Dissolved Organic Matter Contains Previously Unidentified Protein-like Fluorophores in Old Woman Creek

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Introduction

Dissolved organic matter (DOM) constitutes a significant carbon pool in the global carbon cycle and is influential in many other processes e.g., altering the fate of contaminants, acting as redox and pH buffers, etc. DOM in the aqueous environment originates from sources ranging from autochthonous (microbial) to allochthonous (terrestrial) precursors. Lake Fryxell Fulvic Acid (LFFA) and Pony Lake Fulvic Acid (PLFA) represent Antarctica derived microbial end-member standards and Suwannee River Fulvic Acid (SRFA) represents a terrestrial end-member standard (origin in the Okeefenokee Swamp, GA). McKnight et al. (2001) developed a fluorescence index (FI) to determine DOM precursor material. The FI for the corrected spectra is calculated by determining the ratio between emission intensities at 470nm and 520nm at an excitation wavelength of 370nm. The FI for the uncorrected spectra is calculated by determining the ratio between emission intensities 450nm and 500nm at an excitation wavelength of 370nm. McKnight et al., (2001) noted that microbial DOM has more sharply defined intensity maxima at lower excitation and emission wavelengths.

The isolation of DOM from natural waters is a common practice to preserve and concentrate DOM, yet the extraction method may significantly alter its composition. This study explores the characteristics of DOM collected from Old Woman Creek (OWC) National Estuarine Research Reserve, located in Huron, Ohio, isolated using PPL solid phase extraction (SPE). Previous DOM extraction methods such as C-18 or XAD-8 are limited to hydrophobic compounds, while the novel PPL SPE cartridges can capture more polar and non-polar components. PPL and previously collected XAD-8 isolated OWC DOM was characterized using fluorescence spectroscopy. Fluorescence analysis revealed protein-like components not present in previously collected XAD-8 isolated OWC DOM, demonstrating the significance of extraction method on the composition and potential reactivity of isolated DOM.

Methods

- **Extraction and Isolation**
  - *In situ* filtration via Balston filter (0.5 µM)
  - Acidify the sample to pH 2-3 with HCl
  - Gravity filtration through styrene divinyl benzene polymer type sorbents (PPL) cartridges (Dittmar et al., 2008)
  - Clean with one cartridge volume of 0.01 N HCl
  - Elute DOM with one cartridge volume of methanol
  - Concentrate DOM with Rotavapor and then freeze dry
- **Fluorescence Spectroscopy**
  - Put DOM into solution (0.007g DOM in 200ml of Milli-Q water)
  - Run check scans on Varian Cary Eclipse Fluorescence spectrophotometer
  - Check the absorbance at 254nm on UV-vis spectrophotometer
  - Dilute to below 0.05 absorbance
  - Scan dilute sample with excitation 240-450nm every 5nm and emission from 300-600nm every 2nm (Agrawal, 2009)
- **Excitation Emission Matrices (EEMs) Correction**
  - Correct samples with manufacturer-provided instrument correction factors and subtraction of Milli-Q blank with Matlab
  - Plot corrected data on SigmaPlot (Fig. 2-5)*
  - Calculate Fluorescence Index (Table 1)

*Note the varying intensity spectra for each graph.

Figure 1: Aerial view of Old Woman Creek. National Estuarine Research Reserve

Fluorescence Spectroscopy Results

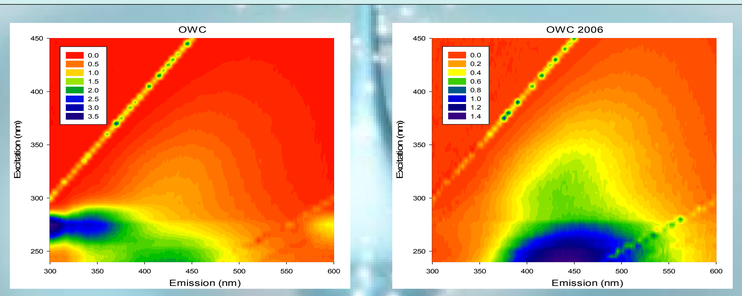

Figure 2&3: Old Woman Creek corrected EEMs from 2012 (left) and 2006 (right). Note high microbial peaks around 300nm and 350nm emission at 275nm excitation in the 2012 results.

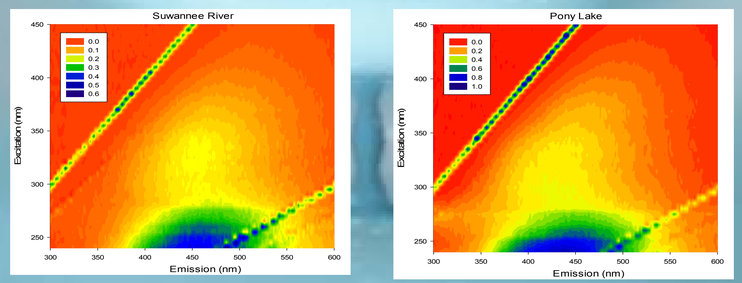

Figure 4&5: Corrected EEMs for the two end-member DOM standards: terrestrial Suwannee River Fulvic Acid (left) and microbial Pony Lake Fulvic Acid (right).

	This Study		McKnight et al. (2001)		Cory et al. (2010)	
Old Woman Creek (2012)	1.47	1.41	-	-	-	-
Old Woman Creek (2006)	1.24	1.40	-	-	-	-
Suwannee River Fulvic Acid	1.10	1.29	1.40	-	1.17-1.37	1.21-1.28
Pony Lake Fulvic Acid	1.58	1.47	-	-	1.47-1.75	1.45-1.52
Lake Fryxell Fulvic Acid	-	-	1.9	-	1.68	1.55

Table 1: Fluorescence Indices for a range of DOM sources. The left columns are uncorrected FI and the right columns are corrected FI. Cory et al. (2010) compared three different fluorescence spectrophotometers, hence the range of FIs.

Discussion

The Fluorescence Index provides an analysis of the source material of the DOM. McKnight et al. (2001) found that the FI, calculated on uncorrected emission spectra, ranged from 1.4 for allochtonous organic matter (SRFA) to 1.9 for autochthonous organic matter (LFFA). Further, Cory et al. (2010) concluded that while the Fluoromax-3 provided the most accurate FIs for the corrected emission spectra, our Varian Eclipse instrument provided adequate results. The Fluoromax-3 reported a FI of 1.55 for Lake Fryxell, 1.46 for Pony Lake, and 1.21 for Suwannee River. Despite significant variation in FI values between uncorrected and corrected spectra, a trend can be recognized. For microbial end-member references (PLFA and LFFA) the FI was always greater than 1.4 and for terrestrial end-members (SRFA) the FI was always less than 1.4. Old Woman Creek represents an intermediate example, in which the DOM constitutes both microbial and terrestrial sources, with a FI of roughly 1.4. The DOM from OWC that was extracted in 2012 displays a strong microbial peak that isn't seen in the 2006 extracted DOM. This may indicate that the microbial component is hydrophilic and thus not captured by XAD-8. It is also possible that this previously unseen microbial peak may be the result of temporal variability between 2006 and 2012 sample composition rather than an extraction artifact. Also, the DOM from the 2006 extraction came from pore water, while the DOM from 2012 came from surface water. It's unlikely that this was the reason for the microbial peak, but it may have been a contributing factor.

Conclusion

- PPL SPE is able to capture a broader range of DOM components because it collects hydrophilic and hydrophobic fractions.
- The FI provides a qualitative trend that indicates source material of DOM relative to end-member references (PLFA, LFFA, and SRFA).
- OWC represents an ideal intermediate source of DOM.

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