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Monitoring the natural processing of organic carbon and phenols in selective wetlands within Monroe County, NY

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Monitoring the Natural Processing of Organic Carbon and Phenols in Selective Wetlands within Monroe County, NY

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August, 2013

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Science at Rochester Institute of Technology
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ABSTRACT

Dissolved Organic Carbon (DOC) is one of the largest active organic carbon reservoirs formed from processes that break down terrestrial/aquatic matter and released from plant roots as exudates. In watersheds, DOC is continuously transported and processed through rivers, streams and lakes. DOC is present in wetlands around Monroe County, New York; in areas that function as groundwater recharge, stormwater retention, nutrient retention and habitats for wildlife. The conducted research was an initial step towards a more thorough understanding of DOC’s processing, especially in regard to its phenolic content, in small urban/sub-urban wetlands in Monroe County, NY. DOC and phenol concentrations were assessed primarily through utilizing a Total Organic Carbon (TOC) analyzer and the Folin-Ciocalteau Reagent method. Ultimately, samples of influent flow to, and effluent flow from, natural, man-made and retention pond wetlands were collected and analyzed to help understand how DOC might be processed through these local wetlands. Results show that there was no statistical difference in the DOC and Phenol Net Concentration difference among wetland types and seasons but statistical significance existed only in the comparison of the total overall DOC and Phenol concentrations among Seasons, Wetland Types and Sites. A better understanding in the interpretation of the changes in DOC and phenol concentrations in wetlands could be achieved if other variables such as wetland size, water depth, retention time and landscape are taken into account in future studies.
INTRODUCTION

Overview

In wetlands and watersheds, the substances that are produced by the decomposition of organic matter and are continuously transported to rivers, streams and lakes connected to a community’s water supply can be cause for concern. These dissolved substances, known collectively as Dissolved Organic Carbon (DOC), can affect human health, as well as other organisms within the aquatic ecosystem. In general, DOC supports aquatic organisms as they are a source of carbon and energy for biota; for humans, DOC is an issue is when it comes to a wetland site that serves as source of drinking water supply as they are a major reactant (Leenheer and Crowe, 2003). In part for these reason, this research was conducted in order to increase our understanding of the natural processing of DOC, and its phenolic portions, in wetlands.

Importance of Wetlands

Wetlands are functional areas of water saturated soil, small lakes, floodplains, and marshes. (Kayranli et al., 2001). Some of these wetlands are constructed and engineered (Tront et al., 2006) for the removal of contaminants from wastewater discharge and sediments (McCutcheon and Schnoor, 2003; Cameron et al., 2003; Weaver et al., 2004) and even removal of pollutants such as nitrates from the municipal water supply (Reilly et al., 2000). Wetland areas provide valuable services to both human societies and wildlife that dwell within the region. They also provide other invaluable functions such as flood storage and nutrient cycling and ground-water recharge (Mitsch and Gosselink, 1993; Balcombe et al., 2005). The types of vegetation present are indicators of the wetland’s functions. The greater the structural diversity of the vegetation, the greater the diversity of the wetland’s resident species (MacArthur and MacArthur, 1961;
Evans and Wilson, 1982; Anderson and Smith, 1999; King et al., 2000; Naugle et al., 2000). This diversity is because the composition of wetland vegetation and structure influences the type, quantity and nutritive quality of plant foods available (deSzalay and Resh 1997, Anderson and Smith., 1998); the distribution, density and structure of cover (Hays et al. 1981, McConnell and Samuel 1985, Anderson and Smith, 1999); and water chemistry (Goslee et al., 1997; Castelli et al., 2000) that involves breakdown and reassembling of organic matter, hence transformation of water quality.

The evaluation of wetlands is of vital importance, especially when it comes to assessing the amount of DOC present. Wetlands’ soil reservoirs contain a large proportion of the world’s stored carbon (approximately 15 x 10^{14} kg), and thus have the highest carbon density of all other ecosystems (Kayranli et al., 2010). They play an important role in the carbon cycle of their surrounding ecosystem (Schlesinger, 1991; Amthor et al., 1998; Whitting and Chanton., 2001). Hence, wetlands can be a diffuse source of humic substances to freshwater systems (Stern et al., 2007). The export of DOC from wetlands can have major implications for freshwater systems (Clark et al. 2005). These implications include the processing of drinking water obtained from lakes and rivers (Grieve, 1990; Mitchell, 1990; Worrall et al., 2003) and as a base for microbial activity (Qualls & Haines, 1992) that break down the DOC and produce carbon dioxide (Dawson et al., 2002; Algesten et al., 2003; Sobek et al., 2003), forming other organic matter in the process (sometimes giving off only CO_{2} if completely broken down). Peat soils near wetlands are considered to be major sources of DOC to surface waters (Urban et al., 1989; Hope et al., 1997; Aitkenhead et al., 1999).
The amount of organic matter processed within a wetland can be dependent on its microorganisms and vegetation. Both are critical components when it comes to the development of hydric soil characteristics. Microorganisms that are heterotrophic generally decompose or denitrify organic matter content in the soil. Vegetation form surficial layers that are high in organic matter as a result of accumulated net primary production (Craft, 2001). The balance between decomposition and primary production represents the organic matter accumulation (Schlesinger 1991, Mitsch and Gosselink 1993). Both processes are regulated by nutrient availability, hydro periods, abiotic stressors (salinity and acidity), solar radiation and air/soil temperatures (Craft, 2001). Such processes may determine the differences in the amount of organic matter between influent flow and effluent flow within a single wetland.

Fleck et al. (2004) stated that the differences in carbon sources, decomposition rates and pathways, and carbon availability within wetlands have profound effects on the carbon forms that reach delta channel waters where they are diverted for drinking water. Wetlands contain five main carbon reservoirs, identified by Kayranli et al. (2010) as plant biomass carbon, particulate organic carbon, DOC, microbial biomass carbon and gaseous end-products such as carbon dioxide and methane. The decomposition of the organic matter within wetlands involves both aerobic and anaerobic processes. Under anaerobic conditions, decomposition is often incomplete and causes the plant remains coming from inflow to accumulate within the wetlands (Kayranli et al., 2010), although plants also grow in the wetlands itself. In addition, the low decomposition rates are also the result of waterlogged conditions (anaerobic conditions) and high levels of phenolic substances (Fenner et al., 2005), which might lead to increasing levels of DOC.
**Wetlands in Monroe County**

There are few wetlands in Monroe County, New York that accumulate peat and contribute to mass carbon export. Most of the wetlands in Monroe County are shallow emergent ones that contain primarily mineral soils and serve in the functions of groundwater recharge, stormwater retention, nutrient retention and habitats for wildlife such as muskrats, beavers, ducks and deer (US EPA, 1995; NY Department of Environmental Conservation, 2003). The watershed contributing to most of the natural and man-made wetlands around Monroe County drain urban or sub-urban landscapes, including roadways and parking lots. Some of these wetlands are shallow artificial marshes or stormwater treatment areas (retention ponds) involved in the removal of phosphorous and nitrate from urban and agricultural runoff (Spieles and Mitsch, 2000). In addition, stormwater treatment areas also involve settling sediments (Kadlec and Knight, 1996), sulfate reduction, metal precipitation (Stein et al., 2007) and breakdown of organic compounds (Knight et al., 1999). These characteristics also apply to natural wetlands and are also the reason why stormwater retention ponds are created around Monroe County to serve such purposes.

Natural wetlands and man-made wetlands differ in their soil and vegetation contents, hydrologic characteristics and function. Man-made or created wetlands are constructed either as a new wildlife habitat or as a new addition to an ecosystem, or to replace a lost wetland site. Sometimes, unlike natural wetlands, man-made wetlands may tend to have significantly higher quantities of rock fragments and twice the median bulk density of natural wetlands due to high sand content and compaction at the surface in the wetland's construction (Brooks, 1993). The soil texture of man-made wetlands can also have higher percentages of silt or clay loams than natural
wetlands (Campbell et al, 2002). Plant species richness and vegetation in man-made wetlands may tend to be lower than natural ones (Jarman et al., 1991). Retention ponds are created wetlands that are built in urban or sub-urban environments and along highways and motorways.

**Dissolved Organic Carbon (DOC)**

Natural waters can contain Total Organic Matter (TOM), which in effect contains the dissolved portion, Dissolved Organic Matter (DOM). The DOM is made up of numerous components consisting of Total Organic Carbon (TOC), DOC, humic acid, fulvic acid, dissolved organic nitrogen and phosphorous, humic and non-humic substances and other undissolved organic matter. The DOC component, which contains inherent phenols, is the main focus of this research. It is made up of a complex mixture of aromatic and aliphatic hydrocarbon structures, which are bonded to functional groups (Leenheer and Crowe, 2003; Fabris et al., 2008). A single DOC molecule may also be made up of large amounts of phenolic compounds (Fabris et al., 2008). DOC is a common substance present in nature, operationally defined by Evans et al. (2005) as “comprising of any organic compounds that can pass through a 0.45 micrometer filter.” In the biosphere, DOC is one of the largest active organic carbon reservoirs (Amon and Benner, 1996) that is naturally formed from the processes that break down terrestrial and aquatic matter (Murphy et al., 2011; Evans et al., 2005). DOC is also released from the roots of plants as exudates. DOC contains complex substances that can affect physical, chemical and biological processes that occur within aquatic environments (Hudson et al., 2003) and influence ecosystems (Jaffe et al., 2008). DOC is a vital source (Sun et al 1997., Wetzel et al., 1995) that affects food webs, particularly microbial webs (Jaffe et al., 2008), either by direct uptake from organisms or
by indirect mechanisms such as pH, turbidity, metal chelation and transportation of contaminants (McDonald et al., 2004).

Freeman et al. (2004) stated that there have been observations of rising DOC concentrations in aquatic ecosystems. The possible causes of increase in DOC concentrations are, in accordance to Fleck et al. (2004), flooding of shallow, peat soils from wetlands and changes in land-use by agriculture to freshwater wetlands. However, there are other potential causes that include climate change and changes in acid precipitation/deposition (Erlandsson et al, 2011). Leenher et al. (2003) also stated that the concentration of DOC is highly variable and dependent upon the source, temperature, ionic strength, pH, surface chemistry of sediment sorbents and the presence of photolytic and microbiological degradation processes. Though the degradation of phenolic compounds in DOC can be achieved by the activities of aerobic and anaerobic microorganisms (Fenner et al., 2005), the anaerobic conditions in peat lands can hinder the activity of the enzyme phenol oxidase from decomposing phenolic compounds and prevent release of major store of the global carbon into the atmosphere (Freeman et al., 2001). The export of DOC can be further increased by warming and elevated atmospheric CO$_2$ (Fenner et al., 2007).

Different types of wetlands (natural or man-made) may produce different amounts of DOC in different seasons. Natural wetlands that have higher vegetation composition than some man-made ones may create conditions that either help or hinder the production or degradation of DOC. As mentioned prior, vegetation composition can influence water chemistry in wetlands. Microorganisms within wetlands can also create aerobic or anaerobic conditions that can hinder activity of enzymes that degrade DOC. Seasons too affect decomposition rates due to varying
temperatures throughout the year. In the case of shallow, emergent wetlands in this study, it is in question as to whether or not these wetlands are producing more or less DOC in different seasons and wetland types. Some shallow wetlands may produce more DOC while others produce less. The amount of DOC produced in these wetlands may be dependent on the quantity and characteristic of vegetation present and the seasons that affect the production/decomposition rates by the vegetation and microorganisms since vegetation is adapted to filter nutrients from water (Boyt et al., 1977) and regulate organic matter within the wetland soils (McLatchey and Reddy, 1998). Warm temperatures can speed up chemical reaction rates while colder ones can slow them down. Wetland vegetation can have their production performance affected by changes in environmental factors (such as temperature and ionic/pH changes, which can vary due to climate), seasons, landscapes and abiotic factors (human impacts). Worrall et al. (2004) found compelling explanations of how observed long-term increases in temperatures caused by global warming might cause increases in peat decomposition rates, thereby producing and releasing more organic matter into freshwater.

Phenols

The inherent characteristic of phenols in DOC is that they are highly recalcitrant and only certain microorganisms seem to be able to decompose them (Freeman, 2005). Generally, phenols are less biodegradable and remain in the environment longer than other components of DOC. In wetlands with anaerobic soils, phenols will not be broken down and may therefore be exported from wetlands to downstream water bodies, to areas that may be involved with drinking water sources. It is known that phenols can react with chlorine used in water treatment to produce dangerous disinfection by-products (Rule et al, 2005; Gone et al, 2009). Though the focal point
of this research does not directly involve studying the formation of disinfection by-products, it is nevertheless a motivation for future research to be conducted in wetlands that might be connected to drinking water sources.

**Folin-Ciocalteau Reagent Method**

In accordance with Pagano et al. (2012), the direct measurement of phenols is logistically challenging. However, the most reliable technique for analyzing complex samples is the Folin-Ciocalteau reagent method, which is capable of measuring all forms of phenols (monophenols, polyphenols and lignin phenols); but mechanistically unable to differentiate between these phenol types. The usefulness of this method has been proven in the quantifying of phenols in environmental samples (Thoss et al., 2002; Yu and Dahlgren, 2000; Thoss et al., 2002b, Box 1983). The Folin-Ciocalteau reagent-based assay only requires microliters of sample.

**Research Objectives**

There have been numerous studies conducted to contribute to the understanding of the inherent characteristics of wetlands. However, there is still more to be learned about their natural processing of organic matter. In this study, the wetlands around Monroe County may have inherent characteristics of altering organic matter by its resident microorganisms and vegetations. They may either increase or decrease the amount of DOC present in their waters. If differences in the amount of dissolved organic matter (and its phenolic content) entering and exiting the wetland do exist, how big is this difference and how does it vary in accordance to the seasons and to the type of wetland?
This research study represents an initial step towards an understanding of the natural processing of DOC- and specifically its phenolic content- in small urban/sub-urban wetlands. The overall objective of this study was to assess the variations in the concentration of DOC/phenols present in selected wetlands and retention ponds around Monroe County of upstate New York in three categories: I) influent/effluent flow (net flux) concentration of DOC/phenols; II) concentration variation in accordance with wetland type; III) concentration variation in accordance with season. This approach is aimed to study the processing of DOC/phenolic content by analyzing their concentration differences. Specifically, I hypothesize that there will be variations in the DOC concentration (assessed by the TOC analyzer) and the phenol concentration (assessed by Folin-Ciocalteau Reagent Method) in wetlands around Monroe County, New York, between:

I) Influent flow and effluent flow
   - Predict effluent flow to have a lower DOC concentration after material is processed through the wetland. While this assumes the organisms and vegetations within wetland are feeding on DOC and reducing its concentration in process, it is also noted that DOC can be produced in addition to being broken down.

II) Natural, man-made and retention pond wetland types
   - Predict that natural wetlands will breakdown the DOC to a greater extent- resulting in lower DOC concentrations in the effluent samples, due to natural wetlands having the most abundance of vegetations capable of breaking down DOC.
III) Season

- Predict that the warmest season (summer) will breakdown DOC to a greater extent- resulting in lower DOC concentrations in the effluent samples during summer and higher in winter. This prediction is based on the observation that having high temperatures that favors photosynthesis processes in plants that result more biochemical production of DOC and breakdown of DOC by microorganisms.
MATERIALS & METHODS

Site Selection

There were a total of 15 selected sampling locations: five of them were natural wetlands, five were created (man-made) wetlands and five were retention ponds. All these locations are listed in the table below; the maps of these locations can be found in the Appendix.

Table 1: Sites of Sampling Locations

<table>
<thead>
<tr>
<th>ID</th>
<th>Wetland Type</th>
<th>Name</th>
<th>Location Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>WN1</td>
<td>Natural</td>
<td>Bailey</td>
<td>Red Creek, East River Road &amp; Bailey Road, - East Side of East River</td>
</tr>
<tr>
<td>WN2</td>
<td>Natural</td>
<td>Ballantyne</td>
<td>Beaver Road &amp; Ballantyne Road - SW Corner of intersection, cattail marsh</td>
</tr>
<tr>
<td>WN3</td>
<td>Natural</td>
<td>Allens</td>
<td>Allens Creek, S. Winton Road</td>
</tr>
<tr>
<td>WN4</td>
<td>Natural</td>
<td>Buckland</td>
<td>Buckland Park</td>
</tr>
<tr>
<td>WN5</td>
<td>Natural</td>
<td>Tamarack</td>
<td>Tamarack Swamp @ Durand Park - cattail marsh, the lake trail has a boardwalk through the marsh</td>
</tr>
<tr>
<td>WMM1</td>
<td>Man-Made</td>
<td>Barker</td>
<td>Barker Road Middle School, Pittsford</td>
</tr>
<tr>
<td>WMM2</td>
<td>Man-Made</td>
<td>French Road</td>
<td>Sisters of St.Joseph - French Road Pittsford</td>
</tr>
<tr>
<td>WMM3</td>
<td>Man-Made</td>
<td>Bryden</td>
<td>Bryden Park</td>
</tr>
<tr>
<td>WMM4</td>
<td>Man-Made</td>
<td>Bloomfield</td>
<td>W. Bloomfield Road - Pittsford (Near Canfield Dr., Just north of thruway)</td>
</tr>
<tr>
<td>WMM5</td>
<td>Man-Made</td>
<td>HANA</td>
<td>HANA - Area 1S. (This is at High Acres and managed by Waste Management.)</td>
</tr>
<tr>
<td>WP1</td>
<td>Retention Pond</td>
<td>J Lot</td>
<td>RIT J Lot (campus parking area)</td>
</tr>
<tr>
<td>WP2</td>
<td>Retention Pond</td>
<td>TFH 1</td>
<td>The Father's House 1 - Chili, pond along Paul Road</td>
</tr>
<tr>
<td>WP3</td>
<td>Retention Pond</td>
<td>TFH 2</td>
<td>The Father's House 2 - Chili, pond along Archer road south of driveway</td>
</tr>
<tr>
<td>WP4</td>
<td>Retention Pond</td>
<td>MCC</td>
<td>Monroe Community College - Pond along 590 near Ice arena</td>
</tr>
<tr>
<td>WP5</td>
<td>Retention Pond</td>
<td>Erie</td>
<td>Erie Station Village</td>
</tr>
</tbody>
</table>
**Sampling Procedure**

Water samples were collected from the selected wetland sites once in each of the four seasons during base flow (dry weather flow) at 3 month intervals during a one year period. No sampling was conducted during rainy weather as retention and concentration differential of the flow might vary during those events. Three replicates were collected at each point of sampling in two separate 60 ml syringes (one for influent flow and the other for effluent flow) and were stored in separate Whirl-Pak® bags. About 40 ml of the sample amount was collected in each syringe. The samples were filtered through 0.45 µm filters before being stored into Whirl-Pak® sterile sample bags. The samples were kept in an ice cooler until they were carried to the laboratory, where they were transferred to a freezer at -20°C until ready for analysis. Frozen samples were brought to room temperature to thaw before analysis.

**Sample Analysis**

Each sample was later subjected to TOC and Folin-Phenol analyses. Approximately 9 mL of the samples from each bag were required to conduct all of the analyses (6~8 ml for TOC and less than 0.5 ml for Folin-Phenol). Excess samples were stored in case of accidental spills or if analysis was done incorrectly and must be redone; and also as a backup to any leaks or spills that occur during thawing, where the freezer’s low temperature sometimes caused the lining glue of the bags to come apart.

**A. Total Organic Carbon Analysis**

The procedure for performing DOC analysis in the TOC Analyzer (TOC-V CPH by SHIMADZU Corporation) employed the same protocols by Stedmon et al. (2011). The TOC
Analyzer was run by the software TOC-V Sample Table Editor. Before running the instrument, all water reservoirs within the analyzer were verified to ensure that enough HPLC grade pure water were available to perform analysis. The air tank (adjusted to 50 psi) must be turned on for the instrument to reach a stable baseline (oven temp to 680°C, humidifier around 1.0, having three baseline conditions as indicated by the instrument’s “Background Monitor”). The TOC Analyzer’s pressure gauze was adjusted to 200 psi, while the carrier gas was adjusted between 140 to 160 mL. The baseline fluctuations reached equilibrium an hour after the instrument and air had been turned on. In setting the instrument’s Calibration Curve Wizard, the NPOC for Organic Carbon, Linear Regression option was selected, while the rest of the settings were set to default.

The reagent prepared for each analysis is 1.0628 g of Potassium Hydrogen Phthalate dissolved in 500 mL HPLC ultrapure water. This stock solution (1000 ppm) served as Tannic Acid Equivalent (TAE), as indicator of phenolic concentration. From this stock, 5 mL was mixed with 100 mL HPLC ultrapure water to prepare 50 ppm TAE. This amount was frequently used as the main standard for comparison with sample results in each TOC run. All samples ready for analysis were transferred from their respective Whirl-Pak® bags into separately labeled glass tubes and the tubes were placed into the TOC Analyzer’s tray. The instrument was left to run overnight and the DOC sample data were collected from the running TOC software in the next day.

B. Folin-Ciocalteau Reagent (FCR) Method

The Folin-Ciocalteau Reagent method (hereafter referred to as FCR) used the EON BioTek Microplate Spectrophotometer (Model EONC) and was run by Gen5 software. The
procedures used in this method were similar to the ones that followed Box (1983), Pagano et al. (2012) and Magalhães et al (2010). Procedures by Box (1983) and Pagano (2012) take two hours to complete and it was found that the sodium bicarbonate used as base forms cloudiness when adapted to a microplate format. A more rapid method for detecting total phenol in a micoplate format was found in the study by Magalhães et al (2010).

In the modified procedure, 1:5 v/v dilutions of Folin Reagent were prepared using nanopure water through a micropipetor and a 50 mL centrifuge tube. The mixture was vortexed for 5 seconds. In preparing the sodium hydroxide, about 1.3998 g of ACS-grade sodium hydroxide (NaOH FW=39.9971 g/mol) was transferred to a 100 mL volumetric flask filled with 50 mL nanopure water. The solution was mixed until the solute had fully dissolved. Tannic Acid standards were prepared at 2.5, 3.5, 4.5, 5.5, 6.5 and 7.5 ppm tannic acid equivalent (TAE) in nanopure water. These standards were modified to fit the range of phenols in the samples from previous trial runs. The flasks were inverted several times to thoroughly mix before ready for use.

An "optically-enhanced" plate with a white plastic closing on each well was used to reduce the amount of light scatter and amplify the signals. On deviation from this procedure by Magalhães et al (2010) was that the wells were filled to capacity (380 µL) to reduce the concavity that occurs in liquid-air interface of Box's (1983) method and increase the light’s pathlength for augmented absorption readings. A ratio of 25% sample, 25% 1:5 v/v FCR and 50% 0.35 NaOH was maintained in each well. In this case, 95 µL of each sample and standard were added into each well; then 95 µL of 1:5 v/v FCR is added next into each well, followed by 190 µL of 0.35 NaOH. A blue-violet color was developed in the highest standard immediately upon the addition of the base. The plate was read at 25ºC at 760 nm between 3-5 minutes after
adding the base to the last well. A calibration curve was constructed using absorbance values from standards and the phenolic content of each sample was calculated through using the linear regression equation from the calibration curve. Phenolic content was reported in ppm Tannic Acid Equivalents (TAE).
RESULTS

ANOVA

Data Analysis was performed using one-way Analysis of Variance (ANOVA) to compare the concentrations and the net differences of DOC and phenols among seasons and wetland types. The table below summarizes the results of twelve different ANOVA tests. The Net Difference is the difference in value between Effluent flow and Influent flow DOC/phenol concentration data. The F-Statistic and p-value significance determined whether differences among the Categories exist or not. The Categories marked with a star (*) indicated the data was statistically significant because their p-values were below 0.05.

Table 2: ANOVA Table of DOC and Phenol concentrations and Net Differences by Seasons and Wetland Types.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Category</th>
<th>df</th>
<th>F-Statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOC Concentration</td>
<td>Season*</td>
<td>3</td>
<td>20.87</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DOC Concentration</td>
<td>Wetland Type*</td>
<td>2</td>
<td>9.67</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Phenol Concentration</td>
<td>Season*</td>
<td>3</td>
<td>4.00</td>
<td>0.008</td>
</tr>
<tr>
<td>Phenol Concentration</td>
<td>Wetland Type*</td>
<td>2</td>
<td>19.12</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DOC Concentration</td>
<td>Site*</td>
<td>14</td>
<td>25.38</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Phenol Concentration</td>
<td>Site*</td>
<td>14</td>
<td>24.71</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DOC Net Difference</td>
<td>Season</td>
<td>3</td>
<td>2.01</td>
<td>0.122</td>
</tr>
<tr>
<td>DOC Net Difference</td>
<td>Wetland Type</td>
<td>2</td>
<td>0.75</td>
<td>0.475</td>
</tr>
<tr>
<td>Phenol Net Difference</td>
<td>Season</td>
<td>3</td>
<td>1.28</td>
<td>0.291</td>
</tr>
<tr>
<td>Phenol Net Difference</td>
<td>Wetland Type</td>
<td>2</td>
<td>1.15</td>
<td>0.325</td>
</tr>
<tr>
<td>DOC Net Difference</td>
<td>Site*</td>
<td>14</td>
<td>3.80</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Phenol Net Difference</td>
<td>Site*</td>
<td>14</td>
<td>3.21</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

**DOC and Phenol Bar Charts**

The data is presented in four different groups of bar chart graphs that display the overall DOC and phenol comparisons by seasons, wetland types, wetland sites and net concentration differences.
In the case of Seasonal data, averages of all Summer, Fall, Winter and Spring DOC concentrations (Figure 1) were 11.27 mg/L, 7.10 mg/L, 6.42 mg/L and 7.65 mg/L, respectively. Summer had the highest DOC concentration average of all seasons. There is a statistical difference in the DOC concentration among the seasons (p < 0.0001). However, no statistical differences exist when comparing Fall and Spring DOC averages, as their error bars were within the same range. In the seasonal phenol data (Figure 2), the overall averages of Summer, Fall, Winter and Spring phenol concentrations were 3.55, 3.23, 3.52 and 3.59 ppm Tannic Acid Equivalents (TAE), respectively. Fall season had the lowest phenol concentration, statistically different from the other seasons (p = 0.008). The error bars between Summer, Winter and Spring were within range, meaning no differences in phenolic concentrations exist between them.

In the group of Wetland types, the average DOC in Natural, Man-Made and Retention Ponds (Figure 3) were 9.63, 7.67 and 7.04 mg/L respectively. Natural wetlands had the highest DOC average of all other wetlands while Retention Ponds had the lowest. By its statistical significance (p < 0.0001), there is strong evidence that differences exist between the DOC concentrations among the wetland types. In the case of phenols (Figure 4), the average phenolic concentration of Natural wetlands was 3.68 ppm TAE; for Man-made was 3.61 ppm TAE and for Retention Ponds, 3.13 ppm TAE. Since differences do exist at a statistically significant level (p < 0.0001), the Retention Ponds had the lowest phenol concentration of the other two wetland types. The error bars within Natural and Man-made wetland overlap each other, indicating that no difference in phenolic concentration exists between the two.
In comparing the average DOC concentrations among all 15 wetland sites (Figure 5), Ballantyne site had the highest DOC concentration average of all sites (19.69 mg/L) while Bloomfield had the lowest (5.29 mg/L). There is strong evidence that differences exist between sites because of its statistical significance (p < 0.0001). This is also true in the comparison of average phenol concentrations in Figure 6, with Ballantyne having the highest in 5.24 ppm TAE and both RIT J Lot and MCC having equally lowest concentrations of 2.98 ppm TAE.

The Net Concentration differences represent values obtained by subtracting Influent Flow from Effluent flow of all DOC and phenol data. The DOC Net Concentration difference between seasons in Figure 7 showed that summer was the only season where DOC decreased (value of -1.66 mg/L) while in other seasons, DOC increased (0.29 mg/L for Fall, 0.11 mg/L for Winter and 1.71 mg/L for Spring). However, there is no statistical difference in this data set (p = 0.122). In the case of Phenol Net Concentration differences between seasons (Figure 8), there still appears to be no statistical difference (p = 0.291). For the rest of the graphs (DOC/phenol Net Concentration differences between Wetland Types in Figures 9 and 10), there is also no statistical difference because their p-values are greater than p = 0.05 (p = 0.475 for DOC Net Concentration difference and p = 0.325 for Phenol Net Concentration difference). Graphs for the DOC and Phenol Net Concentration differences for each of the individual sites are shown in the Appendix.
Figure 1: Average DOC concentrations in each season. The error bars represent standard error.

Figure 2: Average Phenolic concentrations each season. The error bars represent standard error.
Figure 3: Average DOC Concentrations between wetland types. The error bars represents standard error.

Figure 4: Average Phenolic Concentrations between wetland types. The error bars represents standard error.
Figure 5: Average DOC concentration in wetland sites. The error bars represents standard error.

Figure 6: Average Phenol concentration in wetland. The error bars represents standard error.
Figure 7: Net Difference between Influent and Effluent DOC concentrations by Seasons. The error bars represents standard error.

Figure 8: Net Difference between Influent and Effluent Phenol concentrations by Seasons. The error bars represents standard error.
Figure 9: Net Difference between Influent and Effluent DOC concentrations by Wetland Types. The error bars represents standard error.

Figure 10: Net Difference between Influent and Effluent Phenol concentrations by Wetland Types. The error bars represents standard error.
Regression

A single regression graph of DOC versus Phenol (Figure 11) has been plotted using all respective concentration data from this study. The correlation coefficient of the graph ($R^2$) is 0.4265 and the equation of the regression line is $y = 0.104x + 2.6242$. This regression graph is an attempt to determine if the DOC measurements correlate with Phenol measurements in the studied wetlands. The squared correlation coefficient indicated that DOC and Phenol had a weak, positive correlation.

![DOC vs Phenol Regression Graph](image)
DISCUSSION

The differences between the overall DOC concentrations in Summer, Fall, Winter, and Spring seasons in Figure 1 could be interpreted to conclude that DOC was present to the greatest extent within wetlands during the summer and least during the winter. The cause of this difference could be due to the production of DOC, with favorable seasonal temperatures and the Summer growing season. Summer, being the warmest season of the year, had the highest temperatures that probably favored the speeding of biological functioning of the microorganism that decompose or build organic matter. It might also be due to wetland vegetative plants having high photosynthetic rates at high temperatures that result in them becoming more productive in forming organic matter and releasing DOC through their roots in the form of exudates. However, other factors that might have affected the increase in DOC concentrations could be sources outside the wetlands' watersheds or the source of the wetlands' influent flow. Potential factors that were outside the scope of this project include landscape and landcover surrounding the watersheds (these places might be where DOC was produced in large amounts before being carried though water into the wetlands). Despite the difference in DOC concentration among all seasons, the difference in phenol concentrations indicated that Fall season had the lowest phenolic concentration of all seasons (Figure 2). It could be possible that certain microorganisms that are capable of breaking down phenolic compounds became active during the Fall season. As for Summer, Winter and Spring, there was no statistical difference among their phenol concentrations- as if the microorganisms capable of breaking them down were less active during these seasons.
Differences in DOC concentrations between wetland types exist at statistically significant levels. As shown in Figure 3, Natural, Man-Made and Retention Pond wetland types all have varying DOC concentrations throughout the year. While it could be inferred that Natural wetlands had higher DOC concentration due to its abundant vegetation and microorganisms present that decompose and reassemble DOC, the increase could also be due to sources outside the wetland's watershed that were not within the scope of this project. Other factors that might have influenced the production of DOC within Natural, Man-made and Retention Ponds could be the wetland size, age, water depth, water volume and surrounding landscape. These variables had not been taken into account at the time of sample collection, but could possibly influence the increase/decrease of the DOC and phenol concentrations within the wetland types. Related to the average phenolic concentrations between wetland types, retention ponds had the lowest phenol concentrations of all the three types during the year. Either retention ponds had a greater amount of microorganisms that breakdown phenols, conditions that support a greater affinity for breaking down phenols, or less phenols came from outside the watershed in contrast to Natural and Man-made wetlands, whose locations might have experienced more phenol intake from outside sources.

An interesting result of this study was the high DOC and phenol concentrations measured at the Ballantyne site compared to all other wetland sites (Figures 5 and 6). Ballantyne does appear to be unique or an "outlier" among the 15 wetland sites in this regard. It is possible that Ballantyne's influent source came from places outside the watershed that produced or accumulated larger quantities of DOC/phenols. Ballantyne was one of the five natural wetland sites where abundant vegetation was observed at the time of sample collection but not recorded; again variables such
as the wetland's size, water depth, water volume and surrounding landscapes could be useful in the interpretation of Ballantyne's high DOC content. Nevertheless, the high DOC/phenol results of Ballantyne suggest that the site would be a good place to conduct more studies in the natural processing of DOC/phenols. Additionally, the Ballantyne site consistently showed a pattern of potential DOC/phenol breakdown, resonating with a hypothesis of the study. The Ballantyne site could be examined in more detail, and with additional methods of analyses, to study changes in the DOC character as it is broken down or processed through its wetland.

The p-values from ANOVA tests revealed that there were no significant differences in the graphs of the Net Concentration differences of DOC/phenol between seasons and wetland types (Figures 7-10). Therefore, it could be assumed that all the Net Concentration differences were statistically similar in all seasons and among wetland types. However, the ANOVA tests for DOC and Phenol Net Concentration differences for individual wetland sites showed significance (p < 0.0001) for both DOC and phenol in regard to individual wetland Net Concentration differences and indicated that differences did exist within the Net Differences of individual wetlands. The graphs of Net Concentration difference between individual wetlands (Appendix- Figure 12) showed that Ballantyne wetland site had the greatest total decrease in DOC concentration compared to all natural wetlands while Tamarack had the greatest total increase in the production of DOC- an interesting, but opposite phenomenon worthy of further examination (Figure 12 a & b). French Road appeared to display the breakdown of Phenols of all Man-Made Wetlands and The Father's House 2 (TFH 2) had the greatest apparent breakdown of DOC in all Retention Ponds. The balance between decomposition and primary production that represented organic matter accumulation were probably different within these wetlands, as previously mentioned,
sites likely have other sources of DOC coming from outside its watershed. The availability of nutrients, hydroperiods, abiotic stressors, retention time and air/soil temperatures might have impacted the breakdowns of DOC and phenols within the individual sites, although no records had been made on those variables as part of this study. Nevertheless, such variables would be an important reference for future analysis of organic matter between influent and effluent flow within similar wetlands.

While the research results have shown that differences in total DOC and phenol concentrations exist between the Season categories and Wetland type categories, the Net DOC and Phenol Concentration Differences among these categories did not statistically differ. This finding, in part, refuted all the three stated hypotheses of this study. However, the results at a Site-specific level (Appendix, Figure 12) might indicate otherwise: since statistical differences did exist in Net Concentration Differences among individual wetland sites. This finding could warrant future and detailed studies at specific sites that are aimed at improving understanding of the processing of DOC in individual wetlands. It might be found that one of these wetlands has the desired characteristics of processing DOC (and its phenolic content) that could be of interest to scientists seeking natural methods for drinking water pre-treatment.

At the Site-specific level, Hypothesis I (Effluent flow having lower DOC concentration after material is processed through wetland) was proven correct in Ballantyne and Buckland sites in Natural wetlands; and The Father's House 2 (TFH 2) in Retention Ponds. The sites that had decreases in phenol concentrations in effluent flow were Ballantyne in Natural wetlands, French Road and Barker in Man-Made wetlands; MCC, Erie and RIT J Lot in Retention Ponds.
Hypothesis II (Natural wetland types have lower DOC concentrations in effluent flow) was proven correct when comparing the individual sites of Ballantyne and Buckland in Figure 12(a) to all the other wetland types in Figure 12 (c) and (e). However, as stated prior, there are likely other factors in Ballantyne's condition of having higher DOC concentration than all other natural wetlands. If Ballantyne is eliminated from the dataset, the results would show that TFH 2 (Retention Pond) has greater decrease in DOC Net Concentration Difference than Buckland Park (Natural Wetland), which would indicate Hypothesis II should be refuted and re-examined.

Hypothesis III (Lower DOC concentrations in effluent flow during Summer) appears to not be validated in general- as no statistical differences exist in the ANOVA tests of the Net Concentration Difference across seasons.
CONCLUSION

Overall, there was no statistical significance in the DOC or Phenol Net Concentration difference or change in influent and effluent flow among wetland types and seasons within Monroe County, New York. The amount of DOC and phenol entering and exiting the wetlands were similar among wetland types and seasons and showed little or no breakdown occurring between influent flow and effluent flow. Statistical significance in the data existed only when comparing the total, overall DOC and phenol concentrations among Seasons, Wetland types and Sites. DOC was found to be present in the largest quantities during summers and in natural wetlands. Phenols on the other hand, were degraded during winters and in Retention Ponds. A better understanding and interpretation of the changes in DOC and phenol concentrations could be achieved if other variables such as wetland size, water depth, retention time, surrounding landscape, land cover and sources of incoming DOC/phenol were taken into account. Such further research would help promote a better understanding of how DOC/phenol concentration differences occur and whether this difference would have any impact on the overall ecosystem cycle the shallow wetlands were a part of. Site-specific data show that DOC may be being broken down during processing in some wetland sites and produced in others. Further examination of the detailed processing of DOC at these sites is warranted.
References


APPENDIX

I. Additional Graphs

Figure 12: Net Difference of DOC/Phenol between Individual Sites Grouped into Natural, Man-Made and Retention Ponds Wetland Types
II. Maps of Sampling Locations
(Courtesy of Mike Burkett)

a) All Sample Locations
b) Bailey Road & Ballantyne
c) Allens Creek & Buckland Park
d) Tamarack Swamp & Barker Road
e) French Road & Bryden Park
f) Bloomfield & HANA
g) RIT J Lot, TFH 1, TFH 2
h) MCC & Erie Station