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Molecular characterization of macrophyte-derived dissolved organic matters and their implications for lakes

Shasha Liu^{a,b}, Tianhui Zhao^b, Yuanrong Zhu^b, Xiaoxia Qu^b, Zhongqi He^c, John P. Giesy^d, Wei Meng^{a,b,*}

^a College of Environmental Science and Engineering, Ocean University of China, Qingdao 266100, China

^b State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Science, Beijing 100012, China

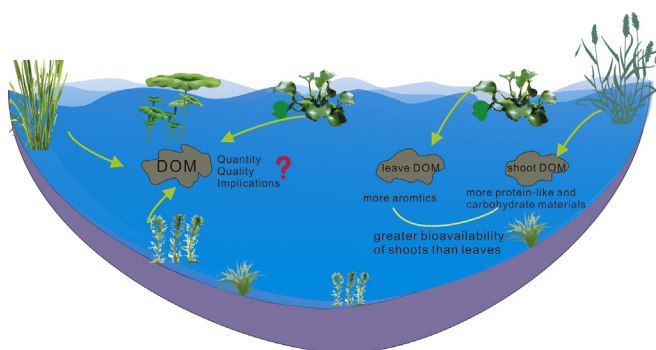
^c USDA-ARS Southern Regional Research Center, 1100 Robert E Lee Blvd, New Orleans, LA 70124, USA

^d Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5B3, Canada

HIGHLIGHTS

- Multiple spectroscopy methods were combined to characterize macrophyte-derived DOM.
- Floating and submerged plants have greater potential to accumulate and return N and P.
- Macrophyte-derived DOM has less aromatic than natural waters and soil leachates.
- Shoots have more protein-like and carbohydrate materials, while leaves are rich in aromatics.
- Nutrients released from macrophytes should pay much attention in P regulation.

GRAPHICAL ABSTRACT



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ABSTRACT

Chemical properties of whole organic matter (OM) and its dissolved organic matter (DOM) extracted from three types of dominant macrophytes in Lake Dianchi were comparatively characterized using elemental analysis, UV, 3D-EEM and ¹³C NMR spectroscopy and their implications for lakes were discussed. Ratios of C/N and C/P were least in the floating water hyacinth and submerged sago pondweed, while total dissolved nitrogen (TDN) and phosphorus (TDP), dissolved organic nitrogen (DON) and phosphorus (DOP) were greatest in those species. In emergent species, C/N, C/P, DON and DON/TDN were less in leaves than in their corresponding shoots. The specific UV absorbance at 254 nm (SUVA₂₅₄) and 280 nm (SUVA₂₈₀) of extracts were in the range of 0.50–1.96 L/mg C · m and 0.40–1.48 L/mg C · m. Both SUVA values were greater in leaves than those in shoots. 3D-EEM spectra showed only a single fulvic-like fluorescence in leaves of emergent macrophytes. In contrast, protein-like peak were observed in spectra of floating and submerged species, as well as the shoot DOM of emergent species. Solid-state ¹³C NMR demonstrated that leaves had greater percentage of recalcitrant alkyl C and aromatic C, while shoots were rich in labile carbohydrates. The overall characterization works suggested that macrophyte-derived DOM has less aromatic constituents than do DOM in natural waters and soil leachates. Also OM and DOM derived from shoots had greater contents of protein-like and carbohydrate materials, while leaves were rich in aromatics. Floating and submerged plants possessed potential to not only accumulate excess N and P, but also for returning them to the lake. Shoots of riparian and emergent species were also an important source of nutrients. Thus, macrophyte biomass should be a great concern in nutrient regulation in Lake Dianchi.

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* Corresponding author at: College of Environmental Science and Engineering, Ocean University of China, Qingdao 266100, China.

E-mail address: mengweicraes@126.com (W. Meng).

1. Introduction

Organic matter (OM) is an important component in the biogeochemical cycles of terrestrial and aquatic ecosystems (Wu and Xing, 2009). It is the major constituent in cycling of elements and flow of energy through ecosystems, and a primary factor influencing biochemical compositions of soils and/or sediments. OM serves as a substrate for binding pollutants, and is closely related to the carbon cycle and global warming (Wu and Xing, 2009). Thus, OM in both terrestrial and aquatic ecosystems is a significant area of research in biogeochemistry.

Dissolved organic matter (DOM) is the most easily released and reactive component of OM, and plays an important ecological role especially in aquatic systems (He and Zhang, 2015). Chemical composition of DOM is a key factor in determining its fate and environmental behaviors in lakes, such as reactions with pollutants, rates of degradation and turnover, and humification (Oades, 1988; Wu and Xing, 2009). Chemical properties of allochthonous DOM composed of humic substances have been more thoroughly studied (He et al., 2006; Kögel-Knabner, 1997; Senesi et al., 2003). However, limited research has focused on non-humic DOM freshly released from autochthonous plants, which is characterized by lesser molecular-weight organic matter (Liu et al., 2016; Zhang et al., 2013).

In lacustrine ecosystems, OM materials are mainly derived from aquatic macrophytes, phytoplanktons, bacteria, and terrestrial inputs from riparian plants or soils (Croué et al., 2003). As one of the primary producers in lake systems, aquatic macrophytes are major autochthonous sources of OM to most lakes, but are more important in shallow lakes with well-developed littoral zones. Macrophyte OM includes a portion that is more easily dissolved and another fraction that is not soluble in water but participates in biogeochemical cycles of lakes after being decomposed by microbes. In aquatic systems, DOM reacts with inorganic and organic substances more frequently and faster than DOM in terrestrial systems (Findlay and Sinsabaugh, 2003). Thus, the types, quantities and possible environmental behaviors of DOM released by autochthonous macrophytes in lakes needed to be addressed.

For better understanding of the quantities and features of DOM released by autochthonous macrophytes, in this study, one riparian species, three emergent macrophytes, one floating species and one submerged species were collected from Lake Dianchi. OM and DOM fractions were characterized by use of ultraviolet-visible (UV-vis), three-dimensional excitation-emission fluorescence matrix (3D-EEM), and solid-state ^{13}C nuclear magnetic resonance (^{13}C NMR) spectroscopic analysis. The objectives of this study were to: 1) characterize OM and DOM from macrophytes of different life forms and also the shoots and leaves of emergent and riparian species; 2) explore relationships between the chemical properties of macrophyte-derived OM and DOM on both qualitative and quantitative aspects; 3) estimate the ratios of N and P derived from macrophytes to total N or P in lake water; and 4) further discuss the degradation of OM/DOM from different species and possible environmental implications on contaminants based on their chemical compositions.

2. Materials and methods

2.1. Study site and sample collection

Lake Dianchi is located in the southwestern plateau of China ($24^{\circ}40' \sim 25^{\circ}02' \text{ N}$, $102^{\circ}36' \sim 102^{\circ}47' \text{ E}$) (Fig. 1). The lake is at an elevation of 1886 m and has a surface area of 330 km². The volume of the lake is approximately $1.2 \times 10^{11} \text{ m}^3$. The mean and maximum depths are 4.4 m and 15.1 m, respectively. The whole lake is divided into two parts: the Caohai section in the north and the Waihai section in the south by the natural dike in northeast of the lake. Dianchi has a subtropical plateau monsoon climate. Vegetation around Lake Dianchi has the typical characteristics of sub-tropical evergreen broad-leaved forest. Annual precipitation is 1036 mm, which is concentrated in the latter half of the year.

Water temperature ranges from 9.8 °C to 24.5 °C, with an annual average of about 16.0 °C. Lake Dianchi is adjacent to Kunming City, the capital of Yunnan Province. Therefore, large quantities of industrial wastewater and municipal sewage have been discharged into the lake. Consequently, a significant amount of nitrogen and phosphorus nutrients enter the lake from the catchment. At present, the lake water is heavily polluted and suffers from eutrophication (Li et al., 2002; Liu et al., 2015). According to data publicly available from the Dianchi management administration, in 2010, concentrations of total phosphorus (TP) and total nitrogen (TN) were 0.28 and 1.06 mg/L, respectively (Table S1).

The distribution of aquatic plant communities in the lake is extremely uneven, and the majority of aquatic plants are concentrated along the coast. Dominant aquatic plants are emergent and submerged plants in the littoral zone. Total biomass of aquatic plants in Dianchi reached up to 41,019.65 Mg (Yu et al., 2000). Based on the dominant species of aquatic plants reported previously (Zhou et al., 2013; Song et al., 2011), six species of macrophytes including riparian, emergent, floating, and submerged types were collected at their maturation stage, from the Waihai section, during October 2011 (Fig. 1). They were one riparian species—crofton weed (*Eupatorium adenophorum* Spreng., Asteraceae), three emergent macrophytes—water oats (*Zizania caduciflora* Turcz., Gramineae), common reed (*Phragmites australis* Trin., Gramineae) and oriental pepper (*Polygonum orientale* L., Polygonaceae), one floating species—water hyacinth (*Eichhornia crassipes* (Mart.) Solms, Pontederiaceae) and one submerged species—sago pondweed (*Potamogeton pectinatus* L., Potamogetonaceae). At least five specimens of each species were collected. In the laboratory, leaves and shoots were separated and washed with deionized water, then killed by heating at 90 °C for 1–2 h and followed by 12–24 h of complete drying at 60 °C. Dry materials were then ground to pass through a 1-mm sieve.

2.2. Extraction of DOM

DOM was extracted by water (ultra-pure, mili-Q) with a 35:1 (ml/g) water to sample mass ratio (He et al., 2009). The suspension was then shaken at 4 °C for 18 h (Brown and Sposito, 1991). After centrifugation for 20 min, extracts were filtered under vacuum through 0.45- μm pore size polycarbonate filters (He et al., 2009). Aliquots of the supernatant were used directly for quantification of carbon (C), nitrogen (N), phosphorus (P) and measurement of UV-vis and 3D-EEM spectra. The remaining supernatant was freeze-dried and kept in a desiccator at room temperature for solid-state ^{13}C NMR analysis.

2.3. Chemical analysis

Before extraction of DOM, total carbon (TC), total nitrogen (TN) and hydrogen (H) in ground dry materials were determined using an elemental analyzer (Elementar vario macro EL, Germany) following an in situ HCl acidification procedure (Nieuwenhuize et al., 1994). Total phosphorus (TP) in ground dry samples was determined by methods described previously (Pardo et al., 2003). In plant extracts, concentrations of total dissolved carbon (TDC), dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were determined by a multi N/C 3100 analyzer (Analytic Jena, German). $\text{NH}_4\text{-N}$ in extracts was measured by Nessler's reagent photometry (Demutskaya and Kalinichenko, 2010). $\text{NO}_3\text{-N}$ in extracts was quantified by use of a UV-vis spectrophotometer at 220 nm and corrected at 275 nm (Agilent Technologies, Wilmington, DE, USA). Dissolved organic nitrogen (DON) in plant extracts was then determined by differences between TDN and the sum of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$. Total dissolved P (TDP) (after digestion with potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$) in an autoclave at 121 °C for 30 min) and total dissolved inorganic P (DIP) in plant extracts were determined by use of molybdenum blue method (Murphy and Riley, 1962). Concentrations of dissolved organic P (DOP) were then determined as the difference between TDP and DIP. Triplicate samples of each species were used in all tests.

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