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# Dissolved Organic Matter Characterization and Impact on Mercury Binding in an Urbanizing Watershed

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# **Dissolved Organic Matter Characterization and Impact on Mercury Binding in an Urbanizing Watershed**

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A Thesis  
Submitted in Partial Fulfillment of the  
Requirements for the Degree of

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**APPROVAL PAGE**

Master of Science Thesis

**Dissolved Organic Matter Characterization and Impact  
on Mercury Binding in an Urbanizing Watershed**

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## Abstract

Dissolved organic matter (DOM) is ubiquitous in aquatic ecosystems, interacting with and controlling the bioavailability and transport of mercury (Hg) and other trace metals through complexation. The complexation of ionic Hg ( $\text{Hg}^{2+}$ ) with DOM can either limit methyl mercury (MeHg) bioavailability or increase MeHg production by stimulating microbial growth. While substantial investigations have examined Hg-DOM interactions in forested ecosystems, little research has been conducted on the Hg-DOM relationship within an urbanized river setting subjected to various anthropogenic sources. The various runoff and pollutant sources vary in DOM quality and character. Three sources of organic matter from the North Branch Park River (NBPR watershed in Hartford, CT (forest, FOR; impervious surface runoff, SWR; and an urban stream, USS) as well as the influent (InfOM) and effluent (EfOM) from the Hartford Water Pollution Control Facility were collected and isolated using Amberlite DAX-8 and XAD-4 resins into operationally defined hydrophobic and hydrophilic acid fractions. Each fraction was characterized for chemical and spectral properties prior to an assessment of Hg-DOM fraction binding by measuring conditional stability constants ( $K_{\text{DOM}^*}$ ) using an equilibrium dialysis ligand exchange (EDLE) method. Molecular weights ranged from 668 Da (forest) to 209 Da (influent),  $\text{SUVA}_{254}$  ranged from 3.17 (forest) to 0.02 (influent)  $\text{L mg}^{-1} \text{ m}^{-1}$ , and FI ranged from 1.2 (forest) to 1.8 (effluent). Spectral properties of the forest site represented those for a terrestrial source while those for the wastewater constituted a microbial end member. The SWR and USS sources were indicative of urban stream organic matter origin.  $K_{\text{DOM}^*}$  values were affected by Hg/DOM ratios and ranged from  $10^{23}$ -  $10^{24}$   $\text{L kg}^{-1}$ , comparable to Hg-DOM binding coefficients from previous studies. NMR and chemical



analysis suggest that binding is controlled by a small number of strong thiol as well as weaker carboxyl functional groups and likely is connected to the low molecular weights. Hg-DOM binding varied over an order of magnitude with source, demonstrating the need for future research to examine the potential implications of shifting DOM contributions in urban ecosystems.

**Key words:** dissolved organic matter, mercury, urban watershed, urban streams, combined sewer overflow, nonpoint source runoff, complexation, hydrophobic acid fraction, hydrophilic acid fraction, wastewater

## Introduction

Mercury (Hg) contamination from atmospheric deposition is of concern due to the toxicity and potential for bioaccumulation, particularly for the methylated chemical species (Stein et al., 1996; Wollenberg et al., 2009). Hg from atmospheric deposition to watersheds or contributions from point source inputs occur primarily as ionic Hg ( $\text{Hg}^{2+}$ ; Stein et al., 1996; Mason and Sullivan, 1997; Ravichandran, 2004). Within anoxic zones (e.g. sediments and wetlands),  $\text{Hg}^{2+}$  is converted to the methylated form in large part by anaerobic sulfate reducing bacteria (SRB; Marvin-Dipasquale et al., 2009), enhancing the potential for bioaccumulation and biomagnification in the food chain (Ravichandran, 2004). However, Hg transport and methylation has been demonstrated to be affected by chemical interactions with inorganic and organic ligands such as hydroxides, chlorines, sulfides, and dissolved organic matter (DOM; Mason and Sullivan, 1997; Dittman et al., 2009; Driscoll et al., 1995).

DOM is a ubiquitous constituent of aquatic ecosystems and can have a significant impact on Hg biogeochemistry through complexation with  $\text{Hg}^{2+}$  to form chemical complexes (Thurman and Malcolm, 1981; Leenheer and Croué, 2003; Hintelmann et al., 1997; Driscoll et al., 1995). Positive correlations between DOM and dissolved Hg at low Hg:DOM ratios have been shown in natural systems (Dittman et al., 2009; Dittman et al., 2010; Aiken et al., 2003; Driscoll et al., 1995; Ravichandran, 2004; Babiarz et al., 1998; Schuster et al., 2008) and significant interaction is evident from the determination of large conditional stability constants  $K$ , reported for  $\text{Hg}^{2+}$ -DOM and MeHg-DOM complexes (Ullrich et al., 2001; Hintelmann et al., 1997). These interactions have been linked to decreased MeHg production, increased  $\text{Hg}^{2+}$  bioavailability (Mierle, 1991) and

increased  $\text{Hg}^0$  production and volatilization (Driscoll et al., 1995; Ullrich et al., 2001; Ravichandran, 2004; Dong et al., 2010). Conversely, DOM also acts as a substrate for SRB by providing organic carbon, stimulating MeHg production (Hintelmann et al., 1997).

DOM is a complex heterogeneous compound composed of aromatic hydrocarbons with amide, carboxyl, hydroxyl, phenol, and, thiol functional groups (Croué et al., 2003; Thurman, 1985), of which 80% are operationally-defined humic substances (Leenheer, 2003). Humic substances are further divided into hydrophobic (HPOA) and hydrophilic (HPIA) acid fractions, the two fractions most reactive in trace metal binding (Ravichandran, 2004). While Hg is attracted to carboxylic and phenolic acid sites (more abundant in the HPOA; Hintelmann et al., 1997; Saar et al., 1982; Thurman, 1985), preferential binding occurs at the thiol sites (Skylberg et al., 2003; Xia et al., 1999; Ravichandran, 2004) due to the high affinity of Hg for sulfur (Haitzer et al., 2002). At low Hg:DOM ratios indicative of concentrations in the natural environment, Hg binds with the stronger thiol binding sites (Haitzer et al., 2002). However, the majority of these investigations, particularly regarding complex Hg-DOM interactions, have been conducted in forested watersheds.

Due to increased development, urbanized watersheds are becoming more prevalent, stirring interest in biogeochemical changes relative to forested processes. Hg flux in urban ecosystems is significantly elevated relative to forested ecosystems, largely due to elevated particulate mobilization during events. However, studies have demonstrated increases in dissolved Hg associated with DOM during events in addition to the particulate fraction. Increases in urbanization and anthropogenic impacts include inputs

from wastewater treatment plant effluent, combined sewage overflows (CSOs), and impervious surface stormwater runoff. These inputs reflect not only altered Hg biogeochemical cycling, but also shifts in DOM characteristics relative to forested aquatic environments (Paul and Meyer, 2008; Baker, 2001; Baker et al., 2003; Hudson et al., 2007). While prior investigations (Imai et al., 2002; Leenheer and Croué, 2003) have demonstrated that urbanization effects do not influence the production or character of natural allochthonous and autochthonous sources of organic matter, we hypothesize that new anthropogenic sources of organic matter will shift the quality in urban streams. Anthropogenic runoff inputs of DOM into rivers are characterized as more labile, more biodegradable carbon (Leenheer and Croué, 2003; Paul and Meyer, 2001; Hudson et al., 2007) relative to forested inputs, higher in HPIA fraction composition (Imai et al., 2002). Conversely, fluorescence has spectrally demonstrated that organic matter from wastewater sources is more recalcitrant, suggesting similarities to microbial autochthonous sources (Hudson et al., 2007). Westerhoff and Anning (2000) concluded that while urban runoff DOC sources had lower aromaticity, the fluorescence was higher relative to natural DOC ( $\text{SUVA}_{254} = 1.2 \text{ mg m}^{-1} \text{ L}^{-1}$  and  $\text{FI} = 2.2$ ). However few studies have been conducted in urban settings specifically on defining urban DOM characteristics.

## **Background**

### *Hg<sup>2+</sup> Cycling in Aquatic Systems*

Mercury (Hg) is a naturally occurring, yet toxic, element existing as three ionic species: elemental ( $\text{Hg}^0$ ), monovalent ( $\text{Hg}^+$ ), and divalent mercury ( $\text{Hg}^{2+}$ ). It is naturally

present at low concentrations in the aquatic environment, but anthropogenic activity has caused increased concentrations in freshwater systems (Ullrich, et al. 2001). The cycling of Hg is complex, with many atmospheric and watershed processes affecting the fate and transport of the various Hg species (Figure 1). Most mercury contributions to the environment come from the wet and dry deposition of mercury due to anthropogenic point sources. Approximately 80% of mercury emissions are due to fossil fuel combustion, 15% are due to direct land application of solid wastes, and 5% are due to commercial effluent discharges to water bodies (Stein et al., 1999). Once released into the atmosphere, mercury primarily (50%) exists as  $\text{Hg}^0$ . Under specific redox conditions  $\text{Hg}^0$  can be oxidized by ozone ( $\text{O}_3$ ) to  $\text{Hg}^{2+}$ , form complexes with other anions (Schroeder et al., 1991) existing in the aqueous phase from reactions occurring within forms of precipitation, and released into the environment as wet deposition. The amount of mercury deposited due to dry deposition is greater than the amount from wet deposition; therefore most mercury that enters a water body is in the form of elemental mercury (Stein et al., 1999).

Mercury watershed cycling is dependent on redox reactions, complexation, wet and dry deposition, bioaccumulation, and soil and sediment sorption (Stein et al., 1999). Sediment accumulation accounts for 98% aquatic mercury with 75% of that bulk adsorbed to humic substances. The remaining amount is bound to sulfides or other metal oxides (Andersson et al., 1990). Once deposited to a watershed, Hg undergoes transformations among chemical species and is impacted by transport processes. Mercury's most toxic form, methylmercury (MeHg), is produced from the reduction of  $\text{Hg}^{2+}$  by sulfate-reducing bacteria (SRB) (Gilmour et al., 1992). MeHg is the most

biologically active form of mercury and is a major environmental concern. Due to its affinity for fatty tissue, MeHg bioaccumulates in biota, specifically fish, resulting in biomagnification through the food chain. Methylmercury production in a watershed is dependent on temperature, pH, organic matter availability, and microbial population. Generally, methylation is enhanced during anoxic conditions, high temperatures, and low pH (Ullrich et al., 2001) with magnification enhanced with food chain length (Evers et al., 2007).

Hg readily forms complexes with many inorganic and organic ligands in the aqueous environment, including chloride, hydroxide, sulfur, and organic matter, that increase the solubility and mobility (Stein et al., 1999).  $\text{Hg}(\text{OH})_2$  is the dominant chemical species in freshwater systems while  $\text{HgCl}_2$  dominates at low pH and high  $\text{Cl}^-$  concentrations (Hudson et al., 1994). Mercury-sulfur complexes have been demonstrated to be significantly stronger than oxygen containing complexes. While Hg-sulfur complexes are more prevalent in reduced sulfidic conditions, these complexes tend to precipitate out into sediments (Bodek et al., 1988). However, Hg also forms very strong complexes with DOM; conditional stability constants reported range from  $(10^{21}-10^{28})$  for soil and aquatic organic matter and  $>10^{30}$  for wastewater humic substances (Skylberg et al. 1997; Lamborg et al. 2003; Haitzer et al. 2002; Hsu and Sedlack, 2003). Hg-DOM complexes often outcompete inorganic ligand complexation (Ravichandran, 2004). Due to the propensity for Hg to bind to humic substances, either in the water column or in sediments, characterizing Hg-OM binding is critical to assess overall fate of Hg in the environment.

### *Urbanization Influences on Mercury Cycling*

Due to the sensitivity and toxic effects of Hg in otherwise pristine ecosystems, watershed cycling studies have focused on forested ecosystems (Babiarz et al., 1998; Hurley et al., 1995; St. Louis et al., 1996). Few studies have been conducted that include urban or suburban areas. Land use change affects chemical cycling through watersheds, either through alteration of ecosystem inputs or function. Mason et al. (2000) demonstrated elevated wet deposition of THg in downtown Baltimore ( $30 \mu\text{g m}^{-2} \text{yr}^{-1}$ ) relative to rural sites ( $\sim 15 \mu\text{g m}^{-2} \text{yr}^{-1}$ ). Results from the Mercury Deposition Network (2006) for Beltsville, Maryland ( $10 \mu\text{g m}^{-2} \text{yr}^{-1}$ ) are also elevated relative to a remote forest site in upstate New York ( $6.6 \mu\text{g m}^{-2} \text{yr}^{-1}$ ).

While atmospheric deposition is generally the primary Hg input, additional sources of Hg in urban and suburban watersheds include leaks from sanitary sewer systems, runoff from impervious surfaces (including litter), and industrial discharges (Paul and Meyer, 2001). In addition to inputs, development also alters watershed processing and transport of Hg. Higher impervious surface cover increases runoff while decreasing riparian groundwater levels. These impacts reduce connections between streams and riparian zones while increasing oxic stream conditions and enhancing runoff (Groffman et al., 2002, 2004; Paul and Meyer, 2001). Total Hg yields (stream outlet flux relative to total atmospheric input) of 40-103% have been estimated for urban ecosystems (Lawson et al., 2001; Mason and Sullivan, 1998), an increase relative to the 10-30% estimated for forested watersheds (Hurley et al., 1995; Mason et al., 1997). Elevated yield is associated with the increased Hg flux (Hurley et al., 1995, 1996), particularly due to substantially higher particulate loading during elevated flow conditions within urban (Lawson et al.,

2001; Mason and Sullivan, 1998) and agricultural (Balogh et al., 2003) areas. Developed ecosystems are particularly affected (Babiarz et al., 1998) due to the high hydraulic runoff percentage and sharp, “flashy” flow response (Hurley et al., 1995; 1998; Mason and Sullivan, 1998) with >94% of annual Hg species flux during high flow periods (Mason and Sullivan, 1998).

## **Objective**

We conducted an investigation to assess the impact of anthropogenic source inputs on DOM characteristics and the subsequent influence on Hg-DOM interactions. Representative water samples were collected from potential inputs in a developing urban watershed, isolated into fractions and characterized for DOM quality using spectral, physical and chemical analyses. The isolated DOM fractions were used in equilibrium dialysis ligand exchange (EDLE) experiments to assess the binding potential of dissolved Hg to the different source of DOM. These efforts strive to assess the potential changes to DOM characteristics in the urban aquatic environment. Additionally, the impact of the shifts in DOM quality on Hg binding will provide a measure of potential changes in bioavailability in the urban environment.

## **Experimental Section**

### *Study Site*

DOC isolates were collected from the North Branch Park River (NBPR) watershed. The NBPR watershed is a subwatershed of the Park River with an area of 28.6 square



miles. The watershed contains 14 subwatersheds of various land cover and land use. About 86% of the NBPR watershed is developed land with 15% impervious surface cover (Fuss & O'Neill, 2010). The area is considered a high density suburban and urban river setting with urbanization generally increasing from the headwaters to the confluence with the South Park River in Hartford. NBPR is a third order stream with a length of approximately 5.9 miles. The NBPR daylight through the northern part of Hartford, CT and flows through underground conduit for 0.5 miles on Farmington Avenue (Fuss & O'Neill, 2010). The watershed is characterized predominantly by Udorthents-Urban soil complexes, Holyoke-Rock outcrop complex, and several silty loam soils (NRCS, USDA, 12 Apr. 2011).

The Park River historically has been polluted, with industrial wastes and sewage discharged directly to the river. In an effort to control and minimize direct access to sewage waste and flooding in the city, the lower reaches of the Park River were buried in 1940 (Bushnell Park Foundation, 1997). While direct industrial point sources have been removed, the sewage system remains combined with overflow events discharging sewage via combined sewer overflows (CSO) to the river during precipitation and snowmelt events (Fuss & O'Neill, 2010). The increased development in the Hartford region has overloaded the combined sewer system and led to sanitary sewage contributions, in addition to impervious surface runoff and industrial contamination, to the NBPR.

### *Sample Collection*

DOM isolates were collected from three locations within the NBPR watershed (Figure 2) as well as the Hartford Water Pollution Control Facility (WPCF) influent and effluent during early 2011. These sites were selected to span the potential range of DOM

sources in developed settings and included forest, impervious surface runoff, raw sanitary sewage, and WPCF effluent. Additionally, a sample was collected from the NBPR during a runoff event to assess contributions to an urban stream from nonpoint sources. The forested site (FOR) was collected in early April in the northwest headwaters of the watershed in Bloomfield, CT, a site preserved to maintain pristine conditions for the nearby drinking water reservoir. Impervious surface stormwater runoff (SWR) was collected during a rain event in May 2011 from runoff entering a storm drain on Route 218 at the intersection with East Lane in Bloomfield, CT. The urban stream sample (USS) represents a stream receiving CSO and impervious surface runoff in addition to forested contributions and was collected at the end of February from the lower reach of the NBPR just before the river enters the underground conduit at Farmington Avenue. Wastewater influent (InfOM) and effluent (EfOM) were collected in January and April 2011, respectively, from the WPCF in Hartford, CT.

### *DOM Isolation*

A 100 L sample was collected from each site, filtered through pre-combusted 0.45  $\mu\text{m}$  glass fiber filters into 50 L carboys and acidified to a pH of 2.0 with HCl. These samples subsequently were fractionated into hydrophobic (humic) and hydrophilic (non-humic) DOM using analytical-scale column chromatography with DAX-8 Supelite resin (40-60 mesh) and XAD-4 Amberlite resin (20-60 mesh), respectively, according to cited methods (Thurman and Malcolm, 1981; Aiken et al., 1992; Hood et al., 2003; Wang, L. et al., 2009). In summary, the sample was pumped through each column in series, and separately each column was back-eluted with 0.1 M KOH. Using Dowex Marathon MSC

Cation Exchange Resin (H form), the eluates were desalted, proton saturated and lyophilized for use in the EDLE sorption experiments. The total DOC concentration of each fraction was determined using a Teledyne Tekmar Apollo 9000 total organic carbon analyzer to assess the relative amount of DOM represented by each fraction. The methods used for the isolation of DOM are explained in more detail by Quaranta (2011).

### *DOM Characterization*

Each isolate was characterized for optical and chemical properties. Spectral analysis of each sample and its isolates was obtained by measuring the specific UV absorbance at 254 nm (SUVA) and fluorescence. SUVA is the absorbance at 254 nm divided by the DOC concentration of the sample. UV-vis was determined on a Cary Bio 50 spectrophotometer. Fluorescence was determined on a Cary Eclipse fluorescence spectrometer equipped with a xenon flash lamp, slit widths of 5 nm and a scan rate of 1200 scans per second. We used fluorescence regional integration (FRI) to quantitatively analyze fluorescence EEMs (Chen et al., 2003) by operationally defining five regions within an EEM based on five distinct fluorescent peaks and integrating the area underneath the EEM spectra for those regions (Supplemental Information, Figure S1). Excitation emission matrix fluorescence spectroscopy (EEMs) contour plots were evaluated using a pre-developed MATLAB code (Quaranta, 2011) to obtain excitation/emission ratios and fluorescence maxima. The EEMs correction method used in this study was modeled after Murphy et al. (2010). The correction matrix was determined by measuring the fluorescence of  $10^{-3}$  M quinine sulfate in 0.1N sulfuric acid at an excitation wavelength of 346.5 nm and emission wavelength interval of 384-667.

The fluorescence intensity at every 1 nm was normalized to a maximum intensity of one and compared to tabulated data (Quaranta, 2011). Instruments equipped with a red photomultiplier tube can provide excitation spectra which are not very distorted (Lakowicz, 1999). Our instrument was equipped with a red photomultiplier tube, so the correction matrix only influenced emission spectra. Fluorescence indices (FI) were calculated for each DOM isolate (McKnight, et al. 2001) as the ratio of emission intensity at 400 nm relative to 500 nm at an excitation wavelength of 370 nm.

High pressure size exclusion chromatography (HPSEC) measured the number ( $M_N$ ) and weight ( $M_w$ ) averaged molecular weight (MW) of the DOM isolate based on methods from Chin et al., (1994) and Zhou et al. (2000) via a Hewlett Packard 1050 series HPLC with polystyrene sulfonic standards (PSS) in sodium salt (Polysciences, Warrington, PA) and acetone as a low MW standard. The system used a Waters Protein-Pak 125 column and phosphate buffer at pH 6.8 with 0.002M  $\text{Na}_2\text{HPO}_4$  and  $\text{KH}_2\text{PO}_4$ , and 0.1M NaCl as the mobile phase.

Additionally, each lyophilized HPOA was analyzed for functional group spectra via nuclear magnetic resonance (NMR) using a Bruker DRX-400 according to standard methods (M. Morton, The University of Connecticut, Chemistry Department) and elemental CNS composition was achieved on a Fisons NA Series 2 Elemental Analyzer at a combustion temperature for sulfur analysis of 1000 °C, according to EPA Method 440 for CHNS determination (C. Koerting, The University of Connecticut, Department of Marine Sciences). Spectra for  $^1\text{H}$  and  $^{13}\text{C}$  were acquired at 400.1 MHz and 100.7 MHz frequency respectively. All spectrums were referenced to a residual water peak at 4.7ppm and externally against TMS (tetramethylsilane).

### *Measurement of Hg-DOM binding*

An equilibrium dialysis ligand exchange (EDLE) experiment was performed with each DOM source hydrophobic fraction to quantify the complexation potential of the freshwater DOM isolates with ionic mercury  $\text{Hg}^{2+}$  using methods developed by Haitzer et al. (2002 et al.; 2003). EDLE experiments were not performed on the hydrophilic isolate of each source because recovery concentrations were too low to generate sufficient lyophilized material. Additionally, the hydrophobic fraction has been suggested to dominate  $\text{Hg}^{2+}$  binding (Ravichandran, 2004; Haitzer et al., 2002; 2003). Spectra/Por Biotech CE 500 MWCO dialysis membranes were used based on concerns over DOM retention and preliminary results with 500 and 3500 Da MW cutoffs. The 3500 Da MWCO membranes allow for a faster diffusion of Hg-EDTA (~48 hrs) but permit leakage of the DOM into the outer solution and result in negative Q values (Equation 2). Equilibrium partitioning experiments were conducted with the CE 500 MWCO to approximate the required diffusion time for Hg-EDTA to reach equilibrium (Supplemental Information, Figure S2). Waples et al. (2005) suggested that CE 500 MWCO membranes reached equilibrium with respect to Hg-EDTA after 9 days while preserving a sufficient DOM gradient. The membranes were cleaned by immersion in DI water overnight followed by rinsing several times with DI water to rid the membrane of sodium azide solution. Cleaned membranes were stored at 4°C in DI water until use.

Sorption experiments were conducted in 125 mL Teflon bottles using approximately 15 cm of dialysis tubing to separate the two solutions, with initial spike concentrations based on the total 100-mL volume of solutions. The 3-mL inner solution contained 1.0  $\text{mg L}^{-1}$  of the DOM isolate. The 97-mL outer solution was spiked with  $\text{Hg}^{2+}$  from a 100

mg L<sup>-1</sup> stock solution of HgCl<sub>2</sub> in 10% HNO<sub>3</sub> (Inorganic Ventures, VA) to resulting concentrations of 0.01, 0.10 and 1.0 µg L<sup>-1</sup>. Each solution contained 0.1 M KClO<sub>4</sub> as an ionic regulator and 0.01 M EDTA adjusted to a pH of 7.0 with NaOH. EDTA was chosen as an auxiliary ligand for the system because it does not interfere with Hg analysis, nor form complexes with Hg-DOM, is suitable at pH values representative of freshwaters (6-8), and has reliable binding constants published for complexes with Hg. Each stock solution was filtered (0.45 µm) prior to use to remove particulate influence. Bottles were equilibrated for nine days on an end-over-end rotator in the dark to reduce the risk of any photodegradation. After equilibrium, aliquots were taken from inside and outside of the membrane bag and analyzed for Hg<sup>2+</sup>. Hg samples were preserved with 0.5% BrCl (v/v). Experiments were conducted in triplicate for each DOM source with a control dialysis set containing only NaClO<sub>4</sub>, EDTA, and Hg<sup>2+</sup>. As EDTA interferes with the analysis of DOC, a parallel set of experiments was assembled to assess the transfer of DOM across the membrane using an initial DOM concentration of 100 mg L<sup>-1</sup> and extrapolated to 1 mg L<sup>-1</sup> to calculate the inner and outer concentrations of DOM in the equilibrium experiments.

Conditional stability constants ( $K_{DOM^*}$ ) were derived for the reaction of Hg<sup>2+</sup> with DOM according to the modified technique developed by Haitzer et al. (2002) and originating from Glaus et al. (1995).  $K_{DOM^*}$  is defined as:

$$K_{DOM^*} = \frac{[HgDOM]}{[Hg][DOM]} \quad (1)$$

where the square brackets represent molar concentrations of Hg:DOM complexes and Hg<sup>2+</sup>. Converting the DOM concentration to kg L<sup>-1</sup> and assuming that the concentration

of  $\text{Hg}^{2+}$  is much smaller than the concentration of DOM, we obtain the following relationship for  $K_{\text{DOM}}$

$$K_{\text{DOM}} = \frac{Q\alpha_{\text{Hg}}}{(\text{DOM})_{\text{in}} - (\text{DOM})_{\text{out}}(Q+1)} \quad (2)$$

where  $Q$  is a measure of the Hg distribution within the system. The  $\text{Hg}^{2+}$  concentration inside the bag indicates the amount of  $\text{Hg}^{2+}$  bound to the respective DOM and the  $\text{Hg}^{2+}$  concentration in the outer solution represents the amount of the total (bound and unbound) Hg.  $Q$  is calculated by:

$$Q = \frac{[\text{Hg}]_{\text{in}} - [\text{Hg}]_{\text{out}}}{[\text{Hg}]_{\text{out}}} \quad (3)$$

and  $\alpha$  is a measure of the complexation of Hg by the auxiliary ligand (L; in our case EDTA) and hydroxide ( $\text{OH}^-$ ). For  $[\text{Hg}] \ll [\text{L}]$ ,  $\alpha$  can be calculated as:

$$\alpha_{\text{Hg}} = 1 + \sum_{i=1}^n \beta_i^{\text{L}} \left( \frac{[\text{L}]_{\text{total}}}{1 + \sum_{j=1}^m [\text{H}]^j \beta_j^{\text{H}}} \right) + \sum_{k=1}^o \beta_k^{\text{OH}} [\text{H}]^{-k} \quad (4)$$

where  $\beta_i^{\text{L}}$  and  $\beta_j^{\text{H}}$  are the stability constants for the stepwise complexation of L to  $\text{Hg}^{2+}$  and  $\text{H}^+$ , respectively, and  $\beta_k^{\text{OH}}$  is the stepwise stability constant for the complexation of  $\text{Hg}^{2+}$  with  $\text{OH}^-$  (Morel and Hering, 1993; Table S1, Supplemental Information).

### *Chemical Analysis*

Total Hg (THg) was analyzed using a Tekran Model 2600 mercury analysis system in water via oxidation, purge, and trap, cold vapor atomic fluorescence spectroscopy (CVAFS) following EPA Method 1631 Revision E. DOC was analyzed via acidification,

purge and combustion according to Standard Method 5310 B (APHA/AWWA/WEF, 2005) using a Teledyne-Tekmar Apollo 9000.

## Results and Discussion

Chemical and spectral characteristic varied with the source of the DOM isolates from the Park River system (Table 1). Recovery from the isolation of the HPOA range from 40-75% of the total sample DOM and were greater than the isolated HPIA for each source. The  $MW_N$  of the five samples ranged from 668 Da (FOR) to 209 Da (InfOM) for the HPOA and 515 Da (USS) to 257 Da (EfOM) for the HPIA. The size of these fractions are a lower MW than expected from previously reported values for aquatic humic substances (Chin et al. 1994; Aiken and Malcolm, 1987; Cabaniss et al. 2000; Zhou et al. 2000), yet, similar to microbially derived organic matter such as HPOA from Lake Fryxell, Antarctica (Haitzer et al., 2003).

The SUVA values measured for the five different sources range from 1.71 – 3.17 L  $mg^{-1} m^{-1}$  and from 0.23 – 1.6 L  $mg^{-1} m^{-1}$  for the HPOA and HPIA fractions, respectively. These values reflect changes in DOC aromaticity with source. Microbially-derived DOM is expected to have a lower aromaticity, and subsequently SUVA, due to lower lignin content compared to terrestrial derived DOM. The SUVA values are similar relative to those reported for Suwannee River fulvic acid (SRFA; 3.2 L  $mg^{-1} m^{-1}$ ), a highly aromatic terrestrially-derived DOM (Dittman et al., 2009; Weishaar et al., 2003; McKnight et al., 2001). SUVA values calculated for the InfOM HPIA isolate are unexpectedly low most



likely due to high concentration interferences because SUVA is normalized by DOC concentrations (Weishaar et al., 2003).

The SWR and FOR sources exhibited the highest  $SUVA_{254}$  value for the HPOA fraction, 3.17 and 3.00 L mg<sup>-1</sup> m<sup>-1</sup>, respectively. The SWR source, a new reporting of the characterization of DOM from impervious surface runoff, comes from impervious storm runoff that is influenced by some grassy terrestrial areas and anthropogenic petroleum hydrocarbon sources (Hook and Yeakley., 2005). The resulting increase concentration of DOC and aromatic carbon is most likely due to the terrestrial contributions and litter and plant residues present on impervious surfaces. The InfOM had the lowest  $SUVA_{254}$  value for both fractions as raw sewage is highly microbially processed and prior studies have indicated high concentrations of labile organic matter in wastewater influent (Park et al., 2010).

The HPOA and HPIA SUVA values were similar for the EfOM and USS sources, with those for USS higher relative to EfOM. The USS source was collected during the early spring snowmelt. Relative to reported data of isolated aquatic DOM, the SUVA value for the USS source suggests influences of high allochthonous DOM input from runoff from organic rich soil layers (Miller et al. 2009; McKnight et al. 2001). The USS source was subject to possible microbial autochthonous DOM inputs from the CSO contributions during the storm that could lower the aromaticity of the isolate. When wastewater effluent is fractionated, the HPIA fraction generally represents a higher percentage of the total DOM relative to the HPOA fraction as wastewater is more biologically and physically processed (Imai et al., 2002). The HPIA generally is lower in aromatic carbon (Aiken et al., 1992), in agreement with the slightly lower SUVA value

between the two isolates for the EfOM sources and lower values relative to the SWR and FOR sources. Regarding the EfOM, our similar SUVA results (2.09 and 2.06 L mg<sup>-1</sup> m<sup>-1</sup>, respectively, for the HPOA and HPIA) agree with previously reported values for a similarly sized treatment plant in an urbanized area (Imai et al., 2002). The relatively high values likely result from the consumption of the readily-available DOM during the treatment process, leaving the more recalcitrant humic substances such as aromatic proteins (Park et al., 2010, Hudson et al., 2007, Chen et al., 2003). Although SUVA is only an indicator of the aromaticity of DOM, the results suggest sources in the NBPR watershed representing possible sewage contributions which are lower in aromatic carbon compared to the sources subject to only terrestrial-like DOM inputs.

#### *Fluorescence Characteristics*

The FI values for FOR and USS site are consistent with terrestrial-like sources of organic matter with values closer to 1.3 while SWR and EfOM fall between the spectrum defining terrestrial-like (1.3) and microbial-like (1.9) end members (McKnight et al., 2001). The FI of the HPIA fraction of EfOM indicates microbial-like organic matter (1.8), a value related to the wastewater treatment process and fact that wastewater effluent contains large amounts of microbial products such as aromatic proteins (Park et al., 2010, Hudson et al., 2007, Chen et al., 2003). The SWR FI (1.6) is similar to the FI of fulvic acids from the Shingobee River (1.5; McKnight et al., 2001). Both SWR and Shingobee River DOM were collected during the summer, have high aromaticity values yet low FI. During the summer, fulvic acids display intermediate characteristics from

those of groundwater sources and snowmelt (McKnight et al., 2001) and the SWR FI, like the Shingobee, is indicative of fulvic acids from a mixture of sources.

The EEMs for the source DOMs (Supplemental Information, Figure S3) were converted to a percentage distribution of each region from the EEMs using FRI (Chen et al., 2003; Figure 3). The FOR HPOA fraction is characteristic of naturally-occurring freshwater organic matter. The FOR HPOA fraction predominantly is composed of humic and fulvic acids as evidenced from the intense peaks in Region C and A (57 and 14%, respectively; Figure 3). The FOR HPIA fraction although similar to the FOR HPOA fraction differs from other reported forested DOM because it is slightly lower in composition of fulvic acids and has slightly higher fluorescence in the humic-like and aromatic protein regions. The percentages from Regions A, T1, and C regions are 41%, 22%, and 16%, respectively; Figure 3). According to Aiken et al. (1992), the HPIA fraction contains a large amount of more labile humic-like compounds resulting in a large Region A of the HPIA EEMs, a characteristic normally associated with the HPOA. Both the HPOA and HPIA fractions of the USS source are dominated by Regions A and T1. Higher values for the HPIA fraction relative to the HPOA fraction suggest that the HPIA fraction is primarily readily biodegradable (labile) organic matter from terrestrial and CSO inputs from the storm event. These represent sources with more highly processed lignin.

Both the SWR and USS sources have an intense Region T1 with very little to no intensities measured in Region B, a characteristic of urbanization and anthropogenic inputs as well as seasonal differences (Hudson et al. 2007; Baker and Spencer et al. 2004). A shift in greater fluorescence relative to the HPOA and HPIA fractions in peak

Regions C and B is noticed for both SWR and USS EEMs as well. The SWR source was dominated by Regions T1 and A, consistent with biological activity in natural systems due to the fluorescence of aromatic amino acids (Hudson et al., 2007). The SWR also demonstrated an increase in Region C and decrease in Region T1 for the HPIA relative to the HPOA, consistent with microbial processing of biologic material in drainage systems, (Chen et al., 2003), biological processing of more labile material has shown to produce more humic-like substances. Comparison of the EfOM EEM, HPOA relative to the HPIA showed an increase of intensities in regions B, T1 and T2 and decreased intensities for regions A and C. Compared to the InfOM-HPOA, a shift towards more humic-like organic matter is observed in the EfOM-HPOA EEM. For the InfOM and EfOM HPOA fractions, both EEMs were similar with slight decreases in Regions B and C for the EfOM. These trends are consistent with those reported previously due to biological processes occurring during wastewater treatment (Park et al., 2010; Hudson et al., 2007; Chen et al., 2003), these processes have been suggested to increase humic substances in effluent waters. The similarities between the fluorescence of sources subject to wastewater may result from significant amounts of humic and fulvic material contributing to the high fluorescence observed in Regions A and C in both fractions (Chen et al., 2003), even though wastewater effluent contains more soluble microbial byproducts than terrestrial derived DOM (Drewes et al., 1999).

All sources of organic matter presented are small MW aggregates. The two end members within the NBPR sources presented here, the FOR and sources from the WPCF (InfOM and EfOM), are comparable to reported data for forested and wastewater organic matter (e.g., McKnight et al., 2001 and Hudson et al., 2007). The FOR site, higher in

aromaticity with a FI near 1.3, suggests terrestrial origins and was composed primarily of humic and fulvic acids. Conversely, the InfOM and EfOM are indicative of soluble microbial organic matter sources with the presence of humic substances as well. The other two sources of organic matter within this watershed (SWR and USS) indicate aggregate heterogeneous organic matter sources comparable to assumptions of urban organic matter quality. Both the SWR and USS exhibit high SUVA correlating to aromaticity. The FI of SWR suggests origins between a terrestrial and a microbial source and from EEMs interpretation SWR has a high percentage of aromatic proteins, soluble microbial-like material, and fulvic acids; whereas the USS source is indicative of terrestrial origins and is primarily composed of humic and fulvic acids and very little aromatic protein composition. These characterizations indicate a change in DOM characteristics resulting in humic substances that are high in aromatic content and low in molecular weight, , and suggestive of microbial contributions. It appears that these urban sources trend to lie between characteristics of our organic matter end members, forested aquatic sources and wastewater sources.

#### *Conditional Distribution Coefficients*

The conditional distribution constants determined for each of the isolates displayed little variation between sources (Table 2) and were comparable to reported values of  $K_{\text{DOM}}$  for other sources of aquatic and soil humic substances (Skylberg et al. 1997, et al. 2003; Haitzer et al. 2002, et al. 2003; Lamborg et al. 2003) ranging from  $10^{23}$ - $10^{24}$  L kg<sup>-1</sup>. Quantifiable sorption ( $Q>0$ ) was observed only for 0.01 µg L<sup>-1</sup> of added Hg<sup>2+</sup>. Unfortunately, replicate and measurement errors were of identical order of magnitude as

the estimate  $K_{DOM}$  values (Table 2). The negative  $Q$  values for 0.1 and 1  $\mu\text{g L}^{-1}$  of added  $\text{Hg}^{2+}$  is suggestive of either a slower rate of Hg-EDTA partitioning through the membrane or of DOM. Saturation of the limited strong binding sites, and the associated dominance of Hg-EDTA complexes, or DOM leakage across the membrane would have resulted in  $Q$  values closer to zero. These binding constants suggest that although the MW of the DOM for all five sources is relatively low, sorption of mercury remains of similar magnitude to prior studies. Organic matter with higher MW generally have a greater aromatic carbon content, and greater probability of forming charged binding sites, therefore increasing metal binding potential (Cabaniss et al. 2000).

Although not significantly different ( $\alpha = 0.05$ ) relative to the other sources, the InfOM and FOR HPOA isolates exhibited the greatest binding potential with Hg,  $10^{24.2}$  and  $10^{23.9} \text{ L kg}^{-1}$ , respectively. The  $K_{DOM}$  values for the InfOM and the EfOM are lower compared to values reported of  $>10^{30} \text{ L kg}^{-1}$  for Hg-DOM complexes for wastewater origins (Hsu and Sedlack, 2003). The difference could be due to methodological differences as the NOM isolation techniques used in the current study may alter functional groups from changes in pH. Only organic matter that will dissolve at pH of 13 is captured. Also, NaOH lyses bacterial cells, adding/creating a more microbial contribution (Simpson et al., 2010). The  $^1\text{H}$ -NMR spectrum (Supplemental Information, Figure S4) for InfOM indicated an aggregate aromatic OM mixture with several compounds relating to anthropogenic compounds (e.g., surfactants, pharmaceuticals) while the spectrums for all sources suggested the presence of methoxy groups in the carboxyl-rich alicyclic molecules (CRAM) region (5-3ppm; Hertkorn et al., 2006). Hertkorn et al., 2006, also found that CRAMs are cyclic terpenoids that have been

carboxylated through biological or chemical processes and make up about 50% of DOM. The wastewater sources, InfOM and EfOM exhibited more discrete peaks, not only in the CRAM region but also in the material derived from linear terpenoids (MDLT) and aromatic proton regions, suggestive of shorter sugars and more uniform organic matter relative to the other source sites. Conversely, the FOR and USS sites displayed broad polymerization represented by a lack of sharp peaks in the three regions suggestive, as might be expected, of a large quantity of lignin-like material that has not been processed. The USS polymerization and response was lower relative to the FOR site, particularly for the fulvic acid MDLT region, suggestive of potential inputs from impervious surface storm water runoff and CSOs. The SWR displayed polymerization intermediate to the wastewater and FOR sites, representing shorter, more relatively mobile organic matter which had undergone some breakdown to simpler sugars.

As with the InfOM and FOR sources, the SWR, USS, and EfOM sources, although similar in  $K_{\text{DOM}}$  values ( $10^{23.3}$ ,  $10^{22.9}$ , and  $10^{23.0}$  L kg<sup>-1</sup>, respectively), varied in spectral and physical properties. Soil organic matter (SOM), indicated by the CRAM region, has been demonstrated to contain relatively strong Hg<sup>2+</sup>-thiol binding sites ( $10^{24}$  L kg<sup>-1</sup>; Skjellberg et al., 1997). While the FOR, SWR and USS were expected to contain significant SOM contributions as evidenced by the fulvic acid response, InfOM and EfOM are not expected to reflect such contributions. Additionally, the InfOM sample had a higher percentage of S than FOR (Table 1; 1.7 and 0.8%, respectively), while the SWR had an even higher S content, suggestive that factors other than SOM and thiol are also influencing Hg binding. MW, another property positively correlated with Hg-DOM

binding, was highest for the FOR HPOA (668 Da), but again was not correlated with the  $K_{DOM'}$  values.

As with the InfOM and FOR sources, the  $K_{DOM'}$  differences between the SWR, USS and EfOM sources were not significant ( $\alpha = 0.05$ ) and not correlated with spectral and chemical properties. Both the SWR and USS were collected during stormwater runoff and reflect significant contributions from watershed runoff. However, the USS has higher terrestrial-like and fulvic acid components while the SWR source has the largest S content (2.4%) and the greatest representation by the humic acids region of all the isolates. Meanwhile, the EfOM  $^1\text{H}$ -NMR spectra properties exhibited the presence of more processed organic matter while having a similar S content (0.95%) to the USS. However, the EfOM had the greatest aromatic proton ratios amongst all isolate spectra, a region that includes phenols, quinones, and other oxygen containing hetero-aromatics (Ma et al., 2000) to which Hg is less likely to bind relative to thiols (Stumm and Morgan, 1995).  $^{13}\text{C}$  NMR spectra (Figure S5) would have assisted in elucidating potential relationships of binding potential with particular chemical structure. Unfortunately, we were limited by the mass of available material and the detection limit for NMR.

Our data did not demonstrate a relationship with C/S ratios (Table 1), with the FOR HPOA having the highest ratio at 154 with strong binding. Remaining isolates generally followed previously determined relationships which determined DOM isolate C/S ratio to be inversely proportional to  $K_{DOM'}$  values (Haitzer et al., 2003). Yet, the SWR HPOA fraction exhibited the lowest C/S ratio but had a  $K_{DOM'}$  value  $\sim 10$  times lower relative to that for InfOM. The lower binding capacity relative to the S content may be explained by the composition of the isolate as NMR results indicated the presence of many carboxyl-



rich alicyclic molecules. While suggesting that the % S content affects the binding of isolate DOM with Hg, the data also suggests that in addition to the thiol groups, carboxyl groups also contributed to the overall Hg complexation with DOM.

## Conclusions

The different isolates presented represent aggregate heterogeneous compositions of organic matter of low MW (668 - 209 Da) similar to sources from Lake Fryxell, Antarctica (autochthonous) and Ohio River fulvic acid (allochthonous). The shift towards lower MW may suggest the presence of degradation reactions occurring in the watershed that decrease the average MW as well as ligand binding sites per molecule. The FOR source is characteristic of a terrestrial source of organic matter with a relative equal composition amongst both its HPOA and HPIA fractions. Conversely, both EfOM and InfOM are characteristic of more labile microbial organic matter with higher compositions of aromatic proteins than humic and fulvic acids. The two additional sources, SWR and USS, exhibit characteristics of urban organic matter with EEMs regional intensities intermediate between the FOR and wastewater end members.

We hypothesized that the HPOA isolates with a higher MW and greater S content would possess the greatest potential for Hg binding. Yet, our findings suggest that both thiols and carboxyl groups contribute to Hg binding with  $K_{DOM'}$  values not significantly different amongst sources ( $10^{22.9} - 10^{24.2}$  L kg<sup>-1</sup>). Additionally, a consistent trend was not present between  $K_{DOM'}$  values relative to specific spectral or chemical properties. The organic matter sources with the highest  $K_{DOM'}$  values were the InfOM and FOR HPOA isolates. Opposite in MW and aromaticity, the InfOM and FOR sources exhibited a more

homogeneous  $^1\text{H}$ -NMR-CRAM signal than the other sources, suggesting larger composition of methoxy groups and soil organic matter lignin. The  $K_{\text{DOM}}$  values for EfOM, SWR, and USS sources were slightly lower with SWR containing the highest S of all the isolates (2.4% w/w) and EfOM the greatest aromatic proton ratios, indicative of oxygen containing moieties less favorable for Hg binding. The USS displayed broad polymerization and contained a high aromaticity, suggesting a much larger heterogeneous mixture of DOM being processed with lignin and aromatic protein contributions.

Each  $K_{\text{DOM}}$  is conditional only for the specific fractions of DOM from the NBPR watershed used in this study under the specific experimental conditions set. Yet, the data suggests that although the binding of  $\text{Hg}^{2+}$  to DOM varies over an order of magnitude among urban sources, the value remains relatively consistent regardless of source. In general, each source represented a lower MW DOM relative to that reported for forested sites, which, along with the spectral and physical data was suggestive of more highly processed DOM. However, further research is warranted to more accurately predict potential shifts in Hg binding between urban runoff DOM sources in freshwater aquatic systems. Alterations in chemical source and in biogeochemical cycles in the urban system may impact Hg fate and bioavailability, either in the immediate receiving water or downstream. To assess potential impacts, multiple stormwater runoff sites, urban streams, and wastewater treatment facilities need to be evaluated. As well as conducting seasonal and temporal sampling events to assess the potential impact that DOM alterations in the urban environment may have on Hg cycling.

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**Table 1.** Chemical and spectral characteristics of hydrophobic (HPOA) and hydrophilic (HPIA) acid fractions from the Park River watershed system including: Forest (FOR), the North Park River (USS), stormwater runoff (SWR), wastewater effluent (EfOM) and wastewater influent (InfOM). FOR, USS, and SWR samples were collected during a precipitation event while wastewater samples were collected during baseflow conditions at the Hartford, CT, water pollution control facility. Elemental analysis data not available for the HPIA fraction due to insufficient dry material recovered. % Recovery represents the amount of initial sample DOM collected in the HPOA and HPIA fractions.  $MW_W$  and  $MW_N$  represent the mass-average and number-averaged molecular weight, respectively.

Sample	DOM Fraction	% Recovery: (HPOA + HPIA)	$MW_W$ (Da)	$MW_N$ (Da)	Elemental Analysis (w/w) %			C/S (M ratio)	SUVA (mg m <sup>-1</sup> L <sup>-1</sup> )	FI
					C	N	S			
FOR	HPOA	58.7	1340	668	47.8	1.31	0.83	154	3.00	1.21
	HPIA		799	428	NA	NA	NA	NA	1.66	1.42
USS	HPOA	73.7	918	433	36.0	1.45	0.95	102	2.59	1.36
	HPIA		1000	515	NA	NA	NA	NA	2.38	1.47
SWR	HPOA	42.0	1937	398	49.9	1.41	2.40	56	3.17	1.55
	HPIA		685	485	NA	NA	NA	NA	2.27	1.60
EfOM	HPOA	68.4	532	261	44.7	1.87	0.95	126	2.09	1.57
	HPIA		415	257	NA	NA	NA	NA	2.06	1.83
InfOM	HPOA	41.9	413	209	51.1	2.18	1.73	79	1.71	1.41
	HPIA		11540	464	NA	NA	NA	NA	0.23	1.49

**Table 2.** Conditional distribution constants measured for the binding of  $\text{Hg}^{2+}$  to different DOM isolates within the North Branch Park River watershed. The five organic matter sources are: FOR, forested stream source; USS, urban stream source from the lower reaches of the North Park River; SWR, stormwater impervious surface runoff; EfOM, effluent from the Hartford Water Pollution Control Facility (WPCF); and InfOM, influent from the Hartford WPCF. Log Stdev represents the standard deviation of the two replicates while Log Meas Err is the propagation of error resulting from the Hg analytical method detection limit.

<i><b>SOURCE</b></i>	<i><b><math>K_{DOM}</math></b></i>	<i><b><math>\text{LOG } K_{DOM}</math></b></i>	<i><b><math>\text{LOG STDEV}</math></b></i>	<i><b><math>\text{LOG MEAS ERR}</math></b></i>
<b>FOR</b>	8.24 E+23	23.9	23.9	24.2
<b>USS</b>	8.86 E+22	22.9	22.6	23.6
<b>SWR</b>	2.05 E+23	23.3	23.5	24.2
<b>EfOM</b>	9.66 E+22	23.0	21.4	23.8
<b>InfOM</b>	1.42 E+24	24.2	24.0	24.6

## Figure Captions

**Figure 1.** Diagram of Hg(II) cycling between the atmosphere and aquatic environment indicating the transport processes (arrows) between the two systems. (Sawyer, et al, 2003).

**Figure 2:** The North Branch Park River Watershed with noted locations of the three sources of aquatic organic matter collected within the watershed: Forest (FOR), the North Park River (USS), and stormwater runoff (SWR). The influent and effluent collected from the Hartford Water Pollution Control Facility is not pictured on the map but is located southeast of the USS site. (CTDEP, Fuss & O'Neill, Feb. 2010).

**Figure 3.** Distribution of Fluorescence Region Integration in the hydrophobic acid (a) and hydrophilic acid (b) fractions from Excitation and Emission Matrices of the five organic matter sources: FOR, forested stream source; USS, urban stream source from the lower reaches of the North Park River; SWR, stormwater impervious surface runoff; EfOM, effluent from the Hartford Water Pollution Control Facility (WPCF); and InfOM, influent from the Hartford WPCF.

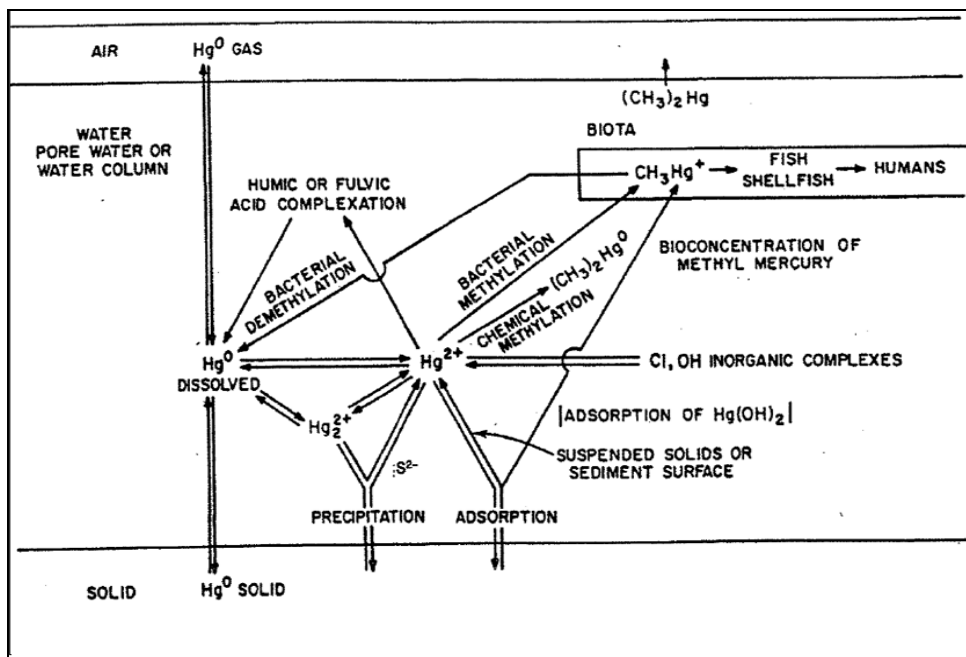
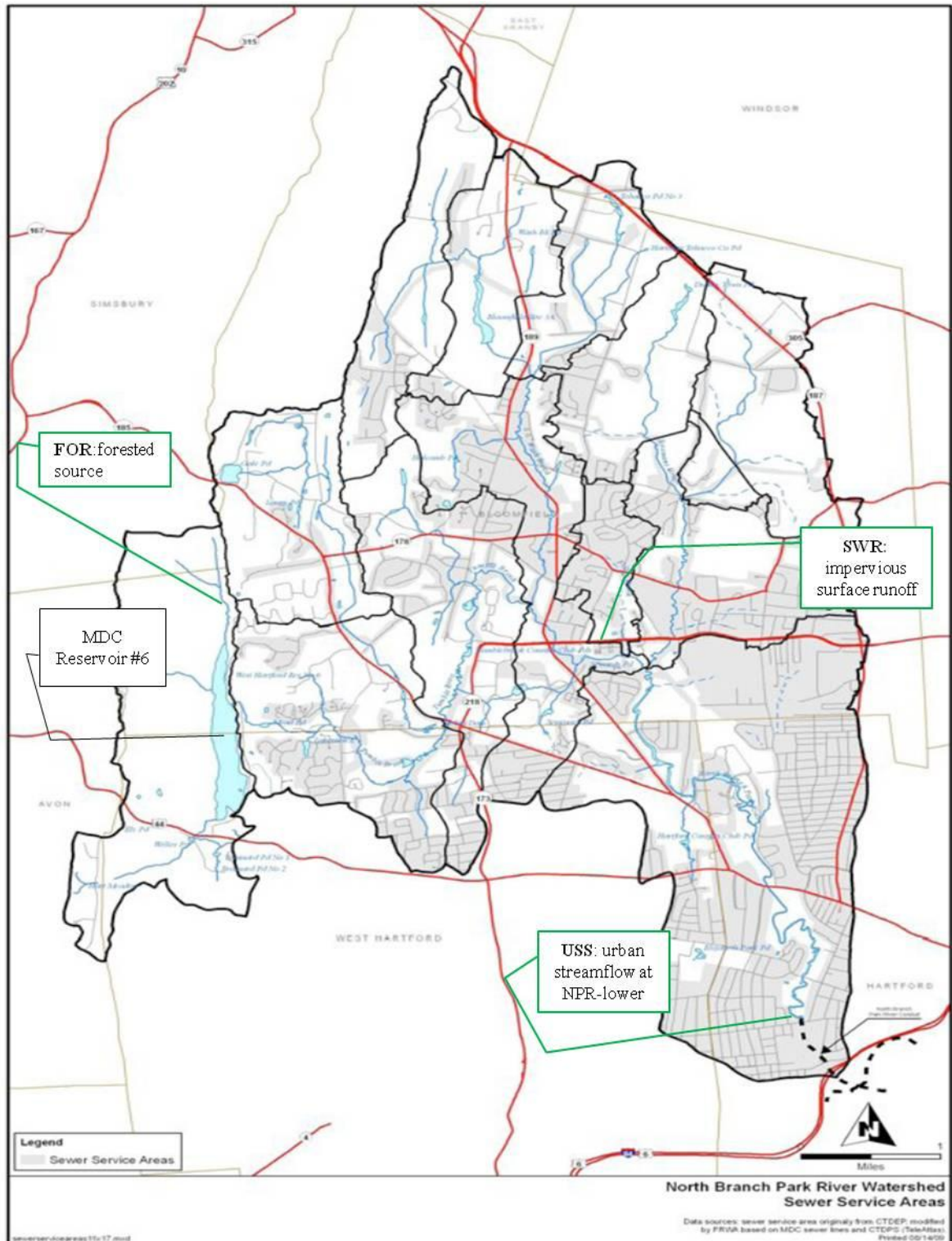
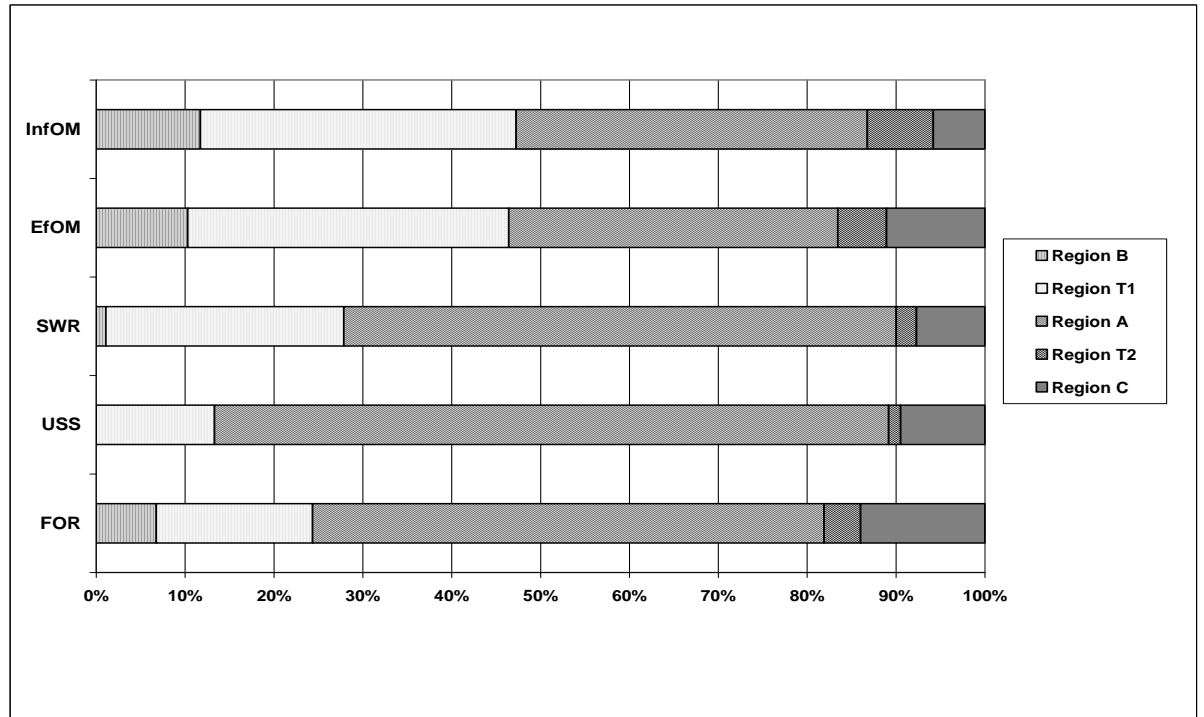


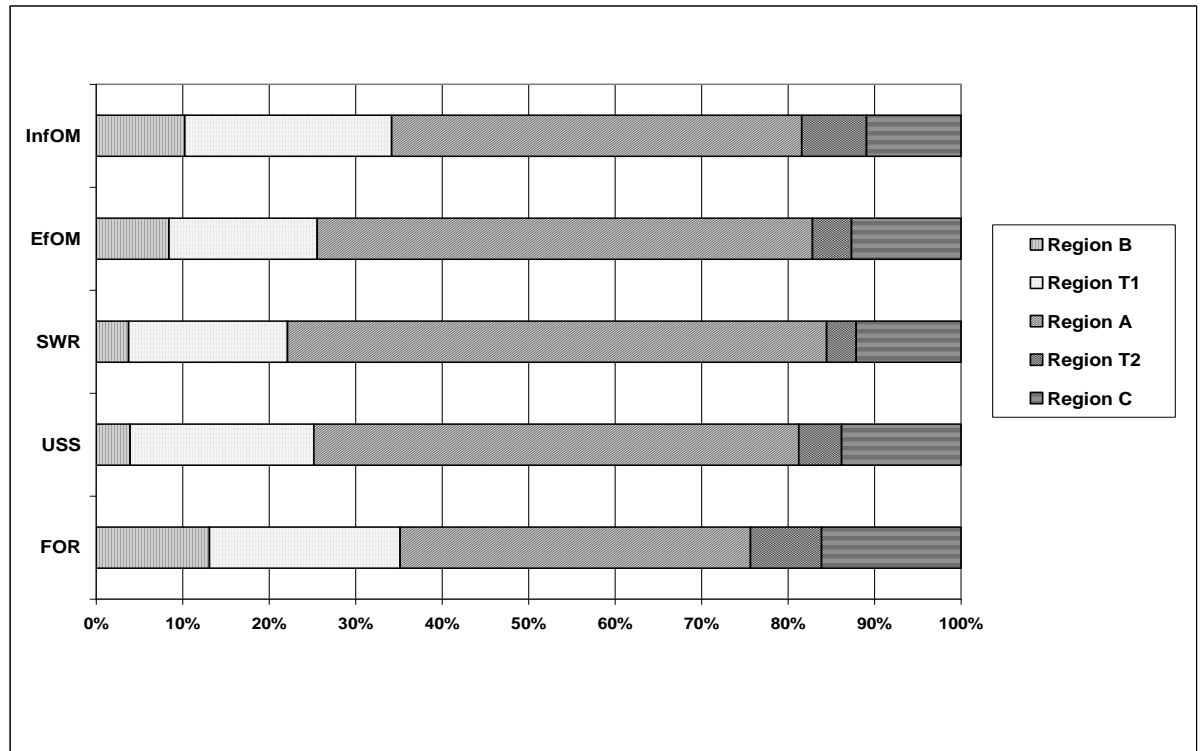
Figure 1



**a.**



**b.**



**Figure 3**

## Supplemental Information

Provided is a collection of additional data and information used for the total analysis of results presented in the paper including:

**Table S1:** Formation Constants

**Figure S1:** Location of the EEMs peaks based on literature reports on DOM characteristics and operationally defined emission and excitation wavelength boundaries. The distinctive peaks are fractionated into five regions within the EEM based on the Fluorescence Regional Integration method.

**Figure S2.** Partitioning of Hg(II) ( $10 \mu\text{g L}^{-1}$ ) from the inside solution using Spectra/Por CE Biotech 500 MWCO dialysis membrane in the absence of DOM.

**Figure S3.** EEMs matrices for the hydrophobic (HPOA) and hydrophilic (HPIA) acid fraction from each source of aquatic organic matter: FOR, forested stream source; USS, urban stream source from the lower reaches of the North Park River; SWR, stormwater impervious surface runoff; EfOM, effluent from the Hartford Water Pollution Control Facility (WPCF); and InfOM, influent from the Hartford WPCF. FOR (a,b); USS (c,d); SWR (e,f); wastewater influent InfOM (g,h), and wastewater effluent EfOM (i,j) from the Hartford WPCF. The red outlines define Peak B, T1, A, C, and T2 regions counterclockwise, respectively.

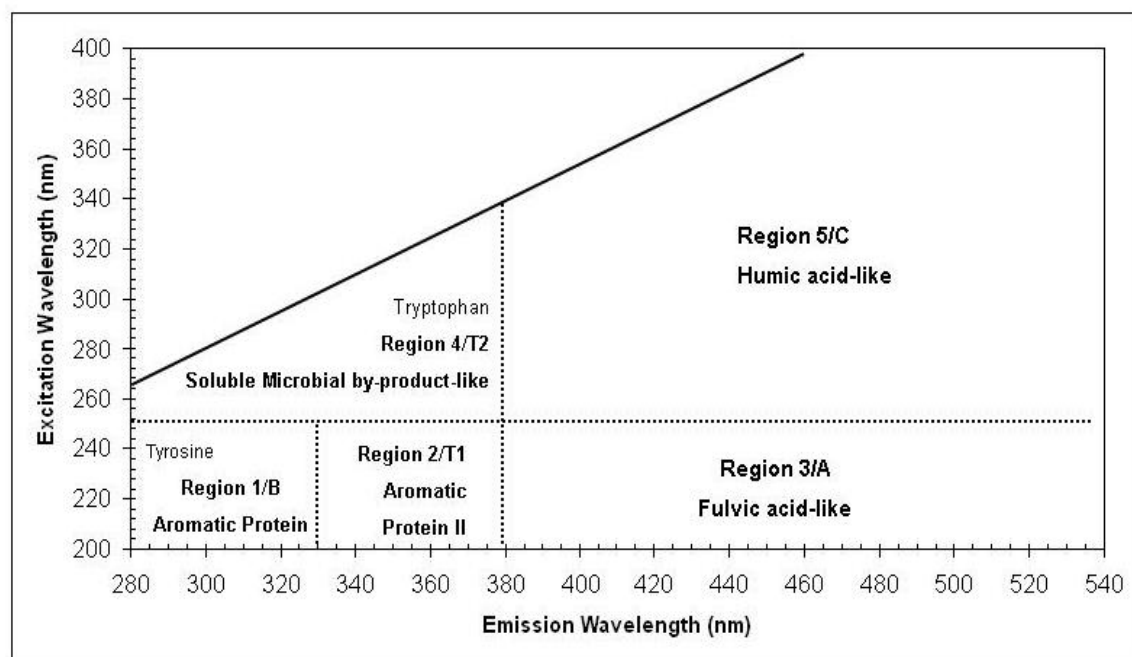


**Figure S4.** 1D  $^1\text{H}$ -NMR Spectrum (9-0ppm) on a Bruker DRX-400 for each source of lyophilized aquatic organic matter from the top: InfOM, influent from the Hartford WPCF; USS, urban stream source from the lower reaches of the North Park River; FOR, forested stream source; EfOM, effluent from the Hartford Water Pollution Control Facility (WPCF); and SWR, stormwater impervious surface runoff. Note: Large peak at 4.8ppm is the reference water peak.

**Figure S5.** 1D  $^{13}\text{C}$ -NMR Spectrum for each source of lyophilized aquatic organic matter. Due to the insolubility of the isolates, no discrete peaks were identified. All sources exhibited noise.

**Table S1.** Summary of the formation constants used for the calculation of  $K_{DOM}$ ’ values (Morel and Hering, 1993).

Reaction	Log $\beta$
$\text{EDTA}^{4-} + \text{H}^+ = \text{EDTAH}^{3-}$	11.12
$\text{EDTA}^{4-} + 2 \text{H}^+ = \text{EDTAH}_2^{2-}$	17.8
$\text{EDTA}^{4-} + 3 \text{H}^+ = \text{EDTAH}_3^{-}$	21.04
$\text{EDTA}^{4-} + 4 \text{H}^+ = \text{EDTAH}_4$	23.76
$\text{EDTA}^{4-} + 5 \text{H}^+ = \text{EDTAH}_5^{1+}$	24.76
$\text{Hg}^{2+} + \text{EDTA}^{4-} = \text{HgEDTA}^{2-}$	23.5
$\text{HgEDTA}^{2-} + \text{H}^+ = \text{HgEDTAH}^{-}$	27.0
$\text{HgEDTA}^{2-} + \text{OH}^{-} = \text{HgEDTAOH}^{3-}$	27.7
$\text{Hg}^{2+} + \text{OH}^{-} = \text{HgOH}^{+}$	10.6
$\text{Hg}^{2+} + 2\text{OH}^{-} = \text{Hg(OH)}_2$	21.8
$\text{Hg}^{2+} + 3\text{OH}^{-} = \text{Hg(OH)}_3$	20.9



**Figure S1**

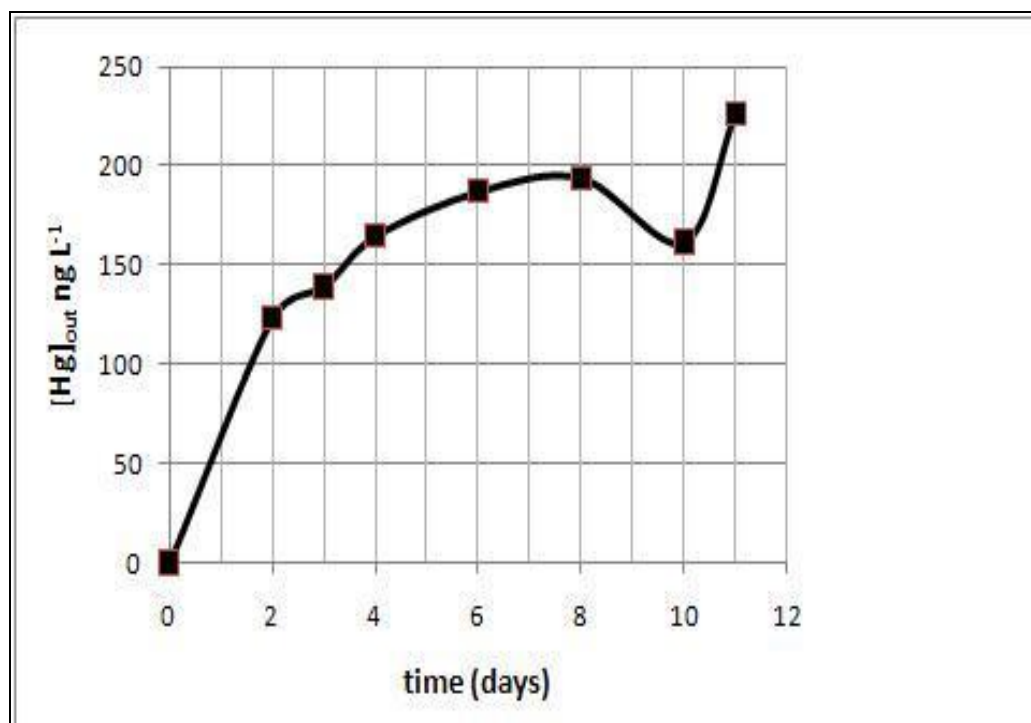
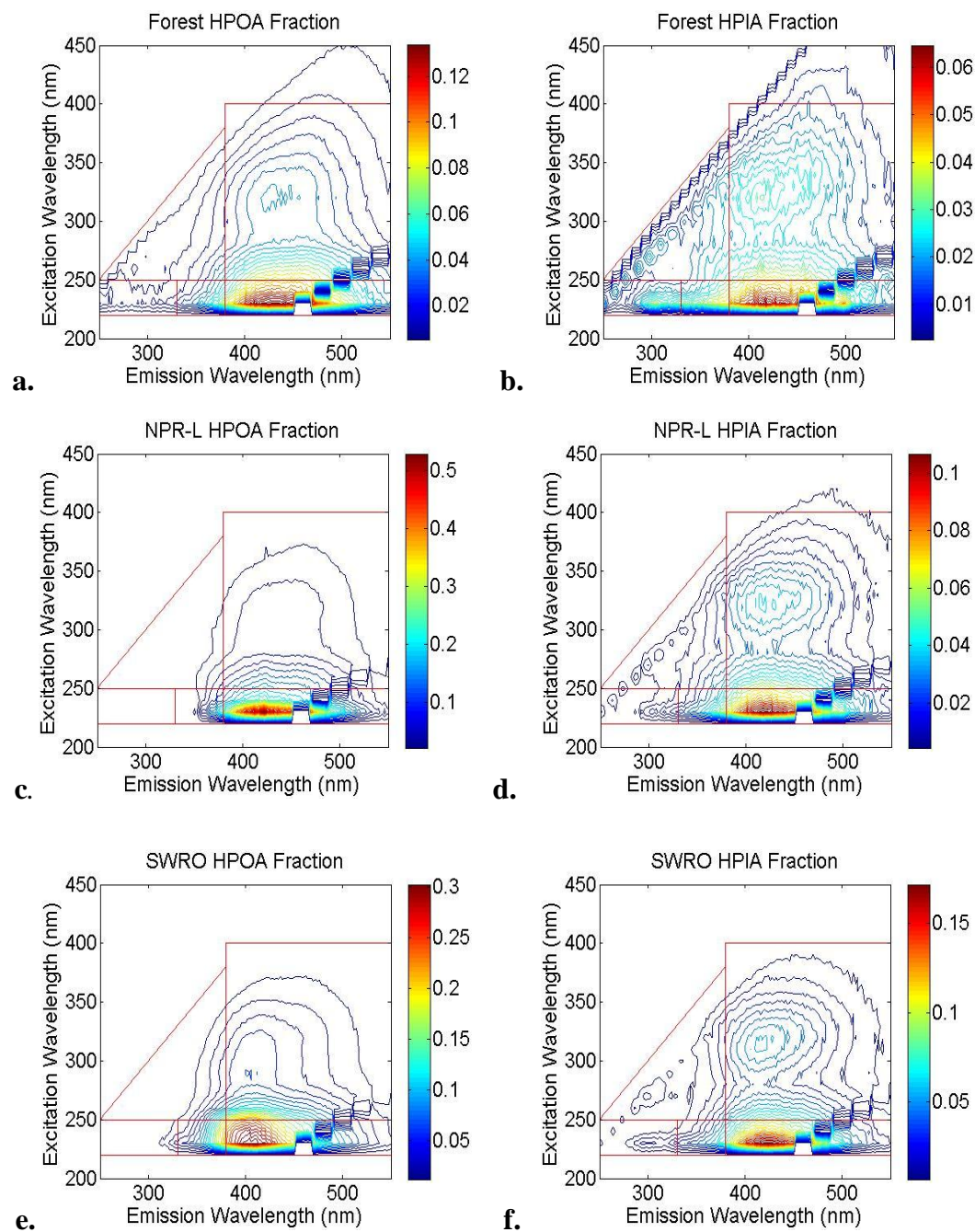
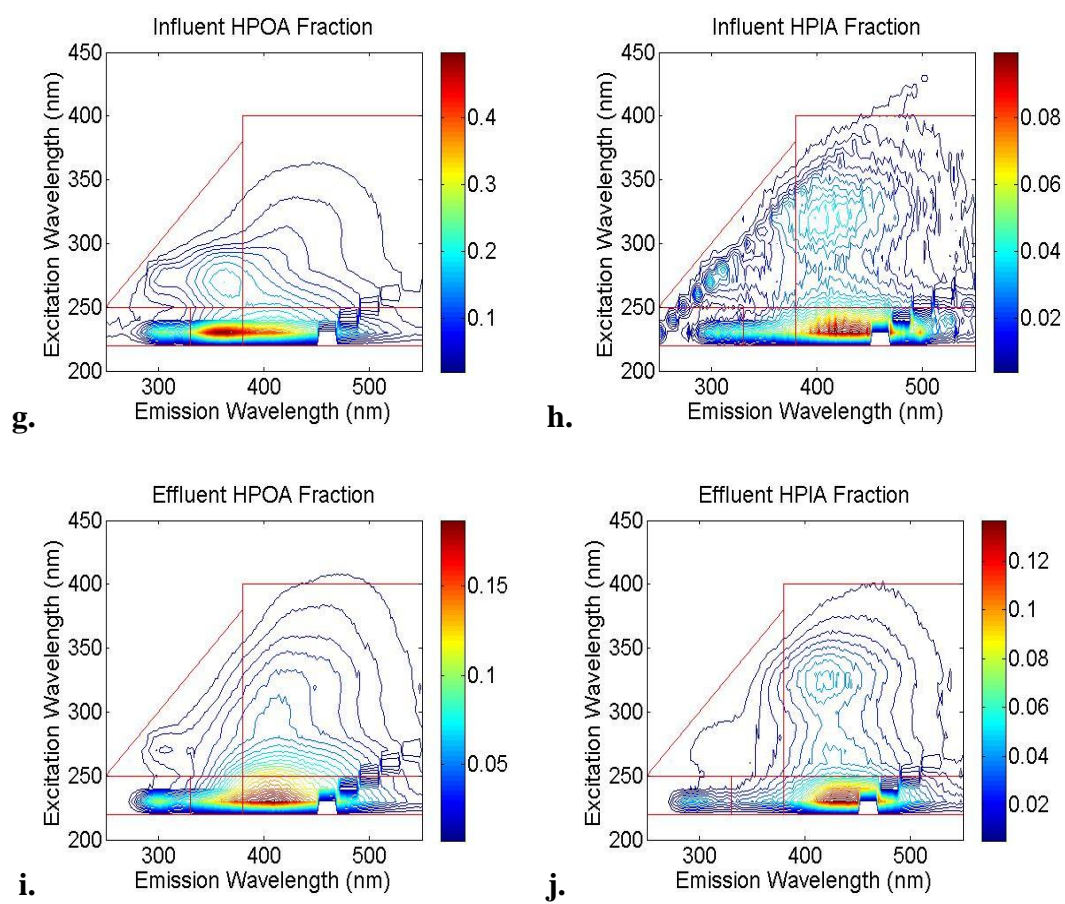


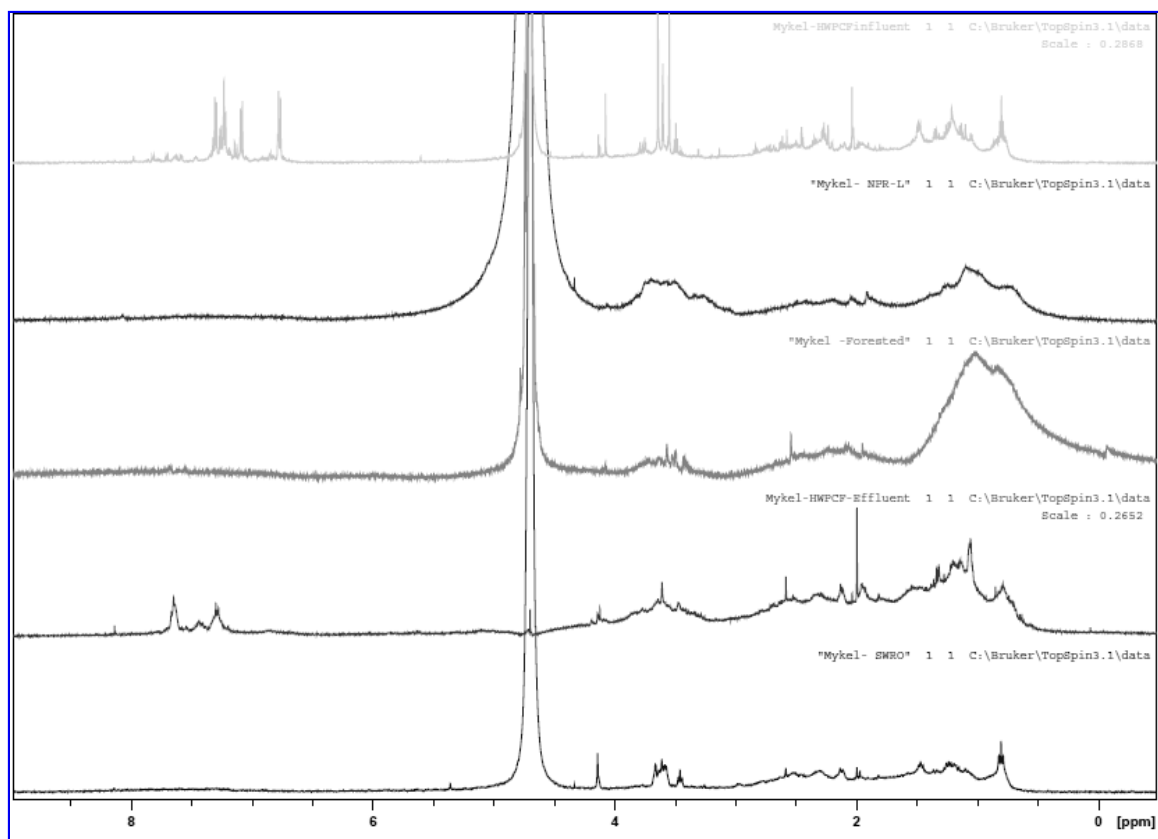
Figure S2



**Figure S3**



**Figure S3 (cont)**



**Figure S4**

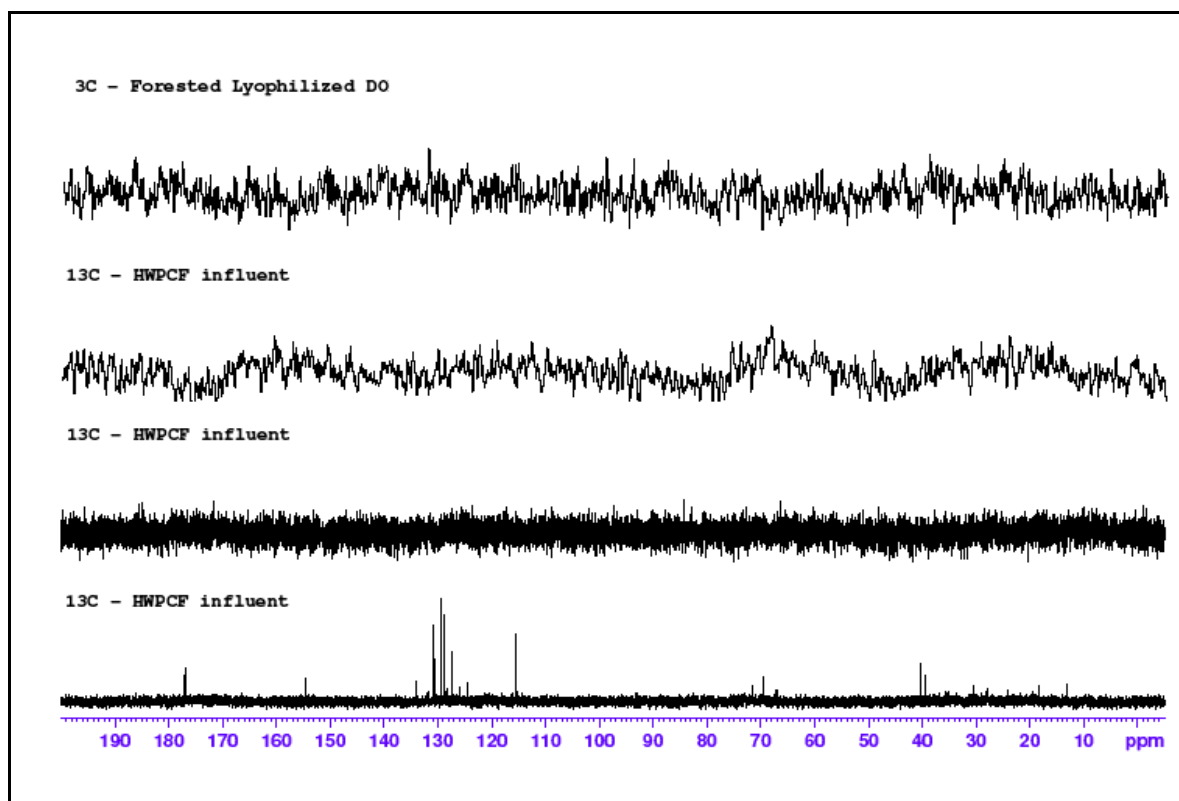


Figure S5