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It is entitled:
Characterizing Variability in Ohio River NOM and Validating Reconstituted Freeze-Dried NOM as a Surrogate for its Aqueous Source

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Characterizing Variability in Ohio River NOM and Validating Reconstituted Freeze-Dried NOM as a Surrogate for its Aqueous Source

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Abstract

Surface water contains natural organic matter (NOM) that reacts with disinfectants creating disinfection byproducts (DBPs), some of which are USEPA regulated contaminants. Characterizing NOM can provide insight with respect to DBP formation and water treatment process adaptation to climate change as the nature of NOM varies. This study collected NOM from the Ohio River over 15 months (April 2010 to July 2011) in order to assess seasonal variability in NOM characteristics. The NOM was characterized using fluorescence spectroscopy, UV$_{254}$, TOC, high performance liquid chromatography – size exclusion chromatography (HPLC-SEC), and elemental analysis. NOM was concentrated, freeze-dried (lyophilized), and validated with the source NOM creating a standardized lyophilized NOM that may be used in water treatment process evaluations investigating utility adaptation to seasonal changes. Additionally, NOM was concentrated at multiple concentration factors, lyophilized, and reconstituted allowing for the determination of optimal NOM concentration and reconstitution conditions. The NOM was characterized using UV$_{254}$, TOC, HPLC-SEC, fluorescence spectroscopy, and DBP formation.

Raw Ohio River water NOM was concentrated in the following order: ultrafiltration (UF), cation ion exchange, reverse osmosis (RO), sulfate removal, and lyophilization. Lyophilization allows for long-term storage of NOM while providing the ability to reconstitute at various NOM concentrations compared to liquid material with a short shelf-life. Lyophilized NOM was used for elemental analysis while UF effluent, concentrate, and reconstituted lyophilized NOM were employed for all other analyses. A single RO concentration factor (150X) was used during the 15-month study while 50X, 100X, 150X, 200X, and 250X were used to determine the optimal RO concentration factor versus reconstitution factor. Parallel factor
analysis (PARAFAC) determined the locations of principle components within fluorescence excitation-emission matrices (EEMs). DBP formation from chlorination was analyzed for the multiple concentration factor concentrate and reconstituted NOM at 1X source, concentrate, and 250X TOC concentrations.

TOC and UV\textsubscript{254} results demonstrated seasonal variation of NOM concentration during the 15-month study. However, PARAFAC determined that the nature of the NOM components were humic-like and constant. The humic characterization was further supported by the humification index (0.79-0.90) determined from corrected fluorescence EEMs. In addition, elemental analysis revealed mid-range oxygen to carbon ratios that are 0.62-0.87, also indicative of humic NOM. Humic substances are composed of naturally-occurring biologically-decayed plant material that can affect the alkalinity, pH, and other treatment characteristics of surface water. Since the humic nature of the NOM was similar over the 15-month study, the primary difference was NOM concentration. Reconstituted lyophilized NOM was validated against source NOM allowing its use as a standardized NOM material in treatment process studies that evaluate effects of changing NOM character and concentration. This temporal library of well-characterized, drinking water relevant NOM is the first of its kind in the drinking water industry and will be a valuable research tool for the drinking water community. The multiple concentration factor study revealed that NOM may be concentrated at RO factors from 50X to 250X, lyophilized, and reconstituted to 1X source, concentrate, and 250X conditions without changing the characterization of the NOM or DBP formation.
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Introduction

Fresh water plays an important role in everyday human life supplying a growing population with potable water while also providing industry, agriculture, and energy companies with a resource to produce their commodities. Aqueous systems, including surface (rivers and lakes) and ground waters (underground aquifers), contain natural organic matter (NOM) whereby the dissolved portion is called dissolved organic matter (DOM). NOM is a complex mixture of organic material including high molecular weight humic and fulvic acids produced by animals, plant life, soils, and waste products from both industry and public services such as wastewater treatment. DOM regulates the speciation of trace elements along with other governing chemical processes in drinking water treatment such as disinfectant decay and disinfection byproduct (DBP) formation (Santschi et al. 2002).

DOM structure, chemistry, and concentration change over time and are dependent on the sources of organic matter (allochthonous or autochthonous influenced) along with other characteristics such as temperature and pH (Leenheer and Croue 2003). Because of its complex nature, neither NOM nor DOM can be directly measured. Instead, surrogates have been adopted in order to more easily track changes in NOM. These include taste, odor, total organic carbon (TOC), and UV$_{254}$ (Thurman 1985). The use of these surrogates is widely accepted and beneficial for water treatment plant operations, which are subject to the U.S. EPA Stage 1 Disinfectants and Disinfection Byproducts Rule requiring TOC reductions based on raw water TOC and alkalinity (USEPA 1998). Furthermore, the use of surrogates allows for simple, cost-effective DOM characterization.
However, a further understanding of DOM character, specifically the humic nature of source water, can improve our understanding of the disinfection byproducts that are formed during treatment (Cowman and Singer 1996). The DOM in freshwater, particularly the chromophoric fraction of DOM (CDOM), can be characterized using various advanced techniques. These include flow field-flow fractionation (Baalousha and Lead 2007), high performance liquid chromatography (Her et al. 2002), elemental analysis (Maurice et al. 2002), molecular weight fractionation (Hua and Reckhow 2007), nuclear magnetic resonance (Aiken 1992), and fluorescence spectroscopy (Pifer et al. 2011).

Previous research efforts have analyzed UV$_{254}$, TOC, and DBP formation in reverse osmosis (RO) concentrated NOM as part of a health effects study evaluating whole mixtures of DBPs (Pressman et al. 2010). RO concentration is beneficial because it results in highly concentrated, complete aqueous NOM whole mixtures (Perdue and Ritchie 2003) which are advantageous for toxicological research. However, the relatively short shelf-life of aqueous concentrated NOM represents a limitation for research purposes. To overcome this drawback, lyophilization (freeze-drying) has been investigated to remove the remaining water from concentrated NOM resulting in a solid with a much longer shelf-life that can easily be reconstituted back to an aqueous state (McCurry et al. 2012). McCurry et al. (2012) formulated a lyophilization method for NOM concentrate expressing 91% UV$_{254}$ and 101% TOC recovery. Afterward, Pressman et al. (2012) validated representative DBPs in NOM concentrate and lyophilized, reconstituted NOM. Despite these advances in NOM research, there remains a lack of data characterizing drinking water relevant concentrated surface water NOM beyond DBP formation, TOC, and UV$_{254}$ surrogates.
In addition to the lack of advanced NOM analysis of concentrated NOM solutions, seasonal variations in source water NOM, including the effects of water cycles and weather events, have not been evaluated. Changes in NOM character may alter the DBPs formed during treatment and an understanding of NOM variability can lead to greater comprehension of DBPs formed during specific seasons or climate events. Furthermore, a library of well-characterized, solid NOM would represent a standardized source for health effects and DBP research. Lyophilization of the NOM would allow for the long-term storage and aid in the creation of a standardized library. This library of NOM could be formed from various public water sources during different seasons encompassing real-world NOM amid changing conditions. Treatment plants from many locations would be able to evaluate their treatment processes for specific seasons far in advance while health effects studies would be relying on a drinking water relevant NOM source. However, an NOM library of drinking water relevant sources has yet to be created and characterized.

Lastly, there is a lack of research analyzing RO concentrated NOM characteristics, at multiple concentration factors, compared to lyophilized NOM concentrations after being reconstituted to the final RO concentrations. Lyophilized NOM has been reconstituted at various TOC concentration factors ranging from source water conditions (1X) to 1000X natural water concentrations and shown to be linear with regards to TOC recovery (McCurry et al. 2012). However, reconstituting at multiple factors has not been compared to concentrating NOM, via RO, at different concentration factors to determine the optimal balance between RO concentration factor compared to reconstitution concentration factor for the retention of the DOM constituents that are important to drinking water treatment and research.
Objectives

To fulfill these research gaps, the objectives of this research are to:

1. Create a library of well-characterized, lyophilized NOM collected monthly from the Ohio River for at least one year. The creation of this library includes: a) evaluating the seasonal variations and characterizing the NOM using advanced CDOM techniques including fluorescence spectroscopy, and b) validating the RO concentration, lyophilization, and reconstitution processes using advanced NOM characterization methods.

2. Determine the ideal RO concentration factor compared to the reconstitution factor of lyophilized NOM with regards to the retention of DOM constituents that affect drinking water processes. This will establish an optimal RO concentration factor compared to reconstituting lyophilized NOM at different concentration factors. Evaluation will be based on advanced CDOM techniques including fluorescence spectroscopy and the evaluation of DBP formation.
Characterizing Variability in Ohio River NOM

Introduction

Aqueous water sources contain natural organic matter (NOM), a complex mixture of organic material produced by animals, plant life, soils, and effluent from industrial and public services. NOM chemistry, concentration, and structure vary over time and are heavily dependent on biological sources and surrounding soil (Leenheer and Croue 2003). Disinfection byproduct (DBP) formation and disinfectant decay in drinking water treatment are governed by NOM, particularly dissolved organic matter (DOM) (Santschi et al. 2002). Due to its complexity, surrogates have been implemented in order to more easily quantify and characterize NOM. Odor, taste, total organic carbon (TOC), and UV_{254} are common surrogates used to measure and characterize NOM (Thurman 1985). Changes in NOM character may affect water treatment DBPs on a seasonal basis. The concentrations of halogenated DBPs increase with the presence of humic NOM (Cowman and Singer 1996). Understanding the variability in NOM characterization, beyond TOC and UV_{254} surrogates, will lead to an increased understanding of the potential DBPs that are formed during particular seasons. Over a period of time, NOM for a source water system can be characterized to observe these seasonal changes.

A further understanding of DOM character allows for an enhanced understanding of DBPs that are formed during treatment, particularly the humic nature of source water (Cowman and Singer 1996). Freshwater DOM, predominantly the chromophoric fraction of DOM (CDOM), can be characterized using various advanced techniques including flow field-flow fractionation (Baalousha and Lead 2007), high performance liquid chromatography (Her et al. 2002), elemental analysis (Maurice et al. 2002), fluorescence spectroscopy (Pifer et al. 2011), molecular weight fractionation (Hua and Reckhow 2007), and nuclear magnetic resonance...
(Aiken 1992). Advanced NOM characterization techniques can further aid in the creation of a standardized library of NOM benefiting drinking water and health effects studies.

Perdue and Ritchie (2003) showed that reverse osmosis (RO) produces highly concentrated, complete aqueous NOM whole mixtures advantageous for drinking water and toxicological research. Previous research efforts relied on UV$_{254}$ and TOC surrogates for NOM characterization in addition to evaluating whole mixtures of DBPs in RO concentrated NOM (Pressman et al. 2010). The limited shelf-life of aqueous NOM samples hinders research efforts as the organic matter degrades overtime. As a result, RO concentrated NOM must be produced prior to each research effort adding to the time and complexity of experimentation. The shorter shelf-life, and thus NOM degradation in aqueous concentrate, can be overcome by lyophilization (freeze-drying). The lyophilization process removes the remaining water from concentrated NOM resulting in a solid with a longer shelf-life that can be easily reconstituted back to an aqueous state (McCurry et al. 2012). Lyophilization and reconstitution methods were developed for concentrated NOM resulting in 101% TOC and 91% UV$_{254}$ recovery (McCurry et al. 2012). Subsequently, Pressman et al. (2012) validated representative DBPs in concentrated NOM and lyophilized, reconstituted NOM promoting the creation of a standardized library of solid NOM for drinking water research. However, despite these advances in NOM research, there is a lack of advanced characterization of RO concentrated NOM beyond UV$_{254}$ and TOC surrogates. Techniques such as fluorescence spectroscopy would not only allow for the characterization of source water NOM but also further validate the RO concentration, lyophilization, and reconstitution processes by characterizing NOM after each stage of treatment. Changes in NOM characterization, along with potential losses due to NOM fractionation, would be evident using more advanced characterization methods.
From the collection of RO concentrated NOM, as previously developed, a library of lyophilized solid can be created. This library would consist of well-characterized NOM from all seasons and conditions such as weather events that a treatment plant would experience. The library represents a standardized source for DBP research, particularly pertaining to health effects studies and water treatment plant process studies. Treatment plants would be capable of conducting DBP studies far in advance of specific seasons in order to potentially modify their treatment processes. Despite these benefits, an NOM library created from RO concentration and lyophilization has yet to be created.

Objectives

The primary objectives of this research are to:

1. Create a library of well-characterized, lyophilized NOM collected monthly from the Ohio River for at least one year. Advanced NOM characterization will be accomplished using fluorescence spectroscopy with parallel factor analysis and high performance liquid chromatography – size exclusion chromatography (HPLC-SEC) in addition to UV$_{254}$ and TOC surrogates.

2. Evaluate the source water NOM for seasonal variations using fluorescence spectroscopy, HPLC-SEC, TOC, and UV$_{254}$.

3. Validate the RO concentration, lyophilization, and reconstitution processes for drinking water studies by characterizing the NOM using fluorescence spectroscopy, HPLC, UV$_{254}$, and TOC after RO concentration and NOM reconstitution using the source water as a baseline for comparison.
Materials and Methods

UF1X and Concentrate Collection

Ohio River NOM was concentrated approximately one week per month starting in April 2010 and ending July 2011. Raw water was processed through a 200-µm cloth bag filter, ultrafiltration (two ceramic 19P37-30 Membralox 0.02-µm, Pall Corp., Port Washington, NY), cation exchange resin columns (Ambersep 200H hydrogen-form, Rohm and Hass, Philadelphia, PA), and RO (three Filmtec BW30-4040 membranes in series, Down, Midland, MI) following previously established methods (Pressman et al. 2010). The RO equipment was operated such that an approximate 165X volumetric concentration factor was maintained throughout NOM concentration which results in an approximate 150X UV<sub>254</sub> and TOC concentration factor. Water quality analyses were conducted for raw water, UF effluent (UF1X), ion exchange effluent, RO concentrate (CONC), and the effluents of the three RO membrane permeates. These included hourly UV<sub>254</sub> analyses as well as TOC, ion chromatography (IC), and inductively coupled plasma (ICP) samples every five hours of operation which was sufficient enough to analyze potential changes in source water and RO concentrate during field collection based on previous research efforts (Pressman et al. 2010).

UF1X and Concentrate Storage and Post-Processing

Approximately 150 liters of RO concentrate was collected each month and stored in high-density polyethylene (HDPE) Nalgene carboys (Thermo Fisher Scientific, Waltham, MA) at 4°C until post processing. UF1X was collected in small one liter aliquots every five hours and batched into a 20-liter HDPE Nalgene carboy (Thermo Fisher Scientific, Waltham, MA) which was stored at 4°C for subsequent analysis. The batched UF1X represents conditions throughout
the period of concentrated NOM collection. Sulfate in the raw water was concentrated within the RO system along with the NOM. Dissolved sulfate negatively impacts lyophilization by producing an NOM solid mixed with sulfuric acid (Koprivnjak et al. 2006; Pressman et al. 2010). Sulfate was removed via precipitation with BaCl₂ (Certified ACS, Thermo Fisher Scientific, Waltham, MA) targeting a 0.86 molar ratio of Ba²⁺ to SO₄²⁻ in order to avoid over applying barium effecting future NOM lyophilization. Samples were diluted for sulfate analysis because they were too concentrated for direct measurement. Using a higher molar ratio of barium-to-sulfate caused TOC and UV₂₅₄ losses along with sulfate removal and was avoided. TOC, IC, and UV₂₅₄ samples were collected after precipitation. The supernatant was stored in HDPE 33-gallon drums (Thermo Fisher Scientific, Waltham, MA) and the precipitated barium sulfate solid was discarded. Each month required a minimum of two precipitation stages while most called for three stages to remove sulfate down to a targeted 10 mg/L. Subsequent BaCl₂ precipitation stages, after the first stage, used a 1:1 molar ratio of Ba²⁺ to SO₄²⁻. The implemented molar ratio was increased to 1:1 because the sulfate concentration in the aqueous concentrated NOM decreased after the first stage of sulfate removal. Sulfate was then directly measured resulting in more accurate results limiting residual barium concentration. The use of a larger ratio after the first stage of precipitation no longer resulted in higher TOC and UV₂₅₄ losses. Concentrate was filtered with pre-rinsed 0.45 µm cartridge filters (AquaPrep 600, Pall Corporation, Port Washington, NY) and stored at 4°C in an HDPE 33-gallon drum. IC, ICP, TOC, and UV₂₅₄ samples were collected after the final stage of precipitation and filtration. Concentrate was then lyophilized in a VirTis Virtual EL pilot scale freeze-dryer (SP Industries, Warminster, PA) following a previously developed procedure (McCurry et al. 2012). Solid NOM was removed
and stored in amber glass bottles, one for each month, in a light-free cabinet along with silica gel packets to absorb excess moisture.

Concentrate Lyophilization and Reconstitution

Lyophilized NOM was reconstituted for experiments following a method outlined by McCurry et al. (2012). Solid NOM was mixed with ultra-pure deionized water ($\rho \geq 18$ M$\Omega$-cm), pH adjusted to ten with 6N NaOH (N.I.S.T. Traceable, Aqua Solutions, Deer Park, TX), and mixed for a 24-hour period. The resulting liquid was filtered with pre-rinsed 0.45-µm PES disc filters (Supor-450, Pall Life Sciences, Port Washington, NY). The volumetric mass-to-volume ratio from lyophilization was used to determine the amount of NOM solid to mix with the ultra-pure water. TOC and UV$_{254}$ samples were collected after reconstitution and filtration.

Water Quality Parameters, Measurement Procedures, and Instrumentation

UV$_{254}$ was measured following Standard Method 5910 for UV-Absorbing Organic Constituents (American Public Health Association (APHA) et al. 2012) with a Nicolet Evolution 300 UV spectrophotometer (Thermo Fisher Scientific, Waltham, MA). ICP analysis was conducted using an iCAP 6000 spectrometer (Thermo Fisher Scientific, Waltham, MA) according to USEPA method 200.7 (USEPA 1994). TOC was analyzed following USEPA method 415.3 (USEPA 2005) using a combustion catalytic TOC analyzer (TOC-Vcph, Shimadzu Corporation, Columbia, MD) and IC analysis was performed using a Metrohm-Peak 761 IC instrument (Metrohm-USA, Riverview, FL) according to EPA method 300.1 (USEPA 1997). Fluorescence spectroscopy was conducted using a Fluorolog3 instrument (HORIBA Instruments Inc., Edison, NJ) with excitation wavelengths of 240 nm to 450 nm (5-nm step), emission wavelengths of 300 nm to 500 nm (2-nm step), and a 5-nm slit width for both aspects.

Fluorolog3 settings were previously determined in an evaluation of instrument settings and
sample conditions. Fluorescence data was corrected for TOC concentration, UV absorbance, Raman integration, Rayleigh scattering removal, and instrumentation settings using MATLAB (Release 2011b, The Mathworks, Inc., Natick, Mass.) and FDOMcorr (Murphy et al. 2010), a toolbox that was customized for this research. Programming errors were discovered in the original FDOMcorr toolbox that were subsequently removed during evaluations of the source code. Furthermore, the code for FDOMcorr was customized to include parameters specific to the Fluorolog3 instrument along with corrections for sample dilutions via TOC measurements.

Parallel factor analysis (PARAFAC) is a tool that allows for the direct comparison of corrected fluorescence excitation-emission matrices (EEMs). PARAFAC implements a series of EEMs modeled after the source data. The model with the lowest error is implemented illustrating the locations of peaks within a set of EEMs. The locations of peaks within EEMs are used to characterize the NOM. PARAFAC analysis was conducted according to a previously established method using the DOMFluor toolbox (Stedmon and Bro 2008). Preliminary fluorescence analysis determined that the TOC concentration and pH of concentrate samples had no effect on peak locations within EEMs (data not shown). Thus, all concentrate and reconstituted NOM samples were volumetrically diluted to approximate UF1X levels and pH adjusted to match the UF1X prior to fluorescence analysis. TOC samples were analyzed after dilution and the fluorescence EEMs were corrected for the TOC dilution factor. The corrected fluorescence EEMs were analyzed for 15 months of UF1X as one sample set, concentrate as one sample set, reconstituted NOM as one sample set, and as a combined sample set using PARAFAC. The corrected EEMs of the UF1X for each month were further analyzed using manual peak picking in order to observe seasonal variations in source NOM. Corrected EEMs produced by the FDOMcorr toolbox were exported into individual spreadsheets for the manual peak picking
process. High-performance liquid chromatography – size exclusion chromatography (HPLC-SEC) was conducted with a Dionex LC30 chromatography oven, a Dionex AD20 absorbance detector set to UV$_{254}$ (Thermo Fisher Scientific, Waltham, MA), a PL aquagel-OH 30 8-µm column (Agilent Technologies, Santa Clara, CA), and a gel filtration standard (Bio-Rad Laboratories, Hercules, CA). Elemental analysis was conducted by Huffman Laboratories, Inc. (Golden, CO).
Results and Discussion

TOC recovery (calculated from RO influent, concentrate, and permeate TOC concentrations in addition to operational flow rates which are not shown) through RO varied from 85% to 95% on a month-to-month basis (data not shown), which is typical of concentrating NOM through RO (Perdue and Ritchie 2003; Pressman et al. 2010). Since the concentration factor was set at 165X based on volumetric flow, and with the observed and expected recovery losses of TOC, the TOC concentration factor (based on UF effluent and RO concentrate effluent TOC concentrations) varied between 140-150X. Similarly, UV$_{254}$ recovery from RO (based on RO influent, concentrate, and permeate UV$_{254}$ values and operational flow rates not shown) fluctuated from 95% to 99% and a UV$_{254}$ concentration factor (calculated with UF effluent and RO concentrate effluent UV$_{254}$ values) of 122-144X was maintained during concentrate collection. There is a notable discrepancy between TOC and UV$_{254}$ concentration factors and recoveries but this has been previously encountered with RO concentrated NOM (Pressman et al. 2012). TOC and UV$_{254}$ results for the 15-months of collection are shown in Figure 1 with concentrate data shown in red empty bars (left axis) and UF1X data in solid blue bars (right axis). No samples were collected in June 2011 due to the extensive schedule upheld during the previous months of collection. The TOC and UV$_{254}$ concentrate data are from the final stage of sulfate removal and filtration prior to lyophilization. UF1X TOC and UV$_{254}$ data are from the batched HDPE carboys collected each month. UF1X data illustrated in Figure 1 were set to a y-axis approximately 165X less than the y-axis for the concentrate data allowing for a direct comparison of monthly changes and fluctuations in RO concentration factors.

Seasonal variations are evident for both the UF1X, which exhibited a TOC range of 1.77 mg/L to 2.86 mg/L, and the concentrate with TOC varying from 214 mg/L to 472 mg/L. The
UF1X and concentrate experienced lower TOC and UV$_{254}$ during the winter season (January 2011 – March 2011) while both the summer (July 2010 – September 2010 and July 2011) and fall (October 2010 – December 2010) produced higher values. These correlate to weather events, rain and snow, that occurred during those seasons. In May 2010, the area surrounding the collection site for the Ohio River experienced 5.66 inches of rain (NOAA 2014) increasing the TOC concentration of the UF1X. Similarly, June 2010 experienced 6.94 inches of rain (NOAA 2014) causing an increase UF1X TOC concentration. Precipitation continued throughout July 2010 and August 2010 further affecting TOC levels of the UF1X. Ohio River turbidity varied from 8.5 NTU to 12 NTU (GCWW 2012) during July 2010 and August 2010 appearing to decrease with rain precipitation events. The winter months experienced precipitation events but in the form of snow. This correlates to the lower UF1X TOC levels for those three months and higher Ohio River turbidity ranging from 42 NTU to 55 NTU (GCWW 2012). UV$_{254}$ and TOC levels increase with rain precipitation while decreasing with snow events. Ohio River turbidity undergoes an inverse relationship, decreasing during rain events and increasing in the months with snow.

Fluorescence spectroscopy was used to characterize the NOM with principle components produced by PARAFAC analysis. Previous literature research (not shown) determined that fluorescence spectroscopy was the next stage in NOM characterization after analysis with TOC and UV$_{254}$ surrogates. The collected NOM was characterized in four different groups: 15 months of UF1X, 15 months of concentrate, 15 months of reconstituted NOM, and all three sources combined as one sample set. Figure 2 represents four sets of two principle components produced from PARAFAC analysis of the fluorescence EEMs for the UF1X (Figure 2A), concentrate (Figure 2B), and reconstituted material across 15 months of collection (Figure 2C). These two
components are in similar locations for the three sources of NOM (UF1X, concentrate, and reconstituted NOM) as demonstrated in Table 1, a summary of principle component locations from Figure 2. Slight variations in peak locations, +/- 14 nm, are evident but expected from optically driven analyses. These small shifts in peak component locations do not substantially alter NOM characterization. Concentrating NOM with RO, lyophilizing the resulting aqueous solution, and reconstituting the solid material do not appear to alter the NOM character with respect to the components resulting from PARAFAC analysis. Because of the similar components in the UF1X, CONC1X, and RECON1X, all three NOM sources were analyzed as one sample set using PARAFAC. The same two principle components are observed, resulting in the same characterization of the NOM when all data were analyzed together (Figure 2D and Table 1).

Both components are representative of humic-like NOM falling within range of previously analyzed humic acids and humic-like NOM excitation and emission wavelengths (Hall and Kenny 2007). The locations of the components indicate the presence of humic acid in the Ohio River, a primary constituent of soil (Stevenson 1994). Component 1 can be further classified as visible humic-like as the peak falls between a previously identified excitation and emission ranges for visible humic-like NOM (Stedmon et al. 2003). Component 2 expresses a longer emission wavelength compared to Component 1, for every sample, suggesting that it contains slightly more conjugated fluorescence molecules (Cory and McKnight 2005). Conjugated molecules are more stable; therefore, the fluorescent organic compound shown with Component 2 should resist degradation slightly more so than Component 1. Disinfection byproduct (DBP) formation may initially be influenced by the visible humic-like NOM shown with Component 1 as it will react faster than the humic-like NOM of Component 2 molecules
(Cory and McKnight 2005). Also, the reconstituted NOM closely represents the NOM characterization of the 1X water suggesting that the performance of lyophilized NOM in drinking water research would be similar to the source NOM. Thus the addition of fluorescence spectroscopy adds an important new characterization process to the previous validations of TOC, UV$\text{_{254}}$, and DBP formation with these lyophilized and reconstituted materials (McCurry et al. 2012; Pressman et al. 2012; Pressman et al. 2010). Chen et al. (2003) analyzed various fluorescent compounds consisting of humic and fulvic acids, proteins, and microbial byproducts. A series of EEM regions were devised for NOM characterization as shown in Figure 3. The locations of principle component locations within EEMs fall within different regions allowing for the characterization of sample NOM. Ohio River NOM is further described as being humic-like as each peak for the UF1X, concentrate, and reconstituted lyophilized material falls within Region V which has been identified as being humic-like (Figure 3). Humic acid is common in upland streams such as the Ohio River (Kraus et al. 2010) where the sources of NOM for this research project were sampled. The locations of Component 1 and 2 correlate to allochthonous, soil, influenced NOM (Coble and Timperman 1998; Mopper and Schultz 1993). Therefore, biological sources such as microorganisms do not appear to be significant components of these Ohio River NOM samples because the samples lacked peaks found in Region I, Region II, and Region IV. Rather, the samples in this research appear primarily influenced by the soil of the riverbed and surrounding areas. The DBPs produced in disinfected drinking water containing this humic NOM will be those that are most affected by humic substances such as trihalomethanes, chloral hydrate, and haloacetic acids (Nikolaou et al. 2004).

Additional analysis was performed on the corrected fluorescence EEMs producing the fluorescence index (FI) and the humification index (HIX) for the UF1X, CONC1X, and
RECON1X from each month. Figure 4 represents the FI and HIX data for the UF1X (solid blue), concentrate diluted to 1X (empty red), and reconstituted material diluted to 1X (gradient green) for the 15 months of collection. The HIX correlates to DOM characterization based on the humic levels of the organic content (Ohno 2002) and is calculated using corrected fluorescence intensities (I) at a single excitation wavelength of 254 nm and multiple emission wavelengths where:

\[
HIX = \frac{\sum I_{435\rightarrow480}}{\sum I_{300\rightarrow345} + \sum I_{435\rightarrow480}}
\]

The HIX values for the three sources of NOM, across the collection period, range from 0.80 to 0.89 (Figure 4A) correlating to previously established HIX data for humic-like NOM (Ohno 2002). Small variations are evident in the HIX values corresponding to the humic level of the NOM possibly due to seasonal variations. The lower rain precipitation, but higher snow levels, for the months of January 2011, February 2011, and March 2011 may be responsible for an increase in the HIX for the UF1X. As snow melts, it percolates through soil and into the river and this may influence the HIX levels for those months. However, months experiencing larger rain events exhibited lower HIX values. This may be caused by the faster rate in which rain runoff enters the river compared to slower snow melt percolation. The FI is a ratio of corrected fluorescence intensities at 370 nm:450 nm and 370 nm:500 nm (excitation:emission) and correlates to DOC organic aromaticity (McKnight et al. 2001). The FI values for the UF1X, concentrate, and reconstituted lyophilized NOM exhibited a range from 1.45 to 1.57 (Figure 4B). These small fluctuations indicate little sequential variability in the DOC of the UF1X, which agrees with the earlier TOC data of the UF1X. Additionally, the FI further identifies the NOM of the UF1X, concentrate, and reconstituted material as being allochthonous falling within
previous FI ranges for soil influenced NOM (Johnson et al. 2011). In general, the FI experiences inverse fluctuations compared to the HIX. Higher values are observed in the winter months, which experienced snow events, compared to the spring and summer months with rain events. The fluctuations in both the HIX and FI were also captured in the concentrate and reconstituted NOM demonstrating the concentration and reconstitution processes have little effect on the characterization of the NOM.

Furthermore, variations were observed when analyzing individual EEMs for the UF1X. Manual peak picking was implemented when evaluating corrected EEMs produced for each month. The locations of the principle components are shown in Table 2 and Figure 5. The summer months of July 2010, August 2010, September 2010, and July 2011 expressed a single peak in the corrected fluorescence EEMs (the highlighted data points and underlined data key entries in Figure 5) while the EEMs from the remaining 11 months featured two peaks. The summer months experienced higher rain precipitation events compared to the spring, fall, and winter months. The observance of a single peak during July 2010, August 2010, September 2010, and July 2011 might be related to those weather events and represent a transition period between the spring and fall/winter months. Overall, the emission location for component 1 ranged from 404 nm to 460 nm. Although the change is greater than 10%, it does not alter the characterization of the component as it still falls within range of previous visible humic-like NOM (Stedmon et al. 2003). Disinfectant reactivity for Ohio River in July 2010, August 2010, September 2010, and July 2011 may be slower due to containing more conjugated fluorescent molecules (Cory and McKnight 2005). Additionally, component 1 for all months fits within Region V (Figure 3) of previously established EEM data (Chen et al. 2003). Peaks in the individual EEMs for all months are comparable to the two main peaks observed when analyzing
all UF1X samples as a single data set. Although small seasonal variations are evident in corrected fluorescent EEMs, the characterization of Ohio River NOM remains constant.

HPLC-SEC analysis was conducted for UF1X and reconstituted NOM from the 15 month collection period. Figure 6 shows two sets of HPLC-SEC elution time graphs for the UF1X and reconstituted NOM. One single peak was observed for both the UF1X (Figure 6A) and reconstituted material (Figure 6B) starting at around 8 minutes of elution time and ending at 10 minutes. While a second peak is visible in the reconstituted NOM at around 10 minutes (Figure 6B), this second peak is believed to correspond to a change in pressure caused by the instrument feed pump after the entire sample had been pumped into the column and does not reflect a separate peak. The HPLC-SEC instrument pump experienced issues providing constant, steady pressure through the gel column and thus, through the UV\textsubscript{254} detector. Changes in pressure are more visible with the RECON because the TOC and UV\textsubscript{254} are approximately 150X that of the UF1X. The elution time between 8 and 10 minutes corresponds to particles with atomic masses between $2.3 \times 10^5$ Da and $1.3 \times 10^5$ Da. Atomic masses in this range are considered high and are correlated with humic acid-like NOM (Piccolo 2002; Tsezos et al. 2011). Additionally, the similarity between UF1X and reconstituted NOM data, across the 15 months of collection, shows that any potential NOM losses that may have occurred during RO concentration, post processing, lyophilization, and reconstitution did not target specific NOM fractions but rather the NOM as a whole mixture. The consistent characterization of the NOM from fluorescence spectroscopy and HPLC-SEC indicate that any NOM losses that occurred during RO concentration and lyophilization may not target specific NOM fractions but rather the NOM as a whole mixture.

The lyophilized solid NOM collected during the 15 month period was analyzed using elemental analysis. Results from elemental analysis are shown in Table 3. All values are
reported as percentages and were corrected using lab measured oxygen content. Small monthly variations in the composition of the NOM are evident in the amounts of measured carbon, hydrogen, nitrogen, oxygen, and sulfur. The carbon composition of the concentrated NOM varied between 49% and 57% with an average of 53% across the 15 months of collection. The composition of oxygen was between 35% and 41% with an average of 37%. Furthermore, the oxygen to carbon ratios (0.62-0.84) fall within range of previously identified humic-like NOM with further identification as being freshwater soil-based (Rice and MacCarthy 1991). The resulting characterization of the NOM from elemental analysis matches well with results from fluorescence PARAFAC analysis, the FI and HIX from corrected fluorescence EEMs, and HPLC-SEC analysis in that the NOM remains humic-like while small fluctuations are observed during different months possibly due to seasonal variability. Overall, Ohio River water appears humic-like and allochthonously influenced over the course of the study and the characterization did not change after RO concentration, post barium removal, lyophilization, and reconstitution.
Conclusions

The locations of principle components from fluorescence EEMs along with the FI and HIX values indicated that the general humic-like characterization of the Ohio River did not change during the 15-month study. Small fluctuations in TOC, UV$_{254}$, FI, HIX, and elemental analysis signified NOM seasonal variability but these were not sufficient to change its overall characterization as evident in peak locations within the fluorescence EEMs. Moreover, the seasonal variability was captured in the concentrate and reconstituted material. Concentrating Ohio River NOM using RO, lyophilizing the resulting aqueous solution, and reconstituting the solid material had no effect on fluorescence and HPLC-SEC NOM characterization and suggests that any NOM losses during RO concentration, lyophilization, and reconstitution are not to specific NOM fractions but rather small amounts of whole mixture NOM. The same two principle components in the EEMs are evident in the source UF1X, concentrate, and reconstituted material. Seasonal variations of the source NOM were illustrated in monthly fluorescence peak EEM shifts. However, the locations of the peaks in the monthly EEMs still expressed humic-like NOM and demonstrated that the source NOM fluorescence characterization did not change much over time. Peak locations shifted during the summer months which served as a transition period between the months in the winter and spring seasons suggesting an influence from the precipitation events that occurred during each season. The FI and HIX data supplemented the lack of change in NOM characterization while expressing seasonality in the source NOM evident by the changes in HIX values which relate to the humic level of the NOM. These changes in HIX values may have been related to the type of precipitation that occurred during each month of NOM collection. Elemental analysis of the lyophilized NOM expressed oxygen-to-carbon ratios that fell within previously established
analyses for humic-like NOM matching well with characterization from fluorescence spectroscopy and HPLC-SEC analysis. The NOM of the source water did not change after processing; lyophilization preserved the humic characterization of the NOM matching well with the UF1X. The consistent advanced characterization of the NOM further validates RO concentration, lyophilization, and reconstitution beyond previous research efforts. A unique library of Ohio River NOM was created over 15 months and it was demonstrated that the characterization, using multiple advanced techniques, of the lyophilized material within the NOM library represents humic-like, allochthonous influenced NOM expressing minor seasonal fluctuations of the source water. The temporal library of well-characterized, standardized Ohio River NOM is the first of its kind leading to beneficial drinking water treatment and research studies.
Future Research

The process of concentrating Ohio River NOM, using RO, lyophilizing the resulting aqueous solution, and reconstituting to source TOC conditions does not change the characterization of the NOM. The temporal library of lyophilized NOM benefits drinking water research in regards to DBP formation and seasonal studies along with health effects studies requiring higher concentrations of NOM. However, the library has a limited application to drinking water systems in the immediate area relying on the Ohio River as a drinking water source. The formation of a temporal library of lyophilized NOM from different surface source waters would benefit multiple drinking water systems outside of the previously examined Ohio River area. RO concentration, lyophilization, and reconstitution can be adapted to other source waters creating multiple libraries of solid NOM material representing different geographical regions, water sources, and climate conditions. The progression of the creation of a temporal library of NOM, benefiting drinking water and health effects studies, would benefit greatly if other sources are were included.
Figure 1 – TOC (A) and UV$_{254}$ (B) for the concentrate and UF1X during the 15 month study, CONC values are on the left ordinate and UF1X values are on the right ordinate for both figures.
Figure 2 – Principle component EEMs for the UF1X (A), concentrate (B), reconstituted NOM (C), and all three sources analyzed as one data set (D) produced by PARAFAC. All samples pH adjusted to match the UF1X for each month and normalized by TOC concentration.
Table 1 – Summary of principle components for each NOM source and all sources analyzed as a single data set.

<table>
<thead>
<tr>
<th>Sources</th>
<th>Component 1</th>
<th></th>
<th>Component 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Emission (nm)</td>
<td>Excitation (nm)</td>
<td>Emission (nm)</td>
<td>Excitation (nm)</td>
</tr>
<tr>
<td>UF1X</td>
<td>416</td>
<td>340</td>
<td>476</td>
<td>370</td>
</tr>
<tr>
<td>Concentrate</td>
<td>420</td>
<td>340</td>
<td>484</td>
<td>370</td>
</tr>
<tr>
<td>Reconstituted NOM</td>
<td>414</td>
<td>345</td>
<td>490</td>
<td>370</td>
</tr>
<tr>
<td>All Sources</td>
<td>420</td>
<td>345</td>
<td>484</td>
<td>375</td>
</tr>
</tbody>
</table>
Figure 3 – The locations of each component from the single data set analysis illustrated in previously established EEM regions (Chen et al. 2003).
Figure 4 – Humification Index (A) and Fluorescence Index (B) results for the UF1X, concentrate, and reconstituted NOM over the 15 month study.
Table 2 – Peak locations in corrected EEMs for individual months.

<table>
<thead>
<tr>
<th>UF1X Source Month</th>
<th>Component 1</th>
<th>Component 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Emission</td>
<td>Excitation</td>
</tr>
<tr>
<td></td>
<td>(nm)</td>
<td>(nm)</td>
</tr>
<tr>
<td>Apr-10</td>
<td>404</td>
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</tr>
<tr>
<td>May-10</td>
<td>410</td>
<td>340</td>
</tr>
<tr>
<td>Jun-10</td>
<td>422</td>
<td>345</td>
</tr>
<tr>
<td>Jul-10</td>
<td>460</td>
<td>360</td>
</tr>
<tr>
<td>Aug-10</td>
<td>458</td>
<td>355</td>
</tr>
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</tr>
<tr>
<td>Nov-10</td>
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<td>340</td>
</tr>
<tr>
<td>Dec-10</td>
<td>408</td>
<td>345</td>
</tr>
<tr>
<td>Jan-11</td>
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</tr>
<tr>
<td>Feb-11</td>
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</tr>
<tr>
<td>Mar-11</td>
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</tr>
<tr>
<td>Apr-11</td>
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</tr>
<tr>
<td>Jul-11</td>
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<td>340</td>
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<tr>
<td>All Months</td>
<td>416</td>
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</table>
Figure 5 – Data from Table 2; peak locations from monthly corrected EEMs. The shadowed data points, along with the underlined data key entries, represent the months that expressed a single peak in their corrected EEMs. All other data points represent months which expressed two peaks in their corrected EEMs.
Figure 6 – High-performance liquid chromatography (HPLC-SEC) with UV$_{254}$ detection for the UF1X (A) and reconstituted NOM (B). All reconstituted NOM samples were pH adjusted to match respective UF1X levels. The TOC of the reconstituted NOM is approximately 150X that of the UF1X; it was not diluted to 1X conditions for analysis.
Table 3 – Elemental analysis of the lyophilized NOM solid. All values corrected for dry conditions and adjusted using measured oxygen.

<table>
<thead>
<tr>
<th>% Composition</th>
<th>Oxygen to Carbon Ratio</th>
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<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>April 2010</td>
<td>49</td>
</tr>
<tr>
<td>May 2010</td>
<td>57</td>
</tr>
<tr>
<td>June 2010</td>
<td>51</td>
</tr>
<tr>
<td>July 2010</td>
<td>52</td>
</tr>
<tr>
<td>August 2010</td>
<td>55</td>
</tr>
<tr>
<td>September 2010</td>
<td>52</td>
</tr>
<tr>
<td>October 2010</td>
<td>54</td>
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<tr>
<td>November 2010</td>
<td>54</td>
</tr>
<tr>
<td>December 2010</td>
<td>54</td>
</tr>
<tr>
<td>January 2011</td>
<td>50</td>
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<tr>
<td>February 2011</td>
<td>53</td>
</tr>
<tr>
<td>March 2011</td>
<td>52</td>
</tr>
<tr>
<td>April 2011</td>
<td>53</td>
</tr>
<tr>
<td>May 2011</td>
<td>54</td>
</tr>
<tr>
<td>July 2011</td>
<td>55</td>
</tr>
</tbody>
</table>
Evaluating RO Concentration Factor and NOM Reconstitution Concentration

Factor

Introduction

Natural organic matter (NOM) is a multifarious mixture of organic polymers found in source waters. The dissolved organic matter (DOM) fraction of NOM reacts with disinfectants and results in faster disinfectant decay along with the formation of disinfection byproducts (DBPs) (Santschi et al. 2002). Because of the complex and ever changing nature of NOM, characterization is difficult and surrogates are often relied upon for quantification and characterization. Total organic carbon (TOC) is commonly used to quantify NOM while UV$_{254}$, odor, and taste are implemented for characterization (Thurman 1985). However, more advanced characterization techniques exist that allow for a more detailed understanding of DOM character. The chromophoric fraction of DOM (CDOM) can be characterized using fluorescence spectroscopy (Pifer et al. 2011), high-performance liquid chromatography (Her et al. 2002), flow field-flow fractionation (Baalousha and Lead 2007), and various other advanced techniques.

Reverse osmosis (RO) has been implemented to produce highly concentrated complete aqueous NOM solutions beneficial for toxicological DBP research and other drinking water research studies (Perdue and Ritchie 2003; Pressman et al. 2010; McCurry et al. 2012). TOC and UV$_{254}$ surrogates were used in previous research efforts characterizing RO concentrated NOM along with the evaluation of DBP mixtures (Pressman et al. 2010). One limitation of using RO for concentrating NOM is that aqueous concentrated NOM degrades over time during long-term refrigerated storage, similar to the storage issues of the 1X source waters. Additionally, RO concentrated NOM must be collected, processed, and implemented in research within a short
time period increasing work efforts. Therefore, concentrated NOM needs to be collected and analyzed prior to every use extending the time required for research efforts. In order to overcome the limited shelf-life, McCurry et al. (2012) developed a lyophilization process to remove the remaining water from concentrated NOM resulting in a solid that can be reconstituted back to an aqueous state at multiple NOM concentration factors. Lyophilization and reconstitution of RO concentrated NOM were validated using $\text{UV}_{254}$ and TOC NOM characterization surrogates with 91% $\text{UV}_{254}$ and 101% TOC recovery (McCurry et al. 2012). The shelf-life of solid NOM stored in sealed bottles in the dark is far longer than the concentrate from which it was made (McCurry et al. 2012). Following development of the lyophilization and reconstitution processes and the validation of those procedures with TOC and $\text{UV}_{254}$ surrogates, an investigation of representative DBPs produced in aqueous concentrated NOM, lyophilized and reconstituted NOM, and 1X source water was conducted as a further means of validating that lyophilized and reconstituted NOM is similar to the source material (Pressman et al. 2012). The concentrations of 96% of measured total DBPs in chlorinated reconstituted NOM were statistically indistinguishable from those in the chlorinated source 1X water while the concentrations of 86% of total DBPs were statistically identical to total DBPs produced in RO concentrated NOM (Pressman et al. 2012).

Previous investigations used a concentration factor of approximately 150X the source water TOC concentrations as a compromise between high concentration and concentrate product flow rate during RO NOM concentration (McCurry et al. 2012; Pressman et al. 2012; Pressman et al. 2010). McCurry et al. (2012) reported on the reconstitution of lyophilized NOM at a higher concentration factor, 1000X, with only a small decrease in $\text{UV}_{254}$ recovery (4.85%). With these advances, lyophilized NOM may be reconstituted to multiple concentration factors higher than
what RO concentration can produce. However, a direct comparison of RO concentration factor and reconstitution factor has not been conducted using UV$_{254}$ and TOC along with more advanced NOM characterization, such as fluorescence spectroscopy, and DBP formation. Historically, a 150X RO concentration factor was selected in order to minimize potential NOM losses such as fractionation due to operating RO equipment at a higher concentration factors and pressures. For higher concentration solutions, such as that required for health effects studies, reconstituted NOM could be used to increase the concentration of the NOM. However, analysis of RO concentrated NOM at multiple concentration factors has not been conducted. Furthermore, concentrate produced from multiple RO concentration factors requires comparison to reconstituted NOM at the same concentration factors using more advanced characterization techniques along with analyzing DBP formation to determine if a preferred concentration factor versus reconstituted concentration factor ratio exists that minimizes overall NOM losses while preserving NOM characterization.

A broad range of RO concentration factors (50X, 100X, 150X, 200X, and 250X) allows for a direct comparison of historical operating conditions (McCurry et al. 2012; Pressman et al. 2012; Pressman et al. 2010) along with determining the most favorable RO concentration factor in comparison to reconstituting lyophilized NOM at the same concentration factors. A lower concentration factor matching source water conditions, 1X, warrants further investigation using reconstituted NOM as this represents a standardized NOM source for treatment studies. Furthermore, 250X NOM benefits health effects research where higher DBP concentrations are desirable (Pressman et al. 2010). More advanced NOM characterization techniques, beyond TOC and UV$_{254}$ surrogates, would allow for the determination of NOM losses during RO
concentration along with lyophilization and reconstitution by potentially tracking any changes in NOM characterization due to fractionation.

Objective

The primary objective of this research is to:

- Determine the most favorable RO concentration factor compared to reconstituted concentration factor for lyophilized NOM by concentrating Ohio River NOM at five different RO concentration factors (50X, 100X, 150X, 200X, and 250X), lyophilizing the resulting aqueous concentrate, and reconstituting the solid NOM matching the source TOC RO concentration factors. The NOM will be characterized using TOC and UV\textsubscript{254} along with fluorescence spectroscopy and high performance liquid chromatography – size exclusive chromatography (HPLC-SEC) in order to observe possible NOM losses and fractionation. Furthermore, DBPs, resulting from chlorination, will be analyzed for the concentrate and reconstituted material at the multiple concentration factors, 1X source TOC conditions, and 250X concentrate TOC conditions.
Material and Methods

UF1X and Concentrate Collection

Ohio River NOM was concentrated and collected over a three week period in September 2012. Water was treated using a 200-µm cloth bag filter, ultrafiltration (two ceramic 19P37-30 Membralox 0.02-µm, Pall Corp., Port Washington, NY), cation exchange resin columns (Ambersep 200H hydrogen-form, Rohm and Hass, Philadelphia, PA), and RO (three Filmtec BW30-4040 membranes in series, Dow, Midlands, MI) following a previously outlined method (Pressman et al. 2010). Five different volumetric concentration factors were used during RO concentration: 50X, 100X, 150X, 200X, and 250X. Hourly UV$_{254}$ and TOC samples were collected for raw Ohio River water, UF effluent (UF1X), ion exchange effluent, RO concentrate (50XCONC, 100XCONC, 150XCONC, 200XCONC, and 250XCONC), and RO permeate effluent. Ion chromatography (IC) and inductively coupled plasma (ICP) samples were collected every four hours at the same sampling points based on previous research efforts demonstrating the appropriateness of the sampling time periods (Pressman et al. 2010).

UF1X and Concentrate Storage and Post-Processing

Approximately 80 liters of concentrate was collected for each concentration factor and stored in 20-liter HDPE Nalgene carboys (Thermo Fisher Scientific, Waltham, MA) at 4°C. UF1X was collected in small one liter aliquots every five hours, combined, and stored in four 20-liter HDPE Nalgene carboys (Thermo Fisher Scientific, Waltham, MA) at 4°C. Because sulfate is concentrated with NOM when using RO, which leads to sulfuric acid formation during lyophilization (Koprivnjak et al. 2006; Pressman et al. 2010), NOM concentrate was subsequently processed for sulfate removal using BaCl$_2$ (Certified ACS, Thermo Fisher
Scientific, Waltham, MA) precipitation with a 0.86 molar ratio of Ba\(^{2+}\) to SO\(_4^{2-}\). Concentrate was separately transferred to a 50-gallon stainless steel drum where it was then mixed with BaCl\(_2\) solid for a one hour period; the concentrate was covered and then left to settle overnight. TOC, IC, and UV\(_{254}\) samples were collected after barium precipitation to assess any losses in the precipitate. The supernatant was pumped back into 20-liter HDPE Nalgene carboys and the settled barium sulfate solid was discarded. A sulfate concentration of 10 mg/L or less was desired and required multiple barium precipitation stages. Subsequent BaCl\(_2\) precipitation stages used a 1:1 molar ratio of Ba\(^{2+}\) to SO\(_4^{2-}\). Each concentration factor required at least two stages of precipitation with most requiring three in order to effectively remove sulfate. The final concentrate was filtered with pre-rinsed 0.45-\(\mu\)m cartridge filters (AquaPrep 600, Pall Corporation, Port Washington, NY) and stored at 4°C. IC, ICP, TOC, and UV\(_{254}\) samples were collected after filtration prior to lyophilization to assess overall losses in precipitation and lyophilization.

**UF1X and Concentrate Lyophilization and Reconstitution**

The concentrate was lyophilized in a VirTis Virtual EL pilot scale freeze-dryer (SP Industries, Warminster, PA) following a procedure developed by McCurry et al. (2012). Concentrate was transferred to HDPE-lined stainless steel 2-liter trays which were then placed in the freeze-dryer. Each concentration factor was lyophilized separately in order to avoid cross contamination. After the three day lyophilization process, resulting NOM solid for each concentration factor was collected and stored separately in amber glass vials with silica gel packets to absorb moisture. Solid NOM was reconstituted, by TOC, such that the NOM that originally came from the 50X sample was mixed to match the TOC of the 50X concentrate (50XCONC) making 50X reconstitute (50XRECON), solid NOM from the 100X sample was
reconstituted to match the TOC of the 100X concentrate (100XCONC) resulting in 100X reconstitute (100XRECON), and so on. To match UF1X TOC, reconstituted samples were diluted so that 50XRECON was diluted to make 50XRECON1X, 100XRECON was diluted resulting in 100XRECON1X, 150XRECON diluted to 150XRECON1X, etc. UF1X was also lyophilized and reconstituted making UF1XRECON. Comparing all the lyophilized concentrates reconstituted at 1X concentrations to the original UF1X (UF1X, UF1XRECON, 50XRECON1X, 100XRECON1X, 150XRECON1X, 200XRECON1X, and 250XRECON1X) facilitates the identification of an ideal concentration factor, if one exists, for the situation where lyophilized NOM would be reconstituted to 1X conditions, such as for water treatment process studies.

Lastly, to compare with the 250XCONC, lyophilized NOM solid from the multiple concentration factors was mixed to 250X TOC conditions. Solid from the 50X sample was reconstituted making 50XRECON250X, lyophilized material from the 100X sample was reconstituted producing 100XRECON250X, NOM from the 150X sample resulted in 150XRECON250X, etc.

Figure 7 represents a schematic of the experimental design. Starting at the top left of Figure 7, raw Ohio River water was filtered and processed through ultrafiltration producing UF1X. The UF1X was then lyophilized and reconstituted resulting in UF1XRECON. During field collection, the UF1X was further processed through RO concentration at five different concentration factors (50X, 100X, 150X, 200X, and 250X) as illustrated in the second row of Figure 7. The resulting aliquots of concentrate were individually lyophilized producing solid NOM from each concentration factor. Separately, the lyophilized NOM from each concentration factor was reconstituted to the TOC (+/- 10%) of the source aqueous concentrate of each concentration factor (second row of Figure 7). The resulting, aqueous reconstituted solution was diluted to within 10% of the TOC concentration of the UF1X and analyzed against the UF1X and
UF1XRECON (third row of Figure 7). As illustrated in the fourth row of Figure 7, the lyophilized NOM from each concentration factor was reconstituted to within 10% of the 250XCONC. The reconstitution design allowed for a direct comparison of concentrates, lyophilized reconstituted NOM to source concentrate (validating the lyophilization process), UF1X (validating RO concentration and lyophilization for water treatment studies), and higher concentration factor concentrates pertinent for health effects research. The lyophilized NOM was reconstituted following a previously outlined method (McCurry et al. 2012). Initially, for each concentration factor, solid NOM was mixed with ultra-pure, distilled, deionized water ($\rho \geq 18$ MΩcm) for a one hour period limiting the amount of NOM solid that would adhere to the glass pH probe. The solution was then adjusted to pH 10 with 6N NaOH (N.I.S.T. Traceable, Aqua Solutions, Deer Park, TX), the sample bottle was capped and covered in foil, and the solution was mixed for a 24-hour period. The resulting liquid was filtered with pre-rinsed 0.45-μm PES disk filters (Supor-450, Pall Life Sciences, Port Washington, NY). Reconstituted 1X samples were made by diluting NOM, reconstituted to its source concentrate TOC, with ultra-pure, distilled, deionized water ($\rho \geq 18$ MΩcm). UV$_{254}$ and TOC samples were collected after final filtrations and dilutions.

**Chlorination**

UF1X, concentrate, reconstituted concentrate, reconstituted 1X, and reconstituted 250X samples were further adjusted with pH 8 borate buffer using twice the borate concentration specified by uniform formation conditions (UFC) (Summers et al. 1996), which was previously determined in 24-hour studies to maintain pH during chlorination (data not shown). 6N NaOH (N.I.S.T. Traceable, Aqua Solutions, Deer Park, TX) and boric acid solid (ACS Certified, Thermo Fisher Scientific, Waltham, MA) were used to make the pH 8 borate buffer. The use of
6N NaOH and boric acid solid in the creation of the borate buffer, instead of the suggested 0.26N NaOH and 1.0 M boric acid, reduced NOM dilution. An approximately 20000 mg/L chlorine stock solution was diluted from sodium hypochlorite solution (Laboratory Grade, 5.65%–6%, Thermo Fisher Scientific, Waltham, MA). Chlorine dosing studies were previously conducted in order to determine a consistent chlorine dose for the UF1X, concentrate, reconstituted concentrate, reconstituted 1X, and reconstituted 250X samples. The dosing studies determined the exact amount of chlorine addition that would result in 0.0 mg/L chlorine residual at exactly 24-hours yielding a Cl₂ to TOC ratio of 0.48. Appendix 1 shows data from this experiment using UF1X. The chlorine dosing experiment was only conducted in the UF1X because previous research demonstrated that chlorine decay is faster in the concentrate compared to the UF1X (Pressman et al. 2012; Pressman et al. 2010). Because equal overall chlorine decay was desired for both the UF1X and concentrate, on a chlorine-to-TOC ratio basis, conducting dosing experiments on the UF1X would guarantee that all the chlorine added to the concentrates would be decayed. As a check, the selected ratio was then tested with 50XCONC and 250XCONC encompassing both the lowest and highest concentrate TOC conditions. The Cl₂ to TOC ratio of 0.48 resulted in a 0.0 mg/L 24-hour chlorine residual for the 50XCONC and 250XCONC (data not shown). NOM chlorination followed previously established methods (Pressman et al. 2010) and free chlorine was measured following Hach Method 8021 using DPD Free Chlorine Reagent Powder Pillows (Hach Company, Loveland, CO). However, a Nicolet Evolution 300 UV spectrophotometer (Thermo Fisher Scientific, Waltham, MA) set to 530 nm was used instead of a Hach colorimeter for free chlorine measurements. See Appendix 2 for the free chlorine calibration curve with the Nicolet Evolution 300 UV Spec and Hach DPD powder pillow packets.
**Water Quality Parameters, Measurement Procedures, and Instrumentation**

UV$_{254}$ was measured according to Standard Method 5910 UV-Absorbing Organic Constituents (American Public Health Association (APHA) et al. 2012) using a Nicolet Evolution 300 UV spectrophotometer (Thermo Fisher Scientific, Waltham, MA). TOC was analyzed following USEPA method 415.3 (USEPA 2005) using a combustion catalytic TOC analyzer (TOC-Vcph, Shimadzu Corporation, Columbia, MD). ICP analysis was conducted according to USEPA method 200.7 (USEPA 1994) using an iCAP 6000 spectrometer (Thermo Fisher Scientific, Waltham, MA). IC analysis was carried out according to EPA Method 300.1 (USEPA 1997) using a Metrohm-Peak 761 IC instrument (Metrohm-USA, Riveriew, FL). Fluorescence spectroscopy was conducted with a Fluorolog3 instrument (HORIBA Instruments IN., Edison, NJ) using excitation wavelengths of 240 nm to 450 nm (5-nm step), emission wavelengths of 300 nm to 500 nm (2-nm step), and a slit width of 5-nm. Fluorescence data was corrected for UV absorbance, instrumentation correction factors, sample TOC concentrations, integrated with the instrument Raman curve, and Rayleigh scattering was removed using MATLAB (Release 2011b, The Mathworks, Inc., Natick, Mass.) and the FDOMcorr toolbox (Murphy et al. 2010) customized for this research. The DOMFluor toolbox was implemented when conducting PARAFAC analysis following a previously outlined method (Stedmon and Bro 2008). All concentrate, reconstituted concentrate, and reconstituted 250X samples with TOC values higher than the UF1X were volumetrically diluted to approximately match 1X TOC levels before fluorescence analysis. Total organic halogen (TOX) was measured following Standard Method 5320B (American Public Health Association (APHA) et al. 2012). All DBPs were analyzed by EE&T, Inc. (Newport News, VA) using USEPA method 551.1 (USEPA 1998) for trihalomethanes (THM4), haloacetonitriles (HAN4), haloketones (HK2), chloral hydrate (CH),
and chloropicrin (CP) while USEPA method 552.3 (USEPA 2003) was used for haloacetic acids (HAA9).
Results and Discussion

In previous research efforts, the volumetric concentration factor was set to 165X during RO concentration resulting in 150X concentrate based on TOC concentration factor (McCurry et al. 2012; Pressman et al. 2012; Pressman et al. 2010). However, due to the lack of research regarding multiple RO concentration factors, the desired concentration factors were set as the volumetric concentration factors. The TOC concentration factors (calculated by dividing the RO concentrate TOC concentration with the UF effluent TOC concentration) were within 15% of each desired value (50X, 100X, 150X, 200X, and 250X) while the UV$_{254}$ concentration factors (calculated by dividing the RO concentrate UV$_{254}$ and the UF effluent UV$_{254}$) were within 22% of the desired factors during RO concentration. TOC recovery (based on RO influent, permeate, and concentrate TOC concentrations as well as operational flow rates not shown) ranged from 86% to 96% for all concentration factors and UV$_{254}$ recovery (calculated using RO influent, permeate, and concentrate UV$_{254}$ in additional to operational flow rates which are not shown) varied from 84% to 99%, typical for RO concentration (Perdue and Ritchie 2003). Overall, RO concentration performance was similar to previous research efforts, based on TOC and UV$_{254}$ concentration factors and recovery, despite the implementation of diverse concentration factors.

Table 4 shows a summary of water quality parameters for the UF1X, 50XCONC, 100XCONC, 150XCONC, 200XCONC, and 250XCONC. Data for the five different concentration factors is from the final stage of sulfate removal prior to lyophilization which also serves as the TOC target during reconstitution. Final TOC concentration factors, after sulfate removal and filtration, for each concentration factor, were within 14% of desired values (Table 4) with 150XCONC representing the largest difference between desired TOC concentration factor and final TOC concentration factor (CF = 173). The observed concentration factors are
higher than the implemented volumetric concentration factors unlike previous RO concentration efforts (Pressman et al. 2010). Previously, a higher volumetric concentration factor (165X) was implemented in order to achieve a 150X TOC concentration factor. The difference in TOC concentration factor performance is possibly due to operating the RO equipment at different concentration factors than previous efforts. Total metals for concentrate at all concentration factors were higher than in UF1X (Table 4). In general, the total metals were concentrated along with NOM during RO collection. However, the concentration factors for metals were lower than that of the TOC which was expected based on previous RO concentration efforts (McCurry et al. 2012; Pressman et al. 2012; Pressman et al. 2010) as metals were lost through RO permeates due to smaller molecular sizes compared to the NOM. The sulfate concentration in the concentrates is lower than that of the source UF1X as a result of barium chloride precipitation. The removal of sulfate is required for concentrate NOM lyophilization (McCurry et al. 2012; Pressman et al. 2012).

Figure 8 shows the TOC concentrations for the UF1X, concentrate, and reconstituted NOM. All samples were reconstituted to within 10% of their pre-lyophilized source TOC concentrations. For example, the reconstituted NOM solutions for each concentration factor were diluted to within 10% of the UF1X TOC (Figure 8A). Next, the lyophilized NOM from each of the concentration factors was reconstituted back to its original concentrate TOC (i.e., the 50X concentrate was reconstituted back to match the 50XCONC TOC conditions) (Figure 8B). Reconstituting to the original concentration factors allows for a direct comparison of 50XCONC to 50XRECON, 100XCONC to 100XRECON, 150XCONC to 150XRECON, 200XCONC to 200XRECON, and 250XCONC to 250XRECON when characterizing the NOM and analyzing DBP formations. Comparing reconstituted NOM with its source concentrate should result in
similar water quality, allowing for validation of the lyophilization and reconstitution processes while observing potential NOM losses at lower or higher concentration factors. Similarly, lyophilized NOM from the 50X, 100X, 150X, and 200X concentration factors was reconstituted to within 10% of 250XCONC TOC producing 50XRECON250X, 100XRECON250X, 150XRECON250X, and 200XRECON250X respectively (Figure 8C). High concentration factor NOM is pertinent for health effects studies where higher DBP concentrations are required (Pressman et al. 2010). Furthermore, the assessment of 250X NOM should help determine if reconstituting lyophilized material at a higher concentration is comparable to direct RO concentration at that concentration or if an ideal combination of concentration factor versus reconstitution concentration factor exists to reach such high NOM concentrations.

HPLC-SEC with UV\textsubscript{254} detection was used to determine the size distribution of the dissolved materials within the UF1X, UF1X RECON, 50XRECON1X, 100XRECON1X, 150XRECON1X, 200XRECON1X, and 250XRECON1X as shown in Figure 9. The peak areas (and peak intensities) were not quantified. Instead the elution time was used to determine atomic mass sizes of dissolved material based on an instrument calibration standard (data not shown). A single peak was observed for all samples, between approximately an 8 minute to 10 minute elution time, which corresponds to particles with atomic masses between $2.3 \times 10^5$ Da and $1.3 \times 10^5$ Da, respectively. Two smaller peaks are visible in Figure 9 at 7.5 minutes and 11 minutes. It is believed that these two additional peaks correspond to a change in pressure in the UV\textsubscript{254} detector caused by the instrument feed pump. The HPLC-SEC instrument pump experienced problems providing steady pressure through the gel column and thus, through the UV\textsubscript{254} detector. Therefore, the two peaks at 7.5 minutes and 11 minutes were not separate. The resulting atomic mass range, $2.3 \times 10^5$ Da to $1.3 \times 10^5$ Da, is considered high and falls within
previously established atomic masses for humic-like NOM (Piccolo 2002; Tsezos et al. 2011). The characterization of NOM does not change with increasing concentration factor or after lyophilization and reconstitution. Moreover, the consistent size distribution of dissolved materials indicates that the NOM is not being fractionated during concentration, lyophilization, and reconstitution. Rather, changes to the NOM were more likely occurring as small whole mixture NOM losses during collection and after post processing as detailed by changes in TOC and UV$_{254}$. HPLC-SEC analysis indicates the lack of an optimal RO concentration factor. Therefore, NOM may be concentrated using RO at any concentration factor, ranging from 50X to 250X, lyophilized and reconstituted to source water conditions without affecting the size distribution of dissolved materials while representing UF1X NOM.

The NOM was further characterized using fluorescence spectroscopy and principle component analysis with PARAFAC. Figure 10 shows four different EEM groups produced by PARAFAC analysis: the UF1X and all RECON1X (Figure 10A), all aqueous concentrate at multiple concentration factors (Figure 10B), NOM reconstituted to match source aqueous concentrate (Figure 10C), and all RECON250X samples (Figure 10D). The individual groups of NOM were chosen in order to create sample sets large enough for PARAFAC analysis and in order to determine if lyophilization and reconstitution change the characterization of the NOM at the lower (50X and 100X) and higher (200X and 250X) concentration factors. The results in Figure 10 were summarized in Table 5. One principle component is produced for each sample set at approximately the same location of 454 nm (emission) and 355 nm (excitation) (Table 5). There were small differences in peak locations, +/- 8 nm for emission ($\sigma = 3$) and +/- 5 nm for excitation ($\sigma = 3$), though these shifts do not change the characterization of the NOM for each sampled group. The principle component locations produced by PARAFAC for all four NOM
sources, shown in Table 5, correspond to humic-like NOM, falling within a previously established component range location for humic acid substances (Chen et al. 2003; Hall and Kenny 2007). The NOM can be further characterized as visible-humic-like fluorescing between an excitation wavelength range of 380 nm to 750 nm, the visible spectrum (Stedmon et al. 2003). The humic-like characterization of the overall NOM fluorescence component found in all the samples agrees with previous discussions of the prevalence of humic acid in upland streams (Kraus et al. 2010). Moreover, the location of the component from the four NOM source groups signifies that the organic matter is allochthonous as opposed to autochthonous (Coble and Timperman 1998). Therefore, components produced by microorganisms were not observed in these Ohio River samples and the NOM appears to be mainly influenced by surrounding riverbed and environmental soil. DBP formation might be influenced by the humic nature of the NOM producing more halogenated species (Cowman and Singer 1996). As with the concentrate prior to lyophilization, these results show that after lyophilization and reconstitution, there is no advantage to any concentration factor since they all result in one fluorescence component.

The single EEM peak for all NOM concentration factors produced in the field (Figure 10B) demonstrates one concentration factor is not better than another with respect to fluorescence analysis. Likewise, the single fluorescence peak demonstrates that lyophilizing and reconstituting to the starting concentrate concentration, the 1X concentration, or the 250X concentration does not appreciably change the NOM. Although UV\textsubscript{254} and TOC measurements showed some NOM losses during RO concentration, lyophilization, and reconstitution, the lack of change in fluorescence EEM location implies those losses were whole-mixture losses and not in specific size or type fractions. As long as potential NOM losses are whole-mixture losses, the
resulting NOM can be reconstituted to any concentration factor while remaining representative of the source NOM.

Figure 11 shows all measured DBP concentrations, which were normalized by sample TOC concentration factor compared to the UF1X TOC allowing for a direct comparison of DBPs produced in concentrate, reconstituted NOM, reconstituted 1X NOM, and reconstituted 250X NOM. Each reported DBP concentration represents an average of triplicate samples of each NOM sample while the bars in Figure 11 represent the low and high reported concentrations. Overall, trihalomethane (THM) concentrations for the 1X sample set (Figure 11A, in solid blue) were within 23 µg/L (51%) of the UF1X. Similarly, THM concentrations for the 250X reconstituted samples (Figure 11A, in solid gray) were with 19 µg/L (41%) of the UF1X. The THMs in concentrate (Figure 11A, in gradient green) and reconstituted NOM (Figure 11A, in unfilled red) samples were lower than both 1X and RECON250X sources. Furthermore, the THM concentrations in the 50XRECON – 250XRECON were within 20 µg/L (50%) of their starting sources (50XCONC – 250XCONC). THM concentrations for the 250XRECON material appeared to match well, within 22 µg/L (52%), with the UF1X. The differences in THM concentrations across all four sample sets may be related to the multiple days in which DBP formation studies were conducted. The concentrate and reconstituted NOM samples were analyzed in one day, the 1X samples on another day, and the 250X samples on an additional day. Samples produced from each day were collected and submitted for analysis separately. Although the samples were chlorinated with the same stock, differences in normalized THM concentrations may be due to experimental error as evident in the concentrate and reconstituted material which were chlorinated on the same day. Further analysis of the THM data is suggested
in order to better understand the differences in THM concentrations across the different NOM sample groups.

Overall, haloacetic acid (HAA9) concentration data appeared to follow a similar pattern to the THM data. All of the reconstituted 1X samples compared well within the UF1X and reconstituted UF1X THM concentrations (Figure 10B) with variability limited to within 5 µg/L (21%). At the opposite end of the NOM concentration spectrum, all of the reconstituted 250X HAA9 samples also compared well with the UF1X and reconstituted UF1X resulting in a maximum difference of 6 µg/L (22%). When compared to the UF1X HAA9 concentration, both the concentrate and reconstituted NOM sample groups exhibited lower HAA9 concentrations. Concentrate HAA9 concentrations were lower by 16 µg/L (57%) and reconstituted HAA9 concentrations were within 9 µg/L (32%) of the UF1X HAA9 concentration. However, when compared to each other, the concentrate samples (red data) and the reconstituted concentrate samples (green data) seemed to compare reasonably well. The HAA9 concentrations of 250X reconstituted samples have a maximum difference of 15 µg/L (78%) compared to the 250XCONC but matched well with the UF1X by 6 µg/L (21%). As with the THM data, HAA9 data would benefit from additional analysis.

Haloacetonitrile (HAN4) concentrations were significantly less than those of THMs and HAAs (Figure 10C). The HAN4 UF1X concentration was the lowest of all the samples from the four NOM groups at 1.1 µg/L. The reconstituted samples, even the reconstituted UF1X, were all at least 4.5 µg/L higher than the UF1X HAN4 concentration. As with previous DBPs, the reconstituted 1X samples appeared to compare reasonably well with those samples reconstituted to 250X (within 2.2 µg/L, 50%). While the concentrate and reconstituted concentrate samples
were lower than the 1X and 250X samples, as with THMs and HAAs, in this case they compare better with UF1X falling within 2.2 µg/L, double the HAN4 concentration in the UF1X. Haloketones (HK2) were very low in three reconstituted samples and below instrument detection limits (0.2 µg/L) for all other samples (Figure 11D).

Total organic halogen (TOX) concentrations are shown in Figure 11E. The trends were quite comparable to much of the DBP data. The 1X reconstitutes were similar to the 250X reconstitutes (within 27%) while the concentrates and reconstituted concentrates were all half as much as the 1X reconstitutes and 250X reconstitutes. The UF1X and UF1XRECON TOX were significantly higher than all the other TOX samples. As with previous DBP analysis, the differences in TOX concentrations may be caused by the differences in experimental days. Additional analysis of the TOX data may reveal potential causes for discrepancies between the results from the different NOM sample groups.

Generally, the concentrations for each type of DBP were similar within the different NOM groups. UF1X and 1X samples were comparable, concentrate and reconstituted concentrate samples were similar, and 250X samples were comparable with each other and 1X samples. Differences in DBP concentrations between the NOM groups were observable but these were expected based on DBP formation results from previous efforts showing that 1X samples compare well with other 1X samples and concentrates with concentrates, but 1X samples and concentrate samples do not closely compare (Pressman et al. 2012). However, the comparability between 250X samples and 1X samples does not agree with previous research efforts using RO concentrated NOM and lyophilized, reconstituted NOM (Pressman et al. 2010; Pressman et al. 2012). According to the overall DBP results, the reconstituted 1X NOM is generally representative of the UF1X regardless of RO concentration factor. Furthermore, the
similarity between concentrate and reconstituted concentrate DBP concentrations suggest that the NOM solid may be used as a surrogate for aqueous concentrated NOM regardless of RO concentration factor. As had been previously suggested by McCurry et al. (2012) based on TOC data, the current DBP results show NOM can be reconstituted to a concentration factor higher than RO concentrate collection. The DBP concentrations suggest that NOM may be concentrated at any factor between 50X to 250X, lyophilized, and reconstituted back to source concentrate conditions, 1X TOC conditions pertinent for water treatment studies, and 250X TOC concentrations benefiting health effects studies.

The prevalence of halogenated DBPs compares well with the humic characterization of the NOM, at every TOC concentration factor, determined from both HPLC-SEC and fluorescence spectroscopy analysis. For THM and HAA9, the 1X reconstituted samples compare well with the original source water (UF1X). Because the 1X reconstituted samples were produced from five different concentration factors, the data demonstrates that there is no particular advantage to selecting one concentration factor over another when using the NOM at the original source water concentrations. Similarly, at 250X, four reconstructions resulted in similar results, but did not match well with the 250XRECON or 250XCONC (both completed on a different day). Potential explanations aside, the data implies there is no benefit to selecting one concentration factor over another prior to reconstituting lyophilized NOM up to 250X. Furthermore, the DBPs produced in reconstituted samples at the five different concentration factors compared with the original concentrates, demonstrating overall that any losses in NOM across concentration and lyophilization appear equally for all concentration factors. Overall, while the DBP results were not perfect, they support the primary objectives of determining the optimal (or lack thereof) RO concentration factor while comparing to reconstituted, lyophilized
material and further validating the RO concentration, lyophilization, and reconstitution processes for both water treatment and health effects studies.
Conclusions

Ohio River NOM was concentrated at five different concentration factors with expected TOC and UV$_{254}$ recoveries. HPLC-SEC data revealed a single peak for UF1X, 50XRECON, 100XRECON, 150XRECON, 200XRECON, and 250XRECON indicating that the size distribution of the dissolved organic matter did not change as RO concentration factor was increased. Furthermore, sulfate removal, lyophilization, and reconstitution did not affect the size distribution either. The constant dissolved particle size distribution resulting from the increasing concentration factors demonstrated that any losses in NOM throughout the processes affected the whole NOM mixture equally. Principle component analysis of fluorescence EEMs showed only a single EEM peak was produced across all four sample sets (1X, RECON, CONC, and RECON250X), further demonstrating that NOM losses during concentration, post processing, lyophilization, and reconstitution were small amounts of whole mixtures and not specific fractions. Therefore NOM should be able to be concentrated with RO and reconstituted at multiple concentration factors without changing the nature of the NOM. Although requiring additional analysis, DBP concentrations produced from chlorinating aqueous RO concentrate and lyophilized NOM reconstituted to source TOC concentrations were similar suggesting that the lyophilized material may be used as a surrogate for its aqueous source regardless of RO concentration factor. Furthermore, the DBP concentrations for the 1X reconstituted NOM validate its use in drinking water research by comparing well with source water DBP concentrations. An optimal RO concentration factor was not evident based on NOM characterization and suggested by DBP concentrations resulting from chlorination. RO concentrate collection can occur between 50X and 250X resulting in an aqueous solution that may be lyophilized and reconstituted back to concentrate TOC conditions, source 1X TOC.
conditions relating to water treatment studies, and 250X TOC conditions valuable for health effects studies.
**Future Research**

Fluorescence characterization and HPLC-SEC analysis of Ohio River NOM demonstrates that the NOM does not change regardless of RO or reconstitution concentration factor based on. Furthermore, the consistent characterization validates the processes of RO concentration, lyophilization, and reconstitution producing NOM sources for drinking water research and health effects studies. Results for DBP formations with concentrate and reconstituted NOM were as expected based on previous research efforts (Pressman et al. 2012; Pressman et al. 2010). However, the discrepancies between DBP concentrations in the CONC, RECON, and RECON250X samples were greater than expected. Additionally, similarities in DBP concentrations with the 1X and RECON250X samples were not expected. The DBP formation data analysis would benefit from statistical analysis along with examining the experimental methods, which occurred during different days, and that of sample measurements. Discrepancies in data points may further be explained through additional statistical analysis or with the examination of experimentation and sample testing methods.
Figure 7 – Schematic of the experimental design. Reverse osmosis concentrated NOM was collected at five concentration factors (in red), lyophilized, and reconstituted to the same five concentration factors (in green). The reconstituted NOM was then diluted to match the UF1X (in blue). The UF1X was also freeze-dried and reconstituted. Finally, the lyophilized NOM was reconstituted to 250XCONC conditions (in black).
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<th>150XCONC</th>
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<td>Sodium (mg/L)</td>
<td>42.15</td>
<td>7.89</td>
<td>2.44</td>
<td>157</td>
<td>156</td>
<td>155</td>
</tr>
<tr>
<td>Strontium (mg/L)</td>
<td>0.225</td>
<td>0.019</td>
<td>0.012</td>
<td>1.56</td>
<td>0.012</td>
<td>1.53</td>
</tr>
<tr>
<td>Sulfate (mg/L)</td>
<td>78.45</td>
<td>33.47</td>
<td>9.73</td>
<td>14.20</td>
<td>16.17</td>
<td>19.92</td>
</tr>
<tr>
<td>Tin (mg/L)</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>&lt;0.1</td>
<td>7.97</td>
<td>7.85</td>
<td>7.73</td>
</tr>
<tr>
<td>Vanadium (mg/L)</td>
<td>&lt;0.001</td>
<td>0.007</td>
<td>0.010</td>
<td>1.58</td>
<td>0.010</td>
<td>1.53</td>
</tr>
<tr>
<td>Zinc (mg/L)</td>
<td>0.002</td>
<td>0.068</td>
<td>0.038</td>
<td>1.53</td>
<td>1.49</td>
<td>1.52</td>
</tr>
</tbody>
</table>
Figure 8 – Total organic carbon (TOC) for the 1X RECON (A), CONC and RECON (B), and 250X RECON (C). All samples pH adjusted to match the UF1X.
Figure 9 – High-performance liquid chromatography (HPLC-SEC) with UV$_{254}$ detection for the RECON1X NOM. All samples were pH adjusted to match the UF1X.
Figure 10 – Principle component EEMs resulting from PARAFAC analysis for the UF1X and RECON1X NOM (A), CONC for the multiple concentration factors (B), RECON for the multiple concentration factors (C), and RECON250X (D). All samples pH adjusted to match the UF1X and normalized by TOC concentration factor.
Table 5- Summary of principle components for the multiple concentration factors and the various NOM reconstitutions.

<table>
<thead>
<tr>
<th>Sources</th>
<th>EEM Principle Component Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>UF1X and RECON1X</td>
<td>Emission (nm) 355</td>
</tr>
<tr>
<td>CONC</td>
<td>450</td>
</tr>
<tr>
<td>RECON</td>
<td>458</td>
</tr>
<tr>
<td>RECON250X</td>
<td>456</td>
</tr>
<tr>
<td></td>
<td>Excitation (nm) 360</td>
</tr>
</tbody>
</table>
Figure 11 – Trihalomethanes (THM) (A), haloacetic acids (HAA9) (B), haloacetonitriles (HAN4) (C), haloketones (HK2) (D), and total organic halogen (TOX) (E) for the various NOM sources. All samples were pH adjusted and chlorinated following Uniform Formation Conditions. Missing values were reported as being below instrument detection limits. Results have been normalized by TOC concentration factor.
Conclusions

Fluorescence EEMs in addition to FI and HIX values indicated that the characterization of the Ohio River remained relatively constant during the 15-month NOM characterization study. The locations of principle components from the EEMs did not change while small variations in UV$_{254}$, TOC, FI, HIX, and elemental analysis demonstrated seasonal variability in NOM. However, these were not significant enough to change its characterization across the 15 months of NOM collection. Concentrating Ohio River NOM using RO, lyophilizing the resulting liquid, and reconstituting the solid material had no effect on fluorescence and HPLC-SEC NOM characterization. Moreover, the consistent NOM characterization suggested that NOM losses occurring during RO concentration, sulfate removal, lyophilization, and reconstitution were not to specific NOM fractions but rather small amounts of whole NOM mixtures. The same two principle components in the fluorescence EEMs were evident throughout the NOM treatment process. Seasonal variations of the source NOM were illustrated in monthly fluorescence data and demonstrated that the source NOM did not greatly change over time. Fluorescence EEM peak locations shifted during the summer suggesting an influence from the precipitation events that occurred during each season. The FI and HIX data supplemented the lack of overall change in NOM characterization while expressing seasonality in the source NOM, further relating to the type of precipitation that occurred during each season of NOM collection. HPLC-SEC data exhibited a single peak for both UF1X and RECON across all months further indicating a lack of fractional NOM losses during concentration and lyophilization. Elemental analysis of the lyophilized NOM expressed oxygen-to-carbon ratios that fell within previously established analyses for humic and soil humic acids agreeing with results from fluorescence spectroscopy and HPLC-SEC analysis. A unique library of Ohio River NOM was created over 15 months and
it was demonstrated that the characterization, using multiple advanced techniques, of the lyophilized material comprising the NOM library represents humic-like, allochthonous influenced NOM expressing minor seasonal fluctuations of the source water. The temporal library of well-characterized, standardized Ohio River NOM is the first of its kind leading to beneficial drinking water treatment and research studies.

Additionally, Ohio River NOM was concentrated at five different concentration factors with expected TOC and UV\textsubscript{254} recoveries. HPLC-SEC data revealed a single peak for UF1X, 50XRECON1X, 100XRECON1X, 150XRECON1X, 200XRECON1X, and 250XRECON1X. HPLC-SEC data indicated that the size distribution of the dissolved organic matter did not change as RO concentration factor was increased. Furthermore, lyophilization and reconstitution also did not affect the size distribution of dissolved particles. The consistent size distribution resulting from the increasing concentration factors demonstrated that any losses in NOM throughout the processes affected the whole NOM mixture equally. Principle component analysis of fluorescence EEMs demonstrated only a single EEM peak across all four sample sets (UF1X and reconstituted 1X, concentrate, reconstituted concentrate, and reconstituted 250X), further demonstrating that NOM losses during concentration, post processing, lyophilization, and reconstitution were small amounts of whole NOM mixtures and not specific fractions. Therefore NOM should be able to be concentrated with RO and reconstituted at multiple concentration factors without changing the nature of the NOM. Although requiring additional analysis, DBP concentrations produced from chlorinating aqueous RO concentrate and lyophilized NOM, reconstituted to source concentrate TOC concentrations, were appeared to be similar suggesting that the lyophilized material may be used as a surrogate for its aqueous source regardless of RO concentration factor. Furthermore, the DBP concentrations for the 1X reconstituted NOM
potentially validated its use in drinking water research by comparing well with source water (UF1X) DBP concentrations. An optimal RO concentration factor was not evident based on NOM characterization and DBP concentrations resulting from chlorination. NOM can be concentrated with RO, between 50X and 250X concentration factors, resulting in an aqueous solution that may be lyophilized and reconstituted back to concentrate TOC conditions, source 1X TOC conditions relating to water treatment studies, and 250X TOC conditions valuable for health affects studies.
Future Research

The temporal library of well-characterized, lyophilized NOM was created from the Ohio River. This library benefits drinking water research and health effects studies for the area surrounding the source input. Multiple libraries, from multiple surface and ground water sources, would benefit drinking water and health effects studies on a more global level. NOM from different geographical regions would allow for the continuation of drinking water research in order to understand how NOM is affected in different weather conditions along with climate changes. Furthermore, additional analysis of DBP results would allow for the determination of optimal RO concentration factor conditions compared to NOM reconstitution concentration factor. Discrepancies in data DBP points may further be explained through statistical analysis or the examination of experimental and sample testing methods allowing for the continuation in creating NOM libraries from multiple sources outside of the previously analyzed Ohio River area.
References


American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF) (2012). Standard Methods For the Examination of Water and Wastewater, Amerian Public Health Association, Washington, DC.


GCWW (2012). "E-mail correspondence with Greater Cincinnati Water Works."


Appendices

Appendix 1

The 24-hour free chlorine data examining UF1X chlorinated with multiple chlorine to TOC ratios.
Appendix 2

Free chlorine calibration curve for the Nicolet Evolution 300 UV Spec and Hach DPD powder packets

\[ y = 0.2276x + 0.0007 \]

\[ R^2 = 0.9991 \]