FLUORESCENCE TECHNIQUES AND DATA TREATMENT METHODS RELATING THE INTERACTION BETWEEN URANYL ION AND FULVIC ACID AND THEIR ENVIRONMENTAL APPLICATION

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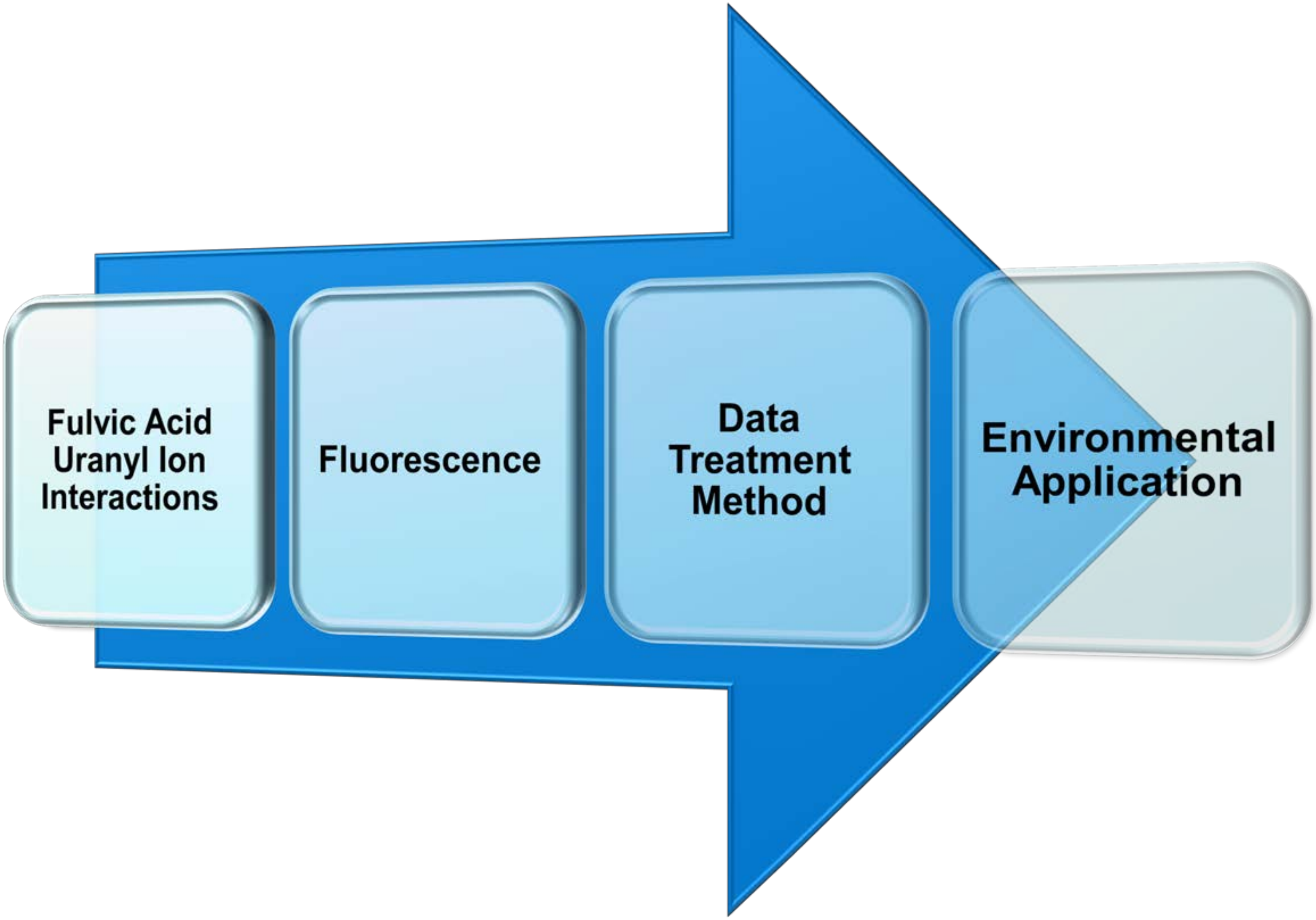

Fulvic Acid
Uranyl Ion
Interactions
Fluorescence
Data
Treatment
Method
Environmental
Application

OUTLINE

Introduction
- Dissolved Organic Matter & Uranium
- Fluorescence, Excitation-Emission Matrix (EEM)

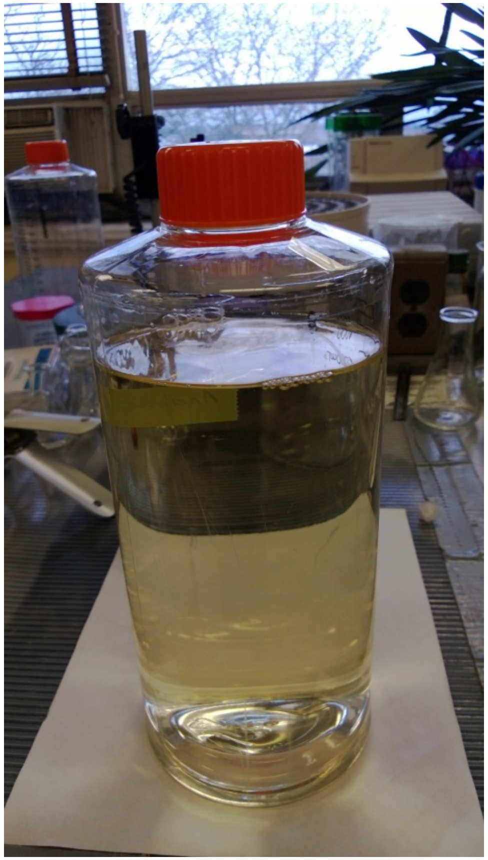
Part One
- Parallel Factor Analysis (PARAFAC)
- Development of Two-Site Model

Part Two
- Regional Integration Analysis (RIA)
- Application of RIA in Fluorescence Quenching Study

Part Three
- Samples from Merrimack River Valley
- Application of RIA in Water Study

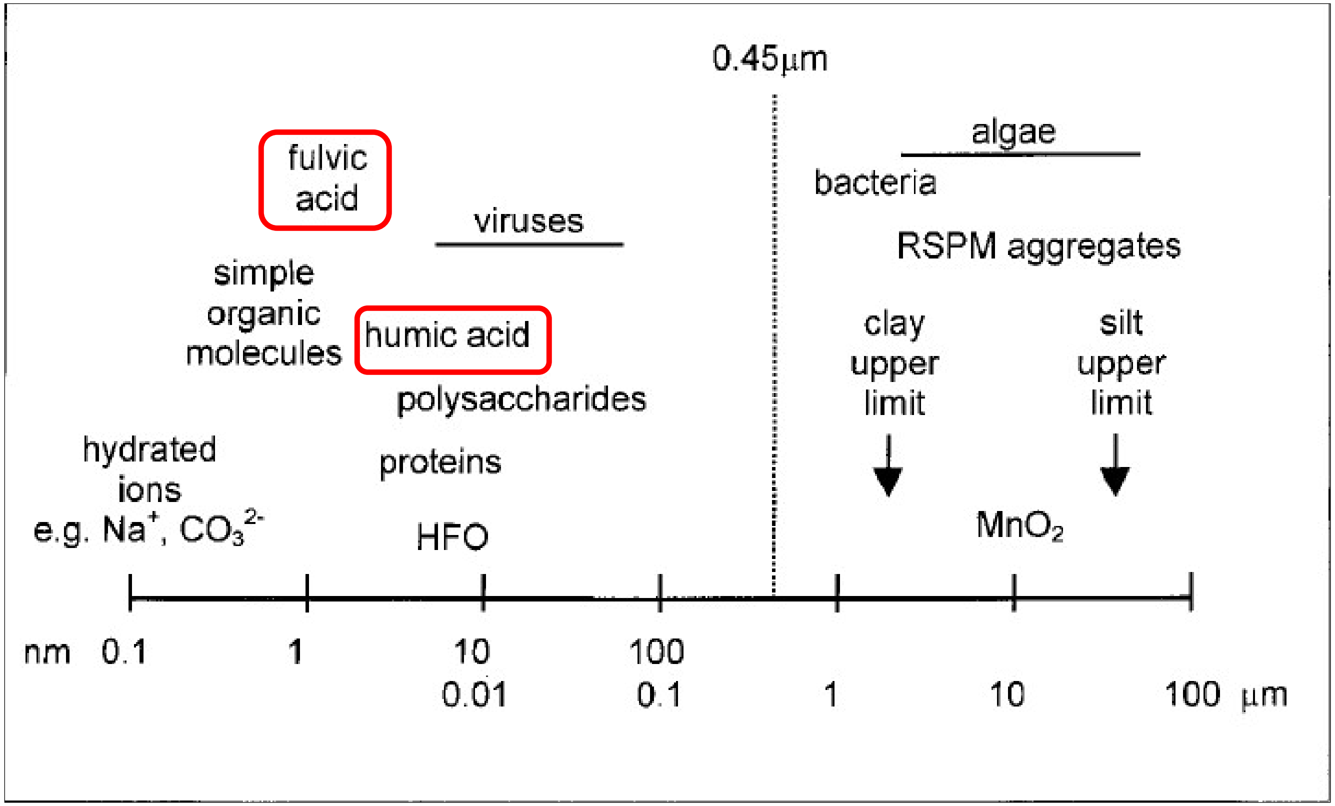
Conclusion
- Conclusion
- Future Work

Dissolved Organic Matter (DOM)



(Left) Pawtucket Falls at Merrimack River, Lowell, MA
(Right) Merrimack River Water

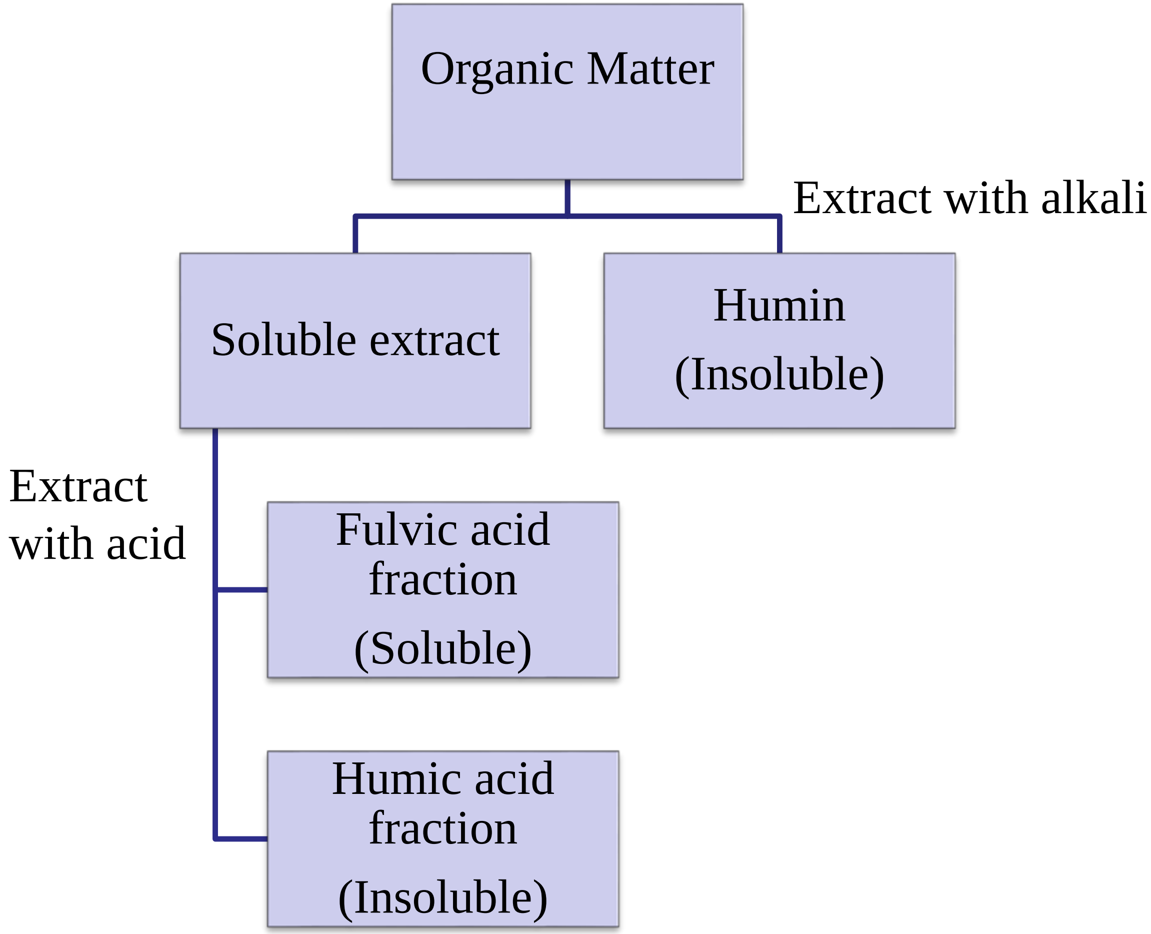
Dissolved Organic Matter



Approximate size of chemical and biological components of natural water

Tipping, E., *Cation binding by humic substances*. Cambridge University Press: 2002

Dissolved Organic Matter

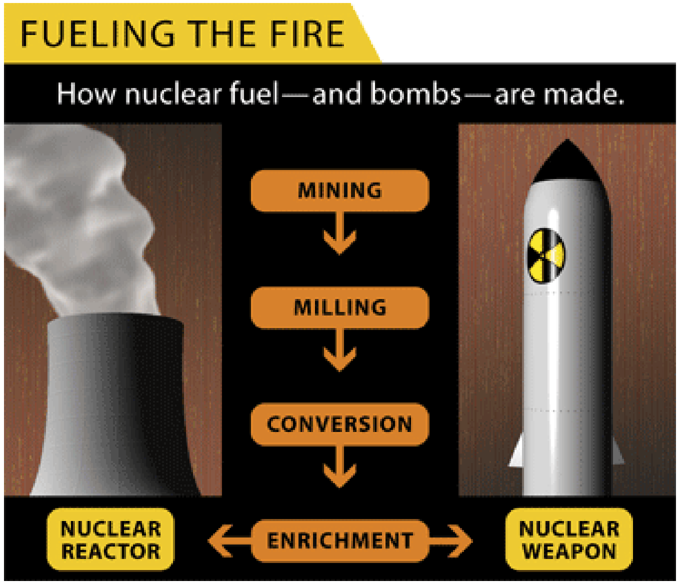


Dissolved Organic Matter

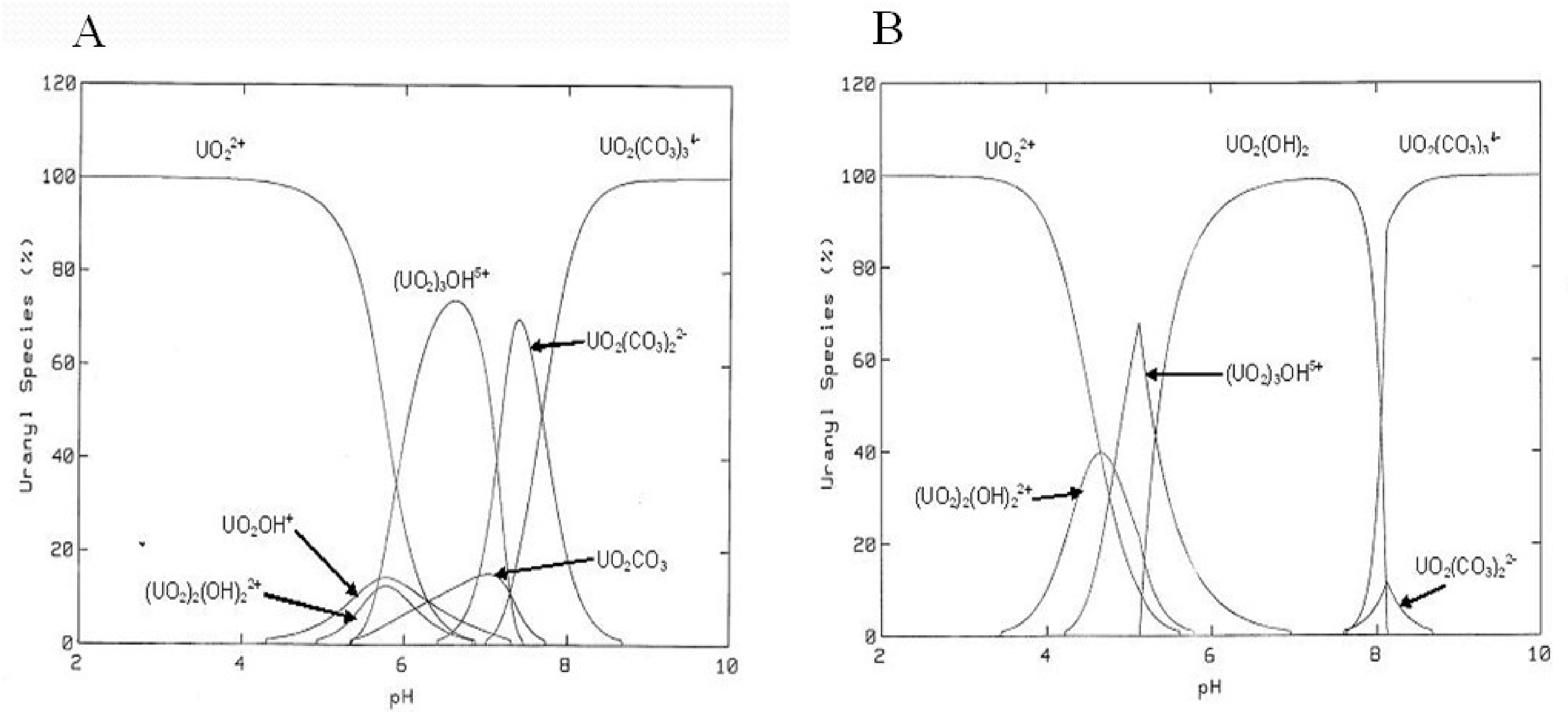
- Decay from dead plants, animals and microorganism
- Biological activity
- Transport toxicants
- Influence in aquatic ecosystem (pH, light)
- Fulvic acid is one of the important components of DOM

Uranium

- Application (Military, Civilian)
- Radioactive (alpha-particle)
- Health influence: internal/external exposure



Uranyl Ion (UO_2^{2+})



Uranyl species distribution (shown as percentage of total Uranyl as a function of pH) for
(A). 1 µmol/L uranyl ion in 0.01 mol/L ionic strength solution
(B). 800 µmol/L uranyl ion in 0.01 mol/L ionic strength solution
Distributions calculated with MINEQL+ ionic equilibrium program.

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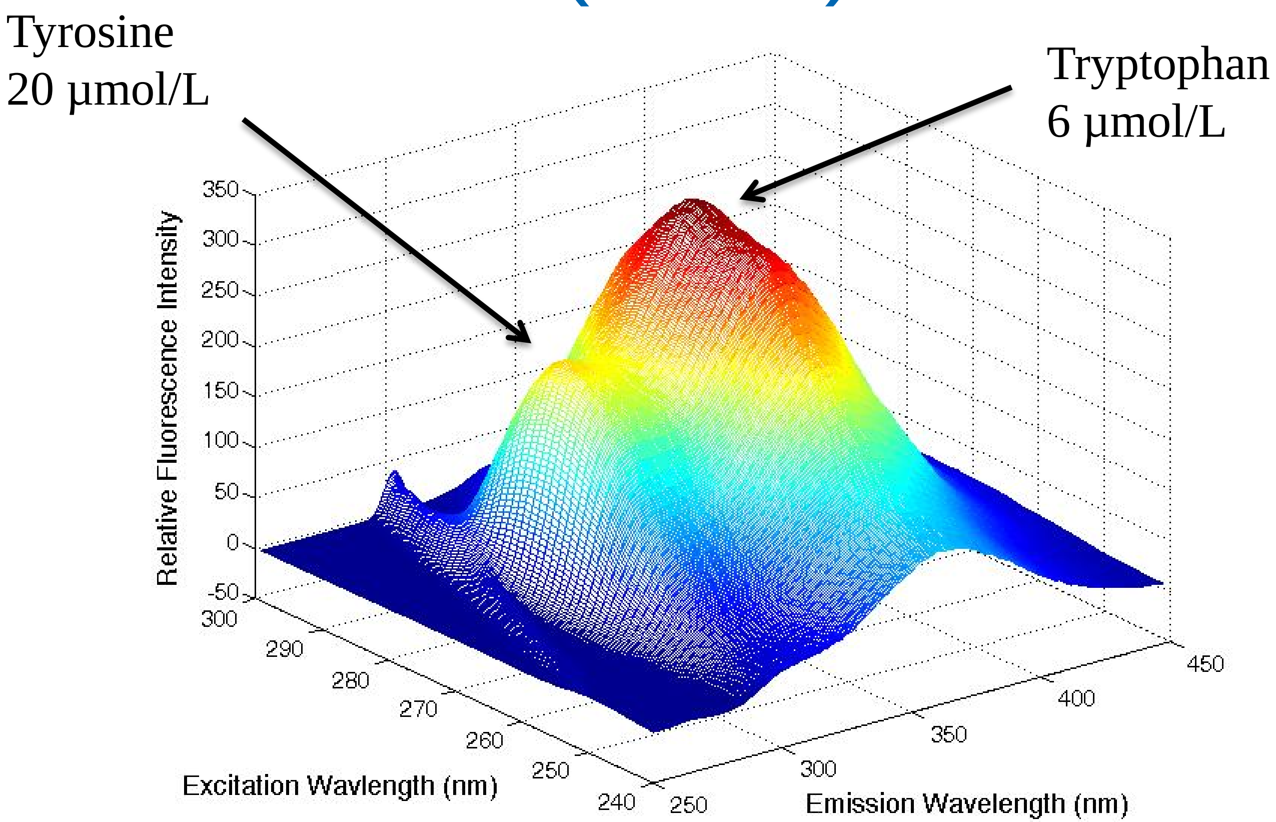
Fluorescence Excitation-Emission Matrix (EEM)



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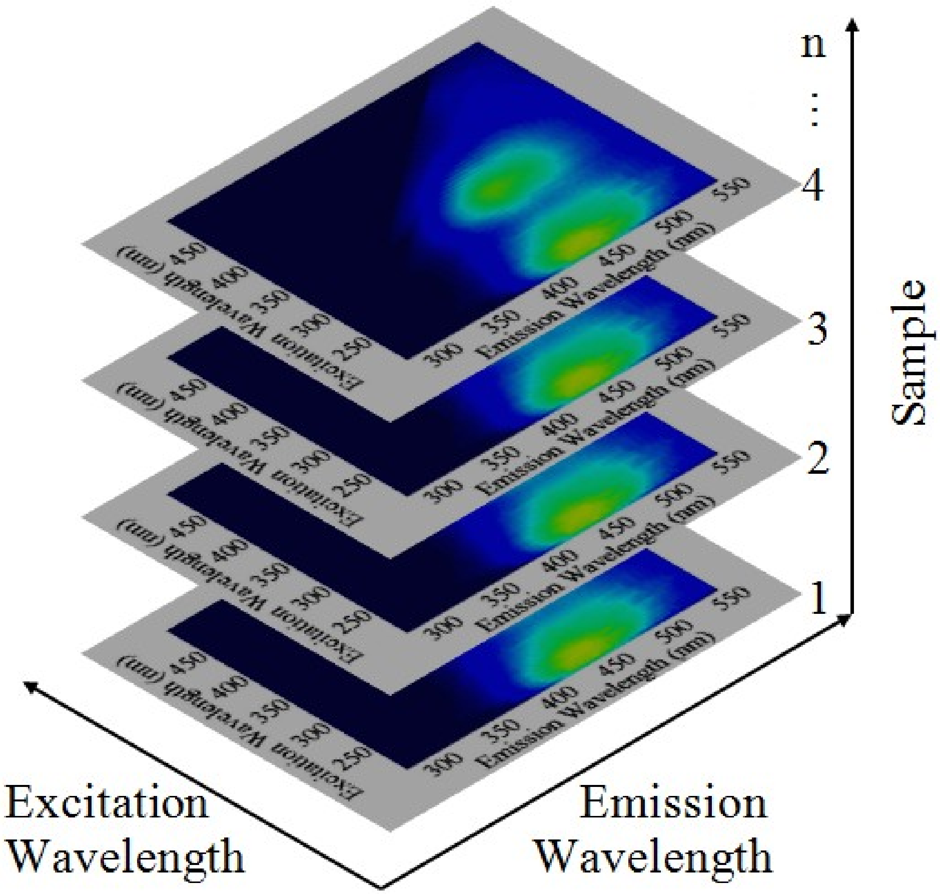
Parallel Factor Analysis (PARAFAC)



Composition of three-array model

Parallel Factor Analysis (PARAFAC)

$$x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + \varepsilon_{ijk} \; , i = 1, \ldots, I; \; j = 1, \ldots, J; \; k = 1, \ldots, K;$$

x_{ijk} Fluorescence intensity

ε_{ijk} Residue values

i Sample #

k Excited wavelength

j Emission wavelength

f Fluorophore

Output:

a Concentration / Intensity

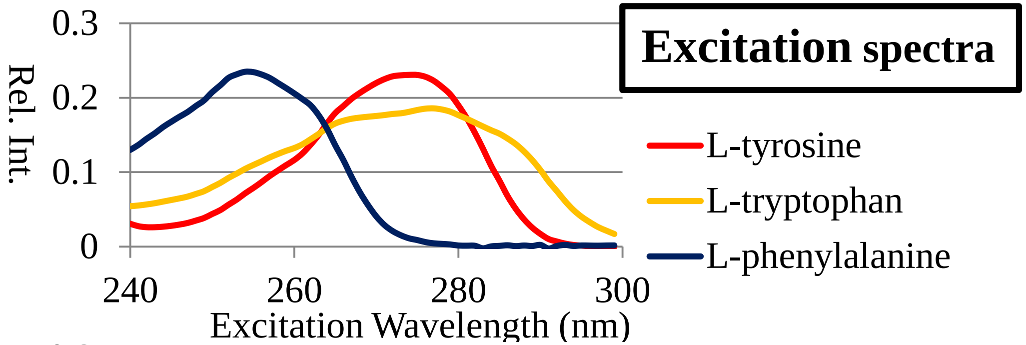
b Emission spectra

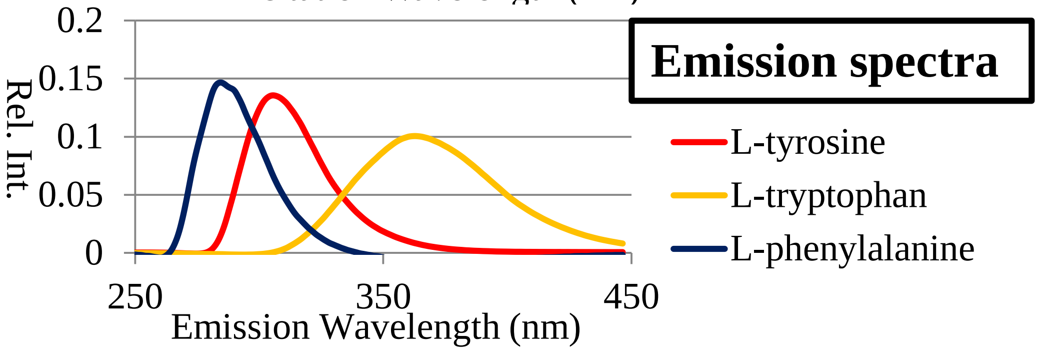
c Excitation spectra

Stedmon, C. A.; Bro, R.,. *Limnol. Oceanogr. Methods* **2008,** *6*, 572-579.

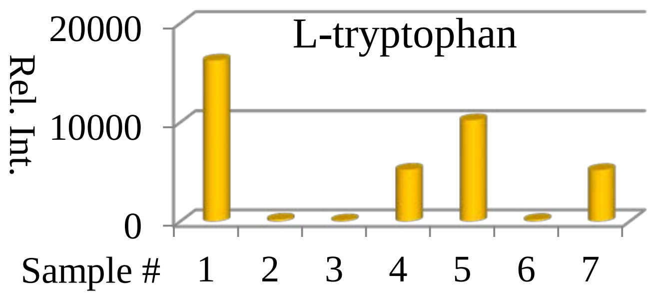
Parallel Factor Analysis

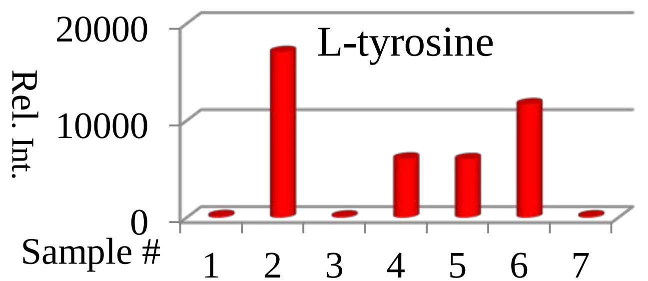
Sample #	1	2	3	4	5	6	7
	Mixture ratios						
L-tryptophan	3	0	0	1	2	0	1
L-tyrosine	0	3	0	1	1	2	0
L-phenylalanine	0	0	3	1	0	1	2

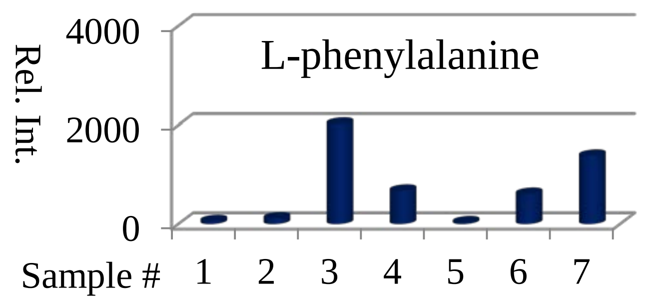




Intensities/ Concentrations







Fluorescence Quenching

M + L <—> ML

$K = [ML]/ [M][L]$

$C_M = [M] + [ML]$

$C_L = [L] + [ML]$

$[ML]/C_L = (I_L - I)/ (I_L - I_{res})$

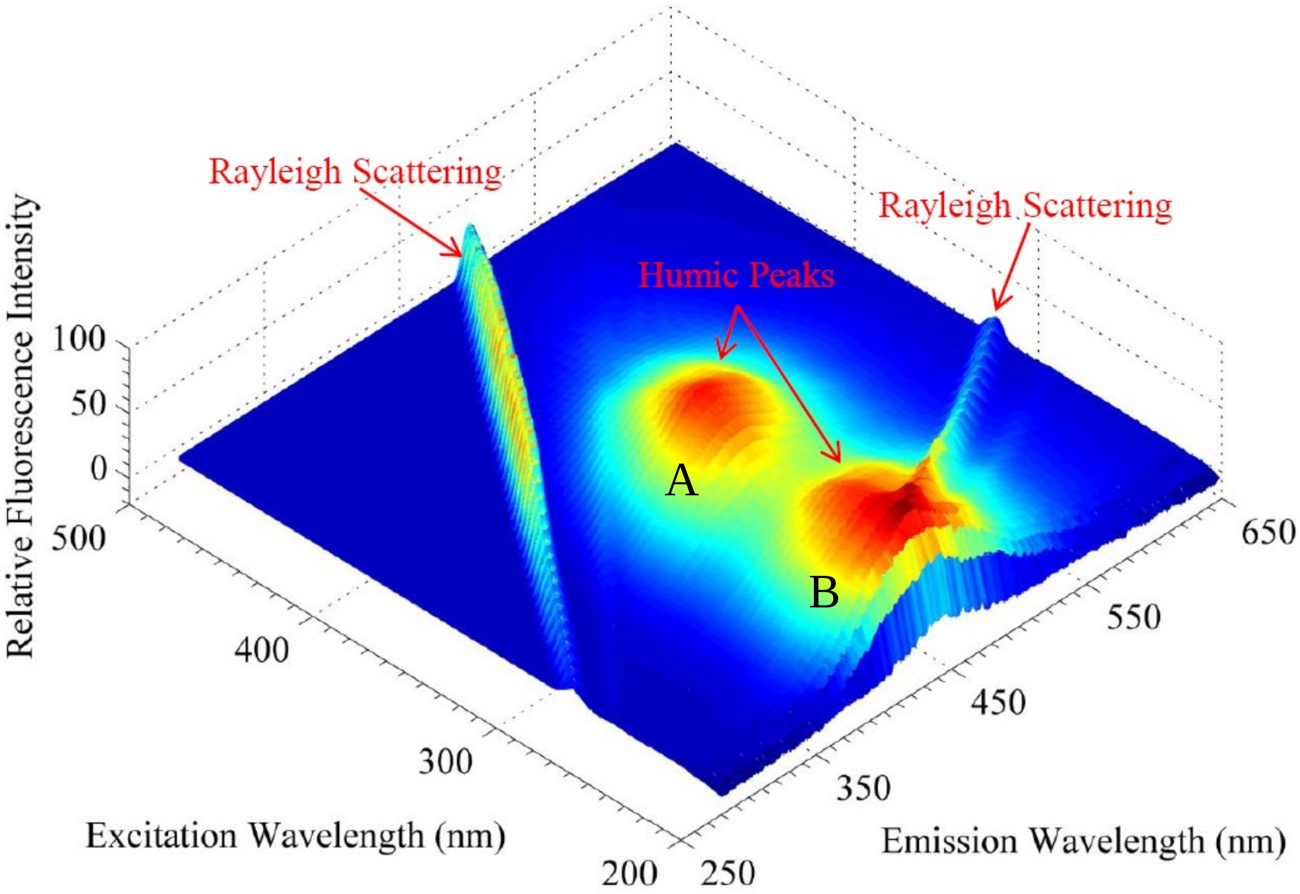
$I = [(I_{res} - 100)/2KC_L]\{(KC_L + KC_M + 1)$

$- [(KC_L + KC_M + 1)^2 - 4K^2 C_L C_M]^{1/2}\} + 1$

K: Conditional stability constant
[M]: Conc. of free metal ion
[L]: Conc. of metal-free ligand
[ML]: Conc. of metal-bound species
C_M: Total conc. of metal
C_L: Total conc. of ligand
I_{res}: Residual fluorescence intensity
I_L: Fluorescence intensity of total ligand
I: Fluorescence intensity during the titration

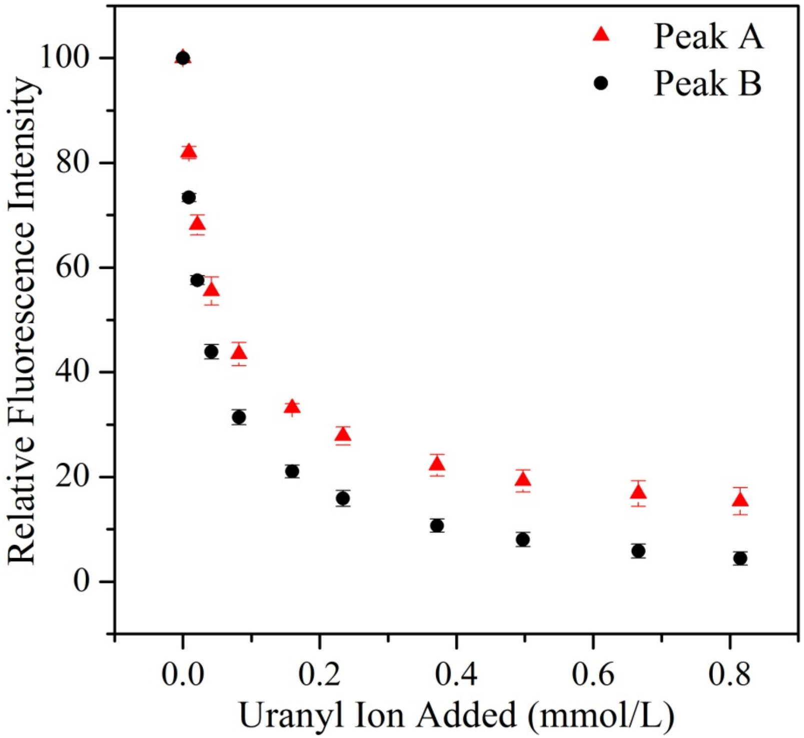
Ryan, D. K.; Weber, J. H., *Anal. Chem.* **1982,** *54*, 986-990.

Fluorescence EEM of SFA



EEM of soil fulvic acid sample 20mg/L

Fluorescence Quenching

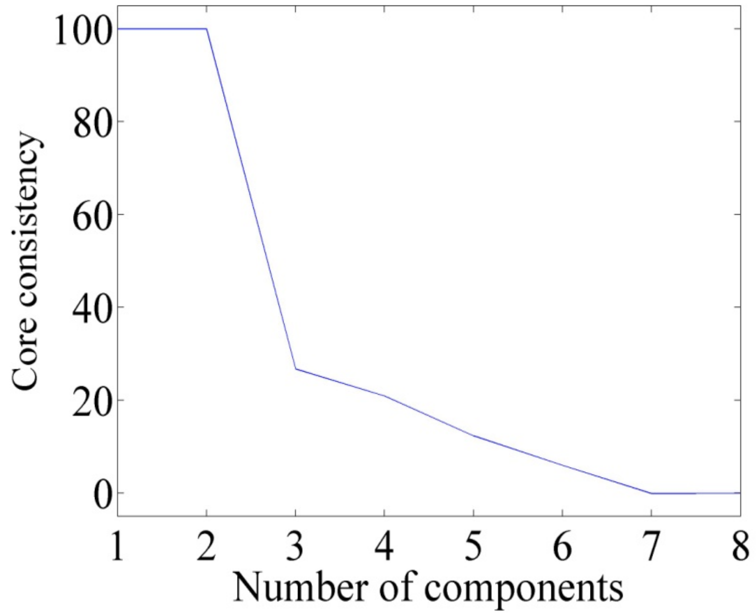


Left: Quenching curves of soil fulvic acid titrated by uranyl ion at 25°C and pH 3.5

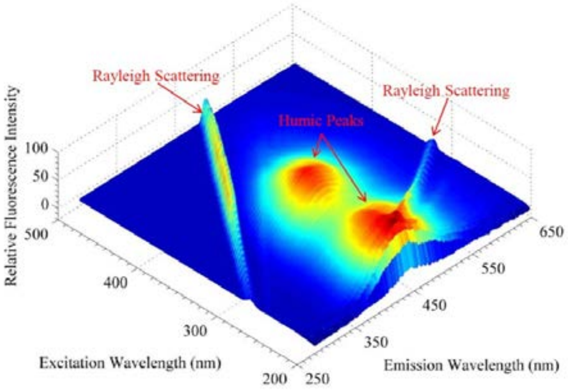
Bottom: Conditional stability constants (log K), SFA ligand concentrations (C_L), and residual intensity values (I_{res}) as determined by application of the Ryan-Weber model.

Sample	Peak	log K (±Std. Dev.)	C_L(μmol/L) (±Std. Dev.)	I_{res} (±Std. Dev.)
SFA	Peak A	4.43 (±0.08)	4.3 (±2.1)	13.8 (±3.6)
	Peak B	4.58 (±0.03)	3.9 (±1.1)	4.3 (±1.5)

Core Consistency Analysis



Core consistency analysis plotted against number of components ranging from one to eight for a titration of soil fulvic acid with Cu^{2+} at 25 °C and pH 6.00.



The Ryan-Weber model: M + L <—> ML

Two-site model: $M + L_1 \longleftrightarrow ML_1$ $M + L_2 \longleftrightarrow ML_2$

Two-Site Model

$$\mathbf{M+L_1 \longleftrightarrow ML_1} \qquad \mathbf{M+L_2 \longleftrightarrow ML_2}$$

$$K_1=[ML_1]/[M][L_1] \qquad K_2=[ML_2]/[M][L_2]$$

$$C_M=[M]+[ML_1]+[ML_2]$$

$$C_{L1}=[L_1]+[ML_1] \qquad C_{L2}=[L_2]+[ML_2]$$

$$[ML_1]/C_{L1}=(I_{L1}-I_1)/(I_{L1}-I_{res1}) \qquad [ML_2]/C_{L2}=(I_{L2}-I_2)/(I_{L2}-I_{res2})$$

$$(I_{L1}-I_1)/(I_{L1}-I_{res1})=K_1[M]/(1+K_1[M]) \qquad (I_{L2}-I_2)/(I_{L2}-I_{res2})=K_2[M]/(1+K_2[M])$$

$$K_1K_2[M]^3 +\{K_1K_2(C_{L1}+C_{L2}-C_M)+K_1+K_2\}[M]^2+\{C_{L1}K_1+C_{L2}K_2-C_M(K_1+K_2)+1\}[M]-C_M=0$$

$$\Rightarrow \mathbf{K_1, C_{L1}, I_{res1}, K_2, C_{L2}, I_{res2}}$$

Amino Acid Model Compound

Comparison of two-site model calculated results to theoretical and fixed values for Cu^{2+} titrations of L-tryptophan and L-tyrosine at pH 6

Compound	Theoretical log K values	Two-site model log K values (±std. dev.)	Fixed C_L (μmol/L)	Two-site model C_L (μmol/L) (±std. dev.)	Two-site model I_{res} (±std. dev.)
Tryptophan	4.86	4.79(±0.05)	10	13.3(±3.9)	0.700(±0.293)
L-tyrosine	4.55	4.69(±0.04)	30	25.6(±5.3)	0.334(±0.577)

Compound	**Two-site model** log K values (±std. dev.)	**The Ryan-Weber model** log K values (±std. dev.)
Tryptophan	**4.79(±0.05)**	**4.70(±0.07)**
L-tyrosine	**4.69(±0.04)**	**4.69(±0.07)**

- **Good results**

  ↓ Two-site model is valid

- **Close results**

  ↓ Large excess of metal ion comparing with ligands (more than 30 : 1)

Results and Discussion

Conditional stability constants (log K), SFA ligand concentrations (C_L), and residual intensity values (I_{res}) as determined by application of the Ryan-Weber model.

	log K (±Std. Dev.)	C_L (μmol/L) (±Std. Dev.)	I_{res} (±Std. Dev.)
Peak A (Ex:330 nm, Em:450 nm)	4.49 (±0.02)	5.05 (±4.19)	15.1 (±2.2)
Peak B (Ex:235 nm, Em:450 nm)	4.56 (±0.07)	4.56 (±3.83)	1.2 (±0.7)

- Log K: Peak A < Peak B
- C_L: Peak A > Peak B
- I_{res}: Peak A > Peak B
- Strong binding ability at pH 3.5

Further Implication

- Three-site model ?

$$\mathbf{M+L_1 <\!\!-\!\!-\!\!> ML_1}$$

$$\mathbf{M+L_2 <\!\!-\!\!-\!\!> ML_2}$$

$$\mathbf{M+L_3 <\!\!-\!\!-\!\!> ML_3}$$

$$[ML_1]/C_{L1}= (I_{L1}-I_1)/(I_{L1}-I_{res1})=K_1[M]/(1+K_1[M])$$

$$[ML_2]/C_{L2}= (I_{L2}-I_2)/(I_{L2}-I_{res2})=K_2[M]/(1+K_2[M])$$

$$[ML_3]/C_{L3}= (I_{L3}-I_3)/(I_{L3}-I_{res3})=K_3[M]/(1+K_3[M])$$

$$C_M=[M]+K_1C_{L1}[M]/(K_1[M]+1)+K_2C_{L2}[M]/(K_2[M]+1)+K_3C_{L3}[M]/(K_3[M]+1)$$

➡ $\mathbf{K_1, C_{L1}, I_{res1}, K_2, C_{L2}, I_{res2}, K_3, C_{L3}, I_{res3}}$

- Even more sites?
- PARAFAC is a good tool for analyzing mixtures

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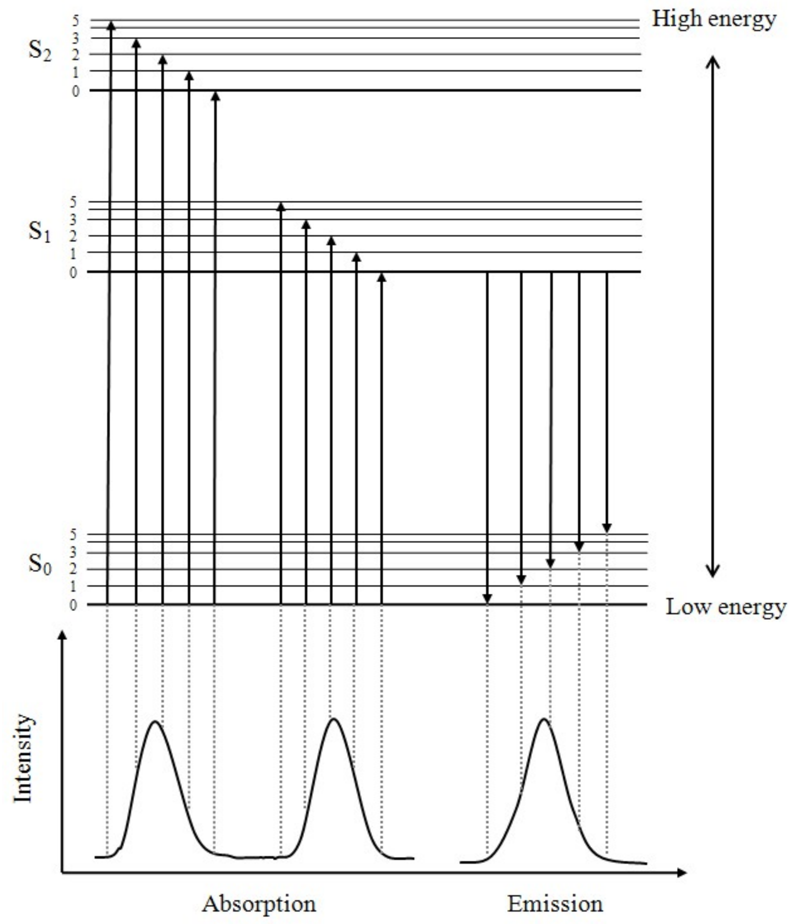
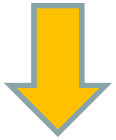
Regional Integration Analysis (RIA)



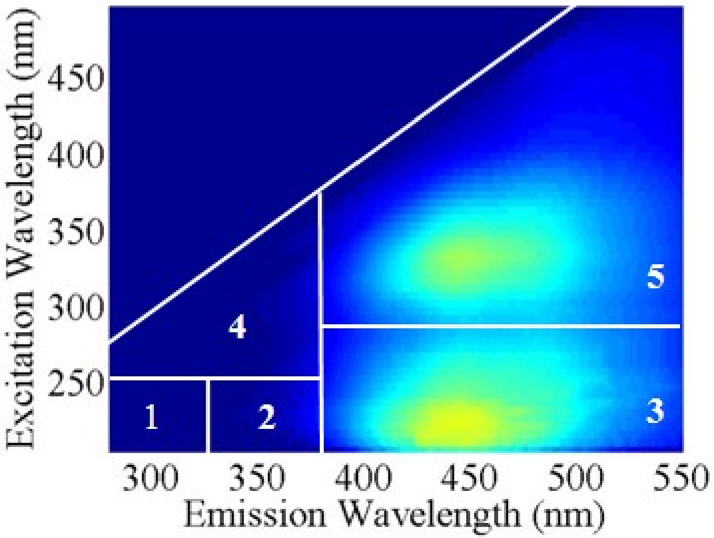
Jablonski diagram

Fluorescence peak height



Peak volume

Definition of Five Regions



EEM Region	Excitation (nm)	Emission (nm)	Description
1	200-250	280-330	Amino-acid-like
2	200-250	330-380	Amino-acid-like
3	200-285	380-550	Fulvic acid-like
4	250-400	280-380	Amino-acid-like & Soluble microbial by product-like
5	285-400	380-550	Humic acid-like

Chen, W.; Westerhoff, P.; Leenheer, J. A.; Booksh, K., *Environ. Sci. Technol.* **2003,** *37*, 5701-5710

Experiment

- Sample: Soil Fulvic Acid (SFA),

  Oyster River Fulvic Acid (ORFA),

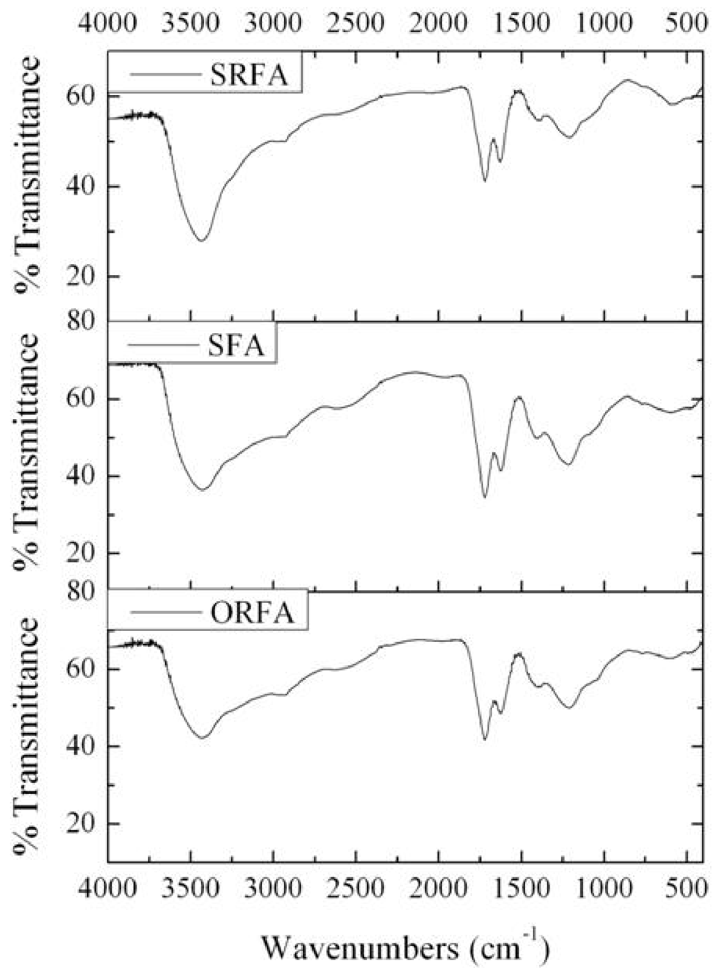
  Suwannee River Fulvic Acid (SRFA).

- Instruments: Perkin Elmer LS 55 fluorescence spectroscopy,

  Perkin Elmer Spectrum 100 FTIR spectroscopy

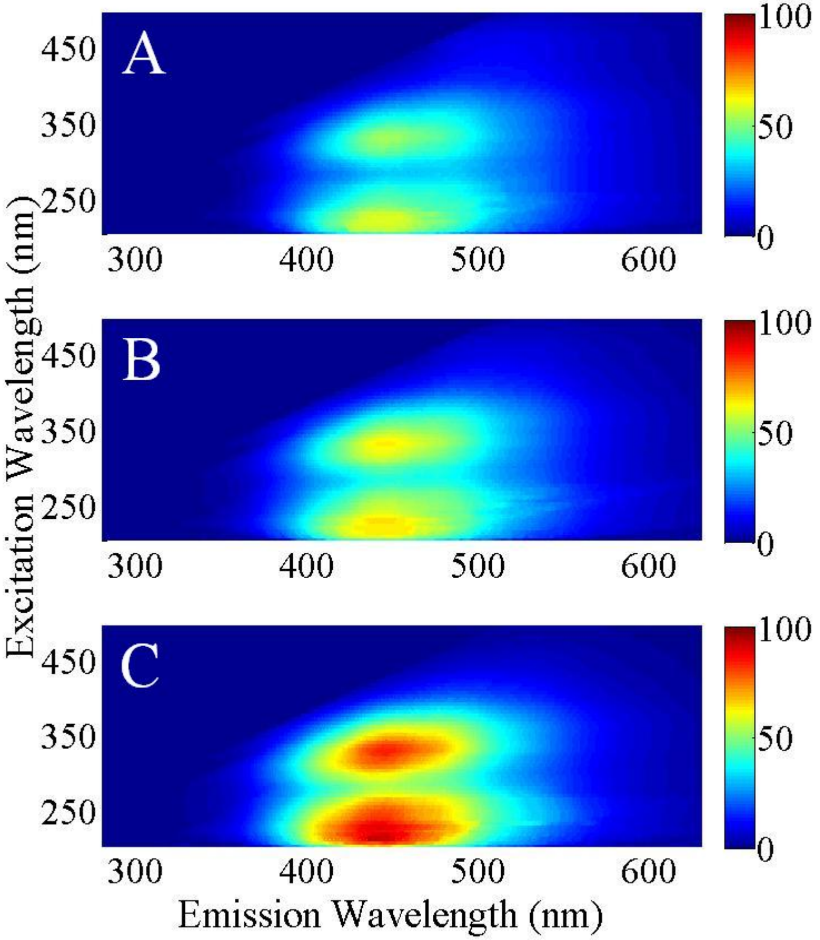
- Titration conditions: pH 3.5, temperature 25 °C, ionic strength 0.01 mol/L

FTIR Results



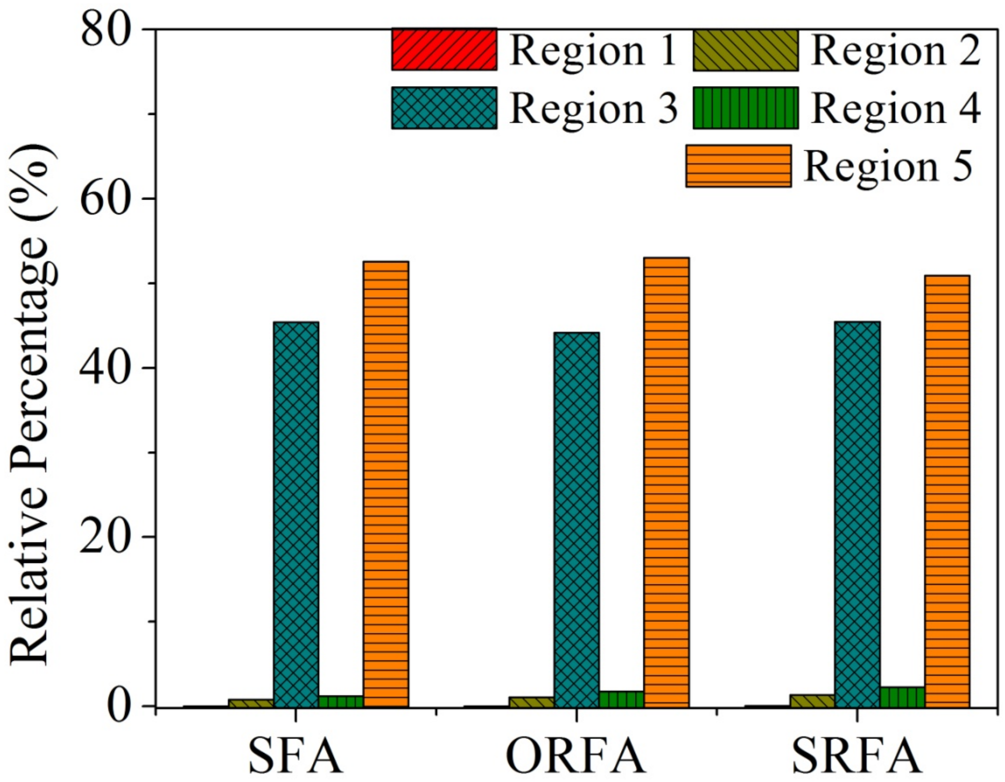
FTIR spectra of SFA, ORFA and SRFA

Fluorescence EEM results



Fluorescence EEM of SFA(A), ORFA (B) and SRFA (C). Concentrations were fixed at 20 mg/L and measured at 25 ° C

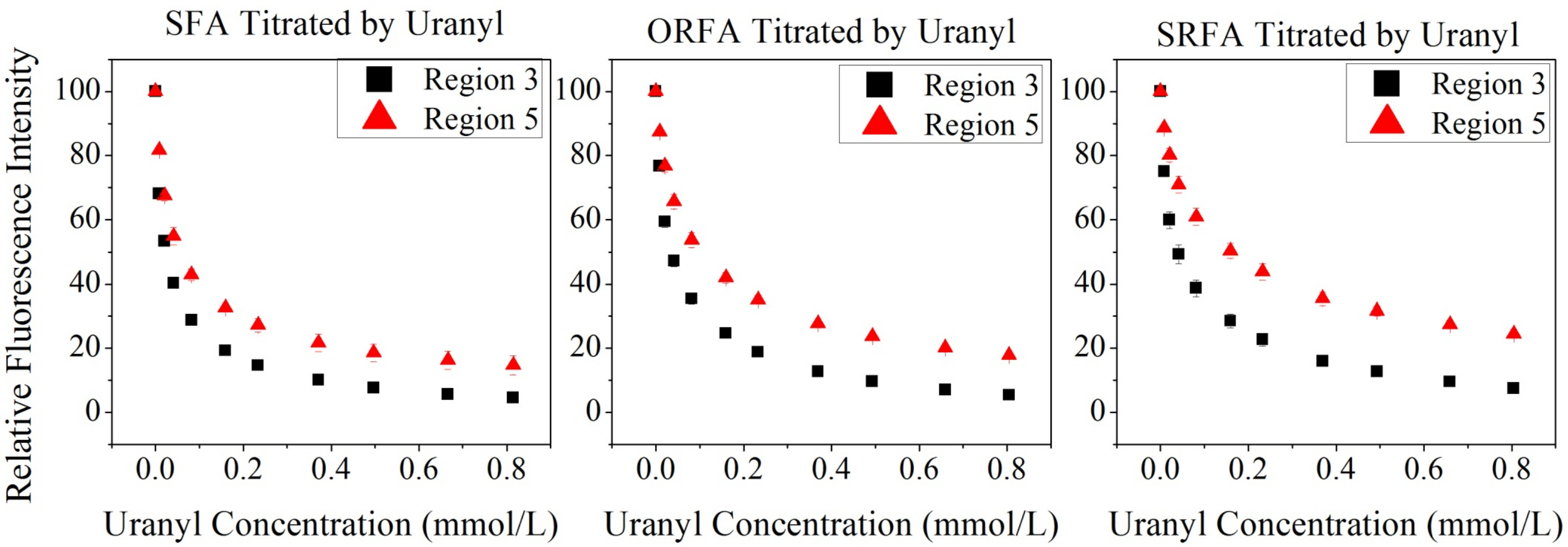
Distribution of Regions



Percentage distribution of five regions in SFA, ORFA and SRFA

High percentage for Region 3 and Region 5
Low percentage for Region 1, Region 2 and Region 3

Fluorescence Quenching



Fluorescence quenching curves of SFA, ORFA and SRFA titrated with uranyl ion at 25 °C and pH 3.5

Results

Equilibrium parameters including conditional stability constant (log K), ligand concentration (C_L) and residual intensity (I_{res}) values determined by the Ryan-Weber model and **RIA** data treatment method

Sample		log K (±Std. Dev.)	C_L(μmol/L) (±Std. Dev.)	I_{res} (±Std. Dev.)
SFA	Region 3	4.67 (±0.03)	3.9 (±1.2)	4.9 (±0.5)
	Region 5	4.44 (±0.07)	4.8 (±1.4)	13.3 (±3.5)
ORFA	Region 3	4.51 (±0.04)	5.3 (±1.0)	4.5 (±0.5)
	Region 5	4.20 (±0.06)	10.3 (±4.1)	13.3 (±1.3)
SRFA	Region 3	4.49 (±0.06)	9.0 (±2.0)	6.9 (±0.6)
	Region 5	4.11 (±0.06)	21.0 (±5.4)	19.0 (±1.9)

Decrease

Increase

Increase

Results

Equilibrium parameters including conditional stability constant (log K), ligand concentration (C_L) and residual intensity (I_{res}) values determined by the Ryan-Weber model and **PARAFAC** data treatment method

Sample		log K (±Std. Dev.)	C_L(μmol/L) (±Std. Dev.)	I_{res} (±Std. Dev.)
SFA	Region 3	4.58 (±0.03)	3.9 (±1.1)	4.3 (±1.5)
	Region 5	4.43 (±0.08)	4.3 (±2.1)	13.8 (±3.6)
ORFA	Region 3	4.34 (±0.05)	7.1 (±1.3)	2.1 (±0.8)
	Region 5	4.17 (±0.07)	9.9 (±3.1)	13.5 (±1.7)
SRFA	Region 3	4.36 (±0.07)	11.7 (±1.9)	4.1 (±0.8)
	Region 5	4.09 (±0.08)	21.2 (±5.6)	19.4 (±2.0)

Results

SFA Results comparison between PARAFAC and RIA data treatment methods

Method		log K (±Std. Dev.)	C_L(µmol/L) (±Std. Dev.)	I_{res} (±Std. Dev.)
RIA	Region 3	4.67 (±0.03)	3.9 (±1.2)	4.9 (±0.5)
	Region 5	4.44 (±0.07)	4.8 (±1.4)	13.3 (±3.5)
PARAFAC	Region 3	4.58 (±0.03)	3.9 (±1.1)	4.3 (±1.5)
	Region 5	4.43 (±0.08)	4.3 (±2.1)	13.8 (±3.6)

Good agreement

↓

RIA method is accurate and valid

Discussion

Limitations

- Region selection in a EEM
- Assumptions should be made in applying fluorescence quenching model

Advantages

- Sensitive and fast for quantitative analysis
- Analysis fluorophores without distinct peaks

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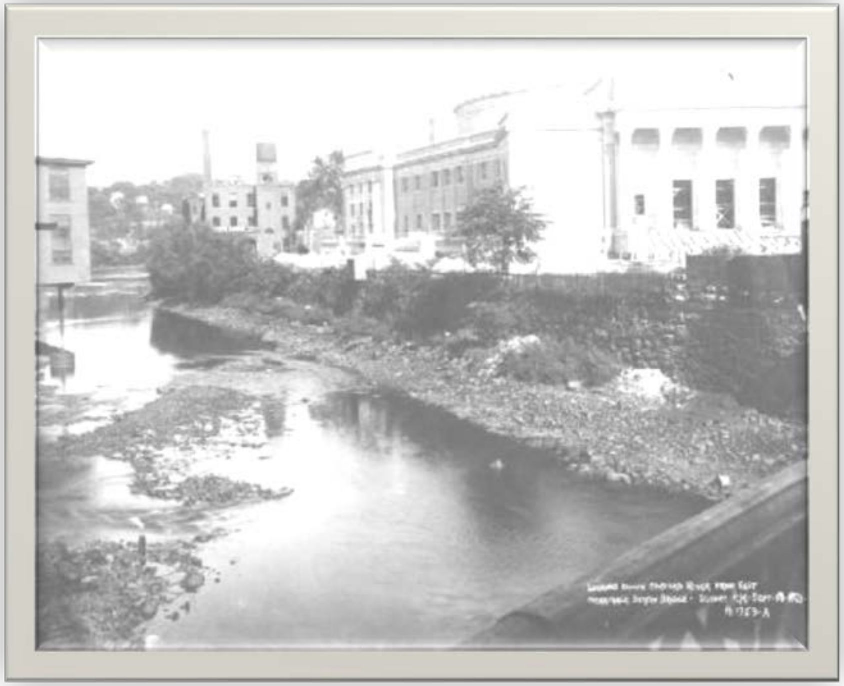
History
River as Sink and Sewer

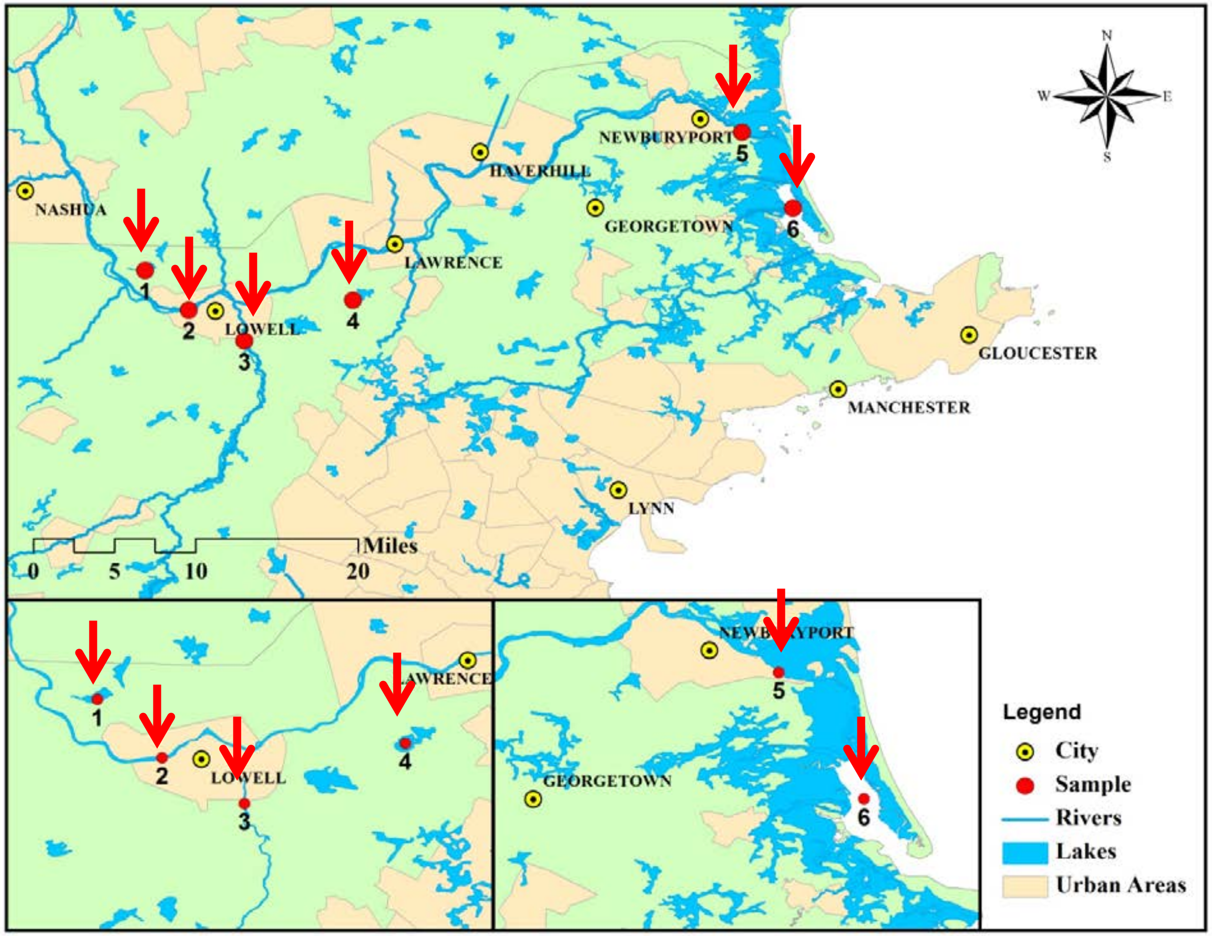


Left: The scum floating at the surface of the intake pond to Ames Worsted Company's turbine building near the Middlesex Dam is visible in this early 1900s photo.

Right: During periods of low flow in the Concord River, the stench of pollution was especially bad.

http://lowelllandtrust.org/greenwayclassroom/history

Sampling Sites



Natural freshwater sampling site:

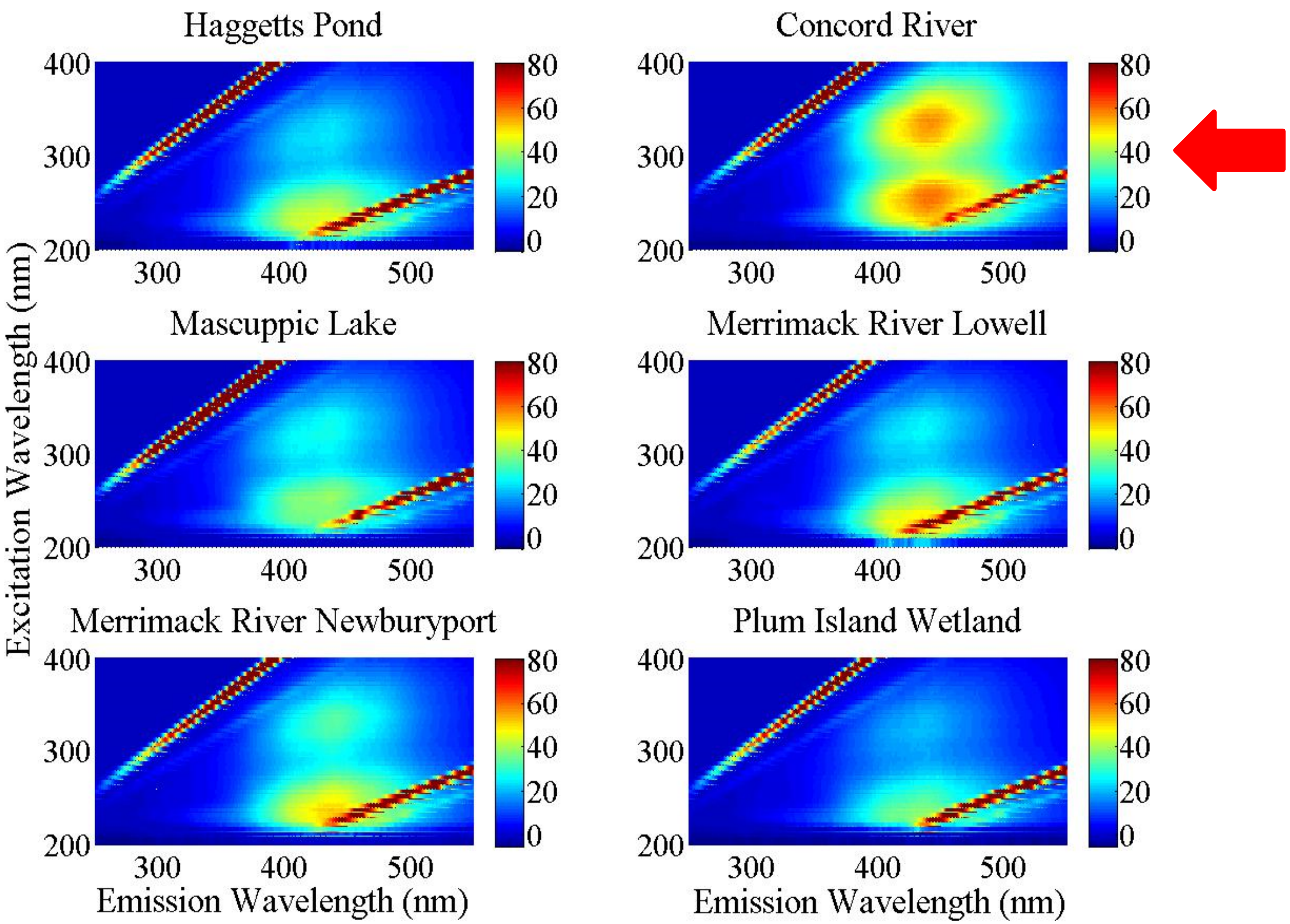
1. Mascuppic Lake (Tynsboro, MA)
2. Merrimack River (Lowell, MA)
3. Concord River (Lowell, MA)
4. Haggetts Pond (Andover, MA)
5. Merrimack River Estuary (Newburyport, MA)
6. Plum Island Wetland (Newbury, MA)

Results

pH values, absorbance and concentrations of common metal ions (in ug/L) of natural water samples

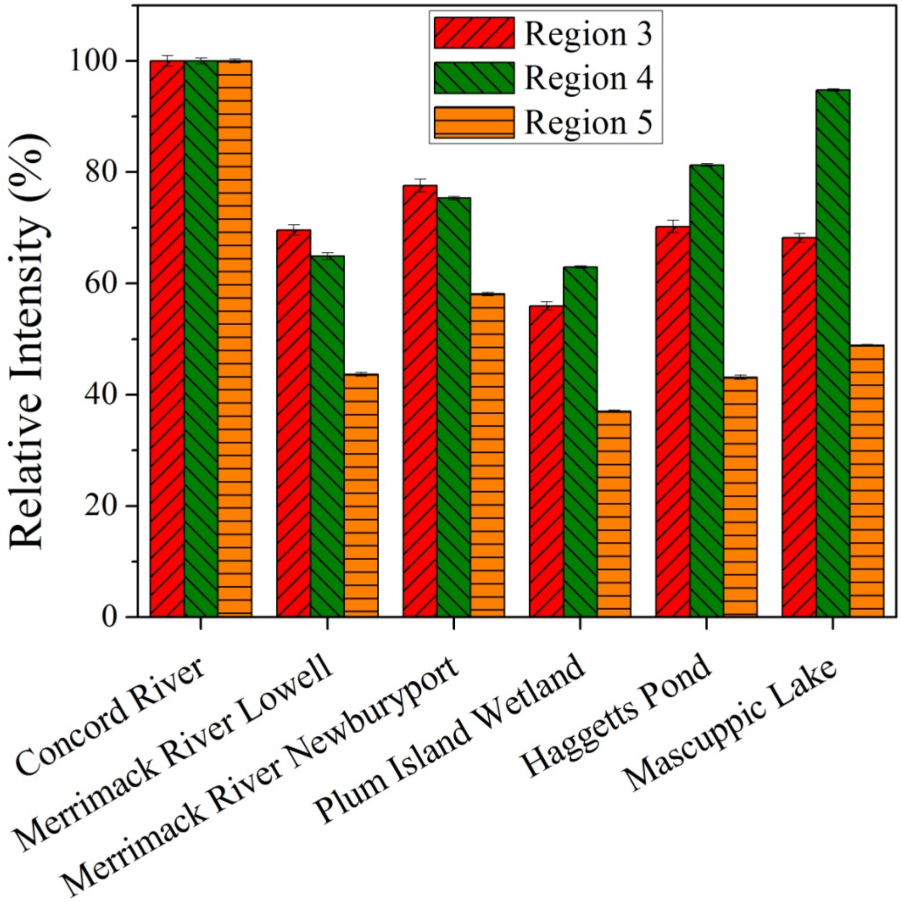
Sample	**pH**	**Absorbance at 254 nm**	Cd (±S.D.)	Cu (±S.D.)	Fe (±S.D.)	Mn (±S.D.)	Ni (±S.D.)	Pb (±S.D.)
Plum Island Wetland	7.4	0.111	145.5 (±9.4)	79.6 (±3.1)	2053.2 (±143.7)	176.7 (±12.9)	1018.5 (±53.7)	N.A.
Haggetts Pond	7.3	0.112	0.2 (±0.0)	8.7 (±0.1)	135.9 (±20.4)	49.2 (±1.6)	10.4 (±1.9)	0.5 (±0.2)
Concord River	7.2	0.304	1.2 (±0.2)	4.8 (±0.1)	860.3 (±93.7)	44.7 (±6.7)	3.8 (±0.7)	0.6 (±0.3)
Mascuppic Lake	7.3	0.118	0.4 (±0.2)	5.4 (±0.4)	131.5 (±22.4)	78.5 (±0.2)	9.7 (±2.1)	0.1 (±0.1)
Merrimack River (Lowell)	7.3	0.091	0.5 (±0.2)	4.3 (±0.1)	195.4 (±17.6)	1.7 (±0.8)	N.A.	N.A.
Merrimack River (Newburyport)	7.3	0.140	4.3 (±0.7)	15.7 (±0.7)	596.4 (±95.4)	79.6 (±3.1)	84.5 (±10.5)	127.0 (±13.5)

Results



Fluorescence EEMs of natural water samples

Results



Relative fluorescence intensity from different regions when the Concord River water is set as 100 %

- Amino acids

Lake, Pond, Wetland > River

- Humic substances

River > Lake, Pond, Wetland

Sample	Region	log K (±Std. Dev.)	C_L(μmol/L) (±Std. Dev.)	I_{res} (±Std. Dev.)
Haggetts Pond	Region 3	4.38 (±0.03)	*	10.1 (±0.4)
	Region 5	3.83 (±0.08)	*	35.5 (±4.9)
	Region 4	3.68 (±0.08)	*	8.6 (±3.3)
Mascuppic Lake	Region 3	4.01 (±0.03)	*	3.4 (±3.34)
	Region 5	3.93 (±0.07)	4.32 (±0.93)	34.2 (±1.3)
	Region 4	3.74 (±0.11)	2.65 (±2.17)	2.4 (±4.1)
Concord River	Region 3	4.26 (±0.02)	*	6.7 (±0.4)
	Region 5	3.93 (±0.04)	*	23.6 (±1.7)
	Region 4	3.65 (±0.03)	*	4.8 (±1.5)
Merrimack River Lowell	Region 3	4.49 (±0.05)	*	8.1 (±0.2)
	Region 5	3.97 (±0.03)	*	28.0 (±1.7)
	Region 4	3.73 (±0.02)	*	0
Merrimack River Newburyport	Region 3	4.08 (±0.03)	*	3.4 (±2.3)
	Region 5	3.91 (±0.04)	4.83 (±3.26)	29.3 (±0.5)
	Region 4	3.91 (±0.03)	4.46 (±2.78)	6.3 (±0.1)
Plum Island Wetland	Region 3	3.99 (±0.02)	*	0.9 (±1.1)
	Region 5	3.67 (±0.05)	0.62 (±0.44)	25.6 (±1.4)
	Region 4	3.88 (±0.22)	0.73 (±0.80)	0
*are the values not provided due to extreme small values obtained				

Discussion

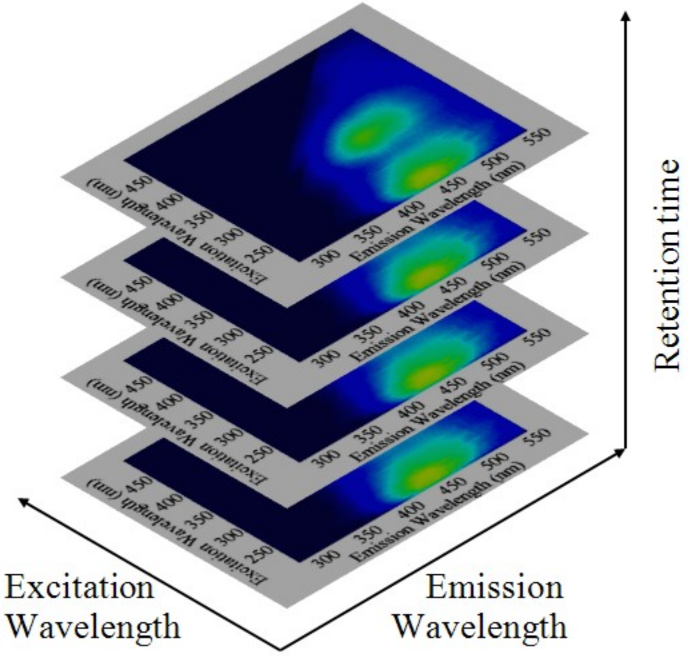
- Stability constants: Region 3 > Region 5 > Region 4
  Merrimack River, Lowell is highest
  Plum Island Wetland is lowest
- Residual values: Region 5 > Region 3 > Region 4
- Extreme small values of C_L
  - Low concentration of ligand site in freshwater DOM
  - Complicated integrations with DOM such as self binding and large colloid aggregation
  - Restrictions of using the Ryan-Weber mode

Final Conclusion

- Applications of EEM
- Application of PARAFAC and RIA in DOM-metal ion binding study
- Application of two-sites model
- Environment application (algea activity, waste water evaluation, transport of metal ion)

Future Work

- Neutral pH conditions (e.g., pH 7)
- Other metals (e.g., Al^{3+})
- Apply PARAFAC in Chromatography for DOM study



Publications

- Zhu, Bingqi, Pennell, Stephen A. and Ryan, David K. (2014) Characterizing the Interaction between Uranyl Ion and Soil Fulvic Acid Using Parallel Factor Analysis and a Two-Site Fluorescence Quenching Model. Microchemical J. 115, 51-57.
- Zhu, B. and Ryan, D.K. (2016) Characterizing the interaction between uranyl ion and fulvic acid using regional integration analysis (RIA) and fluorescence quenching. J. Environ. Radioactivity 153, 97-103.
- Zhu, B. and Ryan, D.K. (2017) Complexation Study of Uranyl Ion with Dissolved Organic Matter in Natural Fresh Waters by Fluorescence Quenching Techniques. in Nuclear Engineering, ISBN 978-953-51-5411-2.