NATURAL ORGANIC MATTER RESEARCH

INTERNATIONAL HUMIC SUBSTANCES SOCIETY

APPLICATION FORM IHSS TRAVEL SUPPORT AWARD

For the 17th IHSS Meeting in Ioannina, Greece (September 1st to 6th, 2014)

1. Applicant			
First name	Wentao		
Family name	Li		
Email	liwentaoepa@hotmail.com		
Tel., Fax	Tel: +86 18052093492; Fax: +86 25 86269876		
Address	School of the Environment, Nanjing University, Xianlin Road No.163, Qixia District, Nanjing, China		
IHSS member		Yes	Since:2013 Member ID:10988
Status of applicant		PhD student	Expected Graduation Date:2016
2. Institution			
Name, address	School of the Environment, Nanjing University, Xianlin Road No.163, Qixia District, Nanjing, China		
Supervisor or person giving the recommendation	Rong Ji		
IHSS member		Yes	Since:2012 Member ID:10361

This application form should be followed by:

1. a one-page curriculum vitae (CV) that includes:
   a. Personal data
   b. Education (including a summary of courses taken)
   c. Awards
   d. Publications
   e. Conferences attended
      i. Oral communications
      ii. Poster contributions
2. a letter of evaluation from the applicant's main supervisor,
3. a manuscript of the paper to be presented (4 pages in total).

The entire application must be submitted as a single pdf file by March 14th, 2014 to the Vice-President of IHSS: Prof. E. Michael Perdue (emperdue@bsu.edu).

CV of Wentao Li

Personal Data
Title: Ph.D. student
Date of birth: 1988-05-06
Gender: man
Email: liwentaoepa@hotmail.com

Mobile: +86 1805 209 3492
Address: School of the Environment, Nanjing University, No. 163, Xianlin Road, Qixia District, Nanjing, Jiangsu Province, China, 210023

Education

2011.09-current	Ph.D. Student of Environmental Engineering School of the Environment, Nanjing University
2013.05-2013.09	Exchange student in School of Life Sciences, University of Applied Sciences and Arts Northwestern Switzerland
2012.07-2012.07	Summer School on Environmental Remediation & Biogeochemistry
2007.09-2011.06	Bachelor of Engineering School of the Environment, Nanjing University

Awards in recent 3 years

2012	The National Scholarship for Graduate Students Outstanding Graduate Students of Nanjing University
2011	Outstanding Graduates of Nanjing University

Publications

1. Li, W.-T.; Xu, Z.-X.; Li, A.-M. Comment on “Identifying Well Contamination through the use of 3-D Fluorescence Spectroscopy to Classify Coalbed Methane Produced Water”. Environmental Science & Technology 2012, 47 (3), 1770-1771.
2. Li, W. T.; Xu, Z. X.; Li, A. M.; Wu, W.; Zhou, Q.; Wang, J. N. HPLC/HPSEC-FLD with multi-excitation/emission scan for EEM interpretation and dissolved organic matter analysis. Water Res. 2013, 47 (3), 1246-56.
3. Li, W.-T.; Chen, S.-Y.; Xu, Z.-X.; Li, Y.; Shuang, C.; Li, A.-M. Characterization of dissolved organic matter in municipal wastewater using fluorescence PARAFAC analysis and chromatography multi-excitation/emission scan: a comparative study. Environ. Sci. Technol. 2014. DOI: 10.1021/es404624q

Conferences attended

2013.07	Oral and poster report on International Workshop on Organic Matter Spectroscopy 2013 (WOMS 2013), 16-19th July 2013, La Garde City, France.
2012.10	Oral and poster report on the 6th National Ph.D. Candidates Academic Conference, New theories and New Technologies in Environmental Science and Engineering. Beijing, China.
2012.09	Poster report on **the 16th Meeting of the International Humic Substances Society**, Hangzhou, China

Letter of Evaluation

Dear Prof. Perdue:

I am very glad to recommend my student Wentao Li to apply for the IHSS travel support award.

He joined my research group after he graduated with a Bachelor degree in Environmental Engineering, and he started to pursue a PhD degree in 2013.

Over the past two years, he has focused on the characterization of dissolved organic matter (DOM) in water and wastewater treatment process. Fluorescence Excitation-Emission-Matrix (EEM) has been extensively used to characterize fluorescent DOM components and hundreds of related papers were published in the past decades. As demonstrated in the IHSS website, the EEM spectra are also important chemical properties of IHSS samples. However, it is difficult to interpret the complex information in EEM, thus a series of methods have been developed, including ratios of fluorescence, fluorescence index, fluorescence regional integration and parallel factor analysis (PARAFAC).

The highlight of his work is that he firstly introduced the HPLC/HPSEC multi-excitation/emission fluorescence scan for DOM characterization and EEM interpretation. With this interesting method, he investigated the polarity and molecular weight distribution of fluorescent DOM species in surface water, municipal wastewater and dye wastewater. Especially, his work corrected the traditional inappropriate interpretations of EEM. On the 2nd international workshop about organic matter spectroscopy, he made a comparison between the popular EEM-PARAFAC and Chromatography Multi-Excitation/Emission Scan, which were well received by the participants.

The 16th IHSS conference (2012, Hangzhou, China) is the first international conference that he attended. On the conference, he learned the scientific frontiers of HS/NOM study. He also wrote letters to the famous IHSS professors to ask some questions. It is the IHSS conference that excited his research interests and made him dedicated to pursue a PhD degree. Frankly, the IHSS conference is another important mentor for him.

Another IHSS conference is approaching. I hope he could get the travel support award and meet old friends again. The honor will encourage him to make more progress on the HS/NOM study.

Sincerely yours

Aimin Li
School of the Environment, Nanjing University

Characterization of dissolved organic matter in natural water using HPLC/HPSEC coupled with UV absorbance and multi-excitation/emission fluorescence scan

Wentao Li, Aimin Li*
State Key Laboratory of Pollution Control and Resources Reuse, Collaborative Innovation Center for Advanced Water Pollution Control Technology and Equipment, School of the Environment, Nanjing University, Nanjing, 210023, China
* Corresponding author e-mail: liaimin@nju.edu.cn

Keywords: dissolved organic matter, polarity, molecular size, humic substances

Abstract Dissolved organic matter (DOM) in surface water was characterized by reversed-phase high performance liquid chromatography (RP-HPLC) and high performance size exclusion chromatography (HPSEC) coupled with UV absorbance and multi-excitation/emission fluorescence scan. In the investigated water samples, there were three major fluorescent DOM species fractionated by their differences in polarity and molecular size, which also mainly contributed to the UV_{254} absorbance. The humic-like fluorophores were always eluted together with the protein-like fluorophores, further confirming that the spectrally independent humic-like and protein-like PARAFAC components might not exist independently. By comparison of fluorescence intensity, the polarity and molecular size of fluorescent DOM species in RP-HPLC and HPSEC chromatograms were further related together. The results in this study contribute to a better understanding of the fluorescent DOM in natural water, and have important implications for estimation of their behaviour in water treatment processes.

Introduction

Dissolved organic matter (DOM) in natural water, including humic substances and proteins, plays an important role in water photochemistry, nutrient availability, biological activity, contaminant transport and finally global warming.[1] In water treatment processes, the composition and characteristics of DOM also substantially affect coagulation efficiency, membrane fouling, oxidants demand and formation of disinfection byproducts (DBPs).[2] Therefore, characterization of DOM is very important for improving treatment efficiency as well as control of DBPs formation.

Fluorescence excitation emission matrix (EEM) spectroscopy has been extensively applied for frequent characterization of fluorescent DOM in both natural and engineered water systems.[2] Among a series of mathematical methods for EEM interpretation, parallel factor analysis (PARAFAC) is a multivariate analysis technique, which can decompose the overlapped fluorescence into spectrally independent components.[3] Researchers often relate the location and shape of PARAFAC components to previously identified components for validation of their results, and a general consensus has been reached that there are two major classifications of fluorescent DOM components, i.e., humic-like and protein-like components.[2] Compared with protein-like (tyrosine or tryptophan-like) components, humic-like components exhibit more temporal or spatial variability, which could be further classified by possible origin (e.g., terrestrial and marine) or EEM location (e.g., Peak A, C and M).[4]

However, notice that the fluorescence characteristics of DOM might be mainly decided by minimal fluorescent structures. The polarity and molecular size are important properties of the bulk DOM molecules, which can affect their behavior in both natural and engineered systems. Recently, we introduced reversed-phase high performance liquid chromatography (RP-HPLC) and high performance size exclusion chromatography (HPSEC) coupled with multi-excitation/emission fluorescence scan for EEM interpretation and DOM characterization, which could reflect relationships between DOM's fluorescence spectra and their physicochemical properties.[5] In a comparative study, we also demonstrated that the current EEM-PARAFAC analysis could not reflect the variety of DOM species with similar fluorescence,[6] which might result in the inconsistent behavior and chemical interpretations assigned to the similar-appearing fluorescent components across studies.[2, 7] Therefore it is important to study the fluorescent DOM species in combination with their polarity and molecular size.

In this study, we firstly report an investigation of surface water with chromatography multi-excitation/emission scan, which focuses on identifying the major fluorescent species, reflecting relationships between protein-like and humic-like fluorophores, and relating UV absorbance, fluorescence, polarity and molecular weight together.

Experimental

Tongyu River, which runs from south to north and across watersheds of Huai River and Yangzi River in China, serves as the important drinking water sources for the north cities of Jiangsu Province. Herein water samples were collected from the Tongyu River across

eight cities. The detailed sampling information is not shown here.

The fluorescence EEM analysis was conducted on a Hitachi F-7000 spectrofluorometer. Water samples were further analysed by the Agilent 1200 LC systems coupled with UV absorbance and fluorescence detectors. For the RP-HPLC application, the Eclipse XDB-C18 column (150×4.6 mm, 5 μm) was applied. The mobile phase was a mixture of acetonitrile and ammonium acetate solution (10 mmol) using modified elution procedure. The HPSEC operation and molecular weight (MW) calibration was according to our previous demonstration.[5] According to results of EEM, FLD parameters were setup as Em=340/Ex=220~300 or Em=430/Ex=220~400 for multi-excitation scan of protein-like or humic-like fluorophores, respectively. HPLC or HSEC multi-emission scan was setup as Ex= 230/Em=300 ~ 500 nm. The UV absorbance spectra from 200 to 300 nm were also conducted in tandem with the fluorescence scan for each sample.

Results and Discussion

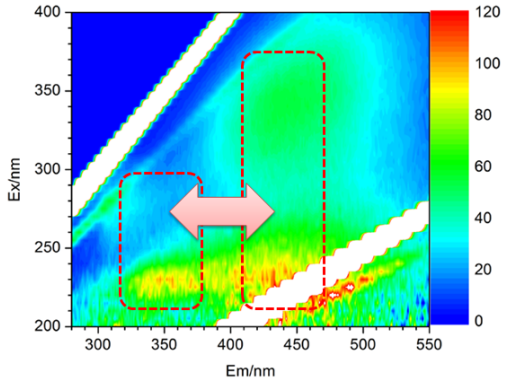

Figure 1. The typical EEM spectra of water samples

Identification of fluorescent DOM

The typical EEM fluorescence spectra are given in the [Figure 1]. Four major peaks were found with the peak-picking function of the instrument FL solution software, which are roughly at Ex230/Em340, Ex280/Em330, Ex240/Em430 and Ex340/Em435 nm. Despite minimal shifts in peaks' locations, similar EEM spectra have also been reported in literature.[8] Generally, fluorescence peaks with Em>370 nm represent the humic-like fluorophores, whereas the fluorescence peaks with Em<370 nm represent the protein-like fluorophores. Inferred from the RP-HPLC multi-excitation scan at Em340 nm or Em430 nm (not shown here), both protein-like and humic-like components display dual-excitation peaks, which is well consistent with our previous demonstration. The multi-excitation phenomena of fluorophores can be elucidated by the rapid internal conversion of excited electrons to the lowest vibration level of the first excited state.[9] Based on the multi-excitation property, all fluorophores can be reflected by chromatography multi-emission scan from 300 nm to 500 nm at Ex230 nm.

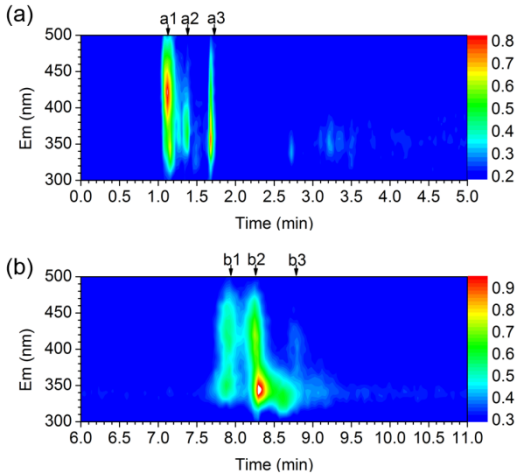

Figure 2 Emisstion-time-maps of (a) HPLC and (b) HPSEC.

The typical RP-HPLC emission-time-map is shown in [Figure 2-a]. There were mainly three fluorescent DOM species with elution time roughly at 1.2 min (Peak a1), 1.4 min (Peak a2), 1.8 min (Peak a3), respectively. With C-18 column, the relatively hydrophilic species will be eluted with shorter retention time. According to the elution procedure applied in this study (not shown here), all the fluorescent DOM species were quickly eluted with acetonitrile content lower than 35%, indicating that they are quite hydrophilic. In the typical HPSEC emission-time-map [Figure 2-b], there were also three major fluorescent species with elution time roughly at 8.0 min (Peak b1), 8.4 min (Peak b2), 9.0 min (Peak b3), respectively. With size exclusion column, DOM species with shorter retention times are of higher molecular size. Inferred from the calibration kit, the apparent molecular weight distribution of such fluorescent DOM were as the following: Peak b1 over than 21.3 kDa, Peak b2 about 21.3 kDa and Peak b3 around 12.3 kDa. In summary, three major fluorescent DOM species were identified by both HPLC and HPSEC, despite the spatial variation of sampling sites in Tongyu River.

Relationships between humic-like and protein-like fluorescence

The current PARAFAC analysis prefers a large EEM dataset to mathematically decompose EEM spectra into spectrally independent fluorescent components,[2] whereas the HPLC or HPSEC multi-emission scan separates the fluorescent DOM species in each sample according to their differences in polarity or molecular size.[6] In both HPLC and HPSEC emission-time-maps [Figure 2], it is worth noticing that all the humic-like fluorescence (Em>370 nm) were simultaneously eluted with the protein-like fluorescence (Em<370 nm), that is, each of the major fluorescent DOM species contained both humic-like and protein-like fluorophores. Similar phenomena were also observed in the humic-like DOM species in municipal effluents.[6] Such phenomena might arise from the relatively strong interaction between humic-like and protein-like fluorophores, which could not be

further separated by C-18 and size exclusion columns. However, humic substances are complex and heterogeneous supramolecules with a high content of aromatic structure. It is well known that tryptophan and tyrosine are the major fluorescent amino acids that result in related protein-like fluorescence. And aniline is the critical fluorescent structure of tryptophan, similar to the function of phenol to tyrosine. In addition, lignin phenols have been demonstrated with protein-like fluorescence.[10] Because proteins, plant lignin and its transformation products are important precursors taking part in humification process,[11] it is more likely that the humic supramolecules directly contain the derived structure of phenol or aniline. The results in this study further confirmed that the spectrally independent humic-like and protein-like PARAFAC components might not exist independently. Additionally, there were some independent protein-like species with minimal fluorescence, which are not combined with humic-like fluorophores [Figure 2].

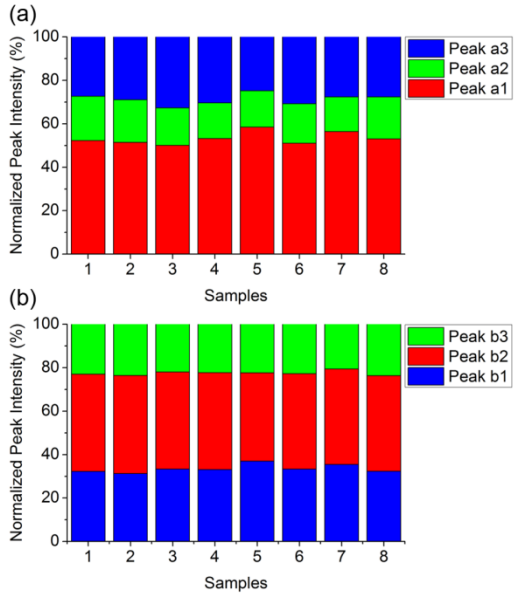

Figure 3. The normalized intensity of fluorescence peaks extracted from (a) HPLC and (b) HPSEC.

Relating UV absorbance, fluorescence, polarity and molecular size together

The polarity and molecular size of fluorescent DOM species could be related together via comparison of fluorescence peaks' intensity between RP-HPLC and HPSEC fingerprints. To avoid interference from some independent protein-like species, the peaks' intensity at Em430 nm was extracted from all emission-time-maps to represent the major fluorescent DOM species [Figure 3]. Despite spatial variation, the normalized percentage of fluorescence peaks didn't vary significantly. In HPLC results, Peak a1 contributed the largest portion to total humic-like fluorescence (50%~57%); Peak a2 and a3 accounted for 16%~20% and 23%~33%, respectively. Whereas in HPSEC results, Peak b2 was responsible for 41%~50%; Peak b1 and Peak b3 took up 29%~37% and 21%~24%, respectively. Assuming that the fluorescence peaks represent tightly associated DOM species that no rearrangement occurred during separation, by comparison of their normalized percentage, it is obvious that the relationships between HPLC and HPSEC fluorescence peaks are as the following: Peak a1 related to Peak b2 (DOM a1-b2), Peak a2 related to Peak b3 (DOM a2-b3), and Peak a3 related to Peak b1 (DOM a3-b1). The results also indicate that the contribution of DOM species to the total humic-like fluorescence in EEM are in the order of DOM a1-b2 > DOM a3-b1> DOM a2-b3.

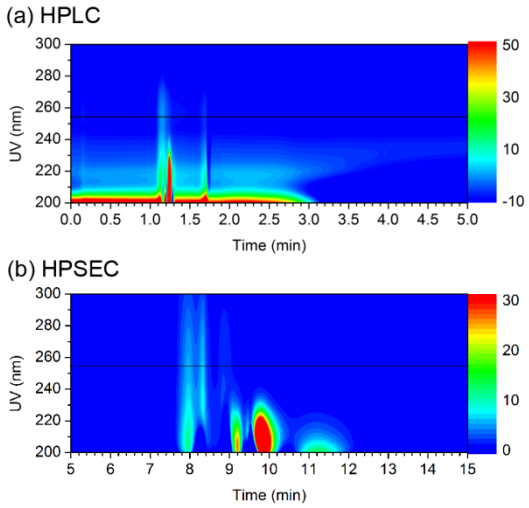

Figure 4. (a) HPLC UV absorbance spectra and (b) HPSEC UV absorbance spectra.

The UV absorbance spectra have also been used for frequent characterization of DOM. However, due to the high heterogeneity, the UV absorbance spectra of DOM are quite featureless. Herein some phenomenological parameters (e.g., adsorption coefficient, absorption ratio or spectral slope ratio at certain wavelengths) have been applied for estimation of DOM's properties.[12] Especially, the UV absorbance at 254 nm (UV254) is often used as an indicator for DOM's concentration and aromaticity. To identify the relationship between UV absorbance and fluorescence spectra, the HPLC or HPSEC UV absorbance spectra were conducted in tandem with fluorescence scan for each sample (Figure 4). By comparison of the corresponding UV absorbance and fluorescence spectra (Figure 4-a vs Figure 2-a; Figure 4-b vs Figure 2-b), it is obviously that the three major fluorescent DOM species mainly contributed to the UV254 absorbance. Additionally, the non-fluorescence DOM species showed UV absorbance lower than 240 nm and had apparent molecular weight lower than 12.3 kDa.

Environmental implications

Knowledge of DOM's physicochemical properties is of great importance to estimate their behaviour in both natural and engineered systems. DOM a1-b2 and DOM a3-b1 exhibited similar humic-like fluorescence spectra, suggesting that the humic-like peaks in EEM could be further separated into different species. Compared with municipal effluents,

the surface water in this study contained relatively few independent protein-like fluorophores. The independent and combined protein-like fluorophores are doomed to behave differently in natural and engineered systems. Additionally, the combination of protein-like and humic like fluorophores might indicate their synchronous behaviour in physical treatment processes, such as coagulation, membrane filtration, anion exchange and adsorption. Although EEM spectra have been extensively applied for characterization of DOM in natural water bodies, few works have addressed these factors when interpreting EEM spectra. The HPLC and HPSEC fluorescence fingerprints display that the composition and concentration of fluorescent DOM were quite similar across samples from Tongyu River, indicating that similar water treatment process could be applied to the investigated drinking water sources. The UV_{254} absorbance and EEM analysis have been applied for estimation of DBPs formation. Similar to municipal effluents,[6] the intrinsic relationships could be elucidated as that the fluorescent DOM species containing humic-like fluorophores contributed to the major UV_{254} in surface water. This study further demonstrates that the chromatography multi-excitation/emission scan could be an informative tool for frequent characterization of DOM in surface water.

References

[1] K.G. Mostofa, C.-q. Liu, M.A. Mottaleb, G. Wan, H. Ogawa, D. Vione, T. Yoshioka, F. Wu, Dissolved Organic Matter in Natural Waters, in: K.M.G. Mostofa, T. Yoshioka, A. Mottaleb, D. Vione (Eds.) Photobiogeochemistry of Organic Matter, Springer Berlin Heidelberg, 2013, pp. 1-137.
[2] S.K.L. Ishii, T.H. Boyer, Behavior of Reoccurring PARAFAC Components in Fluorescent Dissolved Organic Matter in Natural and Engineered Systems: A Critical Review, Environ. Sci. Technol. 46 (2012) 2006-2017.
[3] C.A. Stedmon, R. Bro, Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial, Limnol. Oceanogr.:Methods 6 (2008) 572-579.
[4] P.G. Coble, Marine Optical Biogeochemistry: The Chemistry of Ocean Color, Chem. Rev. 107 (2007) 402-418.
[5] W.-T. Li, Z.-X. Xu, A.-M. Li, W. Wu, Q. Zhou, J.-N. Wang, HPLC/HPSEC-FLD with multi-excitation/emission scan for EEM interpretation and dissolved organic matter analysis, Water Res. 47 (2013) 1246-1256.
[6] W.-T. Li, S.-Y. Chen, Z.-X. Xu, Y. Li, C.-D. Shuang, A.-M. Li, Characterization of Dissolved Organic Matter in Municipal Wastewater Using Fluorescence PARAFAC Analysis and Chromatography Multi-Excitation/Emission Scan: A Comparative Study, Environ. Sci. Technol. (2014).
[7] K.R. Murphy, A. Hambly, S. Singh, R.K. Henderson, A. Baker, R. Stuetz, S.J. Khan, Organic Matter Fluorescence in Municipal Water Recycling Schemes: Toward a Unified PARAFAC Model, Environ. Sci. Technol. 45 (2011) 2909-2916.
[8] D.L. Gone, J.-L. Seidel, C. Batiot, K. Bamory, R. Ligban, J. Biemi, Using fluorescence spectroscopy EEM to evaluate the efficiency of organic matter removal during coagulation–flocculation of a tropical surface water (Agbo reservoir), J. Hazard. Mater. 172 (2009) 693-699.
[9] J.R. Lakowicz, Principles of Fluorescence Spectroscopy, 2nd ed., Springer Science, New York, 2006.
[10] P.J. Hernes, B.A. Bergamaschi, R.S. Eckard, R.G.M. Spencer, Fluorescence-based proxies for lignin in freshwater dissolved organic matter, J. Geophys. Res. 114 (2009) G00F03.
[11] IHSS, International Humic Substance Society, in, International Humic Substances Society
[12] M. Yan, G. Korshin, D. Wang, Z. Cai, Characterization of dissolved organic matter using high-performance liquid chromatography (HPLC)-size exclusion chromatography (SEC) with a multiple wavelength absorbance detector, Chemosphere 87 (2012) 879-885.

Acknowledgments: We gratefully thank generous support provided by Program for Changjiang Scholars and Innovative Research Team in University, National Science Foundation of China (51178215, 51290282 and 51308283) and Jiangsu Nature Science Fund for Distinguished Scientists (BK2011032), P. R. China.