea, project manager, project leader, laboratory activity, and methodology were performed by LF. All authors read and approved the final manuscript.

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Declarations

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