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Seasonal and Spatial Variations in Chemical Composition and Fluxes of Dissolved Organic Matter and Nutrients in the Lower Milwaukee River

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SEASONAL AND SPATIAL VARIATIONS IN CHEMICAL COMPOSITION AND FLUXES
OF DISSOLVED ORGANIC MATTER AND NUTRIENTS IN THE
LOWER MILWAUKEE RIVER

by

Tarek A Teber

A Thesis Submitted in

Partial Fulfillment of the

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in Freshwater Sciences and Technology

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ABSTRACT

SEASONAL AND SPATIAL VARIATIONS IN CHEMICAL COMPOSITION AND FLUXES OF DISSOLVED ORGANIC MATTER AND NUTRIENTS IN THE LOWER MILWAUKEE RIVER

by

Tarek A Teber

The University of Wisconsin-Milwaukee, 2016
Under the Supervision of Professor Laodong Guo

Physical, chemical and biological processes directly influence the transport, composition, and fluxes of dissolved organic matter (DOM) in river watersheds. Changes in the abundance and composition of DOM and nutrients (P&N) in the watershed should reflect changes in hydrological cycle, effluent discharge, land-use and land-cover, and anthropogenic activities in the river basin, especially in rivers that run through metropolitan areas such as the Milwaukee River. Despite the importance of DOM to ecosystem health and function, a literature search to date finds no comprehensive accounting of DOM in the Milwaukee River. To examine DOM dynamics, monthly water samples were collected between February 2014 and April 2015 for the measurements of hydrographic parameters, bulk dissolved organic carbon (DOC), chromophoric-DOM and fluorescent-DOM to determine temporal variations in source and composition of DOM in the lower Milwaukee River and fluxes of DOM to Lake Michigan, as well as influence of human activities in the river basin.

Concentrations of DOC varied from 336 $\mu\text{M-C}$ during the winter under ice cover to 1,146 $\mu\text{M-C}$ during the snowmelt in early spring, with an average of $726 \pm 224 \mu\text{M-C}$. DOC abundance in the Milwaukee River was found to be relatively high in comparison to other world rivers.

Absorption coefficients at 254 nm (a_{254}) averaged $71 \pm 31 \text{ m}^{-1}$, and show a significant correlation with DOC ($R^2=0.8370$). Non-chromophoric DOC represented approximately 33% of the bulk DOC in the river, and majority of DOC (67%) was optically active. Specific ultra violet absorbance at 254 nm ($SUVA_{254}$) and spectral slope ($S_{275-295}$) were inversely correlated with an average of $3.6 \pm 0.7 \text{ L mg-C}^{-1} \text{ m}^{-1}$ and $0.0170 \pm 0.0034 \text{ nm}^{-1}$ respectively. $SUVA_{254}$ and $S_{275-295}$ were mostly controlled by hydrological, biological and degradation processes. Applications of parallel factor (PARAFAC) modeling on EEMs data identified three major fluorescent DOM components (C1, C2 and C3) in the river waters, including two terrestrial humic-like components (C1 and C2) and one protein-like component (C3).

In addition to DOM, variations in abundance, chemical speciation, and export fluxes of nutrients (P&N) from the lower Milwaukee River, as well as the influence of hydrology and anthropogenic activities, also have been studied. Nutrient species including nitrate (NO_3^-), dissolved organic nitrogen (DON), dissolved inorganic phosphorus (DIP) or phosphate, dissolved organic phosphorus (DOP), particulate inorganic phosphorus (PIP) and particulate organic phosphorus (POP) were measured. Overall, NO_3^- was the predominant dissolved N species and DON contributed up to ~30% of the total dissolved N transported in the river. Concentrations of NO_3^- ranged from $62 \mu\text{M-N}$ during the warmer season in spring and summer to $259 \mu\text{M-N}$ in the colder months in winter with an average of $125 \pm 60 \mu\text{M-N}$, suggesting that NO_3^- was predominately controlled by biological uptake and hydrology. Average NO_3^- concentration during 2014-2015 was among the highest in comparison with major world rivers, reflecting the anthropogenic impact on nutrient abundances in the Milwaukee River potentially through agricultural activities. Similar to DOC seasonal variations, DON varied from $2 \mu\text{M-N}$ under ice in winter to $254 \mu\text{M-N}$ during the spring snowmelt with an average of $54 \pm 50 \mu\text{M-N}$.

Within the total dissolved phosphorus (TDP) pool, almost 50% were organic phosphorus (DOP) and the other 50% were inorganic (DIP), indicating a potential role for DOP in nutrient cycling. Phosphorus exported from the Milwaukee River to Lake Michigan coastal zone was predominately in the dissolved form (DIP+DOP represented 62% of total P). DIP exhibited large seasonal variability ranging from 0.114 $\mu\text{M-P}$ in January 2015 under the ice to as high as 2.75 $\mu\text{M-P}$ during April 2015 storm event, with an average of $0.970 \pm 0.791 \mu\text{M-P}$. DOP varied from 0.080 $\mu\text{M-P}$ when discharge was high to 4.687 $\mu\text{M-P}$ during the summer when discharge is low and primary productivity is intense, with an average of $1.120 \pm 0.990 \mu\text{M-P}$. PIP and POP abundances were mainly controlled by hydrology. They were low under ice and very high during the April 2015 storm event and uniquely much lower than those of other world rivers. PIP varied from 0.045 to 2.229 $\mu\text{M-P}$ with an average of $0.473 \pm 0.483 \mu\text{M-P}$ and POP varied from 0.224 to 3.791 $\mu\text{M-P}$ with an average of $0.809 \pm 0.648 \mu\text{M-P}$. The average N/P ratios in inorganic and organic nutrient pools are 151 ± 84 and 80 ± 67 respectively, indicating a N- enriched but P-depleted ecosystem in the lower Milwaukee River. Using the interpolation method of regression, the annual export fluxes of DIP, DOP, PIP, POP, NO_3^- , DON and DOC were 63, 14, 47, 64, 1325, 200 and 6710 ($10^3 \text{ kg-P, N or C}$) respectively and the yields were 28, 6, 21, 28, 581, 88 and 2943 ($\text{kg-P, N or C km}^{-2}$) respectively.

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1. Introduction

1.1 Dissolved organic matter

Dissolved organic matter (DOM) is a complex mixture of organic materials with different sources and composition (Huguet et al. 2010). DOM transport in river systems is associated with the mobility and environmental fate of nutrients (C, N and P) and trace metals and plays a key role in the transformation and reactivity of persistent organic pollutants (POP's) such as polyaromatic hydrocarbons (PAH's) and polychlorinated biphenyls (PCB's), and emerging organic pollutants (EOP's) such as pharmaceutical and personal care products (PPCP's). Changes in DOM abundance and composition in the watershed should reflect changes in hydrological cycle, effluent discharge, land-use and land-cover, and anthropogenic activities in the river basin, especially in rivers that run through metropolitan areas such as the Milwaukee River. Chromophoric dissolved organic matter (CDOM) is the fraction of DOM that absorbs UV-visible light and/or fluoresces. Advances in spectroscopic techniques and statistical modeling, parallel factor analysis (PARAFAC), allow the use of CDOM as a proxy for DOM characterization.

The lower Milwaukee River has been an area of concern due to anthropogenic influence for decades. However, the quantity and quality of DOM exported from the Milwaukee River to Lake Michigan coastal area remain poorly understood but is an important aspect in determining ecosystem health. Fluorescence and UV-Vis spectroscopy have been widely used to study the DOM dynamics in aquatic systems. However, application of these techniques to the Milwaukee River system are scarce. In addition, there are very few studies that report the abundance of dissolved organic carbon (DOC) in the Milwaukee River basin. These studies lack in-depth focus and time-series dataset on DOM (e.g. Shafer et al 1997, Hurley et al. 1996 and Steuer et al.

1999). A thorough understanding of the sources and effects of seasonal dynamics on the abundance, composition and fluxes of DOM in the Milwaukee River can potentially support policies aimed at bringing about critical change in the health of freshwater systems.

The purpose of this first chapter is to establish a thorough comprehension of the seasonal and spatial variations in the abundance, composition and fluxes of DOM and their controlling factors in the lower Milwaukee River.

1.2 Nutrients: phosphorus and nitrogen

Nitrogen (N) and phosphorus (P) are essential nutrients to freshwater organisms. They play a critical role in regulating primary production, water quality and ecosystem health (Guo et al. 2004; DeAngelis 2012). Phosphorus, in most cases, is the limiting nutrient in aquatic environments (Finlay et al. 2013 and Lin & Guo 2016). However accelerated input of bioavailable phosphorus and nitrogen through anthropogenic activities may lead to degradation of water quality and catastrophic environmental issues (Rabalais et al. 2002, Hagy et al. 2004, Yates and Johnes 2013). Nutrients' various forms and phases have different biogeochemical reactivity and fates in aquatic environments. Change in the stoichiometry of the bioavailable forms of nutrients may occur while the total nutrient loads remain constant (Lin et al. 2015).

Recently local government agencies and the scientific community expressed concerns about excessive *Cladophora* growth in the near-shore area of Lake Michigan, which can harbor pathogens and result in costly beach closures (Bootsma et al. 2008). In many previous studies and government monitoring programs, only total P and N are measured without an in depth focus on nutrient species and their fluxes in natural waters (e.g. Finlay et al. 2013). The biogeochemical dynamics of nutrients in the Milwaukee River including abundance, chemical and phase speciation, and fluxes remain unclear to date.

The purpose of this second chapter is to examine in depth the seasonal variations in the abundance, chemical speciation and fluxes of nutrients (P & N) in the lower Milwaukee River.

2. Chapter I: Seasonal and spatial variations in the abundance, composition and fluxes of dissolved organic matter in the lower Milwaukee River system

2.1 Background

Dissolved organic matter (DOM) is the product of degradation of plants, animals and microorganisms (Huguet et al. 2010). It is a heterogeneous and poorly understood mixture of organic materials that often originate from allochthonous (e.g. soil leaching, surface runoff) and autochthonous (e.g. *in situ* primary production) sources in water systems (Laird & Scavia 1990). Almost 70% of surface water DOM has not been characterized by molecular structure, mainly due to its chemical complexity and low abundance in each organic compound class (Guo and Santschi 2007; Andrade et al. 2013). Different classes of organic compounds (aliphatic and aromatic) within the DOM pool will have various ecological roles and fates in aquatic systems (Maie et al. 2014). A fraction of the bulk DOM pool is highly reactive and affects aquatic ecosystems by controlling microbial food webs and biogeochemical cycling, such as binding with hydrous metal oxides and serving as electron shuttles under anoxic conditions (Jaffé et al. 2012). DOM controls the mobility and bioavailability of associated carbon, nitrogen, phosphorous and trace metals (e.g., Guo et al., 2001 and Hestir et al. 2015). It is a critical player in the carbon cycle through carbon transport from terrestrial to aquatic systems (Battin et al. 2008). Biological (heterotrophic oxidation) and photochemical degradation of DOM is linked to the production of CO₂ (g) in aquatic systems, which contributes to atmospheric CO₂ (g) thus altering the global carbon budget and ultimately the climate (Mayorga et al. 2005). Accumulation or an excess of DOM in the water column is one of the major causes of water

quality and ecosystem health degradation, since a significant fraction of DOM (12–56%) could be bioavailable and it is estimated that DOM contains 1-3% nitrogen and 0.2% phosphorus (Guo et al. 1999, Kawahigashi et al. 2004 and Dillon & Molot 1997). DOM also plays an important role in the fate and transport of persistent (POP's) such as polychlorinated biphenyls (PCB's) and polyaromatic hydrocarbons (PAH's), and emerging organic pollutants (EOP's) such as pharmaceuticals and personal care products (PPCP's) (Veith et al. 1971 and Ferguson et al. 2013).

Chromophoric dissolved organic matter (CDOM) is a fraction of the DOM pool that absorbs UV-visible light and/or fluoresces and in aquatic systems, CDOM protects benthic microorganisms against UV radiation and is capable of affecting primary production by absorbing UV-visible energy needed for photosynthesis (Laurion et al. 2000 and Williamson et al. 1996). Fluorescent DOM (FDOM) is the sub-fraction of CDOM with fluorescent properties. Advances in spectroscopic techniques and parallel factor modeling (PARAFAC) allow the use of CDOM/FDOM as a proxy for the bulk DOM pool to determine its major components, abundance, composition and sources (e.g., Stedmon & Bro 2008 and Zhou et al. 2013).

Changes in hydrology, effluent discharge, land-use and land-cover, and anthropogenic activities in the river basin should reflect changes in DOM abundance and composition and fluxes, especially in rivers that run through metropolitan areas such as the Milwaukee River. Changes of DOM quantity and quality may have relevant effects on the biogeochemical cycling, ecosystems health and water quality in the Milwaukee River, near shore Lake Michigan and beyond (Hiriart-Baer et al. 2008). Past studies involving DOM in the Milwaukee River watershed focused on the interaction of dissolved organic carbon (DOC) with trace metals (e.g. Shafer et al. 1997 and Hurley et al. 1996) and phase partitioning of POP's within the DOC,

colloidal organic carbon (COC) and particulate organic carbon (POC) pools (e.g. Steuer et al. 1999) without an in depth focus on DOM characterization and transport. Therefore, knowledge of the abundance, composition and fluxes of DOM in the Milwaukee River is important for better understanding the fate and transport of emerging contaminants, toxic metals and POP's in this river basin.

To date, a literature search finds no comprehensive study of bulk DOC or CDOM in the Milwaukee River system. The goal of this study is to establish a baseline dataset and a thorough comprehension of the sources and effects of seasonal and spatial dynamics on the abundance, composition and fluxes of DOM in the Milwaukee River, which can potentially support policy aimed at bringing about critical change to the health of regional and global freshwater systems.

2.2 Hypotheses and objectives

The hypotheses of this study were 1) variations in DOC abundance and composition should be predominately correlated to variations in the hydrological cycle in the Milwaukee River watershed, 2) DOM in the Milwaukee River should be mostly chromophoric and terrigenous in nature, and 3) DOC fluxes during the spring season (ice melt, high rainfall and storm events) should represent a large percentage of the total DOC annual flux to Lake Michigan.

The objectives of this study include 1) to determine DOC abundance, water isotopes and hydrographic parameters at different seasons and during storm events, 2) to elucidate seasonal and spatial variations in CDOM using both fluorescence and UV-Vis spectroscopy, 3) to examine the interrelationship among DOC, CDOM, discharge, water isotopes and other environmental parameters, and 4) to quantitatively estimate the seasonal and annual export fluxes of dissolved organic carbon and DOM components.

2.3 Methods

2.3.1 Study area and sampling site (chapter I and II)

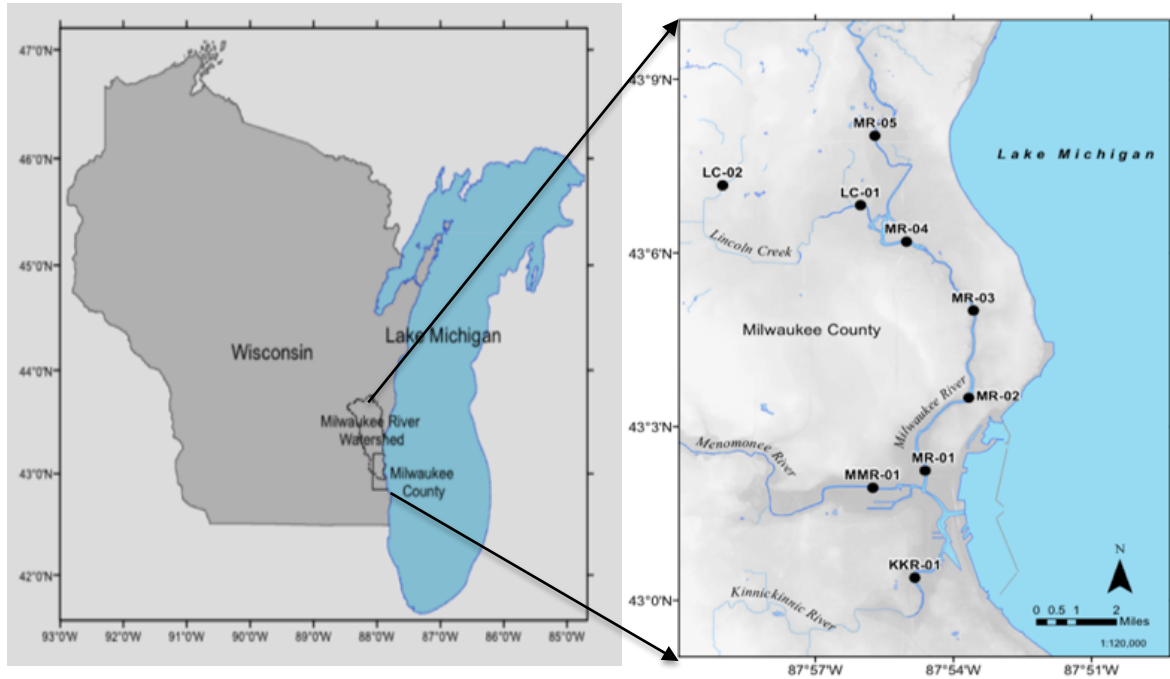


Figure 1. A map of the study area and sampling sites in the lower Milwaukee River during 2014-2015.

The Milwaukee River watershed covers an area of approximately 1813 square Kilometers. The main stem of the Milwaukee River originates in southeastern Fond du Lac County and flows approximately 163 kilometers before it discharges in Lake Michigan in the City of Milwaukee, Milwaukee County, Wisconsin (Figure 1). Most of the river basin is rural and dominated by agricultural land uses. Even though the Milwaukee metropolitan area is highly populated, only 18% of the river basin is developed. Seven percent of the basin is made up of surface water and wetlands and approximately 55% is farmland (see also www.SEWRPC.org).

Sampling sites along the lower Milwaukee River are shown in Figure 1. Monthly water

samples were collected at both MR-01, a downstream station near the delta junction in the Milwaukee River estuary, and MR-05, an upper stream station representing only natural surface runoff, between February 2014 and April 2015 (Figure 1). Transectional sampling along the river was performed on April 10, 2015 (high discharge season) and November 13, 2014 (low flow season) and involved the monthly sampling sites (MR-01 and MR-05), MR-02, MR-03 and MR-04. End-members sampling sites included LC-01, LC-02, MMR-01, KKR-01 (April 10, 2015) and MMR-01, KKR-01 (November 13, 2014) (Figure 1).

2.3.2 Sample collection

Samples were collected and transferred to acid cleaned HDPE (high density polyethylene) bottles and kept on ice at 4 C° in the dark while in transport to the laboratory. At the lab, samples were filtered using a pre-combusted (550 C° for 5 hours) 0.7 µm GF/F filter (Whatman) for DOM analysis and 0.4 µm polycarbonate filters (Whatman) for nutrients analysis using an acid cleaned vacuum filtration apparatus. Samples and filters were refrigerated until analysis. For DOC measurements, samples were kept in pre-combusted glass vials and acidified with concentrated HCl to a pH < 2. At this pH, all inorganic carbon species were converted to CO₂ (g) and escape from the sample during sparging before DOC analysis (next section). DOM photo-degradation and biodegradation are key issues to this analysis, so samples were processed expediently (Dixon et al. 2014). Water samples were filtered immediately right after sample collection and DOC measurements were performed within one week (Zhou et al. 2013 and DeVilbiss et al. 2016).

2.3.3 Measurements of dissolved organic carbon (DOC)

To compare the dynamics of DOC and CDOM, DOC concentrations were determined using a Shimadzu TOC-L VCPN analyzer. Two drops of concentrated HCL were added to the

samples to bring pH <2 to convert all the dissolved inorganic carbon (DIC) to CO₂ (g). Samples were purged with CO₂-free air for 5 minutes to make sure that all CO₂ (g) is removed prior analysis. Three to five replicate measurements were made with a coefficient of variance of <2%. Internal standards from certified DOC sample (Hansell Laboratory, University of Miami) were measured every eight samples for QA and QC (Zhou and Guo 2012).

2.3.4 Measurements of UV-visible absorption

Absorption spectra of CDOM were measured using a UV-VIS spectrophotometer (Agilent 8453). Similar to the fluorescence measurements, samples were diluted with E-pure water to an absorbance (A) of ≤ 0.02 at 260 nm to minimize inner-filtering effects. A one cm quartz cuvette was used to scan water samples with increments of 1 nm over a wavelength range from 200 to 800 nm. Water blanks were measured and the refractive index was corrected for by subtracting the averaged absorbance of the blank between 650 and 800 nm (Zhou et al. 2015). Absorption spectroscopic parameters including absorption coefficient at 254 nm (a_{254}), specific UV absorbance at 254 nm ($SUVA_{254}$), and spectral slope between 275-295 nm ($S_{275-295}$) were further calculated. Specifically, absorption coefficient at 254 nm is calculated as $a_{254} = (A_{254} * 2.303) / L$, where A is absorbance and L is path length of cuvette used, and represents CDOM abundance. On the other hand, $SUVA_{254}$ was calculated as $A_{254} / 2.303 / [DOC]$ and is quantitative linked to DOM aromaticity. $S_{275-295}$ is the slope of the linear regression between 275-295 nm and can be used as a proxy for average DOM molecular weight (Helms et al. 2008, Zhou and Guo 2012).

2.3.5 Measurements of fluorescence EEMs and PARAFAC Modeling

A Horiba Fluoromax-4 spectrophotometer was used to scan the samples with an increment of 2 nm between 220 nm and 480 nm for excitation wavelengths and with 5 nm

intervals between 240 nm and 600 nm for emission wavelengths. A blank was scanned each day of the analysis to remove Raman scattering peaks from 3-D excitation-emission matrix (EEM) spectrums. Samples were diluted with E-pure water to an absorbance of ≤ 0.02 at 260 nm to minimize inner-filtering effects (Zhou et al. 2013). 3D-EEM spectra will locate the Coble peaks A, C, M, T and B that are important to CDOM characterization (Coble 2007). The locations of emission intensities are also used to calculate the humification index (HIX), the biological index (BIX) and the fluorescence index (FIX). FIX was calculated as the ratio of emission intensities at the wavelengths 450 nm and 500 nm with the excitation wavelength of 370 nm. BIX was calculated as the ratio of emission intensity at 380 nm divided by the emission intensity maximum between 420 nm and 435 nm at excitation wavelength of 310 nm. HIX was calculated using the area under the emission spectra between 435 to 480 nm divided by the peak area under the emission spectra between 300 and 345 nm and between 435 to 480 nm at excitation wavelength of 254 nm (McKnight et al. 2001).

These indices are a powerful tool to determine and understand the relative contribution from external and internal CDOM in the river (Zhou et al. 2015). FIX and aromaticity of the bulk DOM as well as the FIX and C/N ratio are inversely correlated. A BIX value of <0.6 will indicate that the bulk DOM has very little autochthonous organic matter. A BIX value between 0.8 and 1.0 is associated with freshly produced DOM from biological origin. Freshly produced DOM will have a HIX value of less than 5. When HIX is greater than 5, the DOM will be considered degraded. HIX is positively correlated to aromaticity and negatively correlated to carbohydrate concentration (Birdwell et al. 2010). To decompose the 3D-EEM spectrums into different fluorescent components without any assumption of their spectral shape and number, parallel factor analysis (PARAFAC) was used. PARAFAC modeling was processed using

MATLAB software and the DOMFlour Toolbox (Stedmon and Bro 2008). Sample EEM's were calibrated, corrected and normalized to maximum fluorescence intensity before analysis. A non-negativity outlier test was performed. To validate model component number, a split-half analysis was used. The fluorescence intensities of each component were quantified during the PARAFAC modeling analysis (Maie et al. 2014). PARAFAC analysis of 3D-EEM spectra will reveal individual fluorescent DOM components including terrestrial humic-like materials, aquatic humic-like materials and protein-like materials (Zhou et al. 2015).

2.3.6 Measurements of hydrographic and water quality parameters

All water quality and hydrographic parameters were measured including pH, alkalinity, temperature and specific conductivity and suspended particulate matter. In order to trace water sources, measurements of major elements/metals were done using methods for environmental samples by simultaneous axially viewed ICP-AES following U.S. EPA guidelines. Measurements of major anions were done using the Dionex ICS-1000 Ion Chromatography System with Ion-Pac AS14A exchange column with Standard Methods for the Examination of Water and Waste Water Method 4110B. Hydrogen and oxygen isotopic composition, including $\delta^{18}\text{O}$ and δD , was measured using a wavelength-scanned cavity ring-down spectroscopy, a water isotope analyzer (L1102-i) (Picarro Inc. Sunnyvale, Calif., USA).

2.3.7 Statistical analysis

Sigma-plot software (version 12.5) was used to perform T-Test significance for all data reported in chapters I and II. Study area and sampling sites maps for this project were generated using ArcGIS at UWM GIS center.

2.4 Results and discussion

2.4.1. Hydrographic and hydrological features of the lower Milwaukee River

Seasonal variations in hydrographic and water quality parameters are shown in Figure 2 and Appendix A. Data of total precipitation (cm) for the Milwaukee River basin from February 2014 to April 2015 were obtained from NOAA web interface at station USW00014839 (<http://www.ncdc.noaa.gov/ghcnd>) close to our sampling sites, while river discharge data were downloaded from USGS National Water Information System at USGS site #04087000 (<http://waterdata.usgs.gov/nwis>). From February 2014 to April 2015, the average discharge was $23.3 \pm 34.6 \text{ m}^3/\text{s}$, slightly higher than the averaged daily discharge during the past 100 years ($13 \text{ m}^3/\text{s}$) (USGS). River discharge peaked during the spring snowmelt ($37.07 \text{ m}^3/\text{s}$, April 2014) and remained approximately three factors lower during the rest of the sampling period. In April 2015 there was a significant storm event due to heavy rainfall combined with snowmelt, creating a remarkably significant discharge peak of $144 \text{ m}^3/\text{s}$ (Figure 2). These hydrological changes will eventually lead to variations in the abundance, composition and fluxes of dissolved organic matter (DOM) in the Milwaukee River. The river water temperature varied from $0.1 \text{ }^\circ\text{C}$ during ice formation in winter to $25.6 \text{ }^\circ\text{C}$ in summer. Generally, no ice cover was observed during the winter at the downstream sampling site (MR-01) near downtown Milwaukee, and its averaged water temperature ($9.8 \pm 9.0 \text{ }^\circ\text{C}$) was slightly higher than that at the upstream sampling site (MR-05, $8.9 \pm 8.7 \text{ }^\circ\text{C}$). The average pH in the Milwaukee River was 8.23 ± 0.22 , with the lowest value during high discharge events likely due to the dilution of low-pH rain and snow waters (pH~7). Similarly, specific conductivity in the Milwaukee River (average of $725 \pm 134 \text{ } \mu\text{S cm}^{-1}$) showed the lowest value during the storm event in April 2015 ($503 \text{ } \mu\text{S cm}^{-1}$) mainly due to dilution, while the highest value was observed during April 2014 after the snowmelt ($952 \text{ } \mu\text{S cm}^{-1}$).

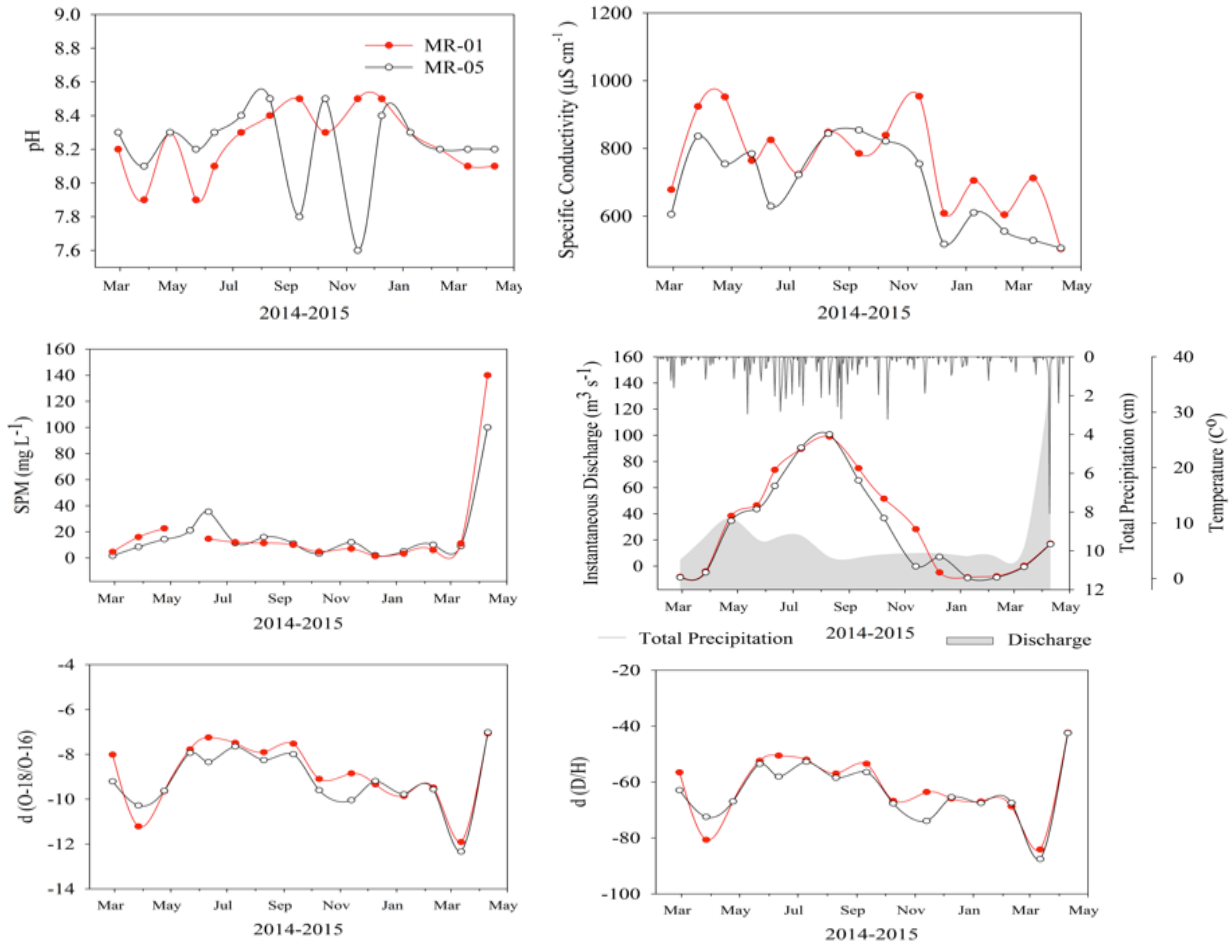


Figure 2. Seasonal variations in pH, specific conductivity ($\mu\text{S/cm}$), suspended particulate matter (mg/L), surface water temperature ($^{\circ}\text{C}$) and water stable isotopes composition ($\delta^{18}\text{O}$ and $\delta^2\text{H}$) at an upper stream station (MR-05) and a down stream station (MR-01) in the lower Milwaukee River during 2014-2015.

Values of both pH and specific conductivity in the Milwaukee River were relatively higher than those of world rivers. This appears to be highlighting the influence of limestone weathering on water chemistry of the basin (sewrpec.org). In contrast to the variations of pH and conductivity, suspended particulate matter (SPM) concentrations ranged from the 1.6 mg/L during ice formation in winter to 140 mg/L during storm event in April 2015, with an average of

18±30 mg/L, showing a positive correlation with river discharge ($R^2=0.917$ and $p<0.001$). Values of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ in river waters were the lightest during snowmelt events for both 2014 and 2015 with an average value of $-8.97\pm 1.37\text{‰}$ for $\delta^{18}\text{O}$ and $-62.74\pm 10.87\text{‰}$ for $\delta^2\text{H}$, indicating the influence of groundwater and low snow accumulation. Additionally, river water isotopic composition under the ice was similar to the averaged composition of groundwater end-member samples ($\delta^{18}\text{O} = -9.04\text{‰}$ and $\delta^2\text{H} = -59.83\text{‰}$).

2.4.2. Variations in DOC abundance in the lower Milwaukee River

Seasonal variations in DOC, CDOM and other derived optical parameters are shown in Figure 3 and Appendix B. Strong seasonality was observed in DOC abundance in both upstream (MR-05) and downstream (MR-01) stations. Concentrations of DOC at the downstream station varied greatly from 318 to 1102 $\mu\text{M-C}$ with an average of $704\pm 220 \mu\text{M-C}$. DOC upstream was slightly higher than that of downstream and ranged from 355 to 1189 $\mu\text{M-C}$ with average of $745\pm 248 \mu\text{M-C}$. There was not a statistically significant difference in DOC abundance between MR-01 and MR-05 ($p=0.633$) (Appendix B). At both sampling sites, DOC concentration peaked during the spring snowmelt ($Q_{\text{max}} = 37 \text{ m}^3/\text{s}$) and was higher than DOC concentrations during the April 2015 storm event ($Q=144 \text{ m}^3/\text{s}$), suggesting DOC sources from soil leaching and a dilution effect during storm events. DOC abundance declined in warmer months in the summer and fall and was the lowest under ice cover during the winter of 2014. This is likely related to enhanced photo-degradation during summer and increased groundwater input during base-flow seasons (Helms et al. 2013). In contrast to the downstream sampling site, the river upstream was mostly ice covered during the winter season. From November 2014 to February 2015, the DOC concentrations downstream were lower than upstream, suggesting a slight DOM exclusion effect during ice formation (Guo et al. 2012) and a small extent of dilution from low DOC ground

water and ice-melt waters along the lower Milwaukee River from station MR05 to MR01. It is also likely that the ice cover prevented terrestrial input of DOM and low temperature minimized *in situ* primary production.

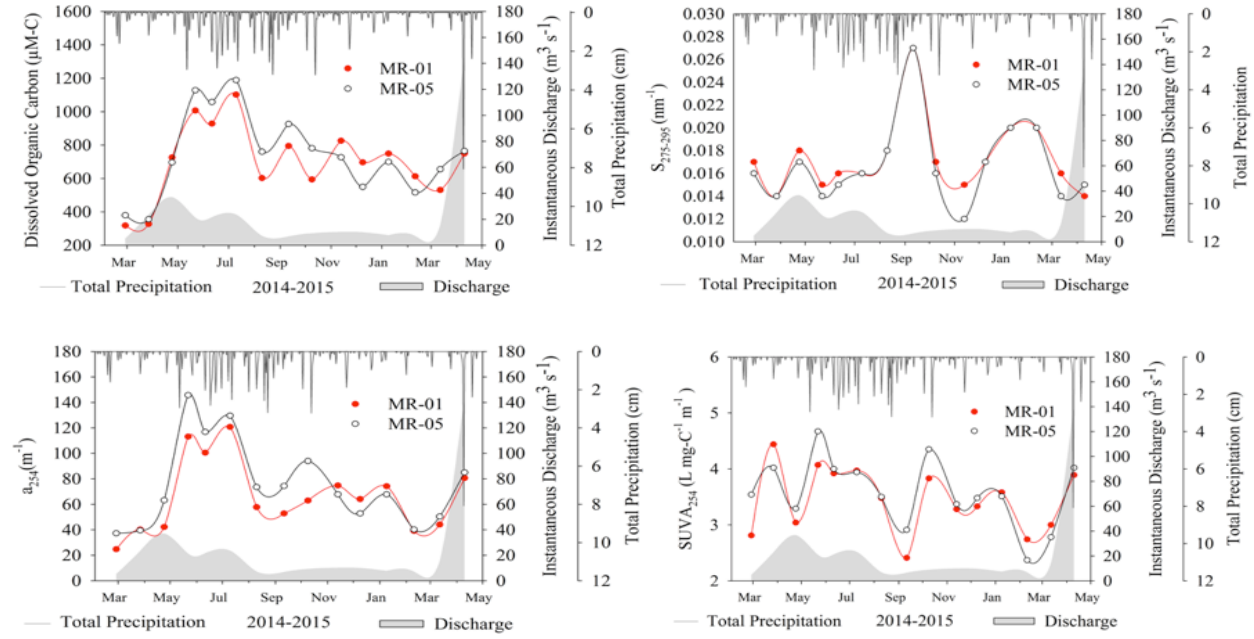


Figure 3. Seasonal and spatial variations of dissolved organic carbon (DOC, $\mu\text{M-C}$), absorption coefficient at 254 nm (a_{254} , m^{-1}), Specific UV absorbance at 254 nm (SUVA_{254} , $\text{L mg-C}^{-1} \text{m}^{-1}$) and Spectral slope between 275 and 295 nm ($S_{275-295}$, nm^{-1}) at an upstream (MR-05) and a downstream (MR-01) stations in the lower Milwaukee River during 2014-2015.

2.4.3. Seasonal and spatial variations in CDOM characteristics

CDOM absorbance has been used as a proxy for bulk DOC in many studies (e.g. Weishaar et al., 2003; DeVilbis et al. 2016; Zhou et al. 2013). In Milwaukee River waters, seasonal and spatial variations of the absorption coefficient at 254 nm (a_{254}) are consistent with seasonal and spatial variations of DOC. During 2014-2015, values of a_{254} at downstream station (MR-01) ranged from 24.7 to 120.8 m^{-1} with an average of $66.1 \pm 28.2 \text{ m}^{-1}$ and from 37.2 to 145.7 m^{-1} with an average of 75.9 ± 33.2 upstream. There was not a statistically significant difference in

a_{254} between upstream and downstream stations ($P = 0.507$). There was, however, a significant correlation between a_{254} and DOC in both upstream ($R^2 = 0.8799$; $p < 0.0001$) and downstream ($R^2 = 0.7875$; $p < 0.0001$) indicating similar DOM sources and CDOM can indeed be used a proxy for the bulk DOC. Non-chromophoric DOC, which can be derived from the intercept DOC value at zero absorbance or $= 0$ (DeVilbiss et al. 2016; Zhou et al. 2016), represented 38% of the bulk DOC downstream and 28% upstream confirming that DOM from upstream is more chromophoric than DOM downstream potentially due to degradation and anthropogenic input of DOM from the Milwaukee metropolitan area. On average, non-chromophoric DOC in the Milwaukee River during our sampling time period was 234 $\mu\text{M-C}$, comprising about 32% of the bulk DOC (Fig. 3), which is slightly lower than that observed for Lake Michigan and other lower Great Lakes (Zhou et al., 2016), supporting my hypothesis that DOM in Milwaukee River waters is mostly chromophoric in nature (over 68%).

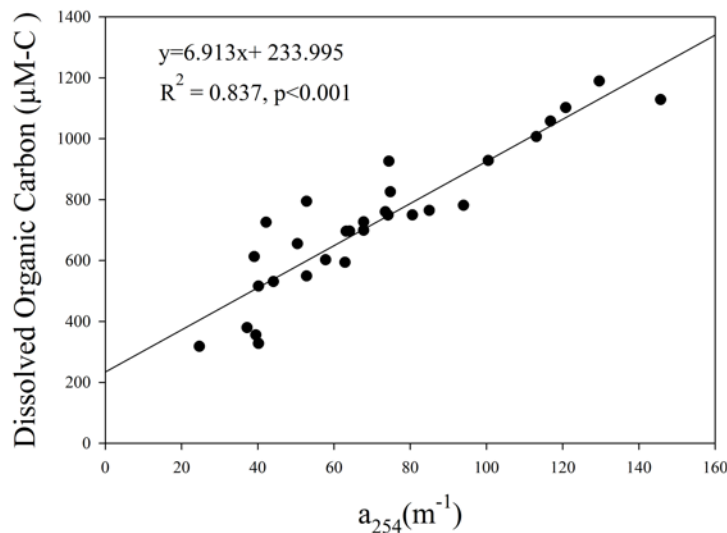


Figure 4. Correlation between DOC and absorption coefficient in the lower Milwaukee River waters during 2014-2015.

Specific UV absorbance at 254 nm ($SUVA_{254}$) is an indicator of bulk DOM aromaticity (Burns et al., 2014; Zhou et al 2013). In lower Milwaukee River waters, $SUVA_{254}$ showed strong seasonal variations that were controlled by water sources and discharge. Downstream $SUVA_{254}$ values varied from 2.4 to 4.4 L mg-C⁻¹ m⁻¹ with an average of 3.5 ± 0.6 L mg-C⁻¹ m⁻¹ while upstream (site MR-05) values varied from 2.4 to 4.7 L mg-C⁻¹ m⁻¹ with an average of 3.6 ± 0.7 L mg-C⁻¹ m⁻¹. There was not a statistically significant difference between $SUVA_{254}$ for both sampling sites during 2014-2015 ($P=0.550$). DOM aromaticity was the lowest during the winter under the ice and during the summer months likely due to the limited terrestrial DOM input in winter months and photochemical and microbial degradations caused by an increase in biological activity, longer residence time, and low suspended particulate matter (~ 12 mg/L) during the summer season. $SUVA_{254}$ was the highest during the spring melt event and relatively high during the April 2015 storm event as terrestrial DOM input peaked during these events, indicating riverine DOM during these sampling periods were mostly derived from fresh soil leachate.

Similar to $SUVA_{254}$, spectral slope between 275 and 295 nm ($S_{275-295}$) is another DOM characterization tool used to determine spatial and temporal variations in DOM molecular weight. Spectral slope has been shown to be negatively correlated to DOM molecular weight (i.e. low $S_{275-295}$ values correspond to higher DOM molecular weight, and vice versa) (Helms et al., 2008). During 2014-2015 sampling seasons, $S_{275-295}$ ranged from 0.0270 to 0.0140 nm⁻¹ with an average of 0.0173 ± 0.0032 nm⁻¹ in the downstream sampling site (MR-01) and from 0.0120 to 0.0270 nm⁻¹ with an average of 0.0167 ± 0.0035 nm⁻¹ in the upstream sampling site (MR-05) suggesting a higher molecular weight DOM pool in the upstream waters, consistent with its fresher DOM source at the upper stream station. However, there was not a statistically significant difference in the spectral slope values between upstream and downstream in the lower

Milwaukee river waters ($P=0.450$). Consistent with low $SUVA_{254}$, $S_{275-295}$ peaked in the late summer and early fall indicating a lower molecular weight and less aromatic bulk DOM pool, potentially due to high *in situ* production of low molecular weight DOM and photobiodegradation of allochthonous DOM in the river waters.

2.4.4. Seasonal variations in FDOM characteristics and fluorescence indices

Examples of EEMs at different seasons in the lower Milwaukee River during 2014-2015 are shown in Figure 5. Similar to many aquatic environments, the Milwaukee River EEM spectra included terrestrial humic-like peaks A and C and protein-like peak T. A protein-like peak T typically existed in all river sample EEM spectra, however, the high abundance of the terrestrial humic-like peaks A and C during high discharge period masked the protein-like peak T (Coble 2007). Excitation emission matrices (EEMs) showed similar trends for both the upstream and downstream sampling sites for all seasons (Figure 5).

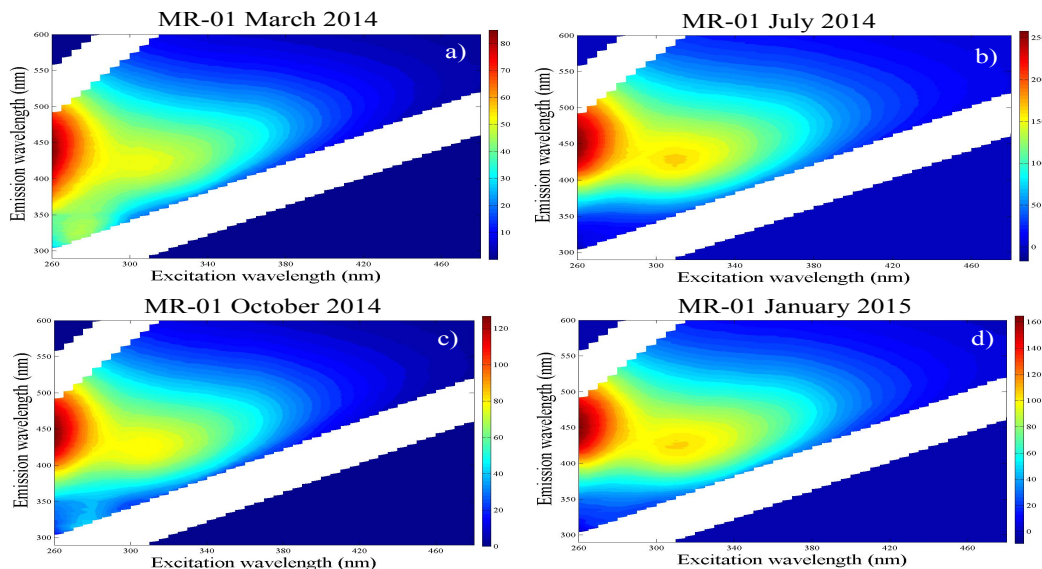


Figure 5. Examples of excitation-emission matrices (EEMs) at different seasons in the lower Milwaukee River during 2014-2015.

Based on EEMs fluorescence intensities data, the highest abundance of FDOM was found in the summer with a maximum of ~ 250 ppb-QSE likely due to an increase in primary productivity and heavy rainfall in warmer months (note that maximum fluorescence intensity is different for each EEM in Figure 5). Limited sources of FDOM (~90 ppb-QSE) were observed before the spring melt event. In winter months, groundwater was the dominant source for the Milwaukee River as a result of ice cover, which limited primary productivity.

FIX values provide information about the source of DOM such as microbial or terrestrial plant material as well as the degree of degradation of bulk DOM (Mcknight et al. 2001). FIX ranged from 1.13 to 1.28 with an average of 1.19 ± 0.05 upstream and from 1.15 to 1.24 with an average of 1.19 ± 0.03 downstream ($p = 0.719$). In both sites FIX values are less than 1.4 indicating that the majority of the bulk DOM in the river was from terrestrial origin. BIX represents biological or autochthonous DOM with values between 0.8 and 1 indicating freshly produced DOM of biological or microbial origin and values below 0.6 is indicating minimal autochthonous DOM in the bulk DOM (Birdwell et al. 2010). BIX values in the river varied from 0.55 to 0.71 with an average of 0.64 ± 0.05 upstream and from 0.56 to 0.73 with an average 0.65 ± 0.05 downstream ($p=0.708$). This is an indication that there is little autochthonous DOM production in Milwaukee River. HIX is strongly correlated with aromaticity and increases with the degree of decomposition or fractionation of DOM by sorption with higher HIX values ($HIX > 5$) indicating degraded DOM (Birdwell et al 2010). Upstream HIX values ranged from 2.02 to 8.83 with an average of 4.918 ± 1.96 and from 1.86 to 7.6 with an average of 4.95 ± 1.98 downstream ($P= 0.961$), consistent with FIX and BIX indicating that DOM in the Milwaukee River water is aromatic and terrigenous in nature.

2.4.5. Seasonal variations in fluorescent DOM components derived from PARAFAC analysis

Excitation-emission plots of fluorescent-DOM components and their characteristics are shown in Figure 6 and Table 1. Three DOM components (C1, C2 and C3) were identified via PARAFAC analysis. Component 1 (C1) was spectrally similar to peak M, which recently had been related to microbial-altered terrestrial DOM with low molecular weight. Component 1 (C1) is common in marine environments but can be found in wastewater, wetlands, and agricultural environments. Component 2 (C2) showed characteristics of terrestrial humic-like DOM with high molecular weight with the highest abundance in wetlands and forested environments. Component 3 (C3) is a protein-like component involving amino acids, free or bound in proteins, fluorescence resembles free tryptophan and may indicate intact proteins or less degraded peptide material (Gueguen et al 2011; Coble 2007; Yamashita et al 2008, Cory and McKnight 2005 and Stedmon and Markager 2005).

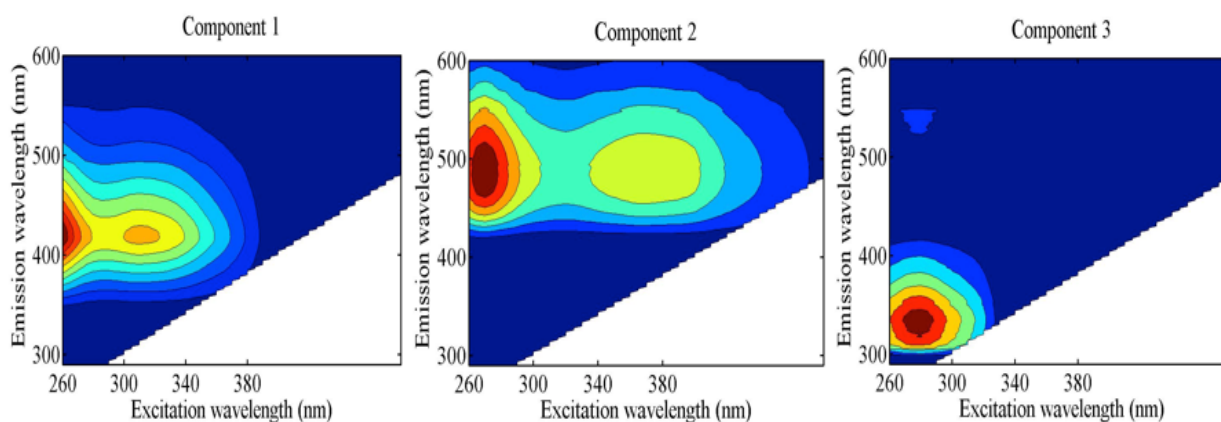


Figure 6. Excitation-emission plots of fluorescent-DOM components derived by PARAFAC analysis in the lower Milwaukee River during 2014-2015.

Table 1. Characteristics of the three components (C1, C2 and C3) identified using PARAFAC analysis in the lower Milwaukee River 2014-2015.

Component	Ex/Em (nm)	Description
C1	240(310)/410	Similar to peak M-marine humic-like. Low molecular weight, common in marine environments associated with biological activity but can be found in wastewater, wetlands, and agricultural environments
C2	265(370)/475	Similar to peak A-UVC humic-like. High- molecular- weight humic, widespread, but highest in wetlands and forested environments
C3	275/330	Similar to peak T- UVB protein-like. Amino acids, free or bound in proteins, fluorescence resembles free tryptophan, may indicate intact proteins or less degraded peptide material

A more detailed description of the PARAFAC derived DOM components is shown in Table 1. Components C1 and C2 were highly correlated to each other ($R^2= 0.945$, $p<0.001$) and correlated to DOC ($R^2= 0.837$, $p<0.001$ and $R^2= 0.827$, $p<0.001$ respectively) confirming that both components originated from the same terrigenous source. On the other hand, component 3 (C3) was not correlated to C1, C2 or DOC ($R^2=0.1222$, $p>0.05$, $R^2=0.110$, $p>0.05$ and $R^2=0.057$, $p>0.05$ respectively) further suggesting that C3 is mostly derived from an aquagenic source.

Seasonal variations of fluorescent-DOM components are shown in Figure 7 and Appendix C. In the downstream station (MR-01), C1 abundance sharply increased after the snowmelt event into the summer with the highest values in July, then declined toward the fall

with lowest values in winter under the ice cover, consistent with the variation trend of bulk DOC.

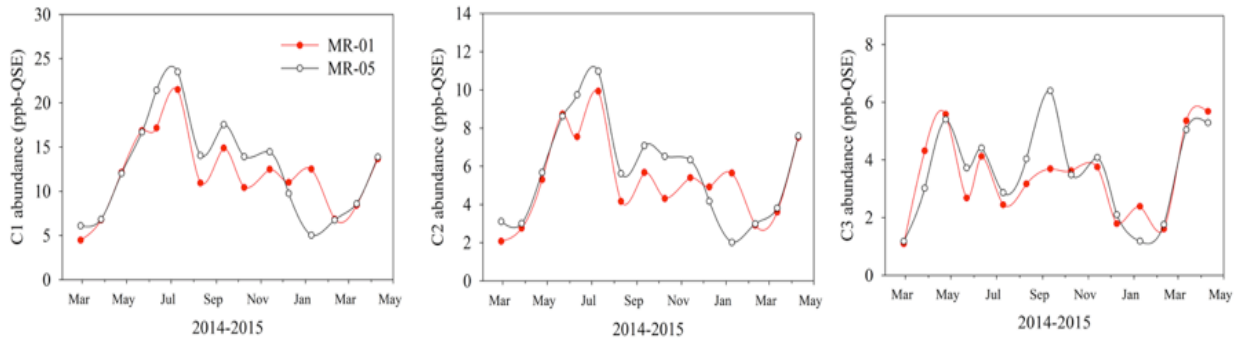


Figure 7. Seasonal variations of fluorescent-DOM components derived by PARAFAC analysis in an upstream (MR-05) and a downstream (MR-01) stations in the lower Milwaukee River during 2014-2015.

Component 1 abundance ranged from 4.5 to 21.5 ppb-QSE with an average of 12.0 ± 4.5 ppb-QSE and from 5.0 to 23.5 ppb-QSE with an average of 12.7 ± 5.6 ppb-QSE in the upstream station (MR-05) ($p=0.469$). Component 2 showed similar seasonal variations as C1 further confirming that both C1 and C2 are from the same terrigenous sources. Component 2 ranged from 2.1 to 9.9 ppb-QSE with an average of 5.4 ± 2.3 ppb-QSE downstream and from 2.0 to 11.0 ppb-QSE with an average of 5.8 ± 2.7 ppb-QSE upstream ($P=0.469$). Component 3 was lowest in the winter months when primary productivity was minimal. C3 abundance sharply increased in the spring and did not show much variability during summer and fall. Downstream C3 abundance varied from 1.1 to 5.7 ppb-QSE with an average of 3.4 ± 1.4 ppb-QSE and from 1.2 to 6.4 ppb-QSE with an average 3.6 ± 1.6 ppb-QSE upstream ($P=0.469$).

2.4.6 DOM Spatial variations in the lower Milwaukee River during high flow

(April 14, 2015) and low flow (November 13, 2014) seasons

Comparisons in DOM characteristics and spatial variations during low-flow and high-flow scenarios are shown in Figure 8 and Appendix D. During the low-flow season, instantaneous

discharge in the Milwaukee River was factors of 14 lower than that of high-flow during the storm event, $10 \text{ m}^3 \text{ s}^{-1}$ and $144 \text{ m}^3 \text{ s}^{-1}$ respectively. During low flow, DOC concentration ranged from $757 \text{ } \mu\text{M-C}$ at MR-05 to $828 \text{ } \mu\text{M-C}$ at MR-01 with an average of $776 \pm 29 \text{ } \mu\text{M-C}$. DOC concentration during high flow ranged from 749 (MR-01) to 798 (MR-03) $\mu\text{M-C}$ with an average of $775 \pm 19 \text{ } \mu\text{M-C}$. Knowing that the downstream (MR-01) is much deeper than other upstream stations, DOC abundance was remarkably higher during low flow than during the storm event (high flow) in the downstream station (MR-01), potentially due to higher dilution effect of DOC during the storm event (Figure 8a) in the downstream than the upstream station. In contrast, DOC abundance during high flow in the upstream station, where the river is much shallower, was relatively higher than that of low flow, suggesting higher input of terrestrial DOM during the storm event.

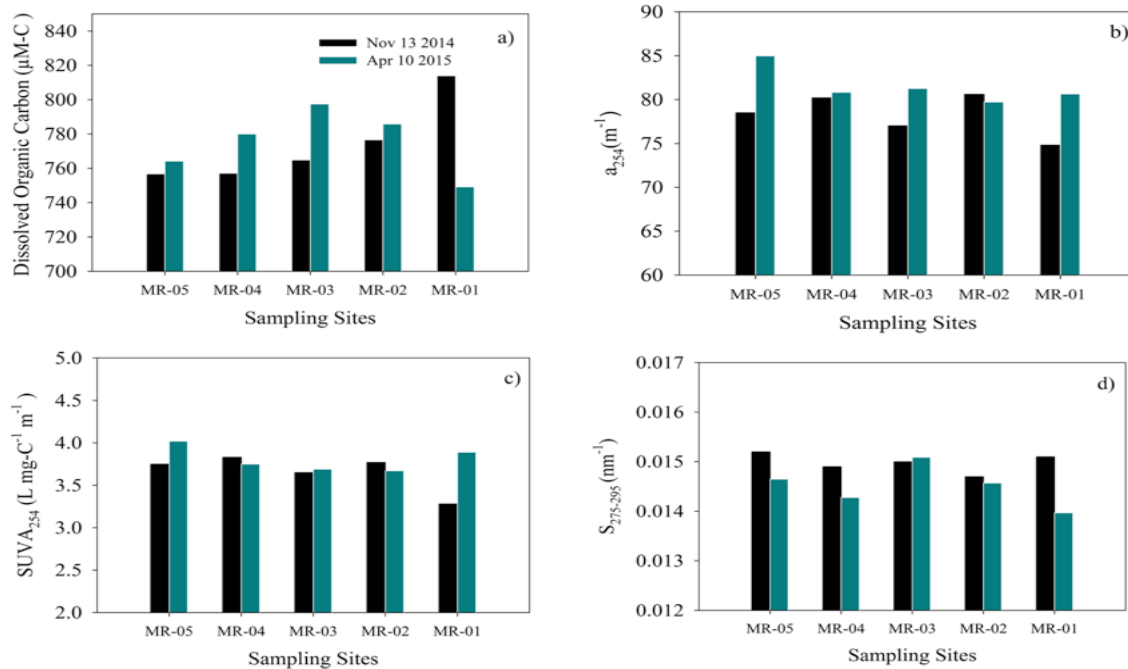


Figure 8. DOM characteristics spatial variations along the lower Milwaukee River during low flow (November 13, 2014) and high flow (April 14, 2015) seasons.

During low flow, absorption coefficient (a_{254}) ranged from 74.8 m^{-1} (MR-01) to 80.6 m^{-1} (MR-02) with an average of $78.2 \pm 2.9 m^{-1}$. During the storm event a_{254} varied from 79.7 m^{-1} (MR-02) to 85.0 m^{-1} (MR-05) with an average of $81.48 \pm 2.06 m^{-1}$. In contrast to DOC, CDOM abundance is higher in the downstream station during high flow than low flow, suggesting excessive input of non-chromophoric degraded DOM through surface runoff from the urbanized Milwaukee metropolitan area during the storm event. During low flow, $SUVA_{254}$ ranged from 3.28 (MR-01) to 3.85 (MR-05) ($L mg-C^{-1} m^{-1}$) with an average of $3.66 \pm 0.22 (L mg-C^{-1} m^{-1})$. On the other hand, during high flow, $SUVA_{254}$ ranged from 3.67 at station MR-02 to 4.02 at station MR-05 ($L mg-C^{-1} m^{-1}$) with an average of $3.80 \pm 0.15 (L mg-C^{-1} m^{-1})$, indicating higher aromaticity of the bulk DOM during the storm event. During low-flow, $S_{275-295}$ ranged from 0.0147 (MR-02) to 0.0152 (MR-05) nm^{-1} with an average of $0.0149 \pm 0.0001 nm^{-1}$. During the storm, ($S_{275-295}$) ranged from 0.0140 (MR-01) to 0.0151 (MR-03) nm^{-1} with an average of $0.0149 \pm 0.0001 nm^{-1}$. As expected, $S_{275-295}$ was inversely correlated to $SUVA_{254}$ confirming that the bulk CDOM during the storm event at MR-01 was higher in molecular weight. Overall, spectral slope ($S_{275-295}$) was lower during low flow at all sampling stations than high flow, indicating more degraded DOM during low flow season due to longer residence time that helps photochemical and biological degradations.

2.4.7 Comparisons of DOC, CDOM and other optical parameters in the lower Milwaukee River to other rivers.

In comparison, DOC average concentration in the Milwaukee River ($725 \pm 232 \mu M-C$) is relatively higher than other rivers, such as the Mississippi River ($342 \pm 42 \mu M-C$), Potomac River ($275 \pm 50 \mu M-C$) and Hudson River ($442 \pm 83 \mu M-C$), consistent with the fact that the Milwaukee River is less hydrologically altered although influences from agricultural activities in the

farmland existed and suggesting less processed DOM in the Milwaukee River watershed due to its short water residence time. In general, the Milwaukee River DOC abundance resembles that of Pike River ($667\pm 417 \mu\text{M-C}$) and the Yukon River at Pilot Station ($692\pm 417 \mu\text{M-C}$), which are mostly natural rivers with less hydrological alteration due to less dam construction. Similar to Milwaukee River absorption coefficient (a_{254}) is positively correlated with DOC in most rivers in Table 2.

Table 2. Comparison of dissolved organic carbon (DOC), CDOM and other derived optical parameters (a_{254} , SUVA_{254} and $S_{275-295}$) in the lower Milwaukee River to other rivers.

River	DOC ($\mu\text{M-OC}$)	a_{254} (m^{-1})	SUVA_{254} ($\text{L mg-C}^{-1} \text{m}^{-1}$)	$S_{275-295}$ (nm^{-1})
Milwaukee (2014-2015) ^a	725±232	77±31	3.52±0.59	0.0170±0.0034
Mississippi (2008-2010)	342±42	28	2.99±0.23	0.0151±0.0008
Potomac (2008-2010)	275±50	21	2.31±0.33	0.0157±0.0017
Colorado (2008-2010)	258±33	12	1.67±0.22	0.0217±0.0016
Hudson (2005-2009)	442±83	42	3.48±0.22	0.0146±0.0007
Yukon at Pilot Station (2001-2010)	692±417	62	3.08±0.47	0.0153±0.0019
Oak creek (2002-2004)	558±158	46	2.86±0.52	0.0155±0.0012
Pike (2002-2004)	667±417	73	3.71±0.55	0.0143±0.0016
Passadumkeag (2004-2007)	1000±358	116	4.19±0.30	0.0134±0.0059

Spencer et al 2012 and ^athis study

In general, SUVA_{254} in the Milwaukee River is relatively high in comparison with other rivers indicating higher aromaticity. SUVA_{254} in the Hudson River (3.48 ± 0.22) resembles that of the Milwaukee River (3.52 ± 0.59), indicating similar aromaticity of the bulk DOM suggesting similarities in watershed characteristics. SUVA_{254} in the Mississippi River (2.99 ± 0.23) is lower than the Milwaukee River potentially due to a much larger watershed with different characteristics, including longer water residence time, photochemical degradation and anthropogenic influence (Duan et al. 2013 and Cai et al. 2015). Spectral Slope $S_{275-295}$ in the

Milwaukee River (0.0170 ± 0.0034) is higher than most rivers in Table 6 except the Colorado River (0.0217 ± 0.0016) indicating a relatively lower molecular weight, likely containing more bioavailable bulk DOM in the Milwaukee River system than other rivers in the U.S. (Spencer et al. 2012).

2.5 Conclusions

This study showed strong seasonal and spatial variations in the abundance, composition and fluxes (discussed in Chapter II) of DOM in the lower Milwaukee River system. DOC concentration was relatively higher than that of other rivers and strongly influenced by hydrological conditions. There was not a significant correlation between DOC concentration and DOM composition. In contrast, there is a significant correlation among DOM optical parameters (i.e. a_{254} , $SUVA_{254}$ and $S_{275-295}$), which changes in river water due to biophysical controls (e.g. Battin et al. 2008), biogeochemical cycling (e.g. Jaffe et al. 2008) and hydrological processes (e.g. Huang and Chen 2009). DOM in the Milwaukee River during 2014-2015 was mostly chromophoric and terrigenous in nature. On average, non-chromophoric DOC comprised about 33% of the bulk DOC, which is much higher than that observed for Lake Michigan and other lower Great Lakes (Zhou et al. 2016). In general, during the storm event and the spring thaw, large amounts of DOC were exported in a short period of time creating a noticeable pulse of DOM in the coastal area of Lake Michigan indicating DOM flux were primarily controlled by hydrology. Average instantaneous DOC flux during the April 2015 storm event were the highest during 2014-2015 ($112982 \pm 1584 \text{ kg-C day}^{-1}$) and represented almost 17% of the total DOC annual flux ($6710 \times 10^3 \text{ Kg-C year}^{-1}$). Similar to many aquatic environments, the Milwaukee River EEM spectra included terrestrial humic-like peaks A and C and protein-like peak T. Three fluorescent DOM components were derived from PARAFAC modeling. Component C1 was

spectrally similar to peak M, which recently had been related to bio-altered terrestrial DOM with low molecular weight. Component C2 showed characteristics of terrestrial humic-like DOM with high molecular weight. Component C3 is a protein-like component and its fluorescence resembles free tryptophan. For future studies DOM components can be used as geochemical proxies to determine bioavailability (e.g. Balcarczyk et al. 2009), photo-reactivity (e.g Cory et al. 2007) and soil DOM chelating strength (e.g Ohno et al. 2006) in the river . Long term monitoring of DOM composition will help better predict how anthropogenic and climatic perturbations will affect coastal ecosystem in Lake Michigan and the metabolic pathways in the river, and will ultimately improve future watershed management decisions and help establish more successful ecosystem restoration plans (e. g Fellman et al. 2010).

3. Chapter II: Seasonal variations in the abundance, chemical speciation and fluxes of nutrients (phosphorus and nitrogen) in the lower Milwaukee River.

3.1 Background

Phosphorus: Phosphorus (P) is the limiting nutrient in most aquatic environments, especially in oligotrophic lakes (e.g., Hecky and Kilham, 1988, Finlay et al. 2013 and Lin et al. 2016). However, accelerated input of bioavailable phosphorus through anthropogenic activities may lead to degradation of water quality and catastrophic environmental issues in coastal waters (Yates and Johnes, 2013). Therefore, effective management of phosphorus is critical to the health of receiving waters (Bootsma et al. 2012). In recent years, bioavailable P has increased in the environment due to human activities (Howarth & Ramakrishna 2005 and Yates & Johnes 2013) and its abundance in many rivers has been increasing (Conley et al. 2009 and Seitzinger et al. 2010). Similar to nitrogen, phosphorous enrichment may be caused by point sources, usually

associated with wastewater and industrial effluent, which are highly concentrated in the dissolved phase, and non-point sources such as surface and subsurface runoff commonly linked to agricultural activities and urban runoff, and are highly concentrated in the particulate phase (Withers et al. 2008). Furthermore, sources of phosphorous can be very diverse including atmospheric deposition, leaf fall, industrial debris, urban litter, automobiles, domestic waste water, pets and livestock waste, domestic and industrial fertilizers, detergents and lubricants, forestry, cultivated land and pasture, etc. (Bannerman et al. 1993, Némery and Garnier, 2016), Although phosphorus is not well quantified for each sources and their relative importance. During storm events, many combinations of these sources become mobilized providing a large degree of both spatial and temporal variability. Unlike nitrogen and silicon, phosphorus has a high affinity to colloids and particles in natural waters (e.g., Santschi, 1992; Lin et al. 2015).

In aquatic systems, P can occur in many different forms including inorganic, organic, dissolved and particulate phases (Lin and Guo, 2016), with measurable fractions of dissolved inorganic phosphorus (DIP) or orthophosphates, dissolved organic phosphorous (DOP), particulate inorganic phosphorous (PIP) and particulate organic phosphorous (POP). Total dissolved phosphorous (TDP) is considered to be the sum of DIP and DOP and total particulate phosphorous (TPP) is the sum of PIP and POP (Meybeck 1982). Different forms of P should have different bioavailability and environmental fates during their biogeochemical cycling in aquatic ecosystems. For example, organisms prefer the uptake of DIP to support primary production, but they may also use DOP when the abundance of DIP is limited. Therefore not all forms of P have the same biogeochemical reactivity. Bioavailable phosphorous may show seasonal variability even under conditions where total P loading does not change.

Changes in stoichiometry of the bioavailable P species over time could be detrimental to

the water quality in the Milwaukee River and coastal areas of Lake Michigan. However, many previous studies and government agencies only measure concentrations of total P without information of chemical speciation of P in natural waters (e.g. Finlay et al. 2013 and Zou et al. 2011). Detailed studies on P speciation in the Milwaukee River are still lacking. Recently, there has been renewed interest in P dynamics and excessive Cladophora algae growth in the near-shore area of Lake Michigan (e.g. Bootsma et al. 2008 and Tomlinson et al. 2010). Therefore knowledge of abundance, chemical and phase speciation and fluxes of the various forms of P will provide a baseline data set to help evaluate and manage such issues.

Nitrogen: Nitrogen (N) is the other essential nutrient for living organisms and plays a critical role in the biogeochemical cycling pathways in aquatic ecosystems (Lee et al. 2014). Fossil fuel combustion (atmospheric deposition), fertilizer application and cultivation of N-fixing crops will significantly increase the fluxes of the various forms of reactive nitrogen to lakes via waterways (Han et al. 2008). It is estimated that anthropogenic impacts approximately doubled the input of reactive nitrogen to the earth's surface mainly through the application of nitrogenous fertilizers (e.g. Schlesinger 2009). Elevated fluxes of nitrogen species are associated with enhanced coastal eutrophication and anoxia (e.g. Diaz and Rosenberg, 2008 and Kane et al. 2014), alteration of nutrient stoichiometry that in some cases favors toxic phytoplankton, and increases in the production of nitrogenous greenhouse gases (Green et al. 2004).

Globally and locally, public health agencies have long recognized that regions of excessive fertilizer use will often have high concentrations of reactive nitrate (NO_3^-) in surface and groundwater that leads to the degradation of drinking water quality and occasionally to the fatal condition in infants known as methemoglobinemia (Avery et al. 1999). Nitrogen exists in natural waters in many chemical forms including dissolved organic nitrogen (DON), dissolved inorganic

nitrogen (DIN), which includes ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-), and particulate nitrogen (PN). The bioavailable nitrogen species in aquatic ecosystems are NH_4^+ , NO_2^- , NO_3^- , and DON. Nitrate is usually the most abundant among all species in oxic environments and therefore, the most frequently analyzed and correlated to anthropogenic influence (mainly fertilizers). DON is measured with NH_4^+ as Kjeldahl nitrogen. However, in natural waters the ammonia is usually low in concentration and Kjeldahl nitrogen reported is mainly DON. Nitrite is as well very low in oxic river waters (Meybeck 1982).

Unfortunately, a search of recent literature finds no studies that examined the seasonal variation of nitrogen and phosphorous species and their fluxes in the Milwaukee River. The goal in this thesis research is to examine in depth the variability of chemical and phase speciation of nutrients and their fluxes in the lower Milwaukee River to help guide future water policy related to nutrient discharge from the Milwaukee River to Lake Michigan.

3.2 Hypotheses and Objectives

My hypotheses are 1) the abundance of nitrogen and phosphorous species will express a strong seasonality in the lower Milwaukee River, 2) Nutrient speciation is predominately controlled by the hydrological cycle and most of anthropogenic nitrogen and phosphorous levels will spike during storm events and snowmelt and high rainfall, and 3) nutrient fluxes from the lower Milwaukee River to Lake Michigan will be predominately in the dissolved inorganic phase (DIP and NO_3^-).

The objectives of this study were to 1) collect monthly and storm event samples at the lower Milwaukee River along an anthropogenically impacted gradient, 2) determine the abundance of various N and P species in the lower Milwaukee River, 3) examine the influence of

hydrology (discharge) and anthropogenic activities (runoff) on the composition of nutrient species in the Milwaukee River, and 4) quantitatively estimate the seasonal and annual river export fluxes of nutrient species to Lake Michigan.

3.3 Methods

3.3.1 Measurement of phosphorous species

Acid persulfate method with modification was used to determine the concentrations of TDP in all samples collected. In brief, the filtrate samples were digested in acid persulfate solution at 95 °C for 24 h. The standard phosphomolybdenum blue method was used to complex the dissolved phosphorous species. Spectroscopic analysis was conducted at 882 nm using a UV-Vis spectrophotometer (Agilent 8453) and 5 cm quartz cuvette to increase the sensitivity. For DIP, digestion is not needed and measurements using the same method were directly performed. Concentrations of DOP were calculated by subtracting TDP from DIP. In the case of TPP, filter samples were wetted with 0.5 M MgCl₂ and heated in an oven at 95 °C until dry, then ashed in the furnace at 550 °C for 2 hours to decompose all organic phosphorous. Concentrations of PIP were determined by direct extraction from filter samples in 1 M HCl solution at room temperature for 24 h, followed by neutralization and dilution, both TPP and PIP extractions were analyzed using the standard phosphomolybdenum blue method. Concentrations of POP were obtained by subtracting TPP from PIP (Lin et al. 2016).

3.3.2 Measurement of nitrogen species

Nitrate measurements were done using the Dionex ICS-1000 Ion Chromatography System with IonPac AS14A exchange column with Standard Methods for the Examination of Water and Waste Water Method 4110B. In brief, water samples were injected into a stream of eluent

(bicarbonate buffer). The anions of interest were separated on the basis of their relative affinities for a low-capacity, strongly basic anion exchanger. The separated anions were converted to their acid forms and measured by conductivity. They were identified on the basis of retention time and quantified by measurement of peak area or peak height that was compared to standards known solutions (Nemi.gov). Measurements of total dissolved nitrogen (TDN) were performed using high temperature combustion on a Shimadzu TOC analyzer (TOC-V) interfaced with a nitrogen detector. Subtracting NO_3^- from TDN was used to estimate the abundance of DON.

3.4 Results and discussion

3.4.1 Abundance of dissolved and particulate phosphorus species

Concentrations of phosphorus species in Milwaukee River waters between February 2014 and April 2015 are shown in Appendix E and their seasonal variations are depicted in Figure 9. In the lower Milwaukee River, the DIP concentration was slightly higher than that of the anthropogenically influenced Fox River (Lin et al., 2016). DIP exhibited large seasonal variability ranging from 0.114 $\mu\text{M-P}$ in January 2015 under the ice cover to as high as 2.751 $\mu\text{M-P}$ during April 2015 storm event, with an average of $0.970 \pm 0.791 \mu\text{M-P}$. The average DIP concentration in the downstream sampling site ($1.072 \pm 0.862 \mu\text{M-P}$) was found to be higher than upstream sampling site ($0.867 \pm 0.720 \mu\text{M-P}$), suggesting DIP input through urban runoff from the Milwaukee metropolitan area. There is not a statistically significant difference in DIP concentrations between the two sites ($P=0.619$). DOP varied from 0.080 to 2.791 $\mu\text{M-P}$ with an average of $0.897 \pm 0.765 \mu\text{M-P}$ at the downstream station and from 0.085 to 4.687 $\mu\text{M-P}$ with an average $1.336 \pm 1.214 \mu\text{M-P}$ at the upstream station, respectively. There is not a statistically significant difference ($P=0.281$) in DOP concentrations between the upstream and downstream stations although the average DOP concentration was higher at the upstream station. PIP varied

from 0.0445 to 2.229 $\mu\text{M-P}$ with an average of $0.473 \pm 0.483 \mu\text{M-P}$ and POP varied from 0.224 to 3.791 $\mu\text{M-P}$ with an average of $0.809 \pm 0.648 \mu\text{M-P}$. DIP and POP were strongly correlated to discharge ($r^2=0.2705$, $p<0.001$ for the downstream and $r^2= 0.7396$, $p<0.001$ for the upstream station), discharge and suspended particulate matter (SPM) were strongly correlated ($r^2=0.917$, $p<0.001$) indicating that high concentrations of POP and PIP observed during the storm event are due to soil erosion and large fraction of PP is originated from terrestrial input and sediment resuspension.

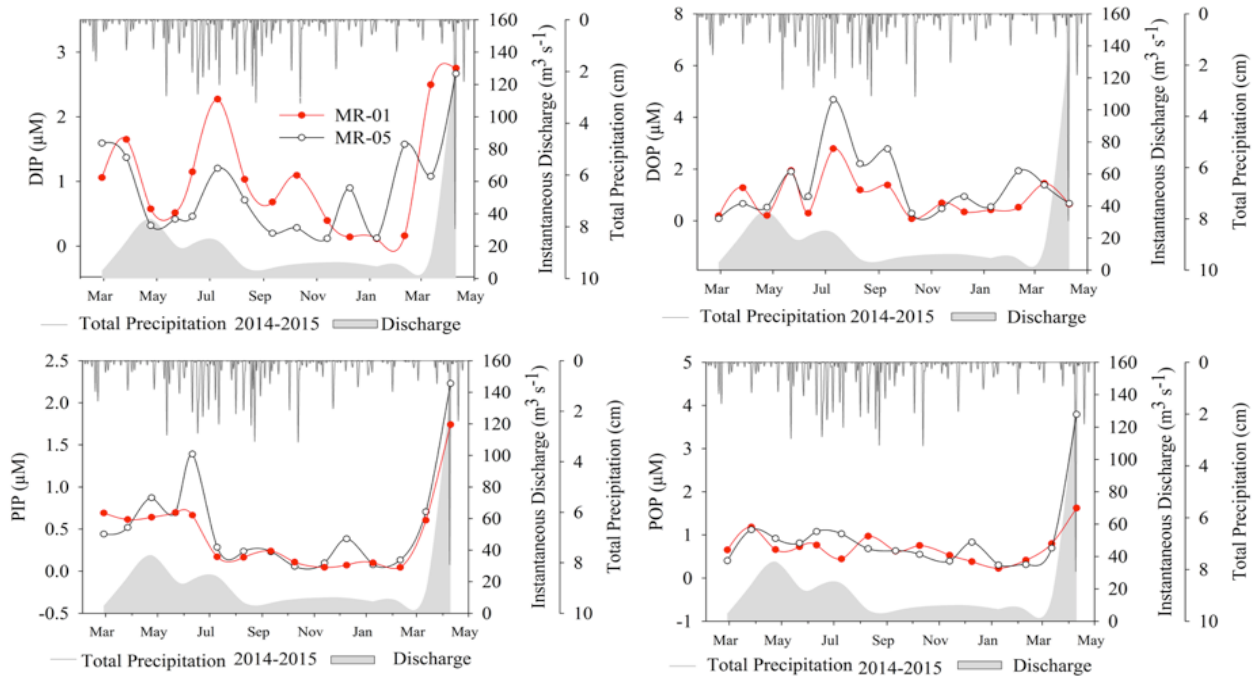


Figure 9. Seasonal variations in abundance of phosphorus species at an upstream (MR-05) and a downstream (MR-01) station in the lower Milwaukee River during 2014-2015.

3.4.2 Seasonal variations of dissolved and particulate Phosphorus species

DIP concentration was lowest in winter season as a result of many factors including co-precipitation of P with Fe oxides and hydroxides, microbial utilization, and dominance of groundwater sources under the ice (Guo et al. 2012). In contrast to nitrate, during the summer

season average DIP concentration peaked ($1.137 \pm 0.531 \mu\text{M-P}$) potentially due to the nonpoint source input from agricultural activities (mainly fertilizers) during the heavy rainfall season and intense DOM photo-degradation. In terms of seasonal variability, DOP was low during the storm event ($0.660 \pm 0.016 \mu\text{M-P}$) potentially due to the dilution effect. Higher abundance of DOP was observed during summer with an average concentration of $2.021 \pm 1.584 \mu\text{M-P}$, suggesting a conversion of DIP to DOP via primary production during warmer months.

PIP seasonal trend mimicked that of SPM and discharge (Figure 9). PIP was highest during the storm event followed by spring snowmelt and low during the base flow season. It was clear that PIP seasonal variation was mainly controlled by hydrology. POP remained relatively unchanged most of the time during this study. POP did not show much of a peak during snowmelt period, potentially due to dilution effect and was the highest during the April 2015 storm event due the intense soil erosion.

3.4.3 Abundance of major nitrogen species

Seasonal variations of nitrogen species are shown in Figure 10 and Appendix F. Upstream NO_3^- concentrations ranged from 70 to 259 $\mu\text{M-N}$ with an average of $131 \pm 64 \mu\text{M-N}$. The downstream NO_3^- concentration was lower and ranged from 62 to 238 $\mu\text{M-N}$ with an average of $119 \pm 55 \mu\text{M-N}$ suggesting dilution of NO_3^- from upstream to downstream. There is not a statistically significant difference between the two sampling sites ($p=0.229$). In Milwaukee River waters, NO_3^- was predominately the major specie in the dissolved nitrogen pool. In comparison with other anthropogenically influenced world rivers, the Milwaukee River NO_3^- concentration is slightly higher than the Mississippi River, Amazon River and Changjiang (Yangtze) River (see also references in Guo et al. 2004). DON concentration was significantly lower than NO_3^- . Upstream DON concentration varied from 2 to 254 $\mu\text{M-N}$ with an average of $55 \pm 59 \mu\text{M-N}$ and

from 11 to 143 $\mu\text{M-N}$ with an average of $54 \pm 59 \mu\text{M-N}$. There is not a statistically significant difference between the two sampling sites ($P=0.619$).

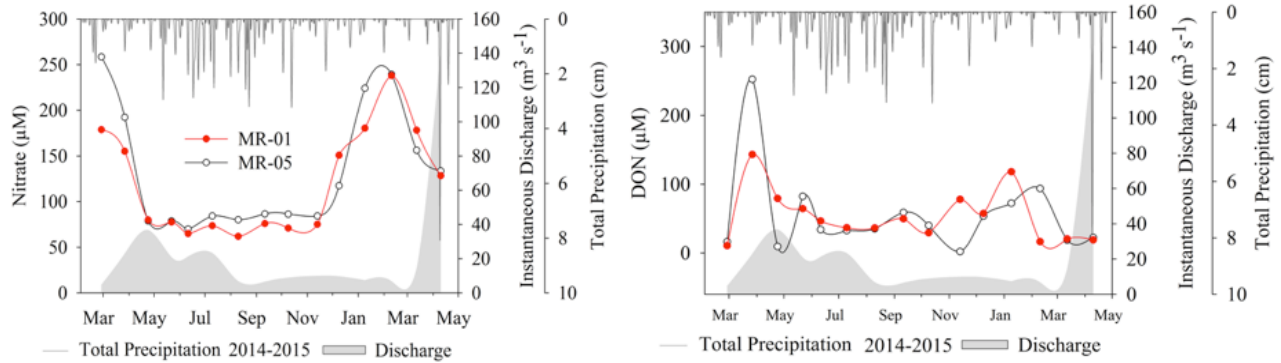


Figure 10. Seasonal variations of nitrogen species at the upstream (MR-05) and downstream (MR-01) station in the lower Milwaukee River during 2014-2015.

3.4.4 Seasonal variation of major nitrogen species

In the Milwaukee River, NO_3^- concentration steeply declined during snowmelt and storm events due to rapid dilution. NO_3^- was relatively low and showed minor seasonal variation most the year and was almost factor of four higher under the ice cover in the winter months (Figure 10), this could have resulted from a combination of many factors including intense NO_3^- fixation by vegetation during the warm season in the spring and summer, slow biological activity in the winter due to freezing temperatures and NO_3^- contaminated groundwater sources during the base flow period under the ice cover in winter.

Similar to DOC seasonal changes, DON concentration in Milwaukee River waters was the highest during the snowmelt event in the spring, where input of terrestrial DOM is high. Lower abundance of DON was observed during a storm event due to dilution effect. DON remained relatively unchanged during late spring and summer and early fall during 2014-2015.

3.4.5 Speciation and average phases partitioning of N and P among dissolved, particulate, organic and inorganic phases

Within the TDP pool, DIP% varied from 20% to 93% with an average of $53\% \pm 25$ downstream and varied from 7% to 95% with an average of $40\% \pm 25$ upstream (Figure 11). Within the TDP pool, DOP% upstream varied from 5.1% to 93% with an average of $60\% \pm 25$ and from 6.9% to 79% with an average of $47\% \pm 25$ downstream. Within the particulate pool TPP, PIP% varied from 8% to 56% with an average of $32\% \pm 15$ and POP% varied from 43% to 92% with an average of $67\% \pm 18$ (Appendix E).

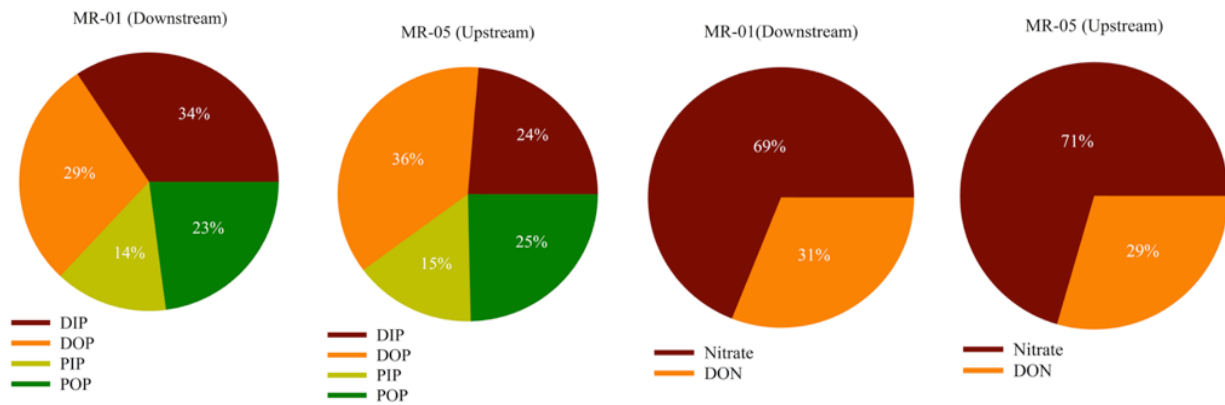


Figure 11. Average phase partitioning of N and P among dissolved, particulate, organic and inorganic forms in the lower Milwaukee River during 2014-2015. (Nitrate; DON, dissolved organic nitrogen; DIP dissolved inorganic phosphorus; DOP, dissolved organic phosphorus; PIP, particulate inorganic phosphorus; POP, particulate organic phosphorus).

Within the total phosphorus pool, average percentage of TDP represented 63% at the downstream station and 60% at the upstream station. Therefore, phosphorus exported from the Milwaukee River to Lake Michigan was predominately in the dissolved form. Average percentage in the downstream DIP (34%) was higher than DIP upstream (24%) and upstream DOP (36%) was higher than downstream DOP (29%), suggesting the addition of anthropogenic phosphorus, mostly DIP, from urban runoffs. Concentrations of TPP are relatively lower than TDP; this is unique to the Milwaukee River. For example, the Mississippi River, Chena River

and Jiulong River all have higher TPP than TDP (Prastka & Malcolm 1994 and Guo et al. 2004). However, the opposite is true for the Milwaukee River. Within the TPP pool, PIP% and POP% were similar in both sampling sites. There was no significant difference in the phase partitioning of dissolved nitrogen between the upstream and the downstream.

Within the total dissolved nitrogen pool, NO_3^- varied from 52% to 94% with an average of $68\% \pm 16$ downstream and varied from 43% to 94% with an average of $73\% \pm 16$ upstream. DON% upstream varied from 6% to 51% with an average of $32\% \pm 16$ and from 6% to 57% with an average of $27\% \pm 16$ downstream. On average, NO_3^- represented ~70% of the TDN in Milwaukee River waters leaving ~30% in the form of DON. In contrast to phosphorus chemical speciation, there was no difference in nitrogen species percentages between the upstream and the downstream stations in the lower Milwaukee River. High nitrate percentages in the Milwaukee River indicate an anthropogenic influence on the drainage basin, including fertilizer applications and urban effluent (e.g. Lee et al. 2014).

3.4.6 Seasonal and spatial variations in the partitioning of N and P species

Seasonal and spatial variations in partitioning of nitrogen and phosphorus in the lower Milwaukee River upstream and downstream stations are shown in Figure 12 and Appendix E. The percent NO_3^- in the total dissolved N pool shows a seasonal variability at both sampling locations. Even though the lower Milwaukee River had similar NO_3^- concentrations trends between the upstream and the downstream during most of the sampling periods, it differed in seasonal NO_3^- trends. For example, NO_3^- made up the majority of the TDN pool; it was very high in winter and early spring (~90%) and lowest during the warmer months (~60%) due to dilution effect and slow vegetation uptake and possibly input of groundwater, which contained low nitrate contamination. In general, DON percentage was highest during the snowmelt event

(~50%) — when the soil leaching of organic materials is the most intense (Guo et al. 2004). In the case of phosphorus, the TDP% expressed higher variability than TPP% between upstream and downstream. In general, DIP% was higher downstream than upstream, indicating changes in source terms for DIP, and DOP% was higher upstream than downstream through out the seasons showing a consistent trend as that of concentrations. Together, changes in DIP and DOP between upstream and downstream stations clearly indicate changes in natural DOP and anthropogenic DIP sources along the Milwaukee River and the influence of DIP from the anthropogenic sources. Overall, the predominant nitrogen and phosphorus species transported from the Milwaukee River to Lake Michigan are DIN (DIN/TDN%=70%), DIP (DIP/TP%=29%) and DOP (DOP/TP%=33%).

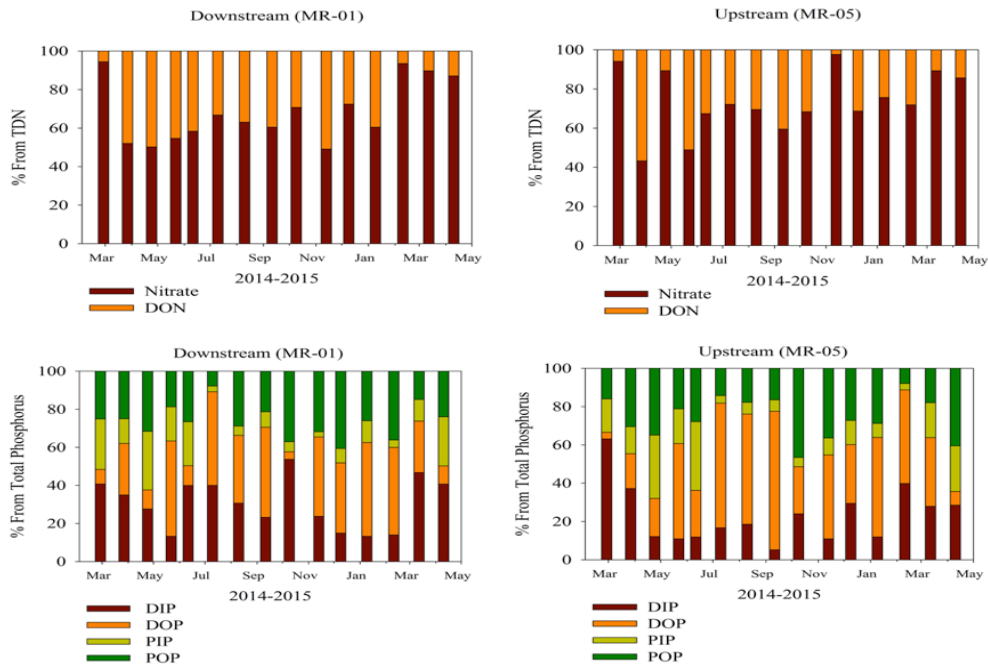


Figure 12. Seasonal and spatial variations of N and P partitioning between dissolved, particulate, organic and inorganic phases at the upstream (MR-05) and downstream (MR-01) stations in the lower Milwaukee River from February 2014 to May 2015. DIN denotes nitrate; DON for

dissolved organic nitrogen; DIP for dissolved inorganic phosphorus; DOP for dissolved organic phosphorus; PIP for particulate inorganic phosphorus; and POP for particulate organic phosphorus.

3.4.7 Nutrient composition in the lower Milwaukee River waters

River waters molar ratio of DOC/DON averaged 19 ± 11 downstream and 46 ± 87 upstream. DOC/DOP averaged 1696 ± 1844 downstream and 1126 ± 1121 upstream. The average DOC/DOP ratio is considerably higher than the Redfield ratio for phytoplankton. However, similar high DOC/DOP ratios have been observed for the Great Lake regions (Lin and Guo, 2016) where P has been a limiting nutrient. DON/DOP molar ratio averaged of 121 ± 126 downstream and 76 ± 100 upstream. In addition DIC/NO_3^- molar ratio averaged 50 ± 24 Downstream and 47 ± 23 upstream. DIC/DIP averaged 14405 ± 19775 downstream and 13012 ± 13024 upstream. NO_3^-/DIP averaged 365 ± 543 downstream and 321 ± 444 upstream (Appendix F).

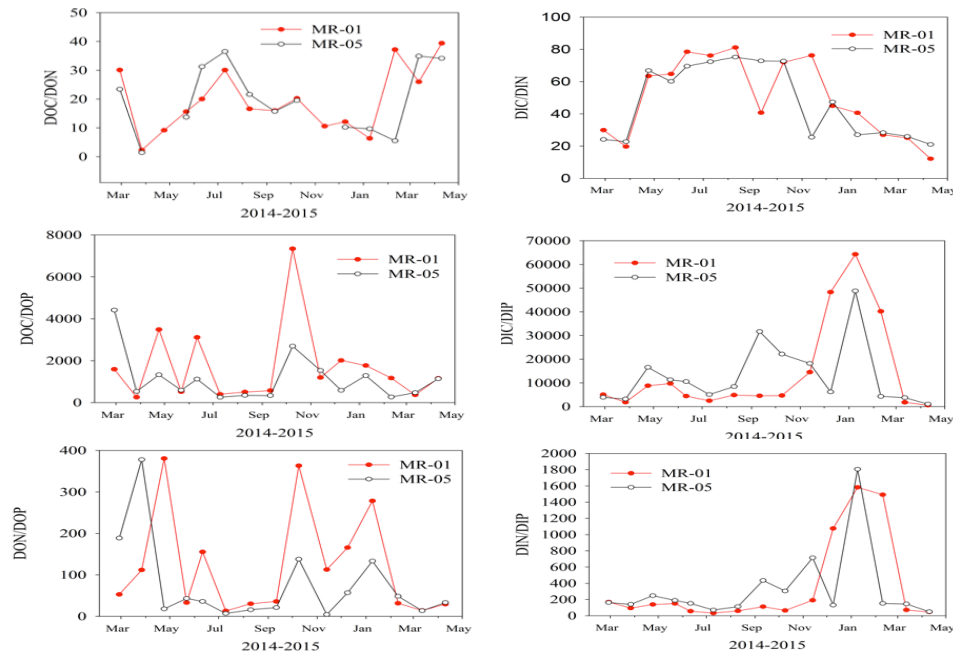


Figure 13. Seasonal variations in nutrient composition at the upstream (MR-05) and downstream (MR-01) stations in the lower Milwaukee River during 2014-2015.

Ratio of DOC/DON was the lowest during winter where most of the DOM is from autochthonous source with fresh DOM and low C/N ratio and high ammonia concentration in the river waters under the ice, likely derived from microbial production and transformation under the ice (Guo et al. 2004). High DOC/DON was observed during summer, snowmelt and storm event potentially due to the dominance of terrestrial DOM and highly degraded soil DOM with low C/N ratio in the bulk DOM. On the other hand, DON/DOP molar ratio was lowest during the summer where DOP concentration was high. DON/DOP was highest during the spring melt period where DON was the highest and DOP was low due to dilution. This is similar to subtropical and temperate rivers such as the Loire River, ~64 (Meybeck et al. 1988) and the Morlaix River, ~97 (Wafar et al. 1989). This indicated similar characteristics of terrestrial DOM in the Milwaukee River watershed (Guo et al. 2004). Nevertheless, the DOC/DOP ratio did not show a clear seasonal variation and remained relatively unchanged during 2014-2015. Consistent with DOC/DON, DIC/ NO₃⁻ was lowest under the ice and higher during the warmer months since NO₃⁻ was lowest during the warmer months due to high biological activities and vegetation uptake.

The DIN/DIP molar ratio in the Milwaukee River waters was highest in winter under the ice and lowest in the summer where primary production is the most intense. This DIN/DIP ratio is almost 10 times higher than the Redfield ratio (N/P=16) and much higher than those of other world rivers (Cai et al. 2008). DIC/DIP was highest in winter under the ice and low in the warmer months, suggesting that DIC/DIP was controlled by hydrology and biological processes. All molar ratios are suggesting that Milwaukee River was an N enriched and P limited ecosystem.

3.4.8 Comparisons in P speciation between the lower Milwaukee River and other rivers

The average DIP concentration in the Milwaukee River during 2014 and 2015 ($0.97 \pm 0.79 \mu\text{M}$) was much higher than those of pristine rivers such as the Chena River in Alaska ($0.030\text{-}0.300 \mu\text{M}$; Cai et al., 2008) and the Jourdon River in Mississippi ($0.030 \mu\text{M-P}$) (Lin et al., 2012). The average DIP concentration was similar to that of the Fox River ($0.828 \pm 0.216 \mu\text{M-P}$), which is heavily polluted as a result of agricultural activities (DeVilbiss et al. 2016). However, DIP in the Milwaukee river waters is less abundant compared to those of polluted rivers, such as the Mississippi River ($2.890 \pm 1.146 \mu\text{M-P}$) and the Jiulong River ($2.140 \mu\text{M-P}$). In contrast, average DOP concentration in the Milwaukee River ($1.12 \pm 0.99 \mu\text{M-P}$) is twice as high as that of the Fox River ($0.599 \pm 0.109 \mu\text{M-P}$) and the Jiulong River ($0.550 \mu\text{M-P}$), indicating the importance of soil DOM and other natural sources in the Milwaukee River. In addition, higher DOP abundance in the Milwaukee River could be related to excess abundance of DIP, especially during summer season where primary production is intense. In contrast to many other river waters, average PIP concentration in the Milwaukee River ($0.50 \pm 0.52 \mu\text{M-P}$) was relatively low in comparison to that of the Fox River ($0.963 \pm 0.128 \mu\text{M-P}$), Mississippi River ($3.450 \mu\text{M-P}$) and Jiulong River ($3.95 \mu\text{M-P}$). Similarly, POP concentration ($0.81 \pm 0.60 \mu\text{M-P}$) was slightly lower than that of the Fox River ($1.020 \pm 0.417 \mu\text{M-P}$), Jiulong River ($1.16 \mu\text{M-P}$), Jourdon River ($0.9 \mu\text{M-P}$) and Mississippi River ($1.81 \mu\text{M-P}$), likely due to the overall low suspended particles and low abundance of P in suspended particles (in terms of $\mu\text{g-P/g-particle}$). In general, phosphorus exported from the Milwaukee River to Lake Michigan was predominantly in the dissolved phase (DIP and DOP).

Furthermore, within the total P pool, DIP/TP% in the Milwaukee River ($29 \pm 27 \%$) is higher than that of most rivers, as shown in Table 4, except that of the Mississippi River (34%).

Uniquely, DOP/TP% in the Milwaukee River (33±34%) is higher than all rivers in Table 4. In contrast, PIP/TP% (14±18%) is lower than that of most rivers except for the Jourdon River (6%). POP/TP% in the Milwaukee River (24±21%) resembles that of most rivers except the Jourdon River (77%). In general, low abundance of TPP in the Milwaukee River waters could affect the extent of P release in to the dissolved phase (Zang and Hung 2007), which is important, knowing that the Milwaukee River is phosphorus limiting aquatic system (Lin et al. 2015).

Table 3. Comparison in dissolved and particulate phosphorous species in the lower Milwaukee River to other rivers.

River	TDP (μM)		%TDP		TPP (μM)		%TPP		Ref.
	DIP (μM)	DOP (μM)	%DIP	%DOP	PIP (μM)	POP (μM)	%PIP	%POP	
Milwaukee	0.97± 0.79	1.12± 0.99	29± 27	33± 34	0.50± 0.52	0.81± 0.60	14± 18	24± 21	This study
Fox	0.828 ± 0.216	0.599 ± 0.109	24 ± 1	18	0.963 ± 0.128	1.020 ± 0.417	29 ± 6	29 ± 5	a
Mississippi	2.890 ± 1.146	0.345 ± 0.148	34 4	4	3.450	1.81	41	21	b,c,d
Jourdon	0.030	0.160	3	14	0.070	0.9	6	77	e
Chena	0.030- 0.300	0.060 ± 0.063	19 ± 9	7 ± 4	0.600 ± 0.300		74 ± 10		f
Jiulong	2.140	0.550	27	7	3.95	1.16	51	15	g

^a. Lin et al. 2016, ^b. Cai and Guo 2009, ^c. Shim et al. 2012, ^d. Duan et al. 2010, ^e. Lin et al. 2012, ^f. Cai et al. 2008 and ^g. Lin et al. 2013

3.4.9 Comparisons in N species between the Milwaukee River and other rivers

As shown in Table 4, the average NO₃⁻ concentration in the Milwaukee River (125 ±61 μM-N) is remarkably higher than that of the lower Mississippi River (103 μM-N), lower Missouri River (88 μM-N), lower Potomac River (11 μM-N) and Amazon River (10 μM-N) suggesting significant influence through agricultural activities, mainly due to intensive application of fertilizers in the Milwaukee River Basin. NO₃⁻ abundance in the Milwaukee River is lower than that of the exceptionally heavily polluted Illinois River (292 μM-N) (Table 4).

Similar to DOP and DOC, the average concentration of DON in the Milwaukee River (54 ± 50 $\mu\text{M-N}$) is considerably higher than that of other major rivers (Table 9). The $\text{NO}_3^-/\text{TDN}\%$ in the Milwaukee River ($70 \pm 55\%$) resembles that of the lower Mississippi River (74%) and lower Missouri River (71%) and much higher than the Lower Potomac (38%) and the Amazon (42%) Rivers, showing a significant human impact on the lower Milwaukee River. In contrast, the $\text{DON}/\text{TDN}\%$ in the Milwaukee River ($30 \pm 45\%$) was generally lower than that of the lower Potomac River (62%) and Amazon River (58%) and higher than that of lower Illinois River (10%), lower Mississippi River (26%), lower Missouri River (29%), again supporting influence of human activities on nutrient speciation in the lower Milwaukee River.

Table 4. Comparison of major nitrogen species (NO_3^- and DON) in the lower Milwaukee River to other rivers.

	TDN		TDN%		
	NO_3^- (μM)	DON (μM)	% NO_3^-	%DON	
Lower Milwaukee River	125 ± 61	54 ± 50	70 ± 55	30 ± 45	This study
Lower Illinois River	292	32	90	10	a
Lower Mississippi River	103	37	74	26	a
Lower Missouri River	88	36	71	29	a
Lower Potomac River	11	18	38	62	b
Amazon River	10	14	42	58	c

^a Goolsby and Battaglin 2001, ^b Morgan and Kline 2011 ^c Meybeck 1982

3.4.10 Fluxes of dissolved organic carbon and nutrient species

Terrestrial export of nutrients species and organic carbon is a critical factor to the biogeochemistry of receiving coastal aquatic systems (Klump et al. 1997). The magnitude of the quantities exported affect aquatic productivity, food web structure and water quality (Vanni et al. 2001). In this study, a strong seasonal variation in concentrations of nutrient species (P and N) and dissolved organic carbon (DOC) was observed. To be able to assess the impacts of

nutrients species and DOC on water quality and ecosystem health in the coastal area of Lake Michigan, it is particularly important to determine contemporary Milwaukee River fluxes. Estimating river fluxes is challenging and fluxes can be accurately characterized only through very intensive sampling (Holmes et al. 2010). In most cases, river export fluxes were estimated from the average concentration measured during warmer months and annual average freshwater discharge. This method assumes similar species abundances during different seasons and assumes permanent linear relationship between daily species fluxes and instantaneous river discharge. Such an approach is associated with many uncertainties. For example, fluxes estimation without snowmelt and storm events sampling will lead to underestimation for organic species and over estimation for inorganic species (Guo et al. 2012). Note that these uncertainties in river fluxes have to be quantified before the export fluxes can be useful to assess freshwater quality status. In this study, annual fluxes of DOC, DON, NO_3^- , DOP, DIP, PIP and POP were estimated using the interpolation method of regression between instantaneous species flux and discharge (Warnken and Santschi et al. 2004). In addition, using the method of regression between instantaneous species fluxes and discharge, fluxes can also be calculated by multiplying the slope of the regression curve to the mean instantaneous discharge. It will be important to compare the two methods uncertainties and biases in order to have an accurate estimation of fluxes. Using the interpolation method of regression, the annual export fluxes of DIP, DOP, PIP, POP, NO_3^- , DON and DOC are 63, 14, 47, 64, 1325, 200 and 6710 $10^3 \text{ Kg-(P,N or C) year}^{-1}$ respectively and the export fluxes of DIP, DOP, PIP, POP, NO_3^- , DON and DOC were 63, 14, 47, 64, 1325, 200 and 6710 $10^3 \text{ Kg-(P,N or C) year}^{-1}$ respectively. When normalized to drainage basin area, the yields were 28, 6, 21, 28, 581, 88 and 2943 $\text{kg-(P,N or C) km}^{-2} \text{ year}^{-1}$ for DIP, DOP, PIP, POP, NO_3^- , DON and DOC, respectively.

Table 5. Nutrient fluxes (10^3 kg-P, N or C year⁻¹) and yields (kg km⁻² year⁻¹) in the lower Milwaukee River during 2014-2015.

Species	DIP	DOP	PIP	POP	NO ₃ ⁻	DON	DOC
Fluxes (10^3 kg-P, N or C year ⁻¹)	63	14	47	64	1325	200	6710
Yields (kg km ⁻² year ⁻¹)	28	6	21	28	581	88	2943

DIP and POP fluxes and yields were the highest among P species in the Milwaukee River waters. In comparison with other world rivers, DIP and DOC yields in Milwaukee River during 2014-2015 were slightly higher than that of other world rivers but NO₃⁻ yield in the river during 2014-2015 were remarkably higher than that of other world rivers. This reflects the anthropogenic impact and the influence of agricultural activities in the river watershed on the lower Milwaukee River and may lead to degradation of freshwater quality and ecosystem health in the river and coastal area of Lake Michigan.

The maximum average instantaneous daily fluxes for all species occurred during the April 2015 storm event. DIP average instantaneous fluxes were similar during spring (55 ± 33 Kg day⁻¹) and summer (58 ± 49 Kg day⁻¹) and five times higher than that of winter and fall. During the April 2015 storm event, DIP flux was 20 times higher than that of the spring and summer including the snowmelt and rain seasons. In general, DOP instantaneous flux was higher than that of DIP except for the April 2015 storm event. Average PIP instantaneous flux was in general lower than that of DIP and DOP except, it was 3 times higher than DOP during the storm. POP averages instantaneous fluxes resembled that of DIP fluxes during 2014-2015. Besides the storm event that was six times higher, NO₃⁻ average daily flux were the highest during the snowmelt in the spring. The average NO₃⁻ daily flux was higher in winter under the ice than fall and summer. Similar to DOC, the flux of DON was high during spring and the storm event. DOC flux during

the storm event was six times higher than that of the spring snow melt season, 19727 ± 9062 and 112982 ± 1584 kg-C day⁻¹ respectively. In general, during the storm event and the spring thaw, large amounts of DOC and nutrients were exported in a short period of time creating an important pulse of DOM in the coastal area of Lake Michigan indicating DOM fluxes from the Milwaukee River to Lake Michigan are primarily controlled by hydrology. Therefore, at different times of the year water sources change from predominately groundwater to rain water and snow melt producing strong seasonality in discharge influencing DOM abundance, composition, and fluxes in the lower Milwaukee River and near-shore Lake Michigan.

Table 6. Seasonal variations in average instantaneous daily fluxes (Kg day⁻¹) of DOC and nutrients species in lower Milwaukee River during 2014-2015.

Species	DIP	DOP	PIP	POP	NO ₃ ⁻	DON	DOC
Spring	55±33	69±37	50±21	65±18	3499±1344	160±97	19727±9062
Summer	58±49	102±112	26±30	38±22	1543±761	66±12	18439±10754
Fall	11±9	20±17	3±1	13±3	842±159	63±31	6946±1235
Winter	11±12	17±11	3±3	9±6	1843±263	93±42	5438±1188
Storm	1044±23	255±6	765±133	1044±591	22808±634	199±3	112982±1584

3.4.11 Comparison of TDN, TDP and DOC yields in the lower Milwaukee River to other rivers (Part I and II)

The Milwaukee River stands out as having almost 10 times higher nutrient yields (flux normalized to drainage area) of TDN than that of the largest arctic rivers such as the Yukon River, Lena River, Yenisey River and Ob' River. In the case of TDP, the yield ranged from 5 to 17 times higher than that of the arctic rivers. On the other hand, DOC yield in the Milwaukee River (2943 Kg km⁻² year⁻¹) was slightly higher than that of the Lena River (2338 Kg km⁻² year⁻¹) and

about two times higher than those of other arctic rivers (Table 7). To date, it is obvious that the Milwaukee River is heavily polluted due to many factors including anthropogenic influence from the urbanized metropolitan area of the city of Milwaukee and agricultural activities considering that over 50% of the Milwaukee River watershed is farmlands. Long term monitoring and observations of DOM and nutrient species dynamics in the Milwaukee River and the coastal area of Lake Michigan is needed to better understand the controlling factors and the potential trend changes due to climate and environmental changes that may affect water quality and ecosystem health.

Table 7. Comparison of TDN, TDP and DOC yields ($\text{Kg km}^{-2} \text{ year}^{-1}$) in the lower Milwaukee River with other rivers.

River	Milwaukee	Ob'	Yenisey	Lena	Kolyma	Yukon	Mackenzie
TDN	718	63	67	69	48	81	36
TDP	34	6	4	2	2	2	2
DOC	2943	1396	1904	2338	1555	1771	820

Milwaukee River data (This Study) and other rivers data (Holmes et al 2010)

3.4.12 Comparison of NO_3^- and DIP in the lower Milwaukee River to other rivers

The average NO_3^- concentration in the lower Milwaukee River ($125 \pm 61 \mu\text{M-N}$) is similar to that of the Mississippi River ($114 \mu\text{M-N}$) and it is surprisingly the highest among all world river waters. The average DIP concentration in the lower Milwaukee River ($0.97 \pm 0.79 \mu\text{M-P}$) resembled that of that of Changjiang River ($0.83 \mu\text{M-P}$) and it is lower than that of the Mississippi River ($2.890 \pm 1.146 \mu\text{M-P}$), Ob's ($2.3 \mu\text{M-P}$) and Trinity River ($1.85 \mu\text{M-P}$). NO_3^- yield in the lower Milwaukee River ($581 \text{ Kg km}^{-2} \text{ year}^{-1}$) is higher than that of the Mississippi River ($294 \text{ Kg km}^{-2} \text{ year}^{-1}$), showing the tremendous addition of anthropogenic nitrate to the Milwaukee River watershed mainly through agricultural activities that may have negative affects

on the ecosystem health in the Milwaukee River and its estuary in coastal Lake Michigan. The second highest NO_3^- yield among all world rivers in Table 12 was the heavily polluted Changjiang River ($392 \text{ Kg km}^{-2} \text{ year}^{-1}$). On the other hand, even though the average concentration of DIP in the Milwaukee River was similar to that of the Changjiang River, the yields were much different, $27.9 \text{ Kg km}^{-2} \text{ year}^{-1}$ and $8.1 \text{ Kg km}^{-2} \text{ year}^{-1}$ respectively. The DIP yield in the Milwaukee River is relatively similar to that of Trinity River ($27.3 \text{ Kg km}^{-2} \text{ year}^{-1}$) and the Amazon River ($21.4 \text{ Kg km}^{-2} \text{ year}^{-1}$). The highest DIP yield among all rivers (Table 8) is the Mississippi River that is almost twice as high as that of the Milwaukee River ($43.4 \text{ Kg km}^{-2} \text{ year}^{-1}$). Even though the Milwaukee River and Lake Michigan are P- limited ecosystems, very high levels NO_3^- could severely deteriorate the water quality and ecosystem health, especially in the coastal waters.

Table 8. Comparison of NO_3^- and DIP concentrations and yields in the lower Milwaukee River to other rivers.

River	Concentration (μM)		Yield ($\text{Kg}/\text{km}^2/\text{yr}$)		References
	NO_3^-	DIP	NO_3^-	DIP	
Milwaukee	125±61	0.97±0.79	581	27.9	This study
Mississippi	114	2.890±1.146	294	43.4	a,b,c
Mackenzie	2.42	0.1	6	0.5	d,e
Changjiang	70.3	0.83	392	8.1	f
Trinity	39.2	1.85	266	27.3	g
Amazon	10	0.7	137	21.4	h
Yukon	2.43 ± 0.63	0.053±0.04	8	0.4	i
Ob'	56	2.3	106	9.6	j
Yenisey	26	0.4	87	2.9	j

^a. Cai and Guo 2009, ^b. Rabalais et al 1996, ^c. Berner and Berner 1996, ^d. Environmental Canada 1978, ^e. Millot et al 2003, ^f. Liu et al 2003, ^g. Warnken 2002, ^h. Demaster and Pope 1996, ⁱ. Guo et al.2004, ^j. Gordeev 2002

3.5 Conclusions

Variations in abundance, chemical speciation, and export fluxes of nutrients (N and P) from the Milwaukee River and the influence of hydrology and anthropogenic activities on nutrient dynamics in river waters were examined during 2014-2015. Nutrient species of nitrogen and phosphorous expressed a strong seasonality in the lower Milwaukee River. DIP concentrations increased during late spring and summer (during heavy rain season), suggesting excess DIP input through agricultural activities and city effluent in the Milwaukee River watershed (including fertilizers). DIP was the highest during the April 2015 storm event. Therefore, P nutrient speciation is predominately controlled by the hydrological cycle. Within the total dissolved phosphorus pool, almost 50% were organic phosphorus (DOP) and the other 50% were inorganic (DIP). DOP was relatively low during the snow melt and storm event potentially due to dilution effect. The highest abundance of DOP was observed during summer, suggesting a conversion of DIP to DOP via primary production. Phosphorus exported from the Milwaukee River to Lake Michigan was predominately in the dissolved form (62% of total P). POP and PIP abundances in the Milwaukee River were uniquely lower than those of the world rivers such as the Fox River, Mississippi River and Jiulong River. In general, particulate P showed strong correlation with discharge and was predominately controlled by hydrology. NO_3^- was the predominant dissolved N species, while DON contributed up to 30% of the TDN transported in the river. NO_3^- abundance was relatively low during the warmer season in spring and summer and peaked under the ice in winter, suggesting that NO_3^- was mostly controlled by biological uptake by vegetation in the watershed and hydrology. In contrast to DIP, the level of NO_3^- was relatively low during the storm mostly due to dilution. In general, the average NO_3^- concentration during 2014-2015 was among the highest in comparison with major world rivers, reflecting the

polluted NO_3^- conditions of the Milwaukee River watershed potentially through agricultural activities. DON abundance on the other hand was much lower than that of DIP and showed similar seasonal variations to DOC. The average N/P in inorganic and organic nutrient pools in the river were 151 ± 84 and 80 ± 67 respectively, indicating a N- enriched but P-depleted ecosystem in the lower Milwaukee River. Except for NO_3^- , the average instantaneous flux was the lowest during winter and high during the spring and the storm event. Therefore, most of anthropogenic nitrogen (NO_3^-) and phosphorous (DIP) fluxes spiked during storm and snowmelt events. The DIP yield in the Milwaukee River ($28 \text{ Kg km}^{-2} \text{ year}^{-1}$) is relatively similar to that of the Trinity River ($27 \text{ Kg km}^{-2} \text{ year}^{-1}$) and the Amazon River ($21 \text{ Kg km}^{-2} \text{ year}^{-1}$) but much higher than that of the heavily polluted Changjiang River ($8.1 \text{ Kg km}^{-2} \text{ year}^{-1}$). Surprisingly, NO_3^- yield in the lower Milwaukee River ($581 \text{ Kg km}^{-2} \text{ year}^{-1}$) was higher than that of the Mississippi River ($294 \text{ Kg km}^{-2} \text{ year}^{-1}$), showing the tremendous addition of anthropogenic nitrate to the Milwaukee River watershed. Controlling factors of nutrients species in the Milwaukee River will keep evolving, as a result of population growth and climate change. Long term monitoring of nutrient dynamics in the Milwaukee River is essential to be able to prevent water quality and ecosystem health perturbations in the river and the coastal area of Lake Michigan.

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5. Appendices

Appendix A. Surface water temperature (Temp), specific conductivity (Sp. cond), pH, suspended particulate matter (SPM) and water stable isotopes composition ($\delta^{18}\text{O}$ and $\delta^2\text{H}$) at an upstream (MR-05) and a downstream (MR-01) stations in the lower Milwaukee River during 2014-2015.

Sampling Dates	Temp C°	Sp. Cond $\mu\text{S cm}^{-1}$	pH	TA ($\mu\text{M kg}^{-1}$)	SPM (mg L^{-1})	d(O-18/O-16) (‰)	d(D/H) (‰)
MR-01 (Downstream)							
2/27/14	0.3	678	8.2	4768	4.6	-8.02	-56.6
3/27/14	1.3	924	7.9	4243	16.0	-11.22	-80.7
4/24/14	11.3	952	8.3	4908	22.6	-9.65	-67.0
5/22/14	13.2	764	7.9	4488	----	-7.78	-52.4
6/11/14	19.6	825	8.1	4864	14.6	-7.25	-50.6
7/10/14	23.4	723	8.3	5300	12.0	-7.49	-52.0
8/10/14	25.6	848	8.4	4932	11.3	-7.91	-57.0
9/11/14	19.9	785	8.5	5360	10.0	-7.53	-53.5
10/9/14	14.4	839	8.3	4956	4.7	-9.10	-66.8
11/13/14	8.9	954	8.5	5876	7.0	-8.85	-63.6
12/9/14	1.1	608	8.5	6547	1.3	-9.34	-65.9
1/9/15	0.2	705	8.3	7217	3.3	-9.86	-67.0
2/10/15	0.4	604	8.2	6354	6.0	-9.49	-68.7
3/12/15	2.3	712	8.1	4384	11.0	-11.91	-84.2
4/10/15	6.3	503	8.1	1450	140.0	-7.07	-42.4
MR-05 (Upstream)							
2/27/14	0.2	605	8.3	6662	1.6	-9.21	-62.9
3/27/14	1.1	836	8.1	5135	8.4	-10.28	-72.5
4/24/14	10.4	754	8.3	4956	14.3	-9.63	-67.0
5/22/14	12.5	784	8.2	4904	21.1	-7.94	-53.6
6/11/14	16.7	629	8.3	4657	35.4	-8.35	-58.1
7/10/14	23.6	722	8.4	5717	11.4	-7.65	-52.7
8/10/14	26	844	8.5	6039	15.9	-8.26	-58.5
9/11/14	17.7	854	7.8	5713	11.0	-8.00	-56.5
10/9/14	10.9	821	8.5	6020	3.3	-9.60	-67.7
11/13/14	2.2	754	7.6	2373	12.0	-10.04	-73.9

Continued appendix A

Sampling Dates	Temp C°	Sp. Cond $\mu\text{S cm}^{-1}$	pH	TA ($\mu\text{M kg}^{-1}$)	SPM (mg L^{-1})	d(O-18/O-16) (‰)	d(D/H) (‰)
MR-05 (Upstream)							
12/9/14	3.9	517	8.4	5411	2.0	-9.19	-65.4
1/9/15	0.1	610	8.3	6032	5.0	-9.78	-67.5
2/10/15	0.2	555	8.2	6583	10.0	-9.56	-67.5
3/12/15	2.1	528	8.2	4056	9.0	-12.34	-87.6
4/10/15	6.2	505	8.2	2785	100.0	-7.02	-42.5

Appendix B. Dissolved organic carbon (DOC) and optical properties including absorption coefficient at 254 nm (a_{254}), specific UV absorbance at 254 nm ($SUVA_{254}$) and spectral slope at 275-295 nm ($S_{275-295}$) at an upstream (MR-05) and a downstream (MR-01) stations in the lower Milwaukee River during 2014-2015.

Sampling Dates	DOC ($\mu\text{M-C}$)	a_{254} (m ⁻¹)	$SUVA_{254}$ (L mg-C ⁻¹ m ⁻¹)	$S_{275-295}$ (nm ⁻¹)
MR-01 (Downstream)				
2/27/14	318	24.7	2.81	0.0170
3/27/14	328	40.2	4.44	0.0140
4/24/14	726	42.2	3.04	0.0178
5/22/14	1007	113.1	4.07	0.0154
6/11/14	928	100.5	3.92	0.0159
7/10/14	1102	120.8	3.97	0.0159
8/10/14	602	57.8	3.48	0.0180
9/11/14	794	52.8	2.41	0.0268
10/9/14	594	62.9	3.83	0.0171
11/13/14	825	74.8	3.28	0.0150
12/9/14	696	64.1	3.33	0.0168
1/9/15	749	74.2	3.58	0.0199
2/10/15	613	39.1	2.74	0.0199
3/12/15	531	44.1	3.00	0.0157
4/10/15	749	80.6	3.89	0.0140
MR-05 (Upstream)				
2/27/14	379	37.2	3.54	0.0163
3/27/14	355	39.5	4.02	0.0143
4/24/14	696	63.2	3.29	0.0173
5/22/14	1128	145.7	4.67	0.0145

Continued appendix B

Sampling Dates	DOC ($\mu\text{M-C}$)	a ₂₅₄ (m ⁻¹)	SUVA ₂₅₄ (L mg-C ⁻¹ m ⁻¹)	S ₂₇₅₋₂₉₅ (nm ⁻¹)
MR-05 (Upstream)				
7/10/14	1189	129.6	3.94	0.0158
8/10/14	760	73.5	3.50	0.0179
9/11/14	926	74.4	2.91	0.0267
10/9/14	781	94	4.35	0.0157
11/13/14	727	67.8	3.37	0.0124
12/9/14	549	52.8	3.48	0.0171
1/9/15	699	67.8	3.51	0.0202
2/10/15	516	40.2	2.37	0.0198
3/12/15	655	50.4	2.78	0.0145
4/10/15	764	85	4.02	0.0147

Appendix C. Fluorescent Component abundance (C1, C2 and C3) and fluorescent indices FIX (fluorescence index), BIX (biological index) and HIX (humification index) in the lower Milwaukee River during 2014- 2015.

Sampling Dates	FIX	BIX	HIX	(ppb-QSE)		
				C1	C2	C3
MR-01 (Downstream)						
2/27/14	1.24	0.70	7.03	4.47	2.07	1.09
3/27/14	1.22	0.73	2.18	6.77	2.76	4.32
4/24/14	1.17	0.68	3.22	12.15	5.30	5.58
5/22/14	1.16	0.58	7.24	16.87	8.73	2.68
6/11/14	1.15	0.62	4.76	17.17	7.54	4.12
7/10/14	1.15	0.56	7.60	21.50	9.93	2.44
8/10/14	1.19	0.64	3.46	10.94	4.16	3.17
9/11/14	1.16	0.64	4.47	14.89	5.67	3.69
10/9/14	1.18	0.65	3.85	10.41	4.31	3.62
11/13/14	1.21	0.66	4.79	12.48	5.40	3.75
12/9/14	1.21	0.64	7.40	11.01	4.92	1.79
1/9/15	1.22	0.64	6.04	12.51	5.64	2.39
2/10/15	1.24	0.69	7.10	6.84	2.92	1.61
3/12/15	1.22	0.68	1.89	8.41	3.61	5.35
4/10/15	1.17	0.59	3.27	13.66	7.51	5.68
MR-05 (Upstream)						
2/27/14	1.24	0.69	8.83	6.10	3.11	1.18
3/27/14	1.23	0.70	2.80	6.83	3.01	3.02
4/24/14	1.19	0.62	3.10	12.02	5.67	5.40
5/22/14	1.14	0.58	5.01	16.70	8.62	3.72
6/11/14	1.13	0.59	4.98	21.43	9.74	4.41
7/10/14	1.16	0.55	5.68	23.51	10.97	2.87
8/10/14	1.17	0.64	3.91	14.05	5.62	4.03
9/11/14	1.17	0.61	2.02	17.55	7.08	6.40
10/9/14	1.17	0.63	4.36	13.91	6.52	3.49
11/13/14	1.19	0.63	6.49	14.47	6.33	4.08
12/9/14	1.21	0.67	6.95	9.75	4.17	2.10
1/9/15	1.28	0.71	5.73	5.03	2.02	1.19
2/10/15	1.28	0.71	7.46	6.75	2.98	1.76

Continued appendix C

Sampling Dates	FIX	BIX	HIX	C1	C2	C3
(ppb-QSE)						
MR-05 (Upstream)						
3/12/15	1.24	0.67	2.55	8.57	3.81	5.04
4/10/15	1.17	0.60	3.90	13.86	7.58	5.29

Appendix D. Optical properties of CDOM during high flow (April 10 2014) and low flow (November 13 2014) in the lower Milwaukee River.

Sample ID	DOC ($\mu\text{M-C}$)	a254 (m^{-1})	SUVA254 ($\text{L mg-C}^{-1} \text{m}^{-1}$)	S275-295 (nm^{-1})	FIX	BIX	HIX
Low Flow (November 13 2014)							
MR-01	825	74.8	3.28	0.0151	1.20	0.66	6.92
MR-02	777	80.6	3.77	0.0147	1.19	0.68	6.21
MR-03	765	77.0	3.65	0.0150	1.24	0.64	6.29
MR-04	757	80.2	3.83	0.0149	1.19	0.67	6.76
MR-05	757	78.5	3.75	0.0152	1.19	0.63	6.49
High Flow (April 10 2014)							
MR-01	749	80.6	3.89	0.0140	1.17	0.59	3.27
MR-02	786	79.7	3.67	0.0146	1.18	0.59	3.36
MR-03	798	81.3	3.69	0.0151	1.17	0.60	2.94
MR-04	780	80.8	3.75	0.0143	1.17	0.58	3.58
MR-05	764	85.0	4.02	0.0147	1.17	0.60	3.90

Appendix E. Concentrations of dissolved inorganic P (DIP), dissolved organic P (POP), particulate inorganic P (PIP), and Particulate organic P (POP), Ratios of DIP to TDP (total dissolved P), DOP to TDP, PIP to TPP (total particulate P) and POP to TPP at an upstream (MR-05) and a downstream stations in the lower Milwaukee River during 2014- 2015.

Sampling Dates	DIP (μM)	DOP (μM)	DIP/TDP (%)	DOP/TDP (%)	PIP (μM)	POP (μM)	PIP/TPP (%)	POP/TPP (%)
MR-01 (Downstream)								
2/27/14	1.06	0.2	84	16	0.691	0.653	51	49
3/27/14	1.649	1.28	56	44	0.613	1.18	34	66
4/24/14	0.576	0.208	73	27	0.641	0.66	49	51
5/22/14	0.515	1.942	21	79	0.696	0.727	49	51
6/11/14	1.149	0.298	79	21	0.665	0.765	47	53
7/10/14	2.271	2.791	45	55	0.17	0.443	28	72
8/10/14	1.032	1.197	46	54	0.164	0.971	14	86
9/11/14	0.681	1.386	33	67	0.239	0.625	28	72
10/9/14	1.094	0.081	93	7	0.107	0.754	12	88
11/13/14	0.394	0.691	36	64	0.046	0.528	8	92
12/9/14	0.14	0.346	29	71	0.071	0.381	16	84
1/9/15	0.114	0.424	21	79	0.099	0.224	31	69
2/10/15	0.16	0.524	23	77	0.045	0.413	10	90
3/12/15	2.496	1.446	63	37	0.605	0.796	43	57
4/10/15	2.751	0.649	81	19	1.741	1.624	52	48
Average	1.072	0.898	52	48	0.44	0.716	31	69
STDEV	±0.862	±0.766	±25	±25	±0.45	±0.347	±16	±16
MR-05 (Upstream)								
2/27/14	1.592	0.086	95	5	0.441	0.403	52	48
3/27/14	1.37	0.668	67	33	0.518	1.121	32	68
4/24/14	0.318	0.525	38	62	0.872	0.918	49	51
5/22/14	0.417	1.906	18	82	0.694	0.811	46	54
6/11/14	0.46	0.943	33	67	1.391	1.078	56	44
7/10/14	1.203	4.688	20	80	0.284	1.025	22	78
8/10/14	0.711	2.209	24	76	0.236	0.681	26	74
9/11/14	0.199	2.782	7	93	0.23	0.633	27	73

Continued appendix E

Sampling Dates	DIP (μM)	DOP (μM)	DIP/TDP (%)	DOP/TDP (%)	PIP (μM)	POP (μM)	PIP/TPP (%)	POP/TPP (%)
MR-05 (Upstream)								
10/9/14	0.283	0.29	49	51	0.057	0.549	9	91
11/13/14	0.118	0.475	20	80	0.096	0.394	20	80
12/9/14	0.9	0.94	49	51	0.384	0.832	32	68
1/9/15	0.124	0.543	19	81	0.077	0.3	20	80
2/10/15	1.575	1.933	45	55	0.132	0.314	30	70
3/12/15	1.078	1.388	44	56	0.705	0.695	50	50
4/10/15	2.668	0.673	80	20	2.23	3.792	37	63
Average	0.868	1.337	40	59	0.556	0.903	34	66
STDEV	±0.721	±1.215	±25	±25	±0.588	±0.843	±14	±14

Appendix F. Concentrations of Nitrate (NO_3^-), dissolved organic N (DON), ratios of NO_3^- to TDN (Total dissolved nitrogen) and DON to TDN at an upstream (MR-05) and a downstream (MR-01) stations in the lower Milwaukee River during 2014-2015.

Sampling Dates	NO_3^- (μM)	DON (μM)	NO_3^-/TDN (%)	DON/TDN (%)
MR-01 (Downstream)				
2/27/14	179	11	94	6
3/27/14	155	143	52	48
4/24/14	80	79	50	50
5/22/14	78	64	55	45
6/11/14	65	46	58	42
7/10/14	74	37	67	33
8/10/14	62	36	63	37
9/11/14	76	50	60	40
10/9/14	71	29	71	29
11/13/14	75	78	49	51
12/9/14	151	57	72	28
1/9/15	180	118	60	40
2/10/15	238	16	94	6
3/12/15	178	20	90	10
4/10/15	128	19	87	13
Average	119	54	68	32
STDEV	± 57	± 38	± 16	± 16
MR-05 (Upstream)				
2/27/14	259	16	94	6
3/27/14	192	252	43	57
4/24/14	79	9	89	11
5/22/14	79	82	49	51
6/11/14	70	34	67	33
7/10/14	84	33	72	28
8/10/14	80	35	69	31
9/11/14	86	59	60	40
10/9/14	86	40	68	32
11/13/14	84	2	98	2
12/9/14	117	54	69	31

Continued Appendix F

Sampling Dates	NO ₃ ⁻ (μM)	DON (μM)	NO ₃ ⁻ /TDN (%)	DON/TDN (%)
MR-05 (Upstream)				
1/9/15	224	72	76	24
2/10/15	239	94	72	28
3/12/15	156	19	89	11
4/10/15	133	22	86	14
Average	131	54	73	27
STDEV	±66	±60	±16	±16

Appendix G. Seasonal variation in nutrients molar ratios at an upstream (MR-05) and a downstream (MR-01) stations in the lower Milwaukee River during 2014-2015.

Sampling Dates	DOC/DON	DIC/DIN	DOC/DOP	DIC/DIP	DIN/DIP	DON/DOP
MR-01 (Downstream)						
2/27/14	30	30	1589	5042	169	53
3/27/14	2	20	256	1851	94	112
4/24/14	9	63	3484	8807	139	381
5/22/14	16	65	518	9755	151	33
6/11/14	20	78	3111	4433	56	155
7/10/14	30	76	395	2467	32	13
8/10/14	17	81	503	4861	60	30
9/11/14	16	41	573	4541	112	36
10/9/14	20	72	7341	4667	65	363
11/13/14	11	76	1194	14529	191	113
12/9/14	12	45	2011	48317	1076	166
1/9/15	6	41	1768	64269	1582	278
2/10/15	37	27	1168	40182	1491	31
3/12/15	26	25	367	1794	71	14
4/10/15	39	12	1154	566	47	29
Average	19	50	1696	14405	356	121
STDEV	±11	±24	±1844	±19775	±543	±126
MR-05 (Upstream)						
2/27/14	23	24	4412	3912	162	189
3/27/14	1	23	532	3189	140	378
4/24/14	74	67	1326	16548	248	18
5/22/14	14	60	592	11339	189	43
6/11/14	31	70	1122	10557	152	36
7/10/14	37	72	254	5062	70	7
8/10/14	22	75	344	8469	113	16
9/11/14	16	73	333	31670	435	21
10/9/14	20	73	2692	22140	305	138
11/13/14	353	25	1530	18194	714	4
12/9/14	10	47	584	6180	131	57
1/9/15	10	27	1288	48803	1805	133

Continued appendix G

Sampling Dates	DOC/DON	DIC/DIN	DOC/DOP	DIC/DIP	DIN/DIP	DON/DOP
2/10/15	6	28	267	4306	152	48
3/12/15	35	26	472	3763	145	14
MR-05 (Upstream)						
4/10/15	34	21	1136	1050	50	33
Average	46	47	1126	13012	321	76
STDEV	±87	±23	±1121	±13024	±444	±100