Comprehensive Analysis of Effluent Organic Matter from Five Wastewater Treatment Plants in Connecticut and Comparison to Natural Organic Matter

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Comprehensive Analysis of Effluent Organic Matter from Five Wastewater Treatment Plants in Connecticut and Comparison to Natural Organic Matter

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Comprehensive Analysis of Effluent Organic Matter from Five Wastewater Treatment Plants in Connecticut and Comparison to Natural Organic Matter

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Table of Contents

Abstract .................................................................................................................. 1

Introduction ......................................................................................................... 2
  Characterization of Natural Organic Matter ....................................................... 2
  Natural Organic Matter ....................................................................................... 7
  Characterization of Effluent Organic Matter ...................................................... 7
  Objectives ........................................................................................................... 10

Overview of Treatment Plants ............................................................... 10
  University of Connecticut, Storrs Campus ....................................................... 10
  Hartford Metropolitan District Commission, Hartford CT .................................. 11
  Heritage Village, Pomperaug CT ...................................................................... 12
  Vernon Treatment Plant, Vernon CT ............................................................... 12
  Stamford Treatment Plant, Stamford CT .......................................................... 13

Methods .............................................................................................................. 14
  Materials ........................................................................................................... 14
  Sample Collection and Preservation ................................................................. 14
  Organic Matter Isolation .................................................................................... 16
  Dissolved Organic Carbon Analysis .................................................................. 17
  Fluorescence and Ultraviolet-Visible Spectroscopy .......................................... 19
  Excitation Emission Matrix Analysis ............................................................... 21
  High Pressure Size Exclusion Chromatography ............................................... 22
  Data Analysis ................................................................................................... 24

Results and Discussion .................................................................................... 24
  Effluent Dissolved Organic Carbon ................................................................. 24
  Organic Matter Size .......................................................................................... 25
  Organic Matter Fluorescence ............................................................................. 31
  Optical Analyses ............................................................................................... 35
  Treatment Plant Intercomparison ...................................................................... 36

Conclusions ......................................................................................................... 38

Environmental Significance .............................................................................. 38
Future Work ........................................................................................................ 39

References ........................................................................................................... 41

Appendices .......................................................................................................... 44
  Appendix A ....................................................................................................... 44
  Appendix B1 ..................................................................................................... 44
  Appendix B2 ..................................................................................................... 54
List of Figures and Tables

Figure 1: Theoretical Aquatic Humic Substances ............................................................. 5

Figure 2: Locations of Peak C and Peak A in Suwannee River Fulvic Acid ..................... 6

Figure 3: Peak locations in an EEM and FRI Regions of Organic Matter ....................... 10

Figure 4: Size Exclusion Chromatograms of Hydrophobic and Transphilic Fractions of UConn, Hartford, Pomperaug, Vernon and Stamford Treatment Plants ..... 27

Figure 5: Numbering Convention of Peaks in Size Exclusion Chromatogram of EfOM .................................................................................................................................. 31

Figure 6: EEMs of UConn, Hartford, Pomperaug, Vernon and Stamford Treatment Plants ............................................................................................................................................................................. 35

Table 1: Overview of Treatment Plants ............................................................................ 16

Table 2: Dissolved Organic Carbon and Percent of effluent organic matter extracted by hydrophobic and transphilic resins and not extracted in hydrophilic fractions ............................................................................................................................................................................................ 26

Table 3: Number averaged and weight averaged molecular weights and polydispersivity excluding 50,000 Dalton polysaccharide peak ......................... 29

Table 4: Molecular Weight at the six peaks of each Size Exclusion Chromatogram ...... 31

Table 5: Absorbance Intensity Ratio ($\text{Abs}_{220}/\text{Abs}_{272}$) at six size exclusion peaks .... 32

Table 6: Percentage distribution of different regions of EEMS by FRI method ............... 34

Table 7: Fluorescence Index, SUVA, pH, and E2/E3 ratio of effluent organic matter ....... 37
ABSTRACT

Natural organic matter is an important driver of biotic and abiotic processes in aquatic environments. Wastewater treatment plants discharge a substantial amount of organic matter into the environment; however effluent organic matter has not been well studied. In this study, traditional organic geochemical techniques were applied to characterize effluent organic matter. Effluent organic matter was isolated by DAX8 (hydrophobic fraction) and XAD4 (transphilic fraction) Amberlite resins. Extraction efficiencies of effluent organic matter by DAX8 resins ranged from 18 to 42 percent as a result of larger content of hydrophilic organic matter than natural organic matter. Average organic matter molecular weights by size exclusion chromatography were from 450-670 Daltons with higher weights for hydrophobic than transphilic fractions. Fluorescence characterization showed both humic and fulvic like fluorescence as well as tryptophan and tyrosine like fluorescence, the latter not commonly observed in terrestrial organic matter. Fluorescence indices were between 1.5 and 1.9 with lower values for hydrophobic organic matter than transphilic. Specific ultraviolet absorbance was measured between 0.8 and 3.0 L mg$^{-1}$ m$^{-1}$. Together these characterization techniques indicate that extracted effluent organic matter is similar in characteristics to microbially derived organic matter. Comparisons of effluent from different plants suggest the characteristics of effluent organic matter are similar regardless of treatment plant.
INTRODUCTION

Dissolved organic matter is a complex and heterogeneous mixture of carbon-based molecules that influences the biotic and abiotic processes in ecosystems. Dissolved organic matter (DOM) is an important carbon source in river systems, supporting microbial growth in the water column, as well as in biofilms[1]. DOM affects the availability of dissolved metals via metal sorption and transport [2-5]. The degradation of many emerging contaminants, such as pharmaceuticals and antibiotics, is greatly enhanced by indirect photolysis in the presence of organic matter [6-8]. Despite the known impact of organic matter in aquatic systems, little work has examined anthropogenic sources of organic matter from wastewater effluent discharges.

The amount of organic matter that wastewater treatment plants contribute to aquatic environments is substantial. In 2008 the estimated flow of wastewater was 32,345 million gallons per day in the US [9]. Treated wastewater is often discharged into rivers where it can constitute a large fraction of flow. For example, in arid regions of the US wastewater can contribute up to 100 percent of streamflow [10]. The concentration of DOM in wastewater is commonly between 4 and 20 mg/L as carbon which is comparable to the concentration of DOM in water from natural sources [11]. Thus, ecosystem processes will be dependent on effluent organic matter as a source of DOC.

Characterization of Natural Organic Matter

As a result of complexity and heterogeneity, the exact molecular structures of only about 20% of organic matter can be determined[1], so more qualitative analytical techniques are used to analyze organic matter. The 20% that can be characterized
includes carboxylic acids, amino acids, carbohydrates, volatile hydrocarbons and other simple organic compounds. The remaining 80% is operationally defined as humic acids and fulvic acids. Humic acids are large, sometime colloidal molecules with molecular weights above 2000 Daltons (Figure 1). Fulvic acids are smaller, between 800 and 2000 and are more water soluble because they contain more carboxylic and hydroxyl functional groups [11].

Extraction of NOM by resin sorption is the most widely used method to isolate organic matter. Organic matter isolation by DAX 8 Supelite™ resin is the standard method used by the International Humic Substances Society [12]. DAX 8 is a polymethyl methacrylate non-ionic macroporous resin. The resin is useful because it adsorbs and desorbs organic matter easily and captures a large fraction of organic matter. DAX 8 is hydrophobic and attracts hydrophobic organic matter by weak physical forces [11]. Organic matter is sorbed when the weak physical forces are dominant. At pH 2, the functional groups of organic matter are protonated and organic matter is adsorbed. Desorption is achieved by raising the pH and deprotonating functional groups. The extraction efficiency of NOM on DAX 8 amberlite resin is approximately 50 percent for an average water sample [11].

The analysis of organic matter using fluorescence spectroscopy has become a common method in the past decade. Measuring the Fluorescence Index of organic matter is one way to determine the source of the organic matter. The fluorescence index of an organic matter sample has been correlated to the position of that sample on the organic matter continuum. Fluorescence indices near 1.3 are representative of terrestrial organic matter, and 1.9 are representative of microbial organic matter [13].
Theoretical Humic Acid

Theoretical Fulvic Acid

**Figure 1:** Theoretical Aquatic Humic Substances, (Hudson et al. 2007)

Arrow in Humic acid indicates possible polypeptide chain
Excitation emission matrices (EEMs) provide a more comprehensive way to study the fluorescence of organic matter. EEMs are obtained by scanning across both excitation and emission wavelengths to produce a matrix of fluorescence intensity. EEMs are a fast and non-destructive method of analyzing organic matter and only require a small amount of sample. Previous work has identified two dominant peak areas in fluorescence EEMs of NOM [13-15]. Peaks A and C are broad fulvic and humic like fluorescence commonly observed in NOM EEMs (Figure 2).

![Figure 2: Locations of Peak C and Peak A in Suwannee River Fulvic Acid, (Her et al. 2003)](image)

Accurate measurement of molecular weight is determined by the use of Size Exclusion Chromatography (SEC). Chromatograms of NOM are unimodal, with a broad
and featureless peak. Terrestrial organic matter has a higher average molecular weight than microbial organic matter. Number averaged and weight averaged molecular weights for Suwannee river are 1360 and 2310 Daltons respectively, organic matter from Lake Fryxell are 713 and 1080 Daltons respectively [16].

Specific ultraviolet absorbance (SUVA) is strongly correlated to aromaticity of organic matter[17]. Terrestrial organic matter is derived from plant matter that contains complex aromatic structures like lignin. As a result, terrestrial organic matter has higher aromaticity and hence higher SUVA values than microbial organic matter. SUVA values reported for terrestrial microbial organic matter are 3.8 and 1.7 L mg\(^{-1}\) m\(^{-1}\) respectively [17].

The E2/E3 ratio is a measure of the extent of conjugation of single and double carbon bonds in the organic matter. As the extent of substructure conjugation in organic matter increases, the wavelengths at which organic matter absorbs light also increase. E2/E3 ratio is calculated by dividing the absorbance intensities at 254 and 365 nm. Absorbance of light at higher wavelengths will cause a lower E2/E3 ratio. Therefore a low E2/E3 ratio is indicative of enhanced conjugation among carbon atoms in organic matter structure. The typical range of E2/E3 ratio of organic matter is between 3 and 6. This indicator of organic matter has not been directly correlated to the natural organic matter continuum. Therefore it can only be a measure of the extent of conjugation of the carbon structure.
Natural Organic Matter

As a result of applying these qualitative techniques, two members have emerged that bound OM characteristics for most aquatic systems. Allochthonous organic matter is any material that originated outside the stream such as decaying terrestrial matter, and autochthonous organic matter is material generated inside the stream from microbial growth. Allochthonous or terrestrial and autochthonous or microbial organic matter are the end members of a continuum that include all natural organic matter (NOM). The physical manifestations commonly used for these two end members are Suwannee River and Lake Fryxell. The organic matter of Suwannee River is dominated by terrestrial organic matter due to dense vegetation along its banks. Lake Fryxell in Antarctica is perennially covered by ice, and all organic matter is derived by microbial activity. Most NOM is not at the ends of the continuum but can be compared to the end members with chromatographic and optical spectroscopic analyses.

Characterization of Effluent Organic Matter

The analytical techniques that are used to study effluent organic matter (EfOM) are not consistent between studies. The extraction method is often modified because the DAX8 resin technique used for NOM captures 26-40 percent of organic matter [4, 18-20]. The most common modification to the extraction procedure is the addition of XAD4 as a second adsorbent column. Another modification is the use of anion and cation exchange resins to further isolate the organic matter eluted from the DAX8 resin [21]. Yet another modification is the use of organic solvents to elute organic matter from
Amberlite resins. The use of different extraction methods further limits the possible comparison of the work that has been done.

Previous work to characterize effluent organic matter has identified 5 distinct peak areas in fluorescence EEMs [15]. Peaks A and C are broad fulvic and humic like fluorescence, peak B is similar to the fluorescence of tyrosine and peaks T1 and T2 are similar to the fluorescence tryptophan as shown in Figure 3. The presence or absence of these five peaks gives insight to the type of fluorophores that are present in organic matter. To quantify the magnitude of the peaks in relation to each other, the Fluorescence Region Integration (FRI) method can be used. The FRI method breaks an EEM into five regions corresponding to the five fluorescent peaks, and integrates the volume underneath each region. The percentage of the total volume in each region shows the intensities of the fluorophores[22].
EfOM results are sparse. Number averaged and weight averaged molecular weights of effluent organic matter extracted on DAX8 resins are 400-500 and 650 to 850 Daltons respectively. SUVA values of effluent organic matter range from 1.5 to 2 [21, 23].

The data that is available suggests that effluent organic matter does lie within the bounds of organic matter. However, previous studies of effluent organic matter often only use a subset of the characterization techniques applied to NOM. Without comprehensive analysis of EfOM it is difficult to conclusively determine the similarity to NOM continuum.
Very few studies have directly compared EfOM from different treatment plants. Comparisons between studies are difficult as a result of varied techniques and non-comprehensive analysis. The range of characteristics of EfOM from different treatment plants is not well understood.

**Objectives**

In this study, we investigate effluent organic matter to answer the following questions: Does effluent organic matter fit within the natural organic matter continuum? Does effluent organic matter from different wastewater treatment plants have similar characteristics? To answer these questions, effluent from five wastewater treatment plants in Connecticut was sampled and tested using analytical techniques that applied traditionally to natural organic matter.

**OVERVIEW OF TREATMENT PLANTS**

**University of Connecticut, Storrs Campus**

The UConn treatment plant serves the University of Connecticut Storrs Campus. Wastewater from dormitory buildings, apartment complexes, classroom buildings and dining halls contribute the majority of the flow to the treatment plant. Other contributors to the plant are the University laboratories, and several local restaurants. The average daily flow rate is 1.5 million gallons per day (MGD). The first stage of treatment is the preliminary physical treatment processes which include a bar screen and cyclone degritter. The next stage is biological treatment in the EIMCO™ carousel aeration tanks.
The carousel tanks have an aerobic zone and an anaerobic zone to enhance nitrogen removal. The aerobic zone utilizes mechanical aerators to introduce dissolved oxygen. The water then flows to one of the two secondary clarifiers and finally to the chlorine contact chamber. The UConn treatment plant chlorinates their effluent from April to October.

**Hartford Metropolitan District Commission**

The Hartford Metropolitan District Commission operates a number of treatment plants in Hartford County including the largest treatment plant in the state located in East Hartford which will be referred to herein as Hartford treatment plant. The wastewater infrastructure in Hartford is a combined sewer, meaning all storm drains in the city lead to the treatment plant along with municipal wastewater. The treatment plant treats wastewater from three flow interceptors: Franklin Avenue Interceptor, Connecticut River Interceptor and Connecticut River Relief Interceptor. The average flow through the plant is 60 MGD, but the flow rate fluctuates greatly with precipitation. The treatment at Hartford begins in the headworks building with a bar screen, and degritter. The water then flows to primary settling tanks to remove suspended solids and into the aeration tanks. The aeration tanks use bubble diffusers to regulate dissolved oxygen levels. After the aeration tanks, suspended solids are settled in secondary clarifiers and finally the treated wastewater flows through the chlorine contact chamber before discharge. The Hartford treatment plant chlorinates the treated wastewater starting in May and ending in October.
Heritage Village, Pomperaug CT

The Heritage Village water treatment plant, referred to as Pomperaug treatment plant, serves three residential retirement communities in Pomperaug CT, as well as a restaurant and a bed and breakfast. The treatment plant is small, with an average flow rate of 0.4 MGD. The treatment system at Pomperaug begins with a bar screen and a degritter. The screened wastewater flows into rectangular aeration tanks that use mechanical aeration. To achieve some level of denitrification in the plant, the target dissolved oxygen in the tank is 0.2 mg/L. When temperatures are above freezing, the mechanical aerators are run intermittently at half hour intervals. When temps drop below freezing, the aerators have to be run constantly to prevent ice formation. The water then flows through secondary clarifiers and then to the chlorine contact chamber. Pomperaug effluent is chlorinated from May to October.

Vernon Treatment Plant, Vernon CT

The Vernon treatment plant receives all municipal wastewater from the cities of Vernon, Ellington and Tolland CT, and parts of Manchester and South Windsor CT. The water that is treated at Vernon is 95% residential waste and receives no storm water. The average flow rate for Vernon is 3.5 MGD. Treatment begins with a coarse and fine bar screen to remove large debris, followed by a cyclone degritter. The wastewater then flows to a primary settling tank to remove suspended solids and into the aeration tanks. The Vernon treatment plant is unique in that granular activated carbon is used as a suspended media in the aeration tanks. Historically, the activated carbon was used to remove pigment dyes from the wastewater that were present from several dye factories in
Vernon that have since gone out of business. As the water leaves the aeration tanks a polymer is added to aid the settling in the secondary clarifiers. After settling, the water flows through sand and anthracite filters before moving to the chlorine contact chamber.

Vernon chlorinates their wastewater from May to October. After chlorination, the Vernon treatment plant post-aerates their water to comply with the 7Q10 criteria established by the EPA. If the plant is running at capacity and the river where treated water is discharged is at a 10 year drought, the flow of the river will be 85% treated effluent. Therefore, Vernon is required to post-aerate their effluent year round.

Stamford Treatment Plant, Stamford CT

The Stamford treatment plant serves the city of Stamford Connecticut. The city of Stamford has separate sewer and stormwater infrastructure, so no stormwater is treated at the plant. The city has sewer lines south of the Merritt Parkway, and septic tanks north of the parkway; when emptied, the waste from septic tanks is sent to the Stamford treatment plant. Wastewater pre-treatment starts with bar screening to remove large debris followed by primary clarifiers to remove suspended solids and lastly degritting. As a result of its proximity to Long Island Sound, the Stamford treatment plant has very strict nitrogen regulations. To meet these regulations, the plant has advanced nitrogen removal.

Stamford uses the Modified Ludzack Ettinger MLE method to treat the wastewater and remove nitrogen by denitrification. In the aerobic tanks, fine bubble diffusers are used to introduce oxygen. After biological treatment by the MLE method, the wastewater flows to secondary clarifiers and is disinfected by an array of high powered ultra violet lamps. Stamford disinfects their wastewater year-round.
METHODS

Materials

Methanol (Certified ACS), Acetonitrile (HPLC Grade), HCl (Trace Metal Grade) and KOH (certified ACS) were purchased from Fisher. DAX 8 Supelite™ Resin was purchased from Supelco, XAD 4 Amberlite Resin and Cation Exchange Resin (DOWEX Marathon MSC, H form) were purchased from Sigma Aldrich. Organic Carbon standard (potassium phthalate) was purchased from Ricca Chemical Company.

Sample Collection and Preservation

Final effluent was collected from five wastewater treatment plants in Connecticut: University of Connecticut (Storrs Campus), Hartford Metropolitan District, Vernon, Stamford, and Pomperaug. Samples were collected between November 2010 and April 2011 (Table 1). The Hartford plant was sampled during dry weather to prevent dilution effects by stormwater. Once collected, samples were transported to the lab where they were filtered using Whatman GF/A glass fiber filters (0.45 um), and acidified to pH 2 using HCl within 24 hrs. Samples were stored in the refrigerator at 2 degrees Celsius and isolated on the amberlite resins within one week. The isolated fractions were analyzed within four weeks.
<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Location</th>
<th>Plant Users</th>
<th>Ave Plant Flow Rate</th>
<th>Treatment Technology</th>
<th>Nitrogen Removal</th>
<th>Disinfection</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCONN</td>
<td>Storrs, CT</td>
<td>Mainly residential, small amount of lab waste</td>
<td>1.5 MGD</td>
<td>Grit Screening EIMCO carousel aeration tanks, secondary clarifier, chlorination</td>
<td>Nitrification and Denitrification by aerobic and anaerobic zones of carousel</td>
<td>CI</td>
</tr>
<tr>
<td>Hartford</td>
<td>Hartford, CT</td>
<td>Combined Sanitary Sewers and Stormwater,</td>
<td>60 MGD</td>
<td>Headworks pumping and grit removal, Primary clarifier, aeration tank, secondary clarifier, chlorination</td>
<td>Nitrification, No Denitrification</td>
<td>CI</td>
</tr>
<tr>
<td>Pomperaug</td>
<td>Southbury, CT</td>
<td>100% Residential, mainly retirement communities</td>
<td>0.4 MGD</td>
<td>Screen Grit, Mechanical Aeration at 0.2 mg/L DO, secondary clarifier, chlorination</td>
<td>Nitrification and Denitrification due to low DO in aeration tanks</td>
<td>CI</td>
</tr>
<tr>
<td>Vernon</td>
<td>Vernon, CT</td>
<td>95% Residential, No Stormwater</td>
<td>3 MGD</td>
<td>Screen Grit, Primary Clarifiers, Granular Activated Carbon in Aeration Tanks, Secondary Clarifiers, Chlorination, post aeration</td>
<td>Nitrification, No Denitrification</td>
<td>CI</td>
</tr>
<tr>
<td>Stamford</td>
<td>Stamford CT</td>
<td>90% residential, no stormwater, very little industry, commercial office buildings</td>
<td>24 MGD</td>
<td>Screen Grit, Primary Settling, Biological Advanced Nitrogen (anoxic, aerobic, anoxic aerobic), UV disinfection</td>
<td>Nitrification and Denitrification by Modified Ludzack Ettinger process</td>
<td>UV</td>
</tr>
</tbody>
</table>

Table 1: Overview of Treatment Plants
MGD: million gallons per day
Cl: Chlorine Contact Chamber Disinfection
UV: Ultraviolet Light Disinfection
Organic Matter Isolation

The resin cleaning and organic matter isolation methods using DAX 8 and cation exchange resins were adopted from previous studies [12], and modified to include the use of XAD 4 resin.

To prepare for the extraction of organic matter, the DAX 8 and XAD 4 resins were submerged in 0.1M KOH for 24 hours, followed by Soxhlet extraction with methanol and acetonitrile for 24 hours each. The cation exchange resin was cleaned by Soxhlet extraction in methanol only. The cleaned DAX8 and XAD4 resins were stored in methanol until use; the cation exchange resin was stored in water. The resins were cleaned before each sample was extracted. Glass Chromaflex chromatography columns, purchased from Kontes, with Teflon end fittings and 0.20 µm bed supports were used for the extraction of organic matter.

The XAD 4 and DAX 8 resins were packed into their respective columns as a methanol/water slurry to minimize porosity and preferential flow paths. A small amount of ponded water in the columns when packing aided in creating one continuous bed of resin. The resins were washed with fifteen bed volumes of deionized water to remove any remaining methanol. As a final cleaning step, the resins were rinsed three times with alternating void volumes of 0.1M KOH and 0.1M HCl prior to extraction. It is important that the last rinse is acid, so the first few bed volumes of organic matter will adsorb to the resin. The cation exchange resin was packed in the same manner and also washed with 15 bed volumes of water to remove methanol. To hydrogen saturate the resin, a void volume of 2N hydrochloric acid was pumped through the column, followed by two bed volumes of water to remove chloride and excess hydrogen atoms.
Once the resins were prepared and packed into their respective columns, the DAX8 and XAD4 columns were connected in series using Teflon tubing. The effluent sample was pumped through the DAX8 resin first, followed by the XAD4 resin at a rate of 15 bed volumes per hour using a Masterflex 7550-30 peristaltic pump. C-Flex tubing was used in the peristaltic pump head. Approximately 50 bed volumes of effluent sample were pumped through both resins to ensure that a significant amount of organic matter could be extracted. Before the sorbed organic matter was eluted from the columns, one bed volume of water was pumped through to remove anions. The organic matter was eluted from each column individually using 3 bed volumes of 0.1M KOH. The organic matter eluted from the DAX8 resin is the hydrophobic (HPO) fraction, and the organic matter from the XAD4 resin is the transphilic (TPI) fraction. Any organic matter that was not captured by either column is the hydrophilic (HPI) fraction. Cations were removed from the concentrated organic matter by pumping through the column packed with cation exchange resin. Because the resin was in hydrogen form, the pH of the organic matter was decreased from approximately 13 before cation exchange to approximately 2 after. The resulting isolated and desalted organic matter solutions were used for the subsequent analyses.

**Dissolved Organic Carbon Analysis**

Dissolved organic carbon was measured on a Tekmar Apollo 9000 organic carbon analyzer. Calibration standards of 1, 3, 5, 10 and 20 mg/L were made by serial dilution of organic carbon standard (potassium phthalate). If the organic carbon concentration of a sample was above the calibration curve, the sample was diluted and re-analyzed. Initial
carbon concentrations were measured after the sample was filtered and acidified. Final carbon concentrations were measured after desalting the HPO and TPI fractions of organic matter on the cation exchange resin. To determine the percentage of organic matter that was not retained on either column, a sample of the HPI fraction was collected after the entire sample had been extracted. To calculate the percentage of organic matter in a fraction, the content of organic carbon of that fraction (mg) was divided by the content of organic carbon of the effluent (mg). The volumes of the UConn and Hartford samples were different than Pomperaug, Vernon and Stamford because different sized columns were used. UConn and Hartford were isolated with larger columns, 50L of effluent was isolated and 3 liters was eluted from each column. For Pomperaug, Vernon and Stamford, 20L of effluent was isolated and 1 L was eluted from each column. The percent recovery of the Stamford treatment plant is much lower than the other four plants. This is probably due to an inaccurately low measurement of the HPI fraction.

DOC measurements were also used to determine specific ultraviolet absorbance (SUVA) which is calculated with the following equation.

\[
SUVA = \frac{100 \times A_{254}}{DOC}
\]

Where \(A_{254}\) is the absorbance at 254 nm measured in a 1cm cuvette, and \(DOC\) is measured in mg/L.
Fluorescence and Ultraviolet – Visible Spectroscopy

Samples were analyzed on the fluorometer directly following isolation without dilution or pH adjustment. pH adjustment was not required because the low pH attained from the cation exchange was desired for fluorescence measurements. Sorbed metals have been shown to quench fluorescence, but at low pH, the metals will be free in solution and not affect fluorescence. There is also a direct pH effect on organic matter, as pH is increased, fluorescence is increased[15]. Since metals were removed with the cation exchange resin, the low pH was desired to compare to previously measured data. All data was analyzed on a Cary Eclipse fluorescence spectrometer with a xenon flash lamp, slit widths of 5 nm and a scan rate of 1200 scans per second. The excitation wavelength was scanned from 200-450 nm at 10nm increments, and the emission wavelength from 250-550 nm at 2nm increments.

Many studies have proposed methods for correcting EEMs data [13, 15, 24-27]. These methods all have similar components but differ in the number of corrections and the order in which they are applied. The EEMs correction method used in this study was modeled after Murphy et al 2010. To verify wavelength accuracy, internal instrument tests were run each day before analysis. The internal instrument tests insured that the excitation and emission wavelengths were accurate, however could not correct for changes in the intensity. To account for changes in intensity over time, a correction matrix with the same dimensions as the EEM was created. The emission wavelength component of the correction matrix was determined with a solution of 10^{-3} M quinine sulfate in 0.1N sulfuric acid. The quinine sulfate solution was scanned at excitation 346.5 nm and emission 384-667 at every 1 nm, and compared to tabulated data [28].
Instruments equipped with a red photomultiplier tube can provide excitation spectra which are not very distorted [28]. Our instrument was equipped with a red photomultiplier tube, so the correction matrix was only dependent on the emission correction. The correction matrix was multiplied by the EEM to obtain wavelength accurate data.

To account for the absorbance of the excitation light and emitted light by the organic matter itself, an inner filter effect correction was made. Assuming that the path length for both the excitation and emission wavelengths is 0.5 cm in the 1 cm sample cell, the following equation can be used to correct for the inner filter effect.

\[
F_{\text{corr}} = \frac{F_{\text{meas}}}{10^{-(A_{\text{exit}}+A_{\text{emit}})}}
\]

\(F_{\text{corr}}\) and \(F_{\text{meas}}\) represent the corrected fluorescence and the measured fluorescence respectively and \(A_{\text{exit}}\) and \(A_{\text{emit}}\) represent the absorbance at the excitation and emission wavelength respectively at any given excitation emission pair. The absorbances used in this equation were measured using a Cary Bio 50 spectrophotometer.

To remove the effects of Raman and Rayleigh scatter, DI water was analyzed using the same parameters as the samples. The blank was multiplied by the correction matrix, and the resulting matrix was subtracted from each sample EEM. The subtraction of a water blank was not sufficient to remove scattered light when the excitation and emission wavelengths were equal and when the emission wavelength was twice the excitation. To remove the scatter, a value of zero for intensity was inserted into an EEM
when the two wavelengths were within 8 nm of each other, and when the excitation wavelength multiplied by 2 and the emission wavelength were within 8 nm. Zeros were also inserted into and EEM when the excitation wavelength was longer than the emission wavelength. The last correction was to normalize the EEM intensities to Raman units. To normalize the EEM, each intensity value was divided by the area under the Raman peak of the water blank at excitation 350 and emission 381-426.

**Excitation Emission Matrix Analysis**

To qualitatively analyze an EEM, the Fluorescence Regional Integration (FRI) method was used as developed by Chen et al in 2003. The FRI method separated an EEM into five regions based on observed fluorescence peaks A, B, C, and the two T peaks (Figure 3). The regions were separated with a horizontal line at 250 nm and two vertical lines at 330 and 380 nm. The diagonal line has a slope of 1 and intersects all the points where emission equals excitation. The volume under the EEM in each region was calculated using the following equation for discrete measurements.

\[
\Phi_i = \sum_{ex} \sum_{em} I(\lambda_{ex}, \lambda_{em}) \Delta \lambda_{ex} \Delta \lambda_{em}
\]

Where I is the intensity at any excitation emission pair, \(\Delta_{ex}\) is the excitation wavelength interval (10nm) and \(\Delta_{em}\) is the emission wavelength interval (2nm). Since each region had a different area, the total volume of each region was normalized with the use of a multiplication factor. The multiplication factor for each region is the inverse of: the area of that region divided by the total area (Appendix A). The resulting values were
average fluorescence response per unit area in each region. Lastly, the average fluorescence response of each region was divided by the total fluorescence response of all regions and multiplied by 100 to convert to a percent fluorescence response.

Commonly, EEMS are measured with an excitation wavelength range from 200nm to 450nm, however it was necessary to truncate data below 230nm. At the concentrations of organic matter that were used in this study, the absorbance of light below 230 nm was above 1, indicating that less than 1% of light was transmitted through the sample. This high absorbance caused a very large correction factor in the inner filter effect equation. Combining the large correction factor with the large amount of fluorometer noise at low wavelengths caused large noise peaks in the EEMS. A cutoff at 230nm allowed the B, T1, and A peaks to be measured but prevented excessive noise.

**High Pressure Size Exclusion Chromatography**

Size exclusion chromatography was performed using a Hewlett Packard 1050 series high pressure liquid chromatograph. The system included a quaternary solvent pump, a 21 and 100 tray autosampler, and a diode array detector capable of analyzing 5 wavelengths simultaneously. The mobile phase was a phosphate buffer at pH 6.8 with 0.002M Na₂HPO₄ and KH₂PO₄, and 0.1M NaCl. A Protein Pak 125 column and guard columns were purchased from Waters. Injections were 50 uL and sample runs were 15 minutes each. All samples were run within a month after sample collection.

Polystyrene sulfonic acids (PSS) in sodium salt form (Polysciences) with the following molecular weights were used to generate the calibration curve (1K, 1.8K, 4.6K, 18K, 35K Daltons). Acetone was used as a low molecular weight calibration standard.
The PSS standards were prepared individually in mobile phase for analysis at 100 mg/L. The diode array detector was set to analyze the PSS standards and acetone at 224nm and organic matter samples at 254 nm. Using the calibration curve, the x-axis of the chromatograms was converted from time to log Molecular weight. The average size of the organic matter was determined using the following two equations for number averaged and weight averaged molecular weight.

\[
M_n = \frac{1}{N} \sum_{i=1}^{N} h_i / \frac{1}{N} \sum_{i=1}^{N} (h_i / M_i)
\]

\[
M_w = \frac{1}{N} \sum_{i=1}^{N} h_i (M_i) / \frac{1}{N} \sum_{i=1}^{N} h_i
\]

In these equations, \( h \) is the height of the peak and \( M \) is the molecular weight. To ensure these equations accurately calculate the average molecular weights three data manipulations were necessary. The baseline was corrected by addition or subtraction of a single intensity across the entire chromatogram. It has been shown that the choice of baseline correction does not have a significant effect on average wavelength [29] therefore the simplest correction was chosen. The low and high molecular weight cutoffs were of more importance to accurate data. In particular, the low molecular weight cutoff can have a large effect on molecular weight. The cutoff was set to 50 Daltons for all samples. The high molecular weight cutoff has less impact on the average molecular weight (Zhou et al. 2000). The high molecular weight cutoffs were chosen based on our
results. Depending on each chromatogram, the high molecular weight cutoff was either set to 3200 or 100,000 Daltons.

**Data Analysis**

A Matlab based code was written to correct and EEMs using the method described in the fluorometer section and all EEMs plots were generated using Matlab. The code was also used to calculate SUVA, fluorescence index and E2/E3 ratio, and graph the EEMs as a contour plot. A second code was written to calculate the number and weight averaged molecular weights with the appropriate corrections. Both codes are given in Appendix C.

**RESULTS AND DISCUSSION**

**Effluent Dissolved Organic Carbon**

The percent of hydrophobic (HPO) organic matter in the wastewater samples is less than typically found for NOM in aquatic systems. Between 18 and 42 percent of EfOM was extracted as the HPO fraction (Table 2). HPO organic matter percents are similar to the range of 26 and 40 percent extracted in previous studies [4, 18, 19, 23]. The transphilic (TPI) fraction accounts for 8-12 percent of the total organic matter. Previous research has shown TPI recovery as high as 20 percent for EfOM [4, 23]. These findings agree with previously published data, EfOM has less hydrophobic organic matter than NOM.
Table 2: Dissolved Organic Carbon and Percent of effluent organic matter extracted by hydrophobic and transphilic resins and not extracted in hydrophilic fraction

<table>
<thead>
<tr>
<th>Treatment Plant</th>
<th>WW effluent (mg/L)</th>
<th>HPO (mg/L)</th>
<th>TPI (mg/L)</th>
<th>HPI (mg/L)</th>
<th>%HPO</th>
<th>%TPI</th>
<th>%HPI</th>
<th>% Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>UConn</td>
<td>4.20</td>
<td>29.67</td>
<td>8.31</td>
<td>2.40</td>
<td>42.3</td>
<td>11.9</td>
<td>57.1</td>
<td>111.3</td>
</tr>
<tr>
<td>Hartford</td>
<td>6.22</td>
<td>31.54</td>
<td>10.31</td>
<td>-</td>
<td>30.4</td>
<td>9.9</td>
<td>59.6 *</td>
<td>100.0</td>
</tr>
<tr>
<td>Pomperaug</td>
<td>5.57</td>
<td>21.19</td>
<td>12.52</td>
<td>4.40</td>
<td>19.0</td>
<td>11.2</td>
<td>79.0</td>
<td>109.2</td>
</tr>
<tr>
<td>Vernon</td>
<td>5.93</td>
<td>22.28</td>
<td>7.75</td>
<td>4.74</td>
<td>18.8</td>
<td>6.5</td>
<td>80.0</td>
<td>105.3</td>
</tr>
<tr>
<td>Stamford</td>
<td>5.29</td>
<td>20.42</td>
<td>10.17</td>
<td>2.13</td>
<td>19.3</td>
<td>9.6</td>
<td>40.3</td>
<td>69.2</td>
</tr>
</tbody>
</table>

Stamford HPI fraction not measured
* Estimated by Subtraction

As a result of low percentages in the HPO and TPI fractions, the percent of OM in the hydrophilic (HPI) fraction is the majority. To effectively extract the majority of organic matter from wastewater effluent, extraction by DAX8 and XAD4 resin may not be the most efficient extraction method. Using XAD2 resin has been proposed as a better way to extract HPI organic matter [11], however it is rarely been used. Pre-concentration of organic matter by reverse osmosis has been coupled with the DAX8 and XAD4[4]. This utilizes the resins solely to separate OM between the HPO, TPI and HPI fractions. The benefit of pre-concentration is that the HPI fraction will be concentrated enough to analyze. Even though the majority of the organic matter was hydrophilic for Pomperaug, Vernon and Stamford, we proceeded with the analysis of the hydrophobic and transphilic organic matter that was extracted to compare these fractions with the comparable fractions of NOM.

**Organic Matter Size**

Size exclusion chromatography revealed extracted EfOM has distinctly different characteristics than NOM. Unlike SEC chromatograms of natural organic matter which
are unimodal [16, 29, 30], the chromatograms for the wastewater samples are multimodal, with several peaks (Figure 4).

UConn

Hartford

Pomperaug
Figure 4: Size Exclusion Chromatograms of Hydrophobic and Transphilic Fractions of UConn, Hartford, Pomperaug, Vernon, and Stamford Treatment Plants.

Vertical lines represent high and low molecular weight cutoffs. Hydrophobic fractions are to the left, Transphilic fractions are to the right.

The HPO fraction of UConn and Pomperaug show a small peak at 50,000 Daltons that is discontinuous from the bulk of the organic matter between 50 and 3200 Daltons. To indicate where mass was integrated the two vertical red lines on the chromatograms represent the high and low molecular weight cutoffs. All chromatograms have a low
molecular weight cutoff of 50 Daltons, and a high molecular weight cutoff of either 3200 or 100,000 Daltons. The hydrophobic fractions of the UConn and Pomperaug treatment plant are the only chromatograms with a 100,000 Dalton cutoff. This large molecular weight peak has been observed in previous studies of wastewater organic matter [21, 31] and characterized as polysaccharide like based on the size. The average molecular weights for UConn and Pomperaug were skewed as a result of the polysaccharide peak. We performed molecular weight calculations with and without inclusion of the polysaccharide peak so we could intercompare the continuous region of the organic matter (Table 3).

Table 3: Number Averaged and Weight Averaged Molecular weights, and polydispersivity excluding 50,000 Dalton polysaccharide peak

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fraction</th>
<th>Mn (Daltons)</th>
<th>Mw (Daltons)</th>
<th>Mw/Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>UConn</td>
<td>HPO</td>
<td>287.2</td>
<td>586.6</td>
<td>2.04</td>
</tr>
<tr>
<td>Hartford</td>
<td>HPO</td>
<td>302.9</td>
<td>664.9</td>
<td>2.20</td>
</tr>
<tr>
<td>Pomperaug</td>
<td>HPO</td>
<td>293.5</td>
<td>566.9</td>
<td>2.00</td>
</tr>
<tr>
<td>Vernon</td>
<td>HPO</td>
<td>346.2</td>
<td>669.8</td>
<td>1.93</td>
</tr>
<tr>
<td>Stamford</td>
<td>HPO</td>
<td>359.1</td>
<td>641.4</td>
<td>1.79</td>
</tr>
<tr>
<td>UConn</td>
<td>TPI</td>
<td>300.0</td>
<td>458.4</td>
<td>1.53</td>
</tr>
<tr>
<td>Hartford</td>
<td>TPI</td>
<td>304.5</td>
<td>501.2</td>
<td>1.65</td>
</tr>
<tr>
<td>Pomperaug</td>
<td>TPI</td>
<td>300.5</td>
<td>493.7</td>
<td>1.64</td>
</tr>
<tr>
<td>Vernon</td>
<td>TPI</td>
<td>374.1</td>
<td>557.3</td>
<td>1.49</td>
</tr>
<tr>
<td>Stamford</td>
<td>TPI</td>
<td>391.9</td>
<td>578.9</td>
<td>1.48</td>
</tr>
</tbody>
</table>

UConn with polysaccharide peak: Mn 308.1, Mw 4161.1, Mw/Mn 13.51
Pomperaug with polysaccharide peak: Mn 291.6, Mw 1531.6, Mw/Mn 5.25

The number averaged and weight averaged molecular weights of EfOM are smaller than NOM. Previous studies measured molecular weight of EfOM between 400-500, and 650-850 for number averaged and weight averaged respectively [19, 21]. The
sizes that are measured in this study are comparable to previously measured average molecular weights of EfOM. There is little variation between the sizes of HPO and TPI fractions.

The multimodal distribution of EfOM followed a similar pattern for all wastewater samples. This multimodal distribution has been observed in previous work investigating wastewater organic matter [21, 31]. The chromatograms consistently showed five peaks in the HPO fractions for all samples and six peaks in the TPI fractions (Figure 5). The peaks in both HPO and TPI fractions were between 1000 and 100 Daltons). The molecular weight at the maximum intensity of each peak was calculated to examine whether any trends in peak masses were observed (Table 4). Certain chromatograms do not have a sharp peak at all locations, but a shoulder indicating that a small peak is present. The molecular weights at chromatogram peaks are all very similar across the different treatment plants with the slight deviation of Hartford HPO. This indicates the five treatment plants, with their varying sizes and technologies, discharge treated effluent that all show six distinct size fractions. This is not to say that there are six distinct molecules, or six distinct molecular weights that compose all wastewater organic matter. The peaks do overlap, so there is organic matter with all sizes between the high and low molecular weight cutoff, but there are six sizes that consistently showed local detection maxima.
Figure 5: Numbering Convention of Peaks in Size Exclusion Chromatogram of EfOM

Table 4: Molecular Weight at the Six Peaks of each Size Exclusion Chromatogram

<table>
<thead>
<tr>
<th>Location</th>
<th>Fraction</th>
<th>Peak 1</th>
<th>Peak 2</th>
<th>Peak 3</th>
<th>Peak 4</th>
<th>Peak 5</th>
<th>Peak 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCONN</td>
<td>HPO</td>
<td>800</td>
<td>622</td>
<td>394</td>
<td>-</td>
<td>175</td>
<td>SH</td>
</tr>
<tr>
<td>Hartford</td>
<td>HPO</td>
<td>1090</td>
<td>811</td>
<td>510</td>
<td>-</td>
<td>183</td>
<td>99</td>
</tr>
<tr>
<td>Pomperaug</td>
<td>HPO</td>
<td>868</td>
<td>626</td>
<td>397</td>
<td>-</td>
<td>177</td>
<td>104</td>
</tr>
<tr>
<td>Vernon</td>
<td>HPO</td>
<td>843</td>
<td>622</td>
<td>400</td>
<td>-</td>
<td>176</td>
<td>106</td>
</tr>
<tr>
<td>Stamford</td>
<td>HPO</td>
<td>836</td>
<td>612</td>
<td>397</td>
<td>-</td>
<td>172</td>
<td>104</td>
</tr>
<tr>
<td>UCONN</td>
<td>TPI</td>
<td>SH</td>
<td>614</td>
<td>417</td>
<td>SH</td>
<td>SH</td>
<td>110</td>
</tr>
<tr>
<td>Hartford</td>
<td>TPI</td>
<td>SH</td>
<td>643</td>
<td>430</td>
<td>234</td>
<td>171</td>
<td>103</td>
</tr>
<tr>
<td>Pomperaug</td>
<td>TPI</td>
<td>873</td>
<td>639</td>
<td>421</td>
<td>229</td>
<td>169</td>
<td>96</td>
</tr>
<tr>
<td>Vernon</td>
<td>TPI</td>
<td>822</td>
<td>634</td>
<td>423</td>
<td>234</td>
<td>173</td>
<td>100</td>
</tr>
<tr>
<td>Stamford</td>
<td>TPI</td>
<td>844</td>
<td>632</td>
<td>419</td>
<td>235</td>
<td>164</td>
<td>100</td>
</tr>
</tbody>
</table>

Notes: Peak 4 not apparent in HPO chromatograms, SH indicates shoulder at peak location of chromatogram

To determine differences in absorbance characteristics between subsamples of different size, the HPLC instrument was set to scan absorbance wavelengths whenever a peak was detected. The wavelength scan measured absorbance from 220 to 350 nm. The shape of the absorbance curves for peaks 1 through 4 were very similar, exhibiting a decrease in absorbance as wavelength increases with a shoulder at around 272nm. Peak 5
in both the hydrophobic and transphilic fractions showed a strong absorbance at 220nm with a sharp unimodal decrease in absorbance to 350 nm. To quantify the decrease in absorbance, the absorbance at 220nm was divided by the absorbance at 272nm (Table 5). Peak 6 was not detected by the instrument, so absorption spectra were not available. The absorbance ratios show that at peak 5, the absorbance of light at 220 is much higher than 272. Structurally, this means that the organic matter in peak 5 has less conjugated carbon bonds, which one might expect given that the organic matter in peak 5 is approximately 170 Daltons. Further differentiation between the SEC peaks could not be determined with absorption spectra alone.

*Table 5: Absorbance Intensity Ratio (Abs\textsubscript{220} / Abs\textsubscript{272}) at 6 Size Exclusion Peaks*

<table>
<thead>
<tr>
<th>Location</th>
<th>Fraction</th>
<th>Peak 1</th>
<th>Peak 2</th>
<th>Peak 3</th>
<th>Peak 4</th>
<th>Peak 5</th>
<th>Peak 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCONN</td>
<td>HPO</td>
<td>1.9</td>
<td>2.2</td>
<td>2.2</td>
<td>-</td>
<td>9.7</td>
<td>-</td>
</tr>
<tr>
<td>Hartford</td>
<td>HPO</td>
<td>2.3</td>
<td>2.7</td>
<td>2.3</td>
<td>-</td>
<td>3.8</td>
<td>-</td>
</tr>
<tr>
<td>Pomperaug</td>
<td>HPO</td>
<td>1.9</td>
<td>2.3</td>
<td>2.0</td>
<td>-</td>
<td>8.6</td>
<td>-</td>
</tr>
<tr>
<td>Vernon</td>
<td>HPO</td>
<td>2.0</td>
<td>2.2</td>
<td>2.1</td>
<td>-</td>
<td>32.9</td>
<td>-</td>
</tr>
<tr>
<td>Stamford</td>
<td>HPO</td>
<td>2.0</td>
<td>2.4</td>
<td>2.1</td>
<td>-</td>
<td>5.8</td>
<td>-</td>
</tr>
<tr>
<td>UCONN</td>
<td>TPI</td>
<td>2.1</td>
<td>2.3</td>
<td>2.3</td>
<td>11.1</td>
<td>17.9</td>
<td>-</td>
</tr>
<tr>
<td>Hartford</td>
<td>TPI</td>
<td>2.4</td>
<td>3.4</td>
<td>2.3</td>
<td>2.2</td>
<td>1.7</td>
<td>-</td>
</tr>
<tr>
<td>Pomperaug</td>
<td>TPI</td>
<td>1.8</td>
<td>3.1</td>
<td>2.1</td>
<td>2.3</td>
<td>10.1</td>
<td>-</td>
</tr>
<tr>
<td>Vernon</td>
<td>TPI</td>
<td>2.0</td>
<td>2.6</td>
<td>2.3</td>
<td>4.8</td>
<td>59.3</td>
<td>-</td>
</tr>
<tr>
<td>Stamford</td>
<td>TPI</td>
<td>1.9</td>
<td>3.0</td>
<td>2.2</td>
<td>2.4</td>
<td>4.9</td>
<td>-</td>
</tr>
</tbody>
</table>

Notes: Peak 4 not detected in HPO fractions, No absorption spectra measured at Peak 6

### Organic Matter Fluorescence

The EEMs of wastewater effluent show fluorescence signatures that are not found in NOM. All EEMs of EfOM show fluorescence in regions A and C that are characteristic of NOM, however EfOM EEMs also showed fluorescence in regions B and T1. The two T peaks indicate tryptophan like fluorescence and peak B indicates tyrosine
like fluorescence. There is some evidence indicating that peaks T1 and B are not typically present in NOM. Suwannee River shows very little fluorescence in Peaks B and T1 [22]. Organic matter from rivers in northeastern England and the Bull run tributary in Virginia, also showed marginal fluorescence in peaks B and T1 [24, 32]. To our knowledge, all published EEM data of microbial end members is truncated at excitation wavelengths above 250 nm. Despite the convention of truncating at 250 nm, all studies of NOM without truncation showed low fluorescence in peaks T1, T2 and B.

The EEMs of the HPO fractions and TPI fractions have different fluorescence. The FRI method was used to quantitatively show differences in fluorescence (Table 6). The EEMs spectra of all HPO fractions showed similar characteristics to each other, and TPI fractions showed similar characteristics to each other. However there were differences between HPO and TPI fractions. One region where the difference between HPO and TPI fractions is apparent is peaks A and T. In many of the EEMS, peaks A and T1 do not show distinct separation (Figure 6). This combined A and T1 peak is mainly observed in the HPO fraction EEMS, in the transphilic fraction EEMS, the T1 peak is reduced and the A peak dominates. Another difference that is observed between HPO and TPI fractions is peak C. The HPO fractions have a smaller percentage distribution of peak C as compared to the TPI fraction. Previous work has shown that peaks A and C are the two fluorescence regions for fulvic acids [15, 22, 32]. Since peaks A and C are both larger for the TPI EEMs, two explanations are possible: either fulvic acids are more strongly retained by the transphilic XAD4 resin, or fulvic acids are retained equally by both resins, and other fluorophores are more strongly retained on the hydrophobic DAX8 resin.
Table 6: Percentage Distribution of Different Regions of EEMs by FRI Method

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant</th>
<th>Fraction</th>
<th>B (%)</th>
<th>T1 (%)</th>
<th>A (%)</th>
<th>T2 (%)</th>
<th>C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UCONN</td>
<td>HPO</td>
<td>7.86</td>
<td>34.40</td>
<td>37.88</td>
<td>6.04</td>
<td>13.81</td>
</tr>
<tr>
<td></td>
<td>Hartford</td>
<td>HPO</td>
<td>10.31</td>
<td>36.12</td>
<td>37.03</td>
<td>5.49</td>
<td>11.06</td>
</tr>
<tr>
<td></td>
<td>Pomperaug</td>
<td>HPO</td>
<td>10.81</td>
<td>45.31</td>
<td>27.27</td>
<td>6.07</td>
<td>10.54</td>
</tr>
<tr>
<td></td>
<td>Vernon</td>
<td>HPO</td>
<td>7.85</td>
<td>40.00</td>
<td>35.49</td>
<td>5.11</td>
<td>11.56</td>
</tr>
<tr>
<td></td>
<td>Stamford</td>
<td>HPO</td>
<td>9.46</td>
<td>39.85</td>
<td>34.20</td>
<td>5.42</td>
<td>11.07</td>
</tr>
<tr>
<td></td>
<td>UCONN</td>
<td>TPI</td>
<td>8.52</td>
<td>26.47</td>
<td>44.50</td>
<td>4.83</td>
<td>15.68</td>
</tr>
<tr>
<td></td>
<td>Hartford</td>
<td>TPI</td>
<td>10.41</td>
<td>21.18</td>
<td>47.20</td>
<td>5.57</td>
<td>15.64</td>
</tr>
<tr>
<td></td>
<td>Pomperaug</td>
<td>TPI</td>
<td>11.86</td>
<td>31.85</td>
<td>33.46</td>
<td>7.80</td>
<td>15.29</td>
</tr>
<tr>
<td></td>
<td>Vernon</td>
<td>TPI</td>
<td>9.55</td>
<td>27.53</td>
<td>41.11</td>
<td>6.58</td>
<td>15.24</td>
</tr>
<tr>
<td></td>
<td>Stamford</td>
<td>TPI</td>
<td>10.72</td>
<td>24.87</td>
<td>41.36</td>
<td>6.43</td>
<td>16.62</td>
</tr>
</tbody>
</table>

UConn

[Graphs showing EEM distributions for UConn HPO and TPI fractions]

Hartford

[Graphs showing EEM distributions for Hartford HPO and TPI fractions]
Figure 6: EEMs of UConn, Hartford, Pomperaug, Vernon, and Stamford Treatment Plants. Hydrophobic fractions are to the left, Transphilic fractions are to the right.
Fluorescence index measurements indicate that the EfOM is microbial-like. All fluorescence indices are within the ranges of the natural organic matter continuum (Table 7). In addition, all fluorescence indices are above 1.5, indicating that the organic matter from the all five treatment plants are closer to the microbial end of the continuum regardless of hydrophobicity. The indices of the TPI fractions of organic matter are larger than those of the HPO fraction, thus indicating that the TPI fraction is more microbial like than the HPO fraction.

**Optical Analyses**

Like the fluorescence characterization, the absorbance characteristics of EfOM are consistent with a microbial source. E2/E3 ratios are within the range of NOM and indicate that EfOM is on the high end of the range. The high E2/E3 ratios indicate that EfOM has low extent of conjugation between carbon atoms in its structure.

SUVA values indicate that the EfOM has similar aromaticity to microbial organic matter. SUVA of the all organic matter fractions were between 1.5 and 3 with one outlier at 0.86(Table 7). This result is consistent with published SUVA measurements of EfOM [19, 21, 23]. The SUVA value of the hydrophobic fraction of each treatment plant is larger than its corresponding transphilic fraction. This indicates that the HPO fraction is more aromatic than the TPI fraction. This data is consistent with the fluorescence index data. Transphilic organic matter has low SUVA, indicating that it has low aromaticity, and not similar to lignin rich terrestrial organic matter.
### Table 7: Fluorescence Index, SUVA, pH and E2/E3 Ratio of EfOM

<table>
<thead>
<tr>
<th>Location</th>
<th>Fraction</th>
<th>Fluorescence Index</th>
<th>SUVA (L/mg-m)</th>
<th>pH</th>
<th>E2/E3</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCONN</td>
<td>HPO</td>
<td>1.71</td>
<td>2.09</td>
<td>1.90</td>
<td>5.69</td>
</tr>
<tr>
<td>Hartford</td>
<td>HPO</td>
<td>1.57</td>
<td>2.09</td>
<td>3.93</td>
<td>5.44</td>
</tr>
<tr>
<td>Pomperaug</td>
<td>HPO</td>
<td>1.69</td>
<td>2.08</td>
<td>2.43</td>
<td>4.85</td>
</tr>
<tr>
<td>Vernon</td>
<td>HPO</td>
<td>1.64</td>
<td>2.81</td>
<td>2.57</td>
<td>4.98</td>
</tr>
<tr>
<td>Stamford</td>
<td>HPO</td>
<td>1.57</td>
<td>3.04</td>
<td>3.18</td>
<td>5.33</td>
</tr>
<tr>
<td>UCONN</td>
<td>TPI</td>
<td>1.93</td>
<td>1.71</td>
<td>2.54</td>
<td>5.28</td>
</tr>
<tr>
<td>Hartford</td>
<td>TPI</td>
<td>1.83</td>
<td>2.06</td>
<td>2.92</td>
<td>5.73</td>
</tr>
<tr>
<td>Pomperaug</td>
<td>TPI</td>
<td>1.94</td>
<td>0.86</td>
<td>2.55</td>
<td>4.05</td>
</tr>
<tr>
<td>Vernon</td>
<td>TPI</td>
<td>1.82</td>
<td>2.33</td>
<td>2.57</td>
<td>4.82</td>
</tr>
<tr>
<td>Stamford</td>
<td>TPI</td>
<td>1.70</td>
<td>1.58</td>
<td>3.11</td>
<td>4.79</td>
</tr>
</tbody>
</table>

### Treatment Plant Intercomparison

To determine if differences in treatment plants have a large effect on EfOM characteristics we revisit the data as a whole. In the hydrophobic fractions, Stamford and Vernon have high SUVA values. Treatment plants with advanced nitrogen removal processes have been shown to have higher SUVA values \([21, 33]\) which are confirmed in this study with the Stamford treatment plant. The Vernon treatment plant does not have advanced nitrogen removal, however the SUVA values are high, this may be an effect of the granular activated carbon in the aeration tanks.

The FRI analysis reveals that fluorescence of EfOM is fairly stable regardless of treatment plant. The EEMs from the different treatment plants are fairly consistent with only a few differences. One difference between the five hydrophobic EEMS is peak T1 and its relation to peak A. The UConn and Hartford EEMs have the smallest peak T1 to A ratio, Vernon and Stamford have a slightly larger peak T1 than A and in the Pomperaug sample, peak T1 is much more dominant than peak A. This pattern indicates that the Pomperaug treatment plant effluent contains more organic matter that fluoresces.
like tryptophan; Vernon and Stamford have less, but more than UConn and Hartford. Another difference between the five hydrophobic EEMS is peak B. Hartford and Pomperaug have the highest percent B, followed by Stamford. UConn and Vernon have a low percent B peak. Combining the peak B and T1 results for the hydrophobic fractions, the Pomperaug treatment plant has the largest percent fluorescence similar to tryptophan and tyrosine. The five transphilic fractions do follow similar trends when comparing treatment plants as the hydrophobic fractions, with the shift towards peaks A and C as discussed above. The contribution of fluorescence from each region is very consistent.

EfOM occupies a narrow range of fluorescence index. The fluorescence indices for UConn and Pomperaug in both HPO and TPI fractions are higher than the other treatment plants. Stamford on the other hand has the lowest fluorescence index in both fractions. It is unclear what the cause of the different fluorescence indices are, however EfOM is microbial like regardless of treatment plant.

The one difference in EfOM characteristics that does stand out from our samples is the occurrence of a polysaccharide peak in SEC. EfOM from UConn and Pomperaug show a polysaccharide peak in the SEC analysis. In biological environments, a major source of polysaccharides is extracellular polymeric substances (EPS) [34]. EPS are the structural material in biofilms and bacterial flocs. The one major difference between the UConn and Pomperaug plants and the other three is the use of mechanical aeration. It is possible that the mechanical aerators in UConn and Pomperaug shear more EPS in the aeration tank than bubble diffusers, causing the polysaccharide like peak in the size exclusion chromatograms. The presence of a polysaccharide peak may be a result of aeration technology.
CONCLUSIONS

Our results show that extracted effluent organic matter is similar to the microbial end member of the NOM continuum. EfOM does have a larger HPI fraction, allowing less to be extracted by DAX8 and XAD4 resins. Size exclusion chromatography showed that wastewater organic matter is smaller natural organic matter. Fluorescence EEMs of effluent organic matter are similar to NOM in the appearance of peaks A and C. The FRI method of the ten fractions of wastewater showed enhanced tryptophan and tyrosine like fluorescence, as compared to terrestrially derived OM. Fluorescence indices and SUVA show that effluent organic matter is similar to microbial organic matter.

Effluent organic matter has similar characteristics regardless of plant size or treatment technology. The average molecular weights of the five plants are very consistent. The EEMs of the 10 fractions of effluent showed some differences as quantified by the FRI method. However, all 10 fractions had the same fluorophores and had similar fluorescence contributions from each of the 5 regions SUVA and fluorescence index measurements also confirm that EfOM is consistent.

ENVIRONMENTAL SIGNIFICANCE

Our results have shown that organic matter from wastewater treatment plants is similar to microbially derived organic matter. In small to moderately sized rivers, the majority of the natural organic matter will be terrestrially derived, since the residence time does not allow for significant microbial growth. As a result, effluent organic matter would be in stark contrast to the natural organic matter and possibly influence stream processes.
Wastewater treatment plants are the major source of emerging contaminants to the environment. Once discharged into aquatic environments, a major removal pathway of emerging contaminants is indirect photodegradation by excited organic matter and reactive oxygen species formed by photo-excited DOM. Current research is being conducted to relate photodegradation rates to organic matter characteristics. Our study has shown that EfOM is optically different than the natural organic matter. If further studies show enhanced indirect photolysis by effluent organic matter, the effect of emerging contaminants may be mitigated.

**FUTURE WORK**

Our results demonstrate that EfOM has very similar characteristics regardless of treatment plant. However there are still questions to be answered. Treatment plants are temporally dynamic systems. In warmer months, bacterial growth is enhanced, and treatment plants are required to disinfect effluent. All EfOM in this study was sampled between November and April. Over the course of a day, flow rates in treatment plants are highest in the morning and evening, during peak water use. All samples in this study were collected between 9 and 11 AM. It is unclear if the seasonal and diurnal fluctuations of treatment plants affect the characteristics of EfOM.

In our study, between 60 and 80 percent of EfOM is hydrophilic and not isolated by either resin. Pre-concentration of OM by reverse osmosis or the use of XAD 2 resin may allow the characterization of the hydrophilic fraction.

Additional characterization techniques could provide further insight to the characteristics of EfOM. Pyrene sorption and fluorescence quenching experiments would
characterize the sorption capacity of effluent organic matter. The sorption capacity would help describe the ability of EfOM to transport contaminants. Analysis of the quantum yields of radical oxygen species could determine the capacity of EfOM to degrade emerging contaminants. Parallel factor analysis (PARAFAC) of can be used to deconvolute individual fluorophores from an EEM. Sample manipulation and chromatography methods could be developed to separate peaks in size exclusion chromatography. Peak separation could allow the quantification of different structures of EfOM.
REFERENCES


**APPENDICES**

**Appendix A**

Fractional Region Integration Method Parameters

<table>
<thead>
<tr>
<th>EEM Region</th>
<th>Area of Region</th>
<th>Fractional Area per region</th>
<th>Multiplication Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>2400</td>
<td>0.054</td>
<td>18.44</td>
</tr>
<tr>
<td>T1</td>
<td>1500</td>
<td>0.034</td>
<td>29.50</td>
</tr>
<tr>
<td>A</td>
<td>5100</td>
<td>0.115</td>
<td>8.68</td>
</tr>
<tr>
<td>T2</td>
<td>9750</td>
<td>0.220</td>
<td>4.54</td>
</tr>
<tr>
<td>C</td>
<td>25500</td>
<td>0.576</td>
<td>1.74</td>
</tr>
</tbody>
</table>

**Appendix B1**

Matlab code used to convert raw fluorescence and spectrometer data into corrected EEM, plot the corrected EEM, and apply fluorescence region integration

```matlab
clear, clc
%EEMS Calculation File

%................................
%User input for file names
%................................

%Prompt user for name of Excel File with fluorometer data
fluoroname = 'dax8cec.xls';
graphtitle = 'Stamford HPO Fraction';
fluoroname=input('enter excel file name of the fluorometer data, remember to include .xls for an excel file --> ','s');
clc
%Prompt user for name of the sheet in the Excel File
fluorosheet='Fluoro'; %input('enter the name of the sheet in the fluorometer data you would like to use --> ','s');
%clc

%Prompt user for name of Excel File with spectrophotometer data blank
spectroname = fluoroname;
```
%spectroname=input('enter excel file name containing spectrophotometer data --> ','s');
clc
%Prompt user for name of Excel sheet in fluorometer data blank
spectrosheet= 'Spectro';%input('enter the name of the sheet in the spectrophotometer data that you would like to use --> ','s');
%clc
disp('working');

%Display User inputs to verify
fprintf('These are your filenames to verify, 
 
 ')

.......................... ..........................
...
% 1) File Load

% In this section, the fluorometer data is converted from the excel file
% into the matrix labeled bigmatrix. The columns of bigmatrix are as
% follows. 1:Emission wavelengths, 2:Fluorescence Intensity,
% 3:Excitation
% wavelength
% .................................................. ....................
% ..................

%import excel file from florometer, xf is numerical data, yf is headers
[xf,yf] = xlsread(fluoroname, fluorosheet);

%determine size of excel file matrix
[row1f, col1f] = size(xf);

%convert excel data into continuous columns
Emwavef = xf(:,1:2:col1f);
Emintensf = xf(:,2:2:col1f);
bigmatrix = [Emwavef(:,),Emintensf(:)];

%convert excel headers into rows with just excitation wavelengths
af = yf (1,1:2:col1f);

%determine number of excitation wavelengths
[row2f, col2f] = size(af);

%convert excel hearders from cell array to strings
bf = char(af);

%determine the number of characters in the sample name. In the data from
%the fluorometer, the last 6 characters of the name will be the excitation
%wavelength.
cf = size(bf);
df = cf(1,2);
ef = cf(1,2) - 5;

%convert cell array of headers into matrix that only contains the
%excitation wavelengths as numbers not strings
for p = 1:col2f
    gf=af(1,p)(1,ef:df);
    exwavef(1,p) = str2num(gf);
end;

%update matrix to include excitation wavelength data
for q=1:col2f
    for j=1:row1f-1
        r=(row1f*q-(row1f-1));
        bigmatrix(r,3)=exwavef(1,q);
        bigmatrix(r+j,3)=exwavef(1,q);
    end
end

%find size of bigmatrix
[row4, col4]=size(bigmatrix);

%Round Emission wavelengths to nearest 1 wavelength in fluorometer data
bigmatrix(:,1)= round (bigmatrix(:,1));

%Quinine Sulfate intensity correction for sample
for pp=1:row4
    if bigmatrix(pp,1) <=480
        bigmatrix(pp,4) = bigmatrix(pp,2);
    elseif bigmatrix(pp,1) <=490 && bigmatrix(pp,1) >480
        bigmatrix(pp,4) = bigmatrix(pp,2) * 1.1;
    elseif bigmatrix(pp,1) <=500 && bigmatrix(pp,1) >490
        bigmatrix(pp,4) = bigmatrix(pp,2) * 1.2;
    elseif bigmatrix(pp,1) <=510 && bigmatrix(pp,1) >500
        bigmatrix(pp,4) = bigmatrix(pp,2) * 1.3;
    elseif bigmatrix(pp,1) <=520 && bigmatrix(pp,1) >510
        bigmatrix(pp,4) = bigmatrix(pp,2) * 1.4;
    elseif bigmatrix(pp,1) <=540 && bigmatrix(pp,1) >520
        bigmatrix(pp,4) = bigmatrix(pp,2) * 1.5;
    elseif bigmatrix(pp,1) <=550 && bigmatrix(pp,1) >540
        bigmatrix(pp,4) = bigmatrix(pp,2) * 1.6;
    end
end

%.................................................. ....................
% 3) Inner Filter Effect Correction
%.................................................. ....................

46
%import excel file from spectrophotometer, xs is numerical data
xs = xlsread(spectroname, spectrosheet);

%find size of spectrophotometer file
[row1s, col1s] = size(xs);

%Round wavelengths to nearest 1 wavelength in spectrophotometer data
xs(:,1) = round(xs(:,1));

%get the corrected florescence intensity
for i=1:row4
    %set j and k to the emmission and excitation wavelegths
    j = bigmatrix(i,1);
    k = bigmatrix(i,3);

    %find wavelengths j and k from the spectrophotometer data
    m = find(xs == j);
    n = find(xs == k);

    %set c and d to the absorbance values for j and k
    c = xs(m,2);
    d = xs(n,2);

    %correct for the inner filter effect and store in the 5th column of
    %bigmatrix
    bigmatrix(i,5) = bigmatrix(i,2)*(10^((c+d)/2));
    %bigmatrix(i,5) = bigmatrix(i,2);
end

%find values for E2/E3 ratio
for i=1:row1s
    if xs(i,1) == 250
        E2 = xs(i,2);
    elseif xs(i,1) == 365
        E3 = xs(i,2);
    end
end

%.................................................. ....................
% 2) Raman and Rayleigh Scatter Correction

% In this section, the florometer blank is converted from an excel file
to
% the matrix labeled blankmatrix in the same way as section 1 with the
% same
% columns. Then the intensities of the blank are subtracted from the
data
% to illiminate the scatter.
% READ EXCEL FILE

\[ [\text{xb}, \text{yb}] = \text{xlsread}(\text{fluoroblank}, \text{fluoroblanksheet}) ; \]

% DETERMINE SIZE OF EXCEL FILE MATRIX
\[ [\text{row1b}}, \text{col1b}] = \text{size}(\text{xb}) ; \]

% CONVERT EXCEL DATA INTO CONTINUOUS COLUMNS
\[ \text{Emwaveb} = \text{xb}(:,1:2:\text{col1b}) ; \]
\[ \text{Emintensb} = \text{xb}(:,2:2:\text{col1b}) ; \]
\[ \text{blankmatrix} = [\text{Emwaveb}(:,), \text{Emintensb}(:,)] ; \]

% CONVERT EXCEL HEADERS INTO ROWS WITH JUST EXCITATION WAVELENGTHS
\[ \text{ab} = \text{yb} (1,1:2:\text{col1b}) ; \]

% DETERMINE NUMBER OF EXCITATION WAVELENGTHS
\[ [\text{row2b}}, \text{col2b}] = \text{size}(\text{ab}) ; \]

% CONVERT EXCEL HEADERS FROM CELL ARRAY TO STRINGS
\[ \text{bb} = \text{char}(\text{ab}) ; \]

% DETERMINE THE NUMBER OF CHARACTERS IN THE SAMPLE NAME. IN THE DATA
% FROM THE FLUOROMETER, THE LAST 6 CHARACTERS OF THE NAME WILL BE THE
% EXCITATION
\[ \text{cb} = \text{size}(\text{bb}) ; \]
\[ \text{db} = \text{cb}(1,2) ; \]
\[ \text{eb} = \text{cb}(1,2) - 5 ; \]

% CONVERT CELL ARRAY OF HEADERS INTO MATRIX THAT ONLY CONTAINS THE
% EXCITATION WAVELENGTHS AS NUMBERS NOT STRINGS
\[ \text{for p} = 1:\text{col2f} \]
\[ \quad \text{gb}=\text{ab}(1,p)(1,\text{eb:db}) ; \]
\[ \quad \text{exwaveb}(1,p) = \text{str2num}(\text{gb}) ; \]
\[ \text{end} \]

% UPDATE MATRIX K TO INCLUDE EXCITATION WAVELENGTH DATA
\[ \text{for q}=1:\text{col2b} \]
\[ \quad \text{for j}=1:\text{row1b}-1 \]
\[ \qquad \text{r}=(\text{row1b}*\text{q}-(\text{row1b}-1)) ; \]
\[ \qquad \text{blankmatrix}(\text{r},3)=\text{exwaveb}(1,q) ; \]
\[ \qquad \text{blankmatrix}(\text{r+j},3)=\text{exwaveb}(1,q) ; \]
\[ \text{end} \]
\[ \text{end} \]

% QUININE SULFATE INTENSITY CORRECTION FOR BLANK
for pp=1:row4
    if blankmatrix(pp,1) <= 480
        blankmatrix(pp,4) = blankmatrix(pp,2);
    elseif blankmatrix(pp,1) <= 490 && blankmatrix(pp,1) > 480
        blankmatrix(pp,4) = blankmatrix(pp,2) * 1.1;
    elseif blankmatrix(pp,1) <= 500 && blankmatrix(pp,1) > 490
        blankmatrix(pp,4) = blankmatrix(pp,2) * 1.2;
    elseif blankmatrix(pp,1) <= 510 && blankmatrix(pp,1) > 500
        blankmatrix(pp,4) = blankmatrix(pp,2) * 1.3;
    elseif blankmatrix(pp,1) <= 520 && blankmatrix(pp,1) > 510
        blankmatrix(pp,4) = blankmatrix(pp,2) * 1.4;
    elseif blankmatrix(pp,1) <= 540 && blankmatrix(pp,1) > 520
        blankmatrix(pp,4) = blankmatrix(pp,2) * 1.5;
    elseif blankmatrix(pp,1) <= 550 && blankmatrix(pp,1) > 540
        blankmatrix(pp,4) = blankmatrix(pp,2) * 1.6;
    end
    if blankmatrix(pp,2) < 0
        blankmatrix(pp,4) = 0;  %setting negative values to zero
    end
end

% Correct for Raman and Rayleigh Scatter
bigmatrix(:,5) = bigmatrix(:,5) - blankmatrix(:,4);

%...........................................................
% 4) Deleting Raman Scatter Peaks and Negative intensities and normalizing
% to Raman Units
%...........................................................
rama
ramanintens=0;
for ii=1:row4
    %deleting raman scatter
    if bigmatrix(ii,1) <= bigmatrix(ii,3)+8 && bigmatrix(ii,1) >= bigmatrix(ii,3)-8
        bigmatrix(ii,5) = 0;
    end
    % deleting Rayleigh Tyndall effect
    if bigmatrix(ii,1) <= bigmatrix(ii,3)*2+8 && bigmatrix(ii,1) >= bigmatrix(ii,3)*2-8
        bigmatrix(ii,5) = 0;
    end
    % setting negative intensities to 0
    if bigmatrix(ii,5) < 0
        bigmatrix(ii,5) = 0;
    end
    %deleting data below a certain excitation wavelength
    if bigmatrix(ii,3) < 230
        bigmatrix(ii,5) = 0;
    end
    %Calculate Raman Area, Water blank ex350 em 380-426
    if bigmatrix(ii,3) == 350
        if bigmatrix(ii,1) <= 426 && bigmatrix(ii,1) >= 380
            %
        end
    end
end

49
ramanintens = ramanintens + 2*bigmatrix(ii,5); % mult by 2
because 2nm increments
end
end

% find ex/em wavelengths 370/450 and 370/500 for fluorescence index
if bigmatrix(ii,3) == 370
    if bigmatrix(ii,1) == 450
        FlInt1=bigmatrix(ii,5);
    elseif bigmatrix(ii,1) == 500
        FlInt2=bigmatrix(ii,5);
    end
end
end

% Normalize to Raman Units
bigmatrix(:,6) = bigmatrix(:,5)./ramanintens;

% set intensities to zero if emission wavelength is shorter than excitation
for iii=1:row4
    if bigmatrix(iii,1) < bigmatrix(iii,3)
        bigmatrix(iii,6) = 0;
    end
end
end

% store all emission values for excitation 370
j=1;
for iii=1:row4
    if bigmatrix(iii,3) == 230
        scan370(j,1) = bigmatrix(iii,6);
        j=j+1;
    end
end
end

% .......................................................... ....................
% 5) Calculate SUVA, Fluoresence Intensity, E2/E3, and max emission at 370
% .......................................................... ....................
% clc

clc
% Calculate SUVA
SUVANUM = find(xs == 254);
SUVAABS = xs(SUVANUM,2);

DOC = input('what is the DOC concentration of the sample in mg/L ');
SUVA = 100*SUVAABS/DOC;
disp('SUVA = ') disp(SUVA) disp('Absorbance at 254 nm') disp(SUVAABS)

%Calculate Fluorescence Index

FlIndex=FlInt1/FlInt2;
disp('Fluoresence index = ') disp(FlIndex)

%Calculate E2/E3 ratio ---> absorbance at 250 / absorbance at 365
E2E3Ratio = E2/E3

%Calculate maximum emission at excitation 370
[max370,max370loc] = max(scan370);
maxEmissionAtEx370 = bigmatrix(max370loc,1)

%.................................................. .......................
% 6) Plot the 3-D EEMS graph
% .................................................. .....................
%.................................................. .......................
%Convert corrected florometer intensities into a matrix arranged in the %same way that they were imported from the excel file. Also create a %matrix that is the same size, containing excitation wavelengths that %correspond to the corrected intensities

for w=1:collf/2
    t=(w*row1f)-row1f+1;
    EEMSmatrix(:,w) = bigmatrix(t:row1f*w,6);
    exwavefmatrix(:,w) = bigmatrix(t:row1f*w,3);
end

%graph the EEMS contour plot
figure(1)
contour(Emwavef,exwavefmatrix,EEMSmatrix,25)
grid, xlabel('Emission Wavelength (nm)')
ylabel('Excitation Wavelength (nm)')
title(graphtitle)

figure(2)
contour3(Emwavef,exwavefmatrix,EEMSmatrix,25)
grid, xlabel('Emission Wavelength (nm)')
ylabel('Excitation Wavelength (nm)')
title(fluoroname)

figure(3)
%surf(Emwavef,exwavefmatrix,EEMSmatrix) %grid, xlabel('Emission Wavelength (nm)') %ylabel('Excitation Wavelength (nm)')
figure(3)
plot(Emwavef(:,1),scan370)
xlabel('Emission Wavelength (nm)')
ylabel('Intensity')
title(fluoroname)

%.................................................. ....................
% 7) Find the wavelengths for the Local Maximum Peaks
% Note: This code can only find the A and C peaks (Hudson 2007)
%.................................................. ....................
%find the max emission for each excitation
[maxx,III] = max(EEMSmatrix);
%find local maximum in the max emissions
[pks,locs] = findpeaks(maxx);

%convert the counters to wavelengths
maxExcitation1 = round(exwavef(locs(1,1)));
maxExcitation2 = round(exwavef(locs(1,2)));
maxEmission1 = round(Emwavef(III(1,locs(1,1)),1));
maxEmission2 = round(Emwavef(III(1,locs(1,2)),1));

%Display Results
PeakA = [maxExcitation1,maxEmission1]
PeakC = [maxExcitation2,maxEmission2]

for ii=1:row4
    if bigmatrix(ii,3) == maxExcitation1 && bigmatrix(ii,1) == maxEmission1
        PeakAIntensity = bigmatrix(ii,6)
    end

    if bigmatrix(ii,3) == maxExcitation2 && bigmatrix(ii,1) == maxEmission2
        PeakCIntensity = bigmatrix(ii,6)
    end
end
PeakRatio = PeakCIntensity/PeakAIntensity

vol1=0;
vol2=0;
vol3=0;
vol4=0;
vol5=0;

for i=1:row4
    if bigmatrix(i,3) <= 250
if bigmatrix(i,1) < 330
    vol1=vol1+10*2*bigmatrix(i,6);
elseif bigmatrix(i,1) >= 330 && bigmatrix(i,1) <380
    vol2=vol2+10*2*bigmatrix(i,6);
else
    vol3=vol3+10*2*bigmatrix(i,6);
end
else
    if bigmatrix(i,1) < 380
        vol4=vol4+10*2*bigmatrix(i,6);
    else
        vol5=vol5+10*2*bigmatrix(i,6);
    end
end

% Region volume calculation Chen 2003 ES&T
area1=2400;
area2=1500;
area3=5100;
area4=9750;
area5=25500;
areatotal=area1+area2+area3+area4+area5;

fracarea1=area1/areatotal;
fracarea2=area2/areatotal;
fracarea3=area3/areatotal;
fracarea4=area4/areatotal;
fracarea5=area5/areatotal;

vol1=vol1/fracarea1;
vol2=vol2/fracarea2;
vol3=vol3/fracarea3;
vol4=vol4/fracarea4;
vol5=vol5/fracarea5;

voltotal=vol1+vol2+vol3+vol4+vol5;
disp('Percent region1')
(vol1/voltotal)*100
disp('Percent region2')
(vol2/voltotal)*100
disp('Percent region3')
(vol3/voltotal)*100
disp('Percent region4')
(vol4/voltotal)*100
disp('Percent region5')
(vol5/voltotal)*100
Appendix B1

Matlab code to plot size exclusion chromatogram, calculate number and weight averaged molecular weights, and find peak locations

clear
clc

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% %%%%%%%%%%%%%%%
%%%%
%This code is developed to calculate number averaged and weight averaged
%molecular weights of organic matter. The data used in the calculations
%will have two columns stored as a csv file. The first is elution time, 
%and the second is peak height.
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% %%%%%%%%%%%%%%%
%%%%
%Data Input

%Prompt user for name of csv File with data
filename = 'stamxad4.csv';
filename=input('enter csv file name of the fluorometer data, remember to include .csv --> ','s');
M = dlmread(filename);

[row1,col1] = size(M);

%prompt user if there is a peak at 5.5 minutes
min5peak = input('Is there a peak at 5.5 min? 1 for yes 0 for no --> '); 

%Convert Elution time to log Molecular Weight using calibration curve

%slope = -0.4955;
slope = input('enter the slope of the calibration curve -->','s');
%intercept = input('enter the y-intercept of the calibration curve -->','s');
%intercept = input('');

for i=1:row1
    M(i,3) = M(i,1)*slope + intercept;
    M(i,7) = 10^M(i,3);
end

%Baseline Correction

%baseline = input('Baseline shift? -->');

54
baseline = 0.4;

for i=1:row1
    M(i,2) = M(i,2) + baseline;
end

maxheight = 0;
line1=0;
for i=1:row1
    if M(i,2) < 0
        M(i,4) = 0;
    else
        M(i,4) = M(i,2);
    end
    if M(i,2) > maxheight
        maxheight = M(i,2);
    end
end

%attempt to autocalculate high molecular weight cutoff

%for i=1:row1
%    if min5peak == 0
%        if M(i,3) < 5 && M(i,3) > 3.5
%            if M(i,2) < maxheight * 0.01 % high molecular weight cutoff, 1% of max intensity
%                line1=M(i,3);
%            end
%        end
%    end
%    if min5peak ==1
%        if M(i,3) < 3.5 && M(i,3) > 3.5
%            if M(i,2) < maxheight * 0.01 % high molecular weight cutoff, 1% of max intensity
%                line1=M(i,3);
%            end
%        end
%    end
%end

%High and Low molecular weight cutoff

if min5peak == 0
    line1 = log10(3200);  %high molecular weight cutoff is 10^3.5 if no peak at 5 min
else
    line1 = 5;    %high molecular weight cutoff is 10^5 if peak at 5 min
end

line2 = log10(50); %lower molecular weight cutoff of 50 Daltons
line3 = log10(822);

%Calculate number averaged and weight averaged MW and polydispersivity
for j=1:row1
    if M(j,3) > linel || M(j,3) < line2
        M(j,4) = 0;
    end
end
for k=1:row1
    M(k,5) = M(k,4) * M(k,7);
    M(k,6) = M(k,4) / M(k,7);
end
S=sum(M);
Mn = (S(4)/S(6))
Mw = (S(5)/S(4))
Polydispersivity = Mw/Mn

%Plot the chromatogram

linelength = 0:0.01:maxheight;
figure(1)
plot(M(:,1),M(:,2))
title(filename)
xlabel('Time')
ylabel('Response')

figure(2)
plot(M(:,3),M(:,2))
title(filename)
xlabel('Log Molecular Weight')
ylabel('Response')
set(gca,'XDir','reverse')
hold on
plot(line1,linelength,'r')
plot(line2,linelength,'r')
%plot(line3,linelength,'r')
hold off
[pks,locs] = findpeaks(M(:,4),'minpeakdistance',20);
for i=1:length(locs)
    peaklocations(i) = M(locs(i),7);
end
peaklocations