Effects of Reductants on the Fluorescence Spectra of Natural Organic Matter

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Introduction

- Fluorescence Spectroscopy – bulk characterization technique for Natural Organic Matter (NOM)
  - Used for qualitative and quantitative characterization
  - Highly sensitive to the sample conditions
- Thiosulfate (TS) and ascorbic acid (AA) are reductants commonly used to quench oxidation experiments with NOM

Objective

- Analyze fluorescence spectra of NOM with reductants added at a range of concentrations
- Determine absorbance threshold where interference occurs, as well as concentration of preservative for which problems arise
- Make recommendations for preservative use during fluorescence spectra analysis
- Investigate effect of reductants on NOM redox properties with respect to observed fluorescence

NOM Background

- Heterogeneous mixture of organic matter from natural waters, sediments, and soils
- Refractory
- NOM: made of soluble and particulate components (Figure2), affecting:
  - Biogeochemical processes
  - Water quality, contaminant binding, fertility of soil, and carbon cycle
- Composition and function dependent on origin, temperature, ionic strength and composition of cations, pH, microbial activity, and photochemistry
- Concentration expressed as Total Organic Carbon (TOC)

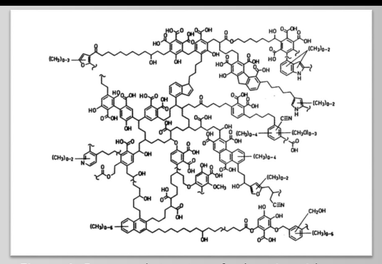

Figure 1: Proposed structure for humic acid fraction of NOM [1]

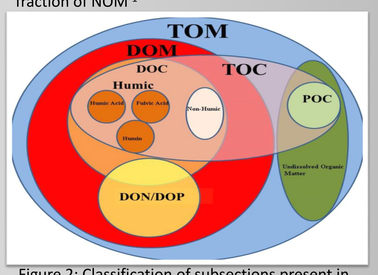

Figure 2: Classification of subsections present in NOM/Total Organic Matter (TOM); Dissolved Organic Nitrogen (DON); Dissolved Organic Phosphorous (DOP); Dissolved Organic Carbon (DOC); Particulate Organic Carbon (POC) [2]

Fluorescence Background

- Absorption of photon -> excitation of electron to higher energy level (Figure 3)
- Relaxation of electron -> emission of fluorescence (fluorophores)
- Composition specific
- Information on redox state, source, and reactivity information for NOM
- EEMs plots: Intensity of energy emitted at particular emission (λ_{EM}) and excitation (λ_{EX}) wavelengths (Figure 4)
- FI assess composition differences
  - $FI=I_{470}/I_{520}$ @ λ_{Ex}=370 nm
  - Microbial FI~1.8
  - Terrestrial FI~1.2
- Peak Picking
  - Humic substance peaks (A&C)
  - Protein/polyphenolic/nitrogen (B&T)

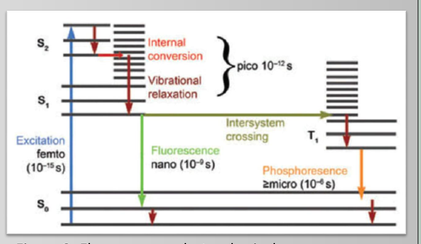

Figure 3: Fluorescence photo-physical phenomena [3]

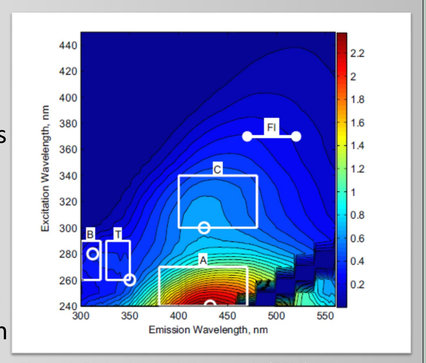

Figure 4. Excitation emission matrix (EEM). Intensity in RU units, Peaks A,B,C and T boxes. FI ratio[4]

Experimental Methods

- **Samples preparation**:
  - 3 mg/L TOC - Suwannee River NOM (SRNOM) 2R101N
  - ~7.5 pH, 10 mM phosphate buffer
  - Reductant concentrations range between 5 and 160 mgAA/L and 5 to 160 mgTS/L in NOM solution (< 2% dilution)
  - Overnight storage at 5˚C
- **Fluorescence Spectroscopy runs:**
  - Jobin Yvon HORIBA FluoroMax – 4
  - λ_{EX} 240 – 450 nm; λ_{EM} 300 – 550 nm
  - 20˚C, duplicates
- **Ultraviolet/visible spectra (UV/Vis)**
  - CARY 100 Bio, VARIAN – 200-800 nm
  - HACH DR 500 – 200- 600 nm
  - TS&NOM, AA&NOM; TS& Distilled Water (DI); AA&DI
- **EEM corrections and analysis in MATLAB®:**
  - Blank subtraction, Raman normalized, instrument correction, light screening correction ; Matlab code
  - Differential EEMs AA&NOM versus AA& DI plots; peaks A, C analysis; FI analysis

General NOM Results

- Control EEM spectra - 0 mg preservative per litter NOM solution (Figure 5)
- Used in order to determine differential fluorescence interference after preservative addition

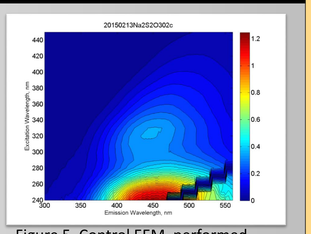

Figure 5. Control EEM, performed under similar experimental conditions as the rest of the samples

NOM + TS Results

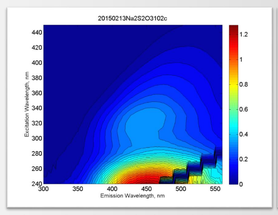

Figure 6. NOM solution with 10 mgTS/L concentration

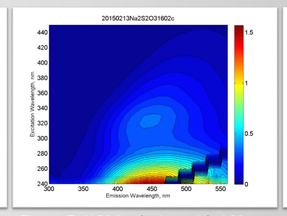

Figure 7. NOM solution with 160 mgTS/L concentration

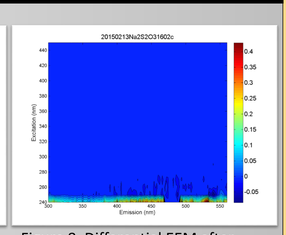

Figure 8. Differential EEM after subtraction of the control EEM from the 160mgTS/L EEM

- The interference observed only affects low λ_{EX} (Figure 8)
- The interference seems to be uniform and independent of λ_{EM}
- FI determined to be statistically similar (p_value=0.74)
- Peak A dependent on TS concentration (Figure 9)
- High absorbance observed (Figure 10) in the UV/Vis Spectra below 270 nm, affecting correction factor:
  - $\frac{F_{corr}}{F_{meas}} = 10^{\frac{A_{EX}+A_{EM}}{2}}$
- Correction factor accounts for light screening

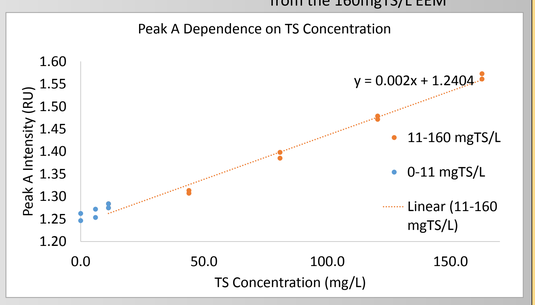

Figure 9. Changes in Peak A intensity with changes in TS concentration

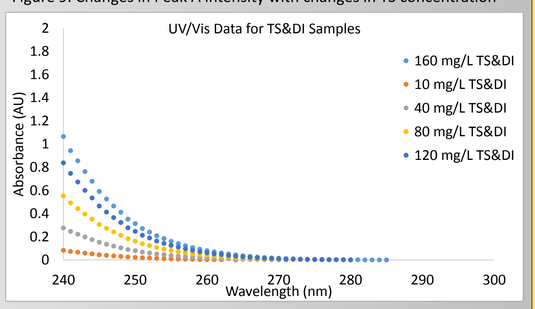

Figure 10. UV/Vis absorbance of TS+DI samples

- 0 to 11 mg/L TS in DI : statistically similar Peak A values (Average=1.3, p_value=0.20)
- 10% screening at =78 mgTS/L
  - $1.27 * 1.1 = 0.002[TS] + 1.2404$

NOM + AA Results

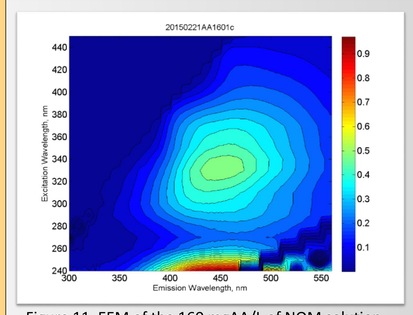

Figure 11. EEM of the 160 mgAA/L of NOM solution

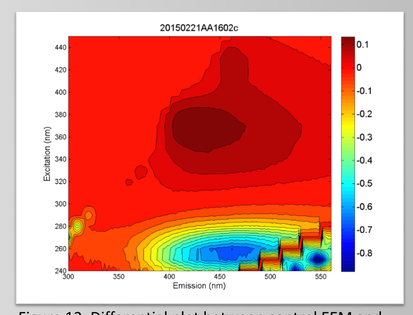

Figure 12. Differential plot between control EEM and the EEM of the 160 mgAA/L of NOM solution

- AA absorbs light between 240 nm and 300 nm
- AA does not fluoresce
- AA presence systematically enhances fluorescence intensity in the Peak C region, and quenches fluorescence intensity of Peak A region (Figure 12)
- FI statistically similar for up to 40 mgAA/L (p=0.15) (Figure 13)
- **Deduced that AA affects the redox properties of NOM which affect fluorescence**

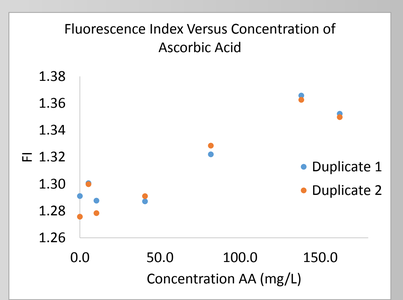

Figure 13. Fluorescence index changes with increase in concentration of AA

Conclusions

- TS and AA are important for oxidant quenching
- During analysis of samples prepared with these preservatives, there is need to consider constraints for fluorescence characterization
- For TS concentrations above 78 mg/L, it is expected to notice 10% screening of light, and along with it strong effects in the EEM plot below 250 nm λ_{EX}
- It is hypothesized that AA can lead to changes in the redox properties of NOM, changing the photo-physics of the sample

Future Work

- Analyze NOM from other sources (different composition)
- Verify all results through the reproduction of the full experiment
- Perform size exclusion chromatography (SEC) for the AA+NOM samples to determine potential size effects due to redox reactions
- Perform Nuclear Magnetic Resonance experiments, to asses transformations in NOM structure after AA addition
- Publish findings, providing concise description of the constraints in oxidant quenching chemicals
- Analyze the fluorescence spectra for additional preservatives

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