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# *Excitation Emission Spectrometry as a Means to Estimate and Semi-Quantitatively Identify Organic Contaminants in Water*

## *NEMC, August 2019*

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We are

One lab and two locations

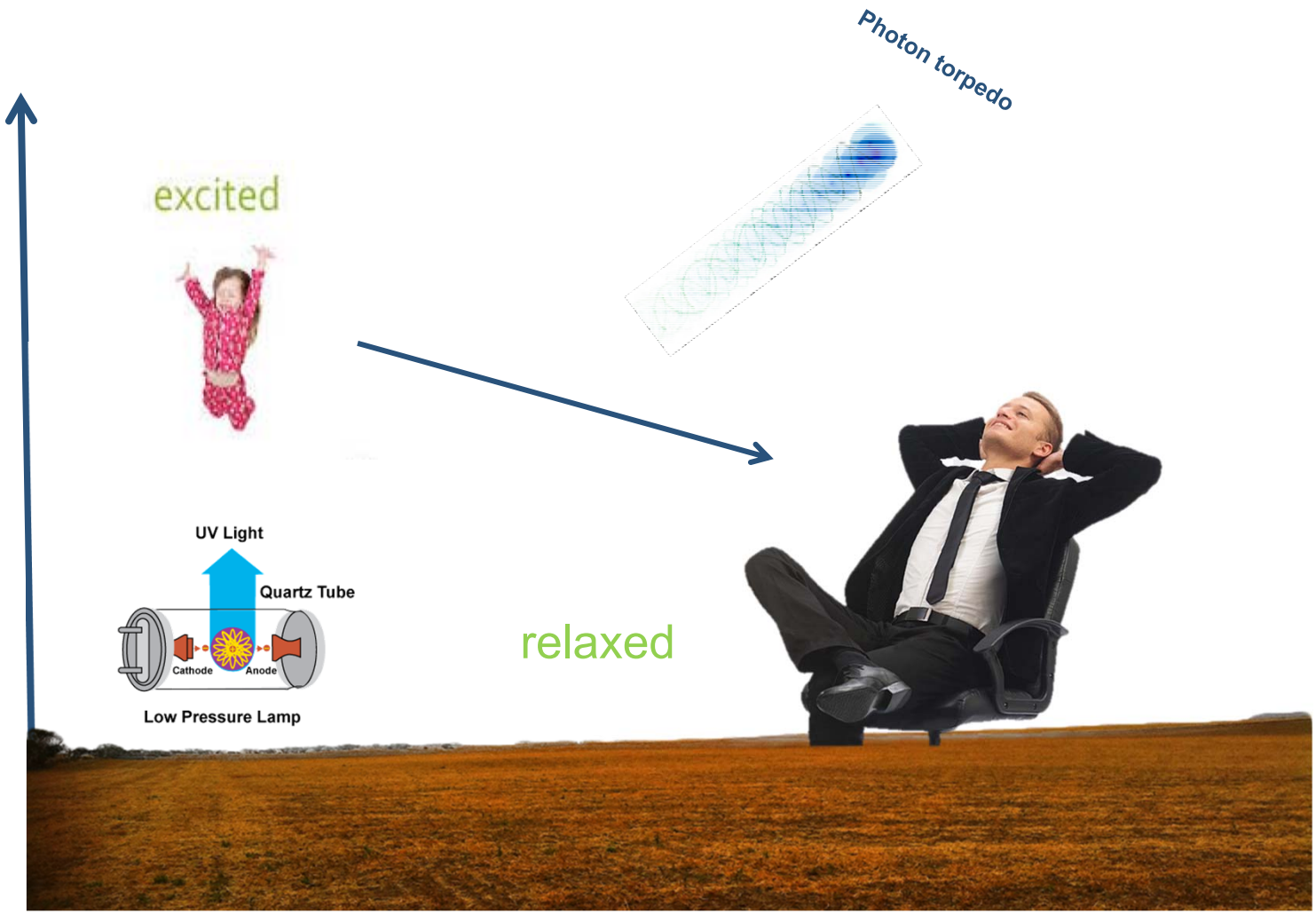
Monrovia

South Bend

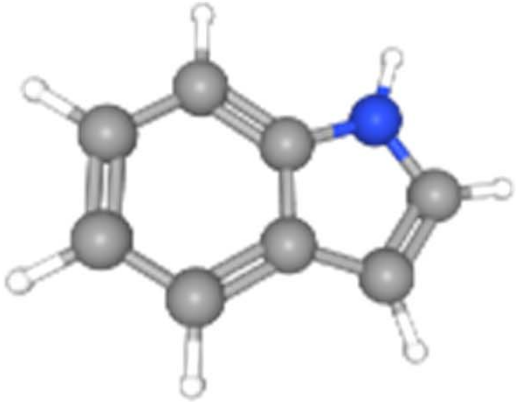
# What is Excitation – Emission (matrix) Fluorescence Spectroscopy (EEM)?

- Three dimensional scan of excitation wavelength, emission wavelength, and color representing fluorescence intensity
- Requires a scanning spectrofluorometer
- Being used to identify pollution sources, or dissolved organic matter
  - Humic acids
  - Fulvic Acids
  - Amino Acids

# Fluorescence involves a beam of UV light emitting light of a longer wavelength

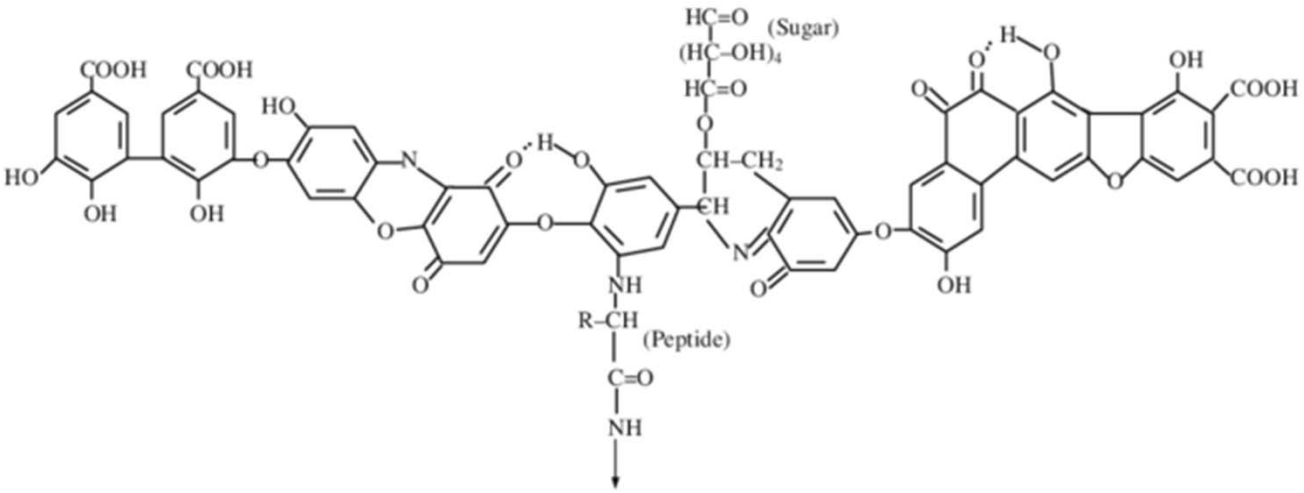


# Typical compounds studied using EEM include those with indole group

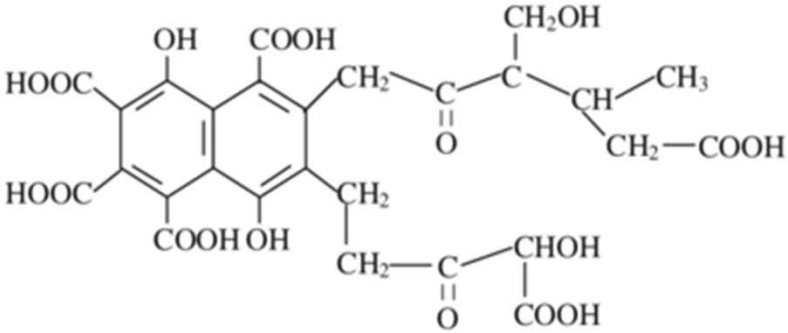


- Tryptophan
- Tyrosine
- Phenylalanine

# Humic and fulvic acids are also aquatic fluorophores

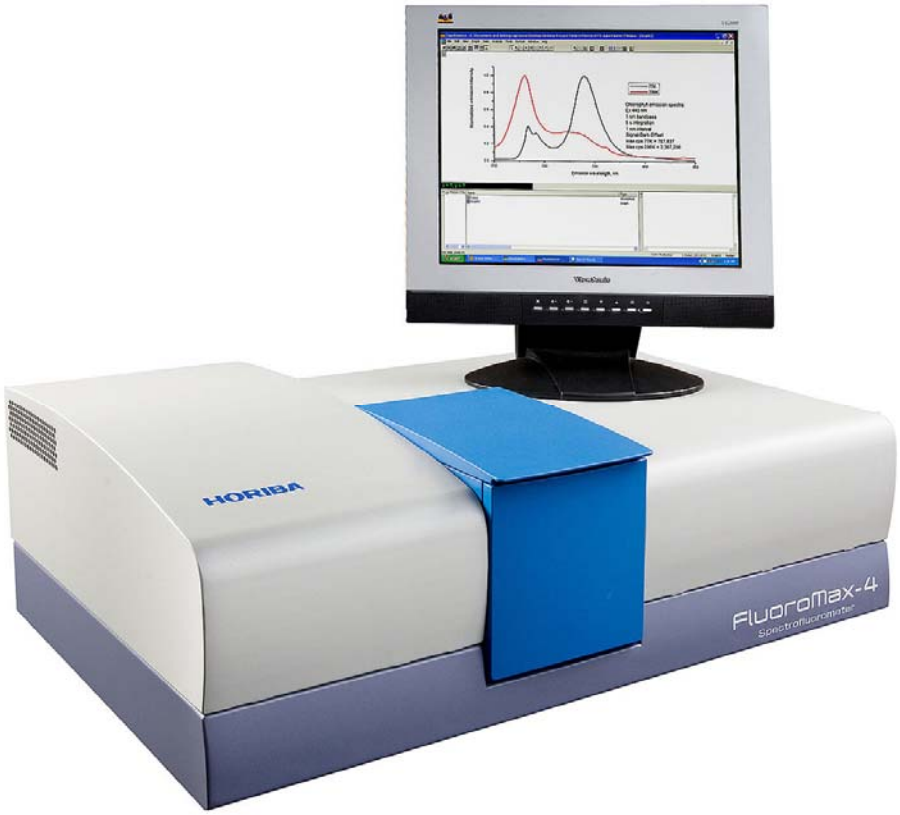



**(a) Humic Acid** (Stevenson 1982)



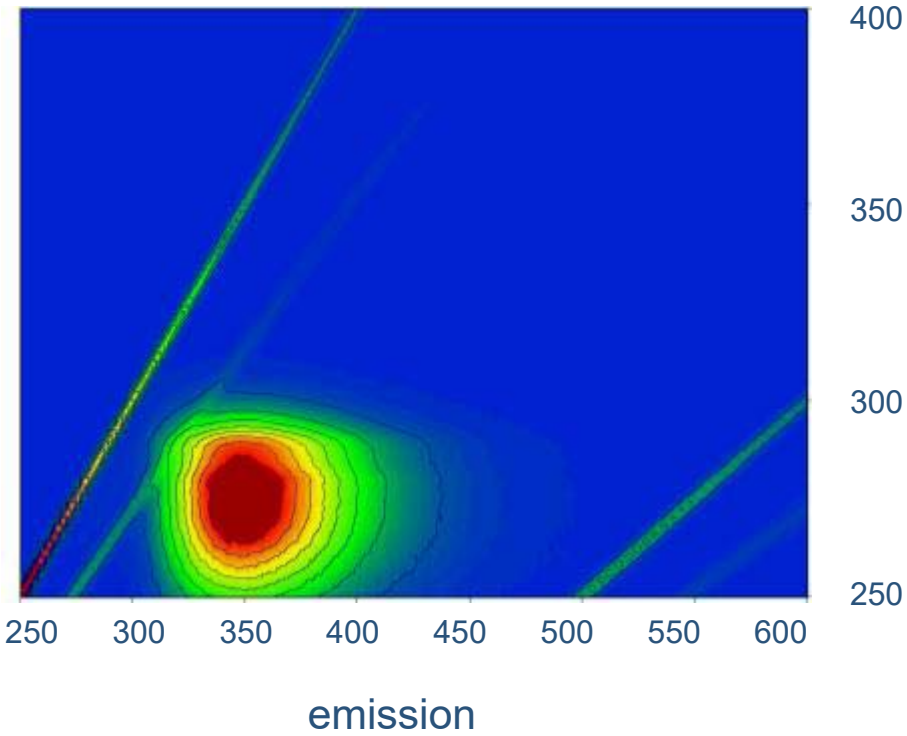

**(b) Fulvic Acid** (Buffle 1977)

# Data can be collected on a scanning spectrofluorometers



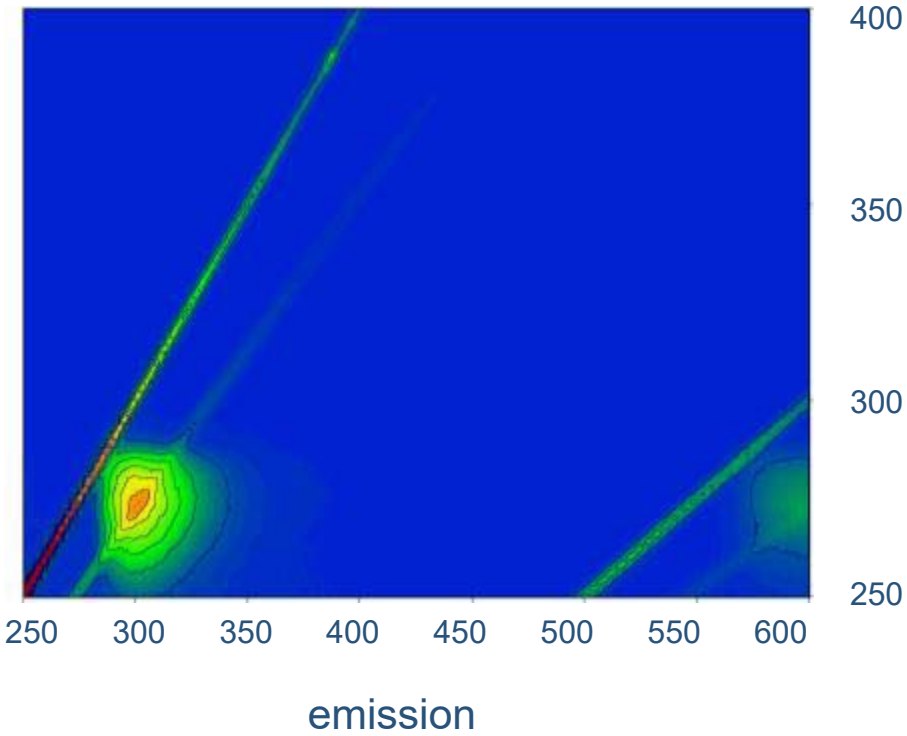
# Some example three dimensional scans of various compounds

# EEM 3D Spectra of tryptophan



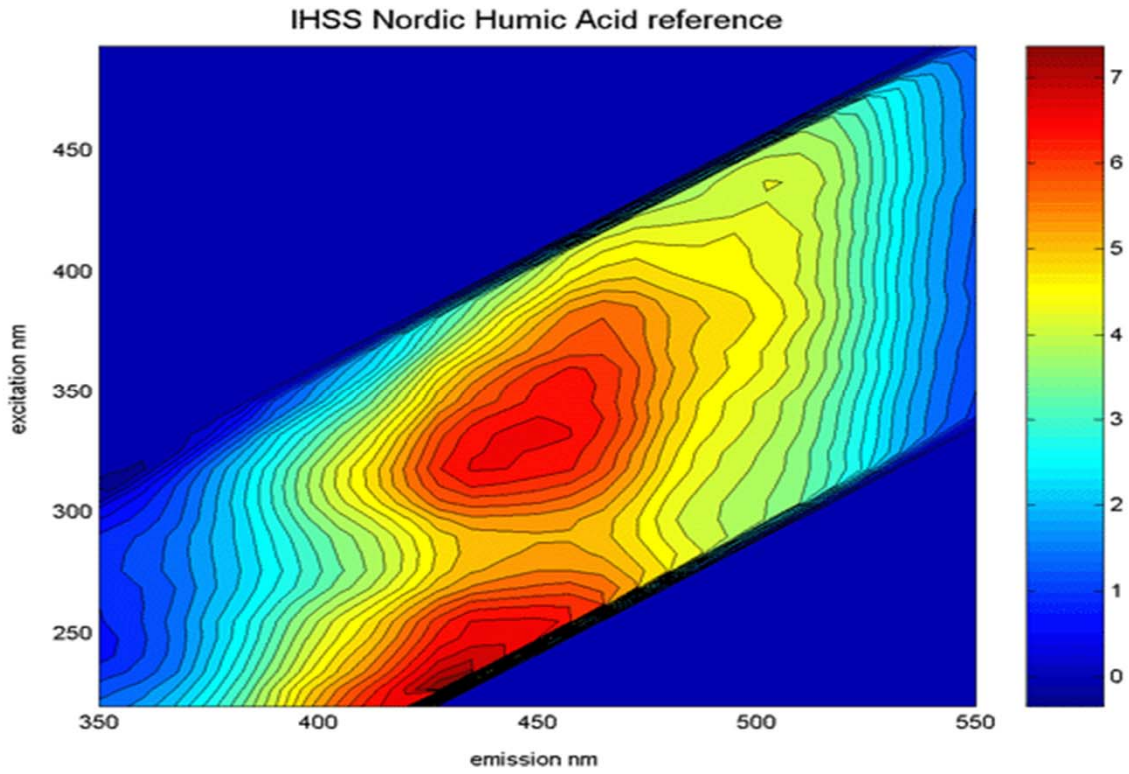

**Shimadzu AD-0133**

# EEM 3D Spectra of tyrosine



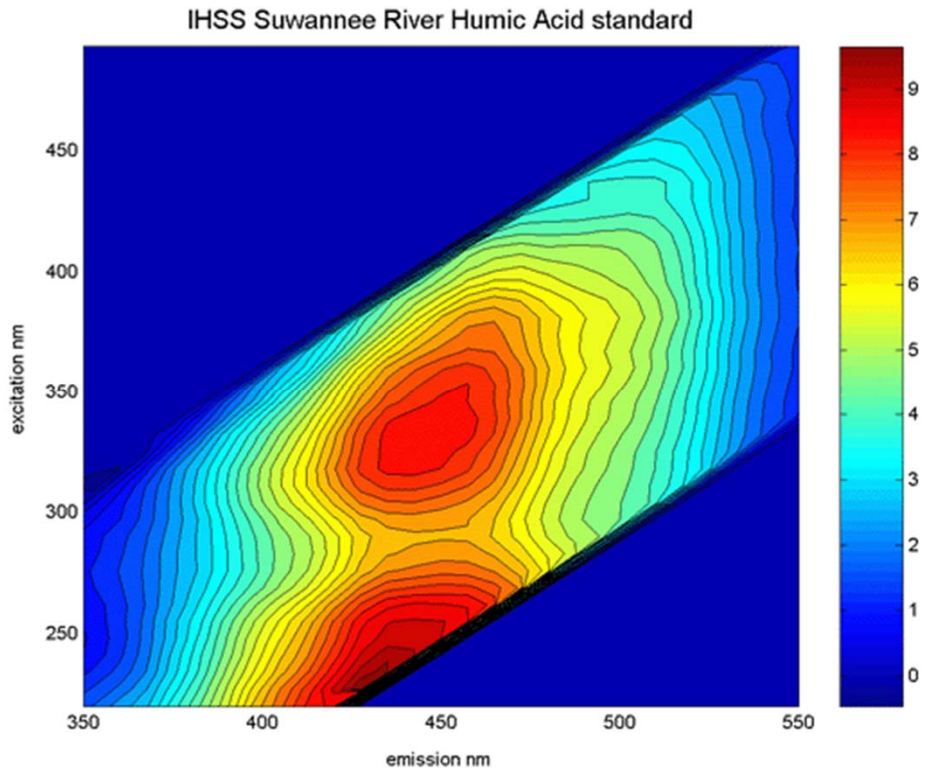

**Shimadzu AD-0133**

# EEM 3D Spectra of Nordic Humic Acid




http://humic-substances.org/

# EEM 3D Spectra of Suwannee River Humic Acid



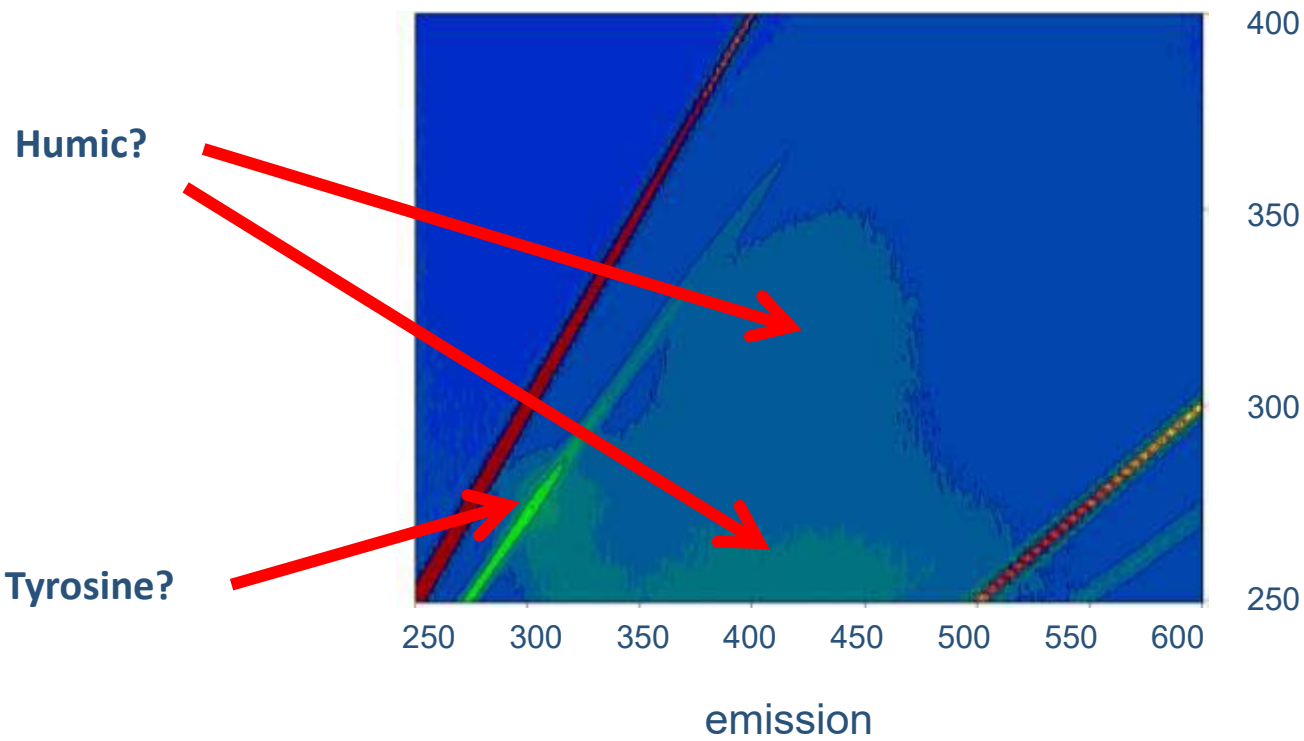

http://humic-substances.org/

# Characteristic Aquatic fluorophores

| Fluorophore | Excitation (nm) | Emission (nm) |
|---|---|---|
| Tryptophan (like) | 225 - 237 | 340 - 381 |
| | 275 | 340 |
| Tyrosine (like) | 225-237 | 309-321 |
| | 275 | 310 |
| Humic (like) | 237-260 | 400-500 |
| | 300-370 | 400-500 |
| | 312 | 380-420 |

Hudson,N.,Baker,A.andReynolds,D.(2007).*Fluorescenceanalysisofdissolvedorganicmatterinnatural,wasteandpollutedwaters-areview.*RiverResearchandApplications23:631-649.

# Estimating unknown compounds with EEM 3D in pond water

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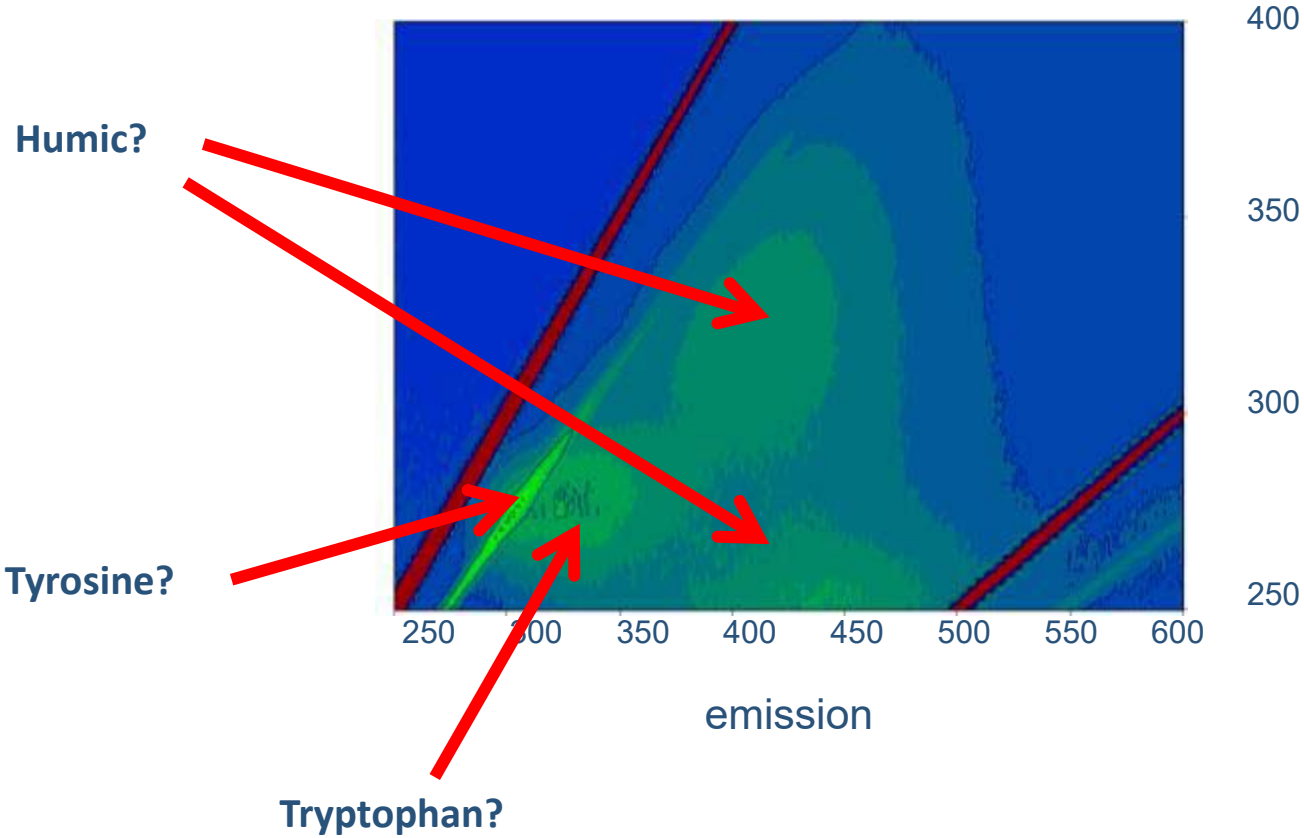
# Estimating unknown compounds with EEM 3D in wastewater

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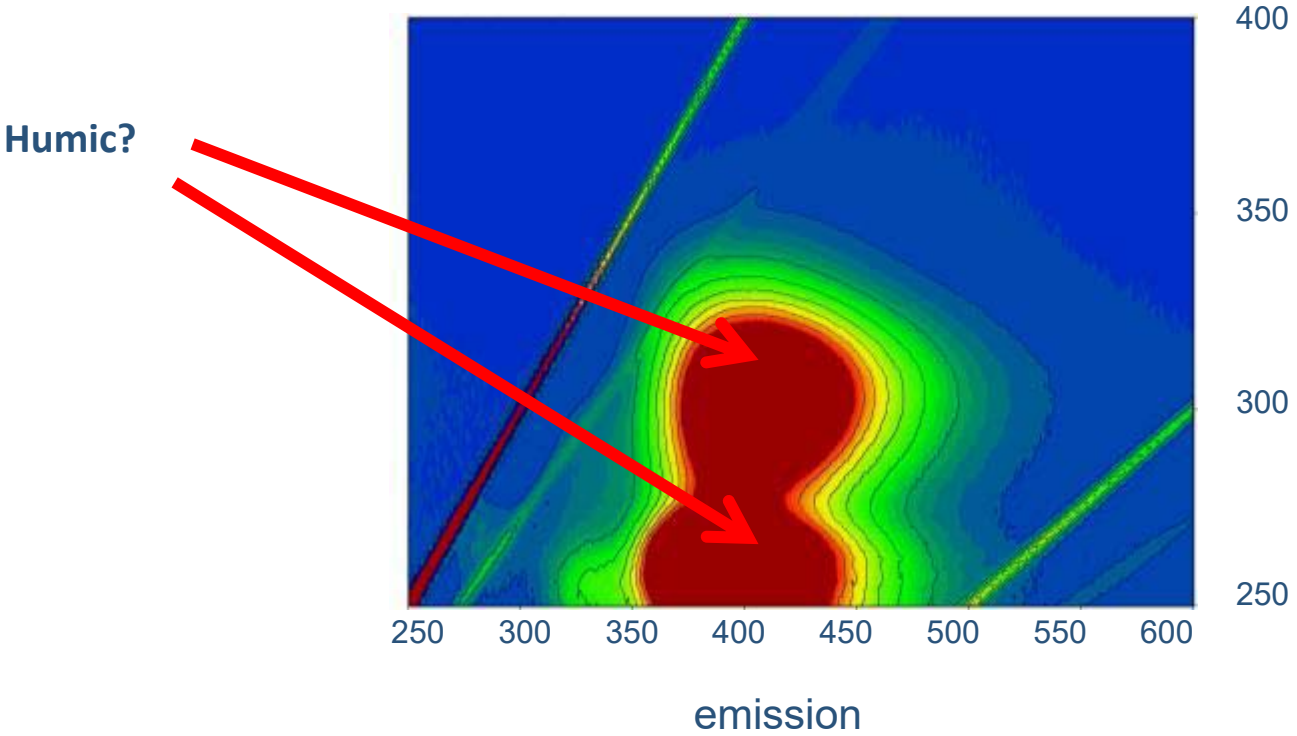
# Estimating unknown compounds with EEM 3D in wastewater

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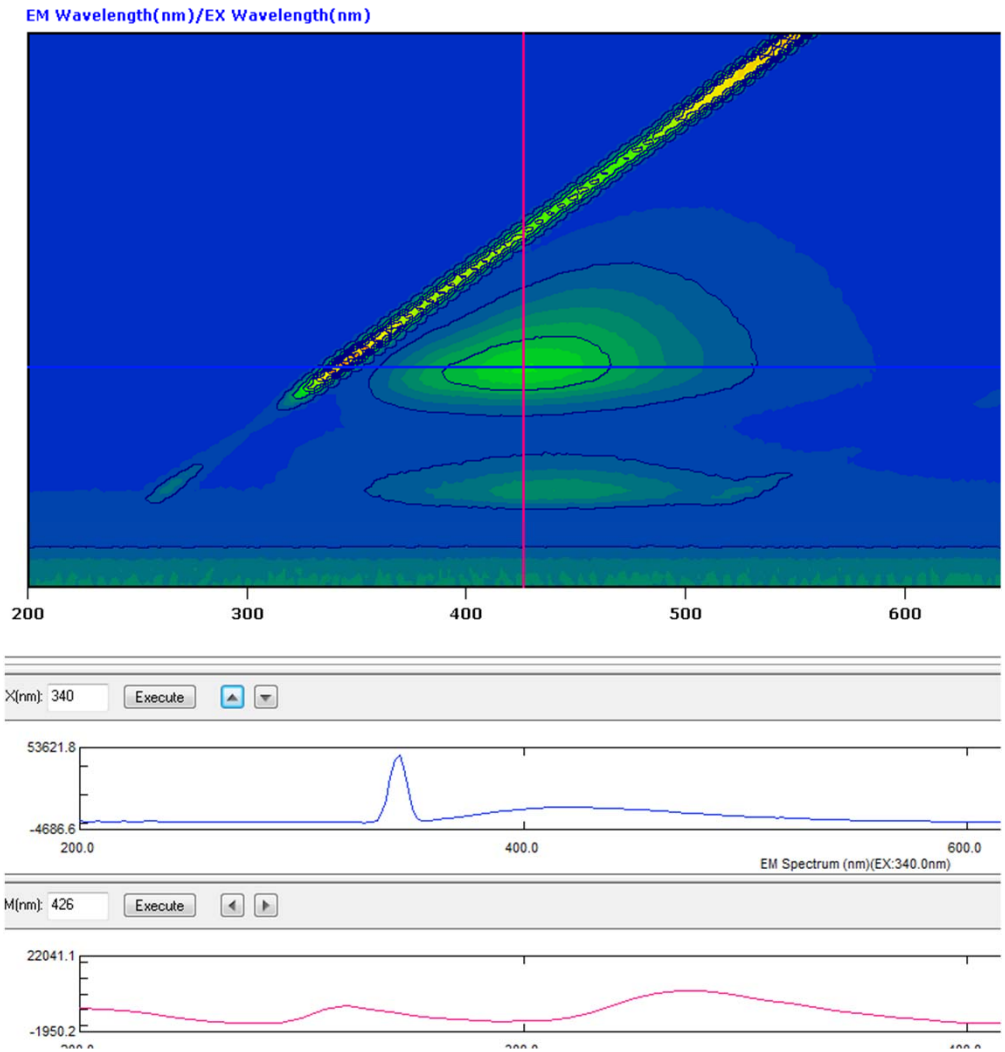
# EEM 3D Fluorescence spectroscopy can be used to characterize organics in water

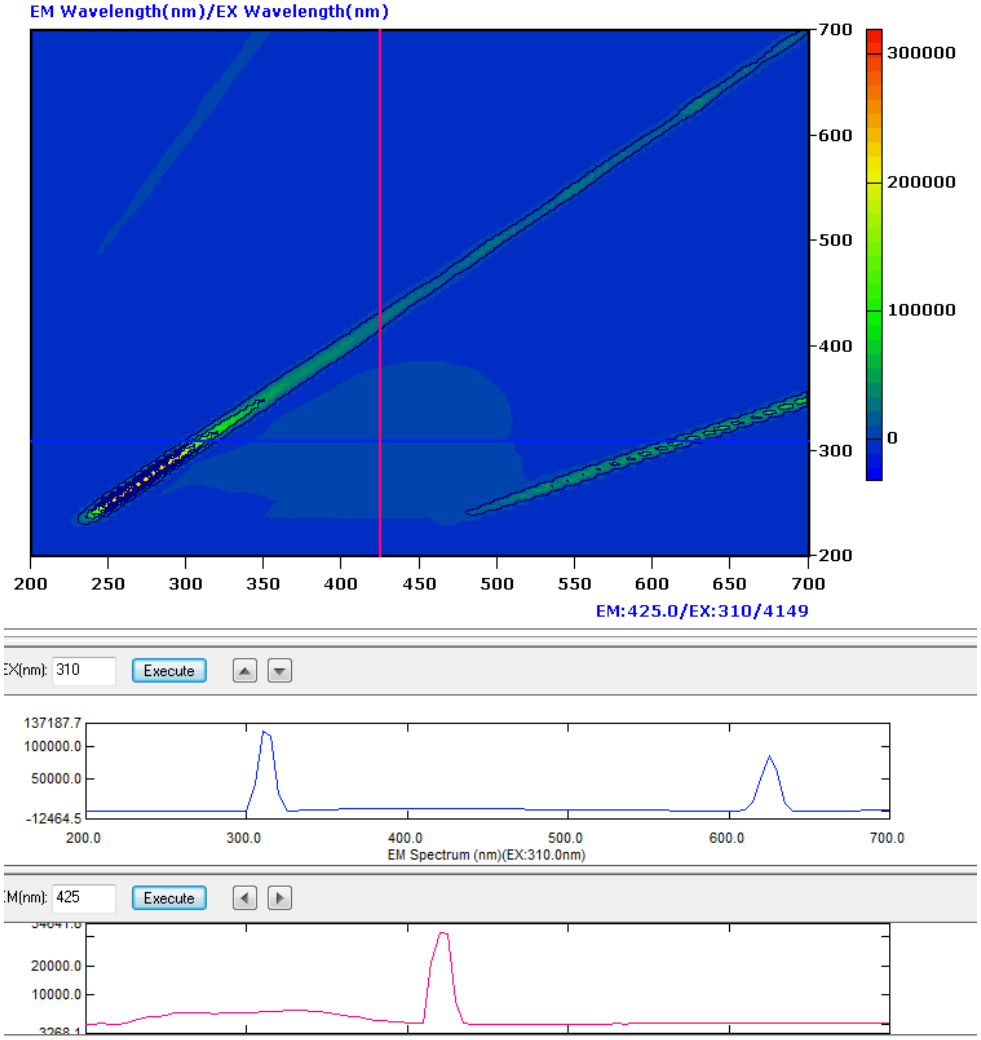
- However, humic acid excitation and emission varies with source
- Concentrations to qualify need to be fairly high
- Analyst training needed and interpretation is subjective.

# Can we use EEM in routine water quality laboratory testing?




**Sample high in algae shows definite pattern**

# Can we use EEM in routine water quality laboratory testing?




**Sample lower in algae shows a less definite pattern**

# Quantitative analysis of Chlorophyll A

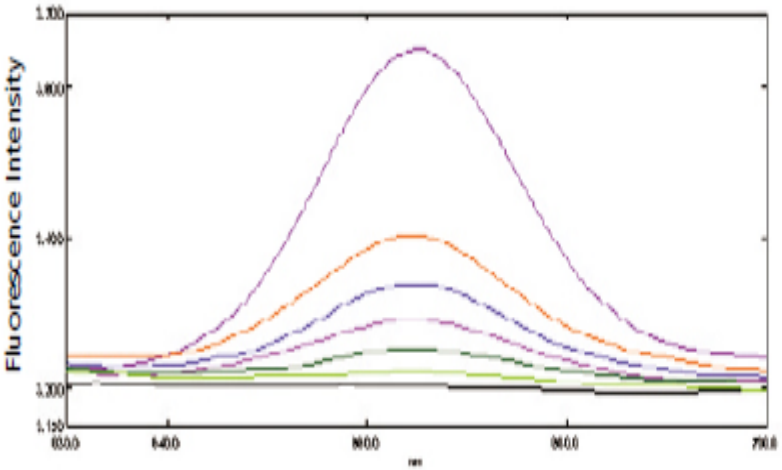



Fig. 3 Chlorophyll-a at Concentrations of 0.75, 1.5, 3.0, 4.5, 7.5, and 15 ppb

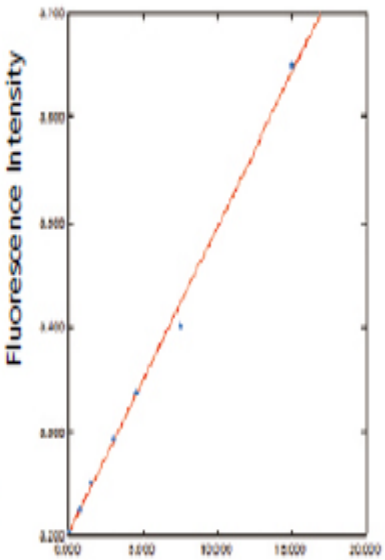
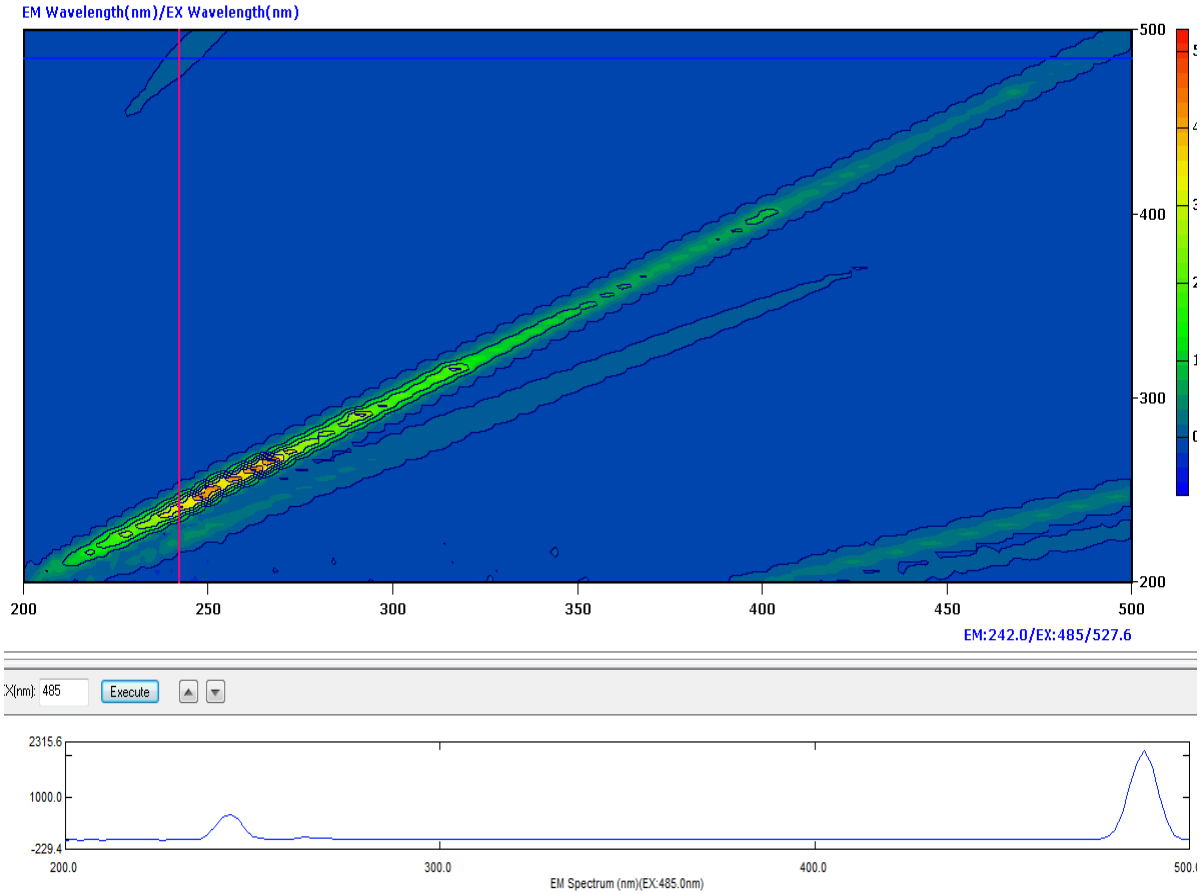



Fig. 4 Calibration Curve

https://www.ssi.shimadzu.com/products/fluorescence-spectroscopy/rf-6000-wide-variety-fields.html

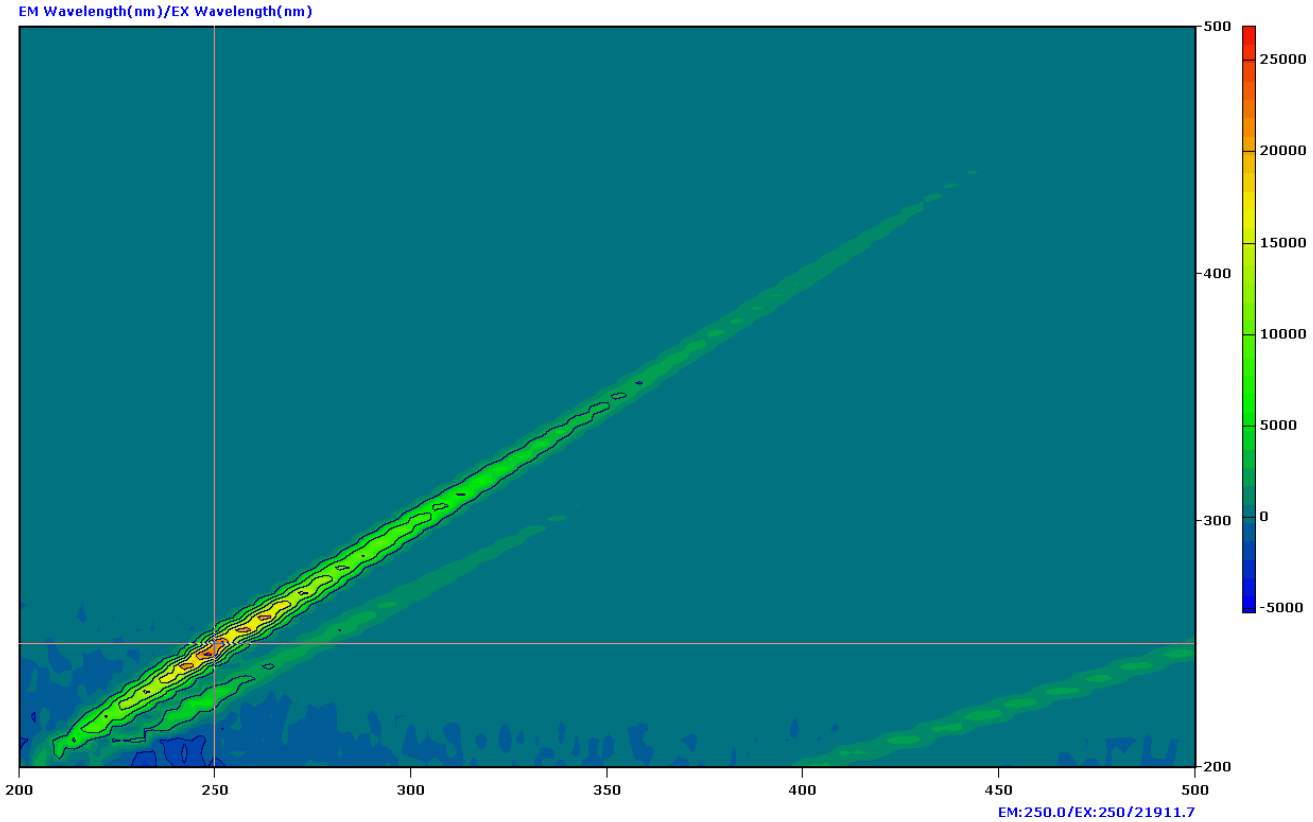
# Source water TOC not high enough for detection of pattern



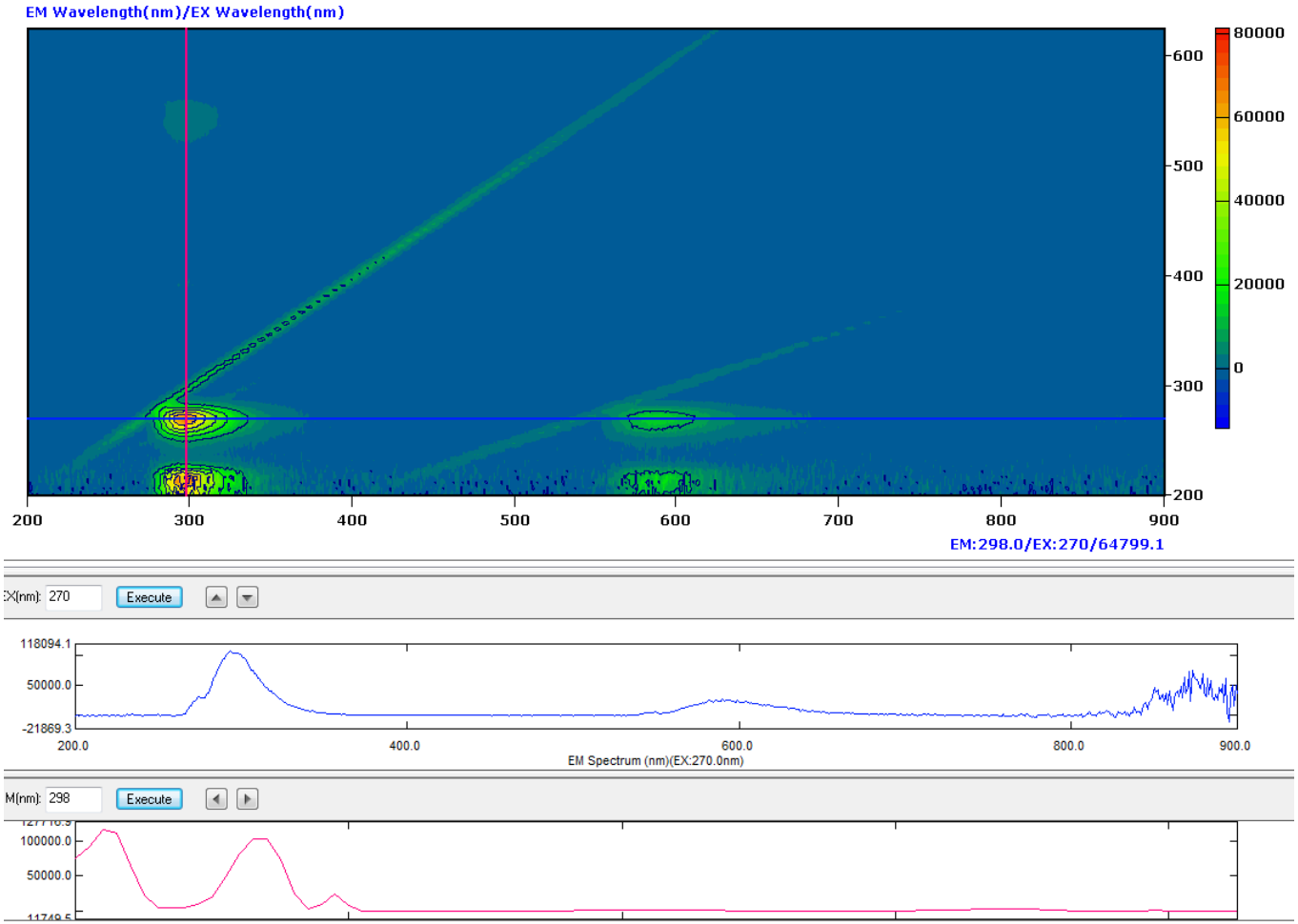

**One source water**

**No real spectra**

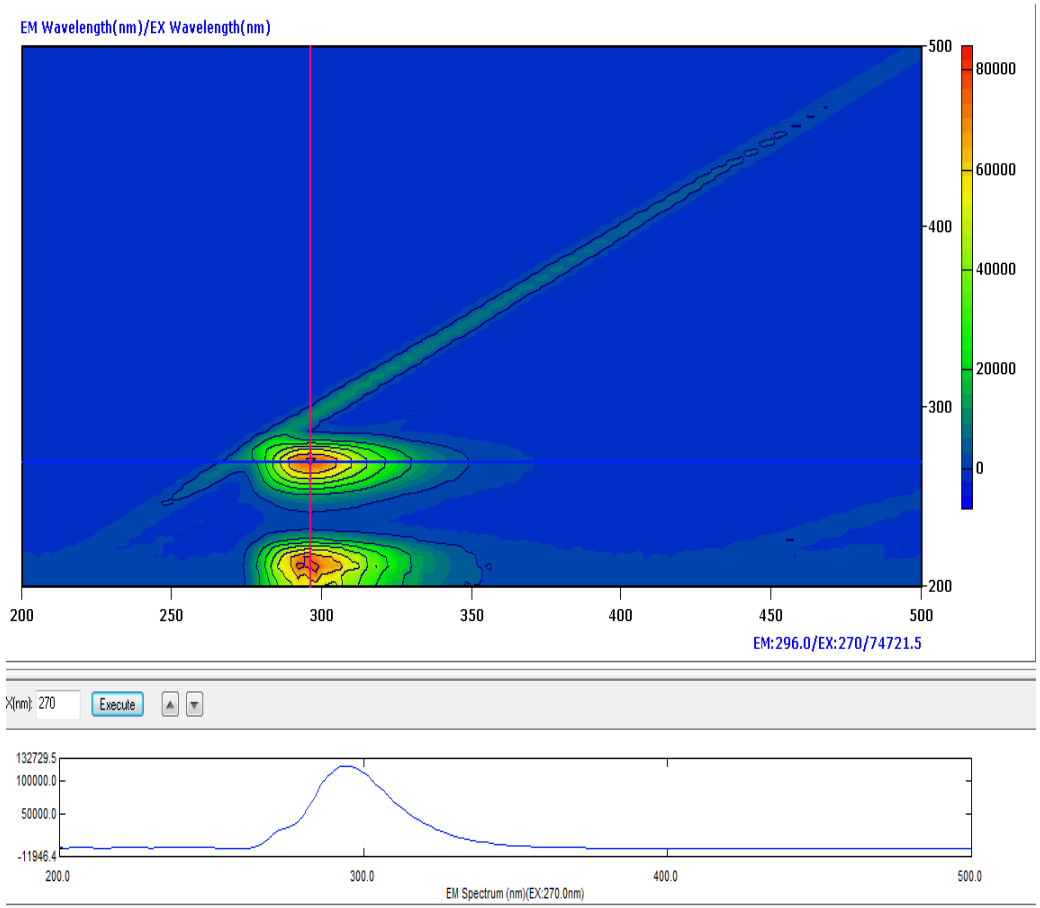
# In addition, TOC is calibrated with KHP. KHP has no peaks

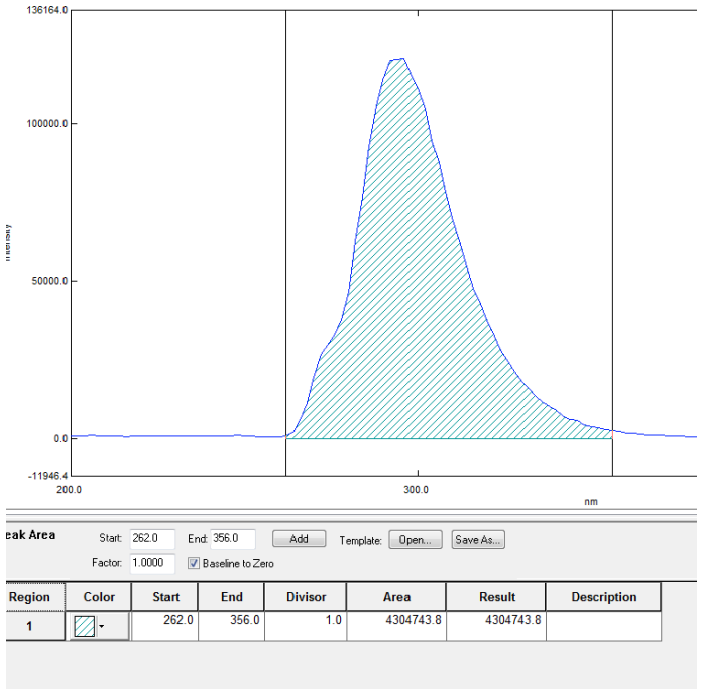


# We did find distinctive peaks for phenol

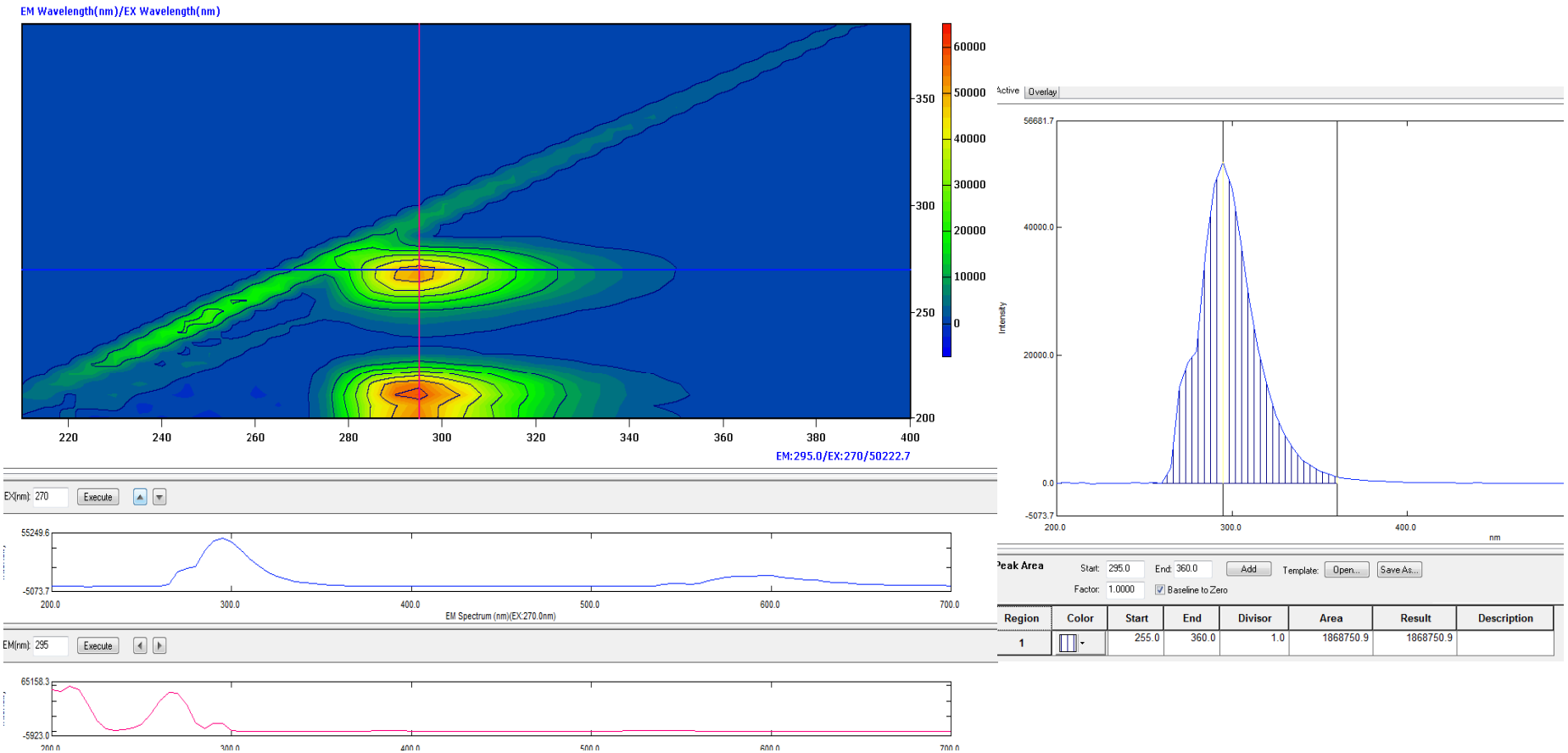


# A closer look at 2.5 ppm phenol

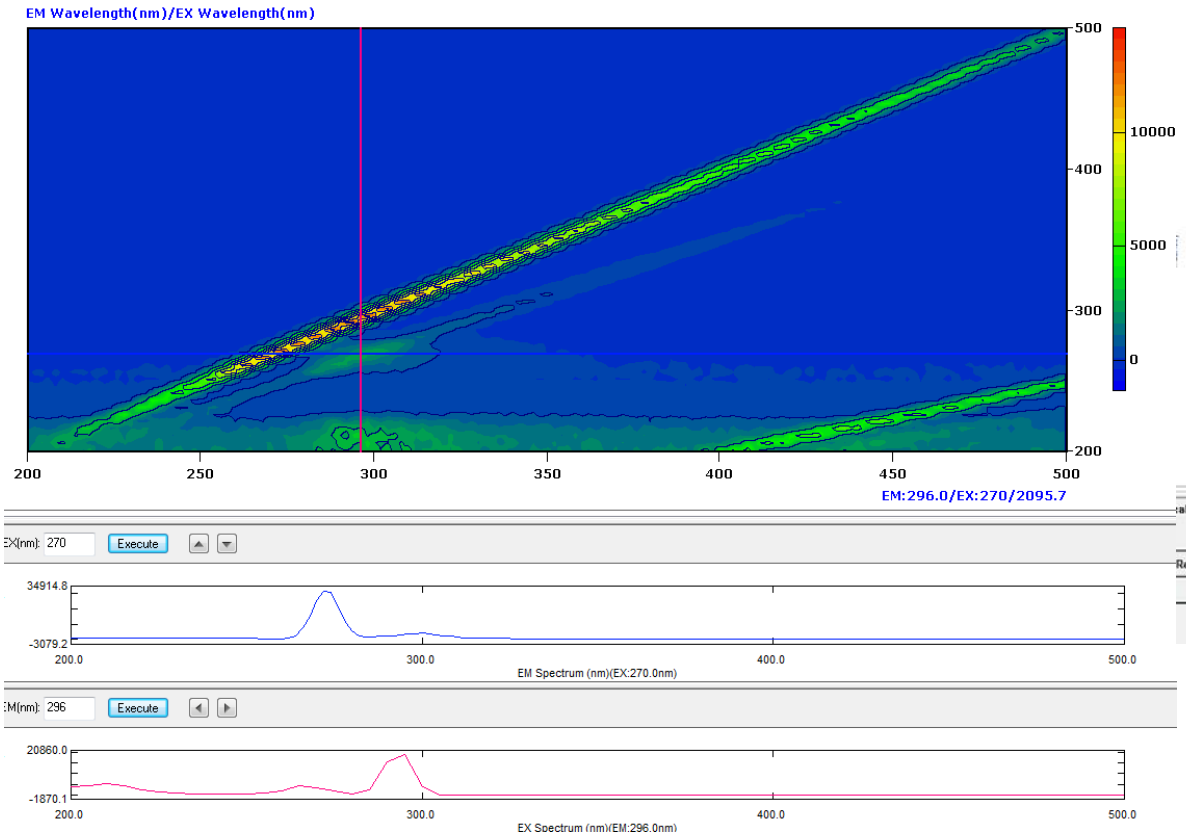


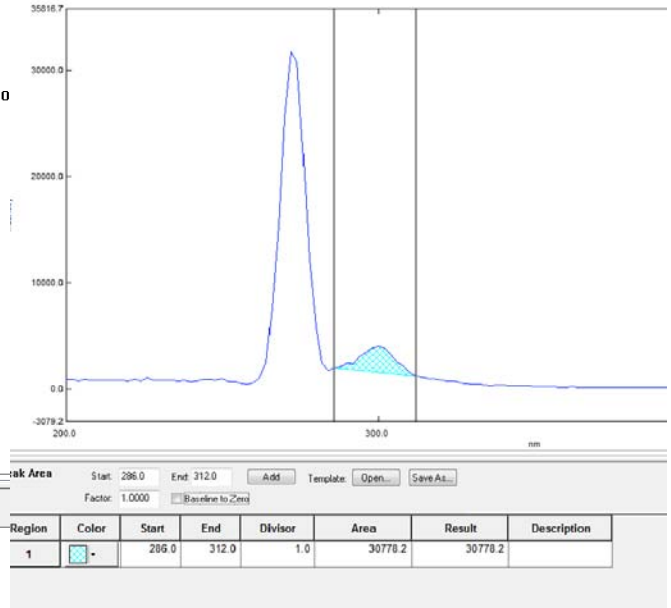



# A closer look at 1.0 ppm phenol

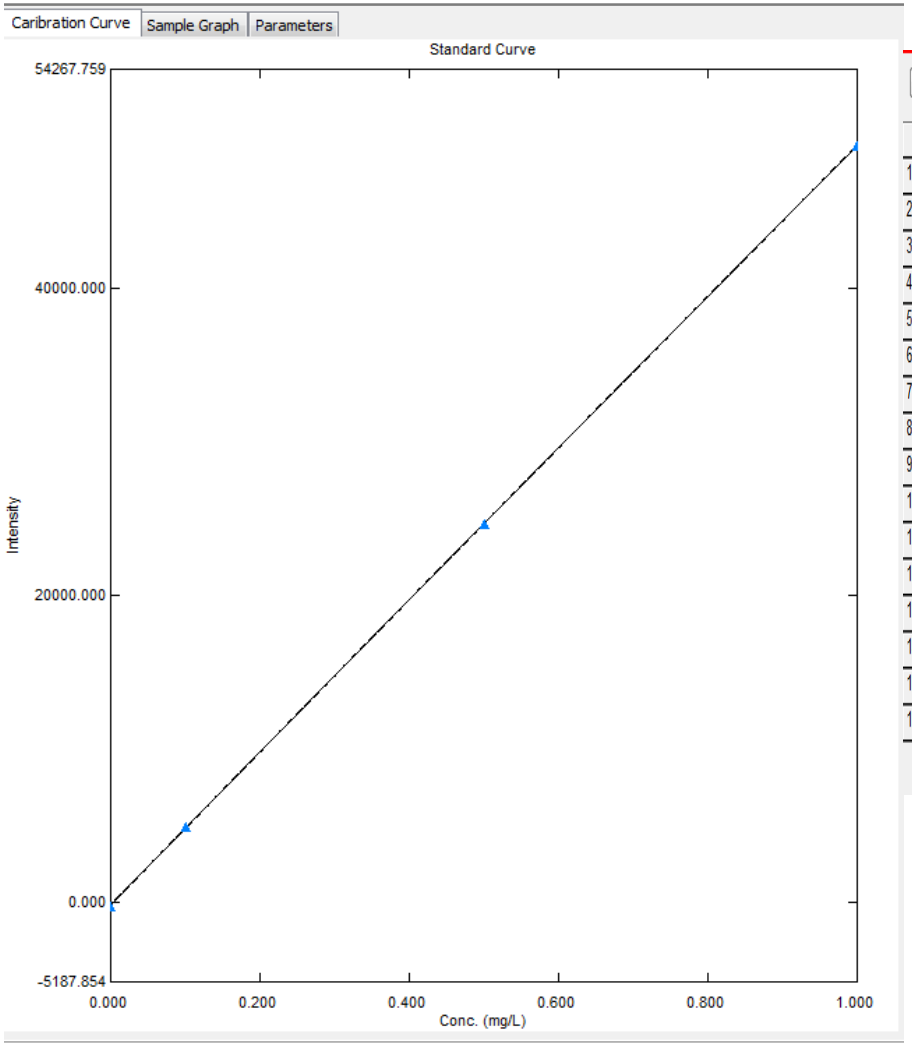


# Shows up even at 0.04 ppm phenol



# Phenol calibration curve (0.1 – 1.0 ppm)




Add Line... Edit... Open... Save As...

| | Sample Name | Sample ID | Option | Type | Ex | Conc | EX270.0_EM295.0 | Wgt.Factor | Comments |
|---|---|---|---|---|---|---|---|---|---|
| 1 | STD1 | SampleID1 | Option1 | Std-Repeat | | 0.000 | -307.862 | 1.000 | |
| 2 | STD1-2 | SampleID1-2 | Option1-2 | Std-Repeat | | 0.000 | -157.212 | 1.000 | |
| 3 | STD1-3 | SampleID1-3 | Option1-3 | Std-Repeat | | 0.000 | -234.585 | 1.000 | |
| 4 | STD1-Avg | SampleID1-Avg | Option1-Avg | Average | | 0.000 | -233.219 | 1.000 | Avg of preceding 3 Samples |
| 5 | STD2 | SampleID2 | Option2 | Std-Repeat | | 0.100 | 5093.724 | 1.000 | |
| 6 | STD2-2 | SampleID2-2 | Option2-2 | Std-Repeat | | 0.100 | 4618.448 | 1.000 | |
| 7 | STD2-3 | SampleID2-3 | Option2-3 | Std-Repeat | | 0.100 | 5032.812 | 1.000 | |
| 8 | STD2-Avg | SampleID2-Avg | Option2-Avg | Average | | 0.100 | 4914.995 | 1.000 | Avg of preceding 3 Samples |
| 9 | STD3 | SampleID3 | Option3 | Std-Repeat | | 0.500 | 24494.881 | 1.000 | |
| 10 | STD3-2 | SampleID3-2 | Option3-2 | Std-Repeat | | 0.500 | 24686.403 | 1.000 | |
| 11 | STD3-3 | SampleID3-3 | Option3-3 | Std-Repeat | | 0.500 | 24727.604 | 1.000 | |
| 12 | STD3-Avg | SampleID3-Avg | Option3-Avg | Average | | 0.500 | 24636.296 | 1.000 | Avg of preceding 3 Samples |
| 13 | STD4 | SampleID4 | Option4 | Std-Repeat | | 1.000 | 49015.505 | 1.000 | |
| 14 | STD4-2 | SampleID4-2 | Option4-2 | Std-Repeat | | 1.000 | 49710.105 | 1.000 | |
| 15 | STD4-3 | SampleID4-3 | Option4-3 | Std-Repeat | | 1.000 | 49213.770 | 1.000 | |
| 16 | STD4-Avg | SampleID4-Avg | Option4-Avg | Average | | 1.000 | 49313.127 | 1.000 | Avg of preceding 3 Samples |

# Phenol P/A data from the previous calibration

| ppm | n | Average ppm | $S_x$ | %RSD | % Recovery |
|---|---|---|---|---|---|
| 0.1 | 4 | 0.101 | 0.002217 | 2.20 | 101 |
| 0.2 | 4 | 0.201 | 0.003464 | 1.73 | 100 |
| 0.05 | 4 | 0.054 | 0.001414 | 2.64 | 107 |
| 0.005 | 7 | 0.0077 | 0.001113 | 14.4 | 154 |
| 0.02 | 7 | 0.0204 | 0.001512 | 7.40 | 102 |
| unspiked | 1 | 0 | | | |
| 0.05 ppm spike | 4 | 0.049 | 0.001258 | 2.55 | 99 |

MDL (calculated) = 0.003 ppm

# Final Observations and conclusions

- The EEM technique can be used to qualitatively estimate pollution at higher concentrations
  - May be good for removal studies
- Pattern recognition requires interpretation
  - Not amenable to routine testing
- Spectrofluorometric Analysis could be used to replace existing methods
  - Chlorophyl-a
  - phenol

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