CHARACTERIZATION OF DISSOLVED ORGANIC CARBON: ASSESSMENT OF COPPER COMPLEXATION AND EXPORT OF CARBON FROM WATERSHEDS AS A FUNCTION OF LAND USE

By

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ABSTRACT

CHARACTERIZATION OF DISSOLVED ORGANIC CARBON: ASSESSMENT OF COPPER COMPLEXATION AND EXPORT OF CARBON FROM WATERSHEDS AS A FUNCTION OF LAND USE

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Dissolved organic carbon (DOC) is a critical component of freshwater ecosystems and influences the transport of many pollutants. The aim of this work is to assess DOC characteristics, to determine if these characteristics influence the complexation of copper, and to identify the influence of land use on DOC characteristics. A method utilizing solid-phase extraction is developed to simultaneously quantify DOC fractions and copper-DOC complexes. Fractions are based on specific bonding mechanisms (hydrogen, donor-acceptor, London dispersion, and ionic bonding) thought to be responsible for stabilizing humic substances in aqueous solutions. This method produces different fractions for a range of natural DOC and standardized humic materials. Using this method, complexation constants for copper are derived for individual fractions of DOC and these are found to be similar to those reported elsewhere for bulk DOC using more intensive analytical techniques. Additionally, the complexation of copper by high and low molecular weight fractions of DOC is related to the amount of aromatic structure and oxygen functional groups present in DOC samples.

The solid-phase extraction method is then used to investigate possible relationships between DOC characteristics and land use. By collecting surface water

samples from sub-watersheds with unique land uses, statistically significant differences in DOC characteristics are observed. Sub-watersheds containing agricultural and forested land are found to produce DOC higher in molecular weight and aromatic structure than urban catchments. DOC from urban landscapes is found to be more hydrophobic than from other types of land use. Forested catchments produced DOC that is consistently different from that derived from the other types of land uses. In addition to land use, a limited number of environmental variables explain variations in DOC aromaticity, molecular weight and hydrophobicity. When combined with land use, the amount of solar radiation, precipitation and water temperature explain up to 80% of the variability observed in DOC characteristics. Results of this work suggest qualitative differences in DOC can influence copper complexation and the nature of DOC may vary significantly between surface waters as a result of land use.

This work is dedicated to my wife, Kim.

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ABBREVIATIONS

AG – agricultural
AHA – Aldrich HA
au – absorbance units
Cu – copper
Da – Daltons (grams $mole^{-1}$)
DDI – deionized distilled water
DOC – dissolved organic carbon
F – fluoride
FA – fulvic acid
FOR – forested
GC – golf course
GLM – general linear model
HA – humic acid
HMW – high molecular weight
HSD – Tukey's honestly significant difference test
IHSS – International Humic Substances Society
LMW- low molecular weight
LS – ligand size
M – molarity (moles $liter^{-1}$)
MSU – Michigan State University
MW – molecular weight
MWBSDR - Molecular Weight Binding Site Density Ratio
NOM – natural organic matter (RO isolated DOC)
NRN – Nordic Reservoir NOM
NUVA – normalized ultraviolet absorbance at 280nm
PL – parking lot
RCR – Red Cedar River
RO – reverse osmosis
SPE – solid-phase extraction
SRF – Suwannee River FA
SRH – Suwannee River HA
SRN – Suwannee River NOM
URB – urban

CHAPTER 1

INTRODUCTION

Dissolved organic carbon (DOC) is known to alter the fate of both organic (Chin, Aiken et al. 1997; Raber, Kogel-Knabner et al. 1998; Burkhard 2000; Lee, Kuo et al. 2003) and inorganic pollutants (Kimball, Callender et al. 1995; Santschi, Lenhart et al. 1997; Linnik 2003) and has been shown to influence redox reactions in surface waters (Cory and McKnight 2005). In addition to influencing geochemistry, DOC plays a significant role in aquatic ecology as the primary food source for heterotrophic bacteria in surface water systems (Ghabbour and Davies 2004; Perga, Kainz et al. 2006). Despite being a fundamental component of surface water ecosystems, scientific understanding of DOC evolution and behavior is limited.

Previous research has primarily focused on DOC quantity, rather than the quality (Hope, Billett et al. 1994). Hydrologic conditions and land cover are two factors that have been shown to influence the amount of terrestrial DOC exported from the watershed system to surface waters (Moore and Jackson 1989; Dillon and Molot 1997; Gergel, Turner et al. 1999; McGlynn and McDonnell 2003). However, concentrations of DOC alone are unable to explain DOC-pollutant interactions (Lee, Gan et al. 2003). The composition, or quality, of DOC also influences the biological growth (Rosenstock, Zwisler et al. 2005; McCallister, Bauer et al. 2006; Christian and Lind 2007). Based on observed variations in the chemical and biological behavior of DOC, there is a need to assess the variability of DOC characteristics.

The focus of this work is on variations in DOC characteristics, the influence of DOC characteristics on copper complexation and the parameters related to DOC characteristics. Due to the importance of land cover and catchment characteristics in influencing the release of DOC from watershed surfaces to aquatic systems, the hypothesis that DOC characteristics are influenced by land use and other environmental factors is investigated. This work was part of a larger project, Michigan State University - Watershed Action Through Education and Research (MSU-WATER), which, among other objectives, aimed to determine the influence of land use on surface water quality.

Results from this work are significant on many levels. At the molecular level, improved understanding of how DOC structure influence DOC behavior (i.e. complexation of trace-metals) allows for a more accurate description of chemical interactions in surface water systems. This provides an improved description of the fate and transport of trace-metals and organic pollutants. Furthermore, by determining environmental variables related to DOC characteristics, possible processes responsible for DOC formation and transformation can be identified. At a systems level, results also help to complete gaps in the carbon cycle. By filling these gaps it is possible to assess interactions beyond chemistry; ecology, geology, biology, etc. Overall, this research provides a better understanding of DOC characteristics and identifies parameters related to these characteristics.

Each proceeding chapter presents original research addressing gaps in understanding related to DOC; with the exception of the Chapter 2, which provides a brief review of pertinent literature that forms the foundation of this work, and Chapter 7, which briefly highlights some of the important results of this work, describes some of the

implications of this work and suggests future research needs. Chapter 3 presents a novel method utilizing solid phase extraction (SPE) for simultaneously quantifying DOC characteristics as well as trace metal complexation. In Chapter 4, this method is used to assess the influence of molecular structure, specifically aromaticity, on the complexation of copper. In Chapter 5, influence of land use on DOC characteristics is evaluated. Chapter 6 explores factors other than land use, such as weather and solution chemistry, which play a role in determining DOC quality.

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CHAPTER 2

LITERATURE REVIEW

Dissolved Organic Carbon

Dissolved organic carbon (DOC) is a heterogeneous collection of diverse, relatively low molecular weight, organic constituents that aggregate through hydrophobic and hydrogen bonding (Sutton and Sposito 2005). DOC is operationally defined as the amount of organic carbon passed through a 0.45μm pore-size filter (Leenheer and Croue 2003). Typically, greater than 90% of the organic carbon present in surface waters is DOC (Thurman 1985). The composition of DOC includes biologically active compounds at various stages of microbial and physico-chemical transformation (Ghabbour and Davies 2004; Wickland, Neff et al. 2007). While there is not a consensus on the dominant source of DOC to surface water systems (Hemond 1990), photosynthetic plants and autotrophic bacteria are identified as two major sources (Hedges 1981; Thurman 1985). Based on the relative influence of terrestrial and aquatic processes, DOC is often classified as terrestrial, derived from terrestrial sources, or aquatic, derived from aqueous biotic processes. Allochthonous, or terrestrial, sources of DOC include plant exudates, organic compounds resulting from litter decomposition, the leaching of soil organic matter, as well as enzymes and biomass produced by microbial growth and decay (Thurman 1985; Wickland, Neff et al. 2007). Autochthonous sources of DOC are the result of the exudation and excretion of biomolecules and the decay of organisms from all trophic levels (Tranvik 1993). The main source of DOC in aquatic systems are aquatic plants, bacteria, phytoplankton, algae, invertebrate excretion and micro-flora associated

with detritus (Otsuki and Wetzel 1974; Naiman, Melillo et al. 1987; Wetzel 1992; McKnight, Andrews et al. 1994; Kaiser, Arscott et al. 2004). In both systems, terrestrial and aquatic, DOC under goes significant biotic and abiotic transformations (Hedges 1981).

The amount of DOC exported from temperate and boreal catchments is 10 to 100 kgC ha-1 yr-1 (Hope, Billett et al. 1994). In most streams, terrestrial sources of DOC are significant (Fisher and Likens 1972). The influence of terrestrial sources are supported by carbon isotope studies that indicated DOC in surface water systems are typically less than 40 years old, while carbon in ground waters and soil organic matter are substantially older (Thurman and Malcolm 1981; Schiff, Aravena et al. 1990). For instance, soil organic matter has an average age of 550-700 years (Campbell, Paul et al. 1967). Based solely on age, the carbon present in groundwater and soil is likely to be highly degraded and altered. DOC on the other hand is relatively less degraded.

Catchment hydrology influences the export of DOC from watersheds (Fisher and Likens 1972; Schlesinger and Melack 1981). The concentration and type of DOC discharged to surface water bodies varies with different stages of the stormwater hydrograph (Schlesinger and Melack 1981; McGlynn and McDonnell 2003; Hood, Williams et al. 2005). The flow path which water travels through the catchment before being discharged to a surface water body is very important in determining organic carbon discharges (Edwards and Cresser 1987; Billett and Cresser 1992). By avoiding potential sorption sites present in deeper soils, laboratory experiments have shown that interflow produces more DOC than water transported through deep soils (Edwards 1984; Moore and Jackson 1989).

Absorption of DOC to soil plays a significant role in regulating the amount and composition of DOC that is exported to surface waters (McDowell and Wood 1984; Hope, Billett et al. 1994). Concentrations and fluxes of DOC from forested and grassland soils has been found to decrease with increasing soil depth (Dawson, Ugolini et al. 1978; Sollins and McCorison 1981; McDowell and Wood 1984; Cronan and Aiken 1985; Hornung, Stevens et al. 1986; McDowell and Likens 1988; Moore and Jackson 1989; Meier, Chin et al. 2004). This decrease in DOC concentration is attributed to the sorption of organic acids to soils (McDowell and Wood 1984; Cronan and Aiken 1985; Thurman 1985). Soils also exhibit chromatographic retention of hydrophobic fractions of DOC while preferentially releasing hydrophilic fractions when flushed (Jardine, Weber et al. 1989; Kaiser and Zech 1998; Meier, Chin et al. 2004). DOC with indirect flow paths through leaf litter and soil is likely to be stripped of hydrophobic DOC (Kawahigashi, Kaiser et al. 2006).

Generally, biotic processes in riverine environments result in a marked decrease in DOC transported downstream, particularly in headwater streams (Wallace, Ross et al. 1982; Naiman, Melillo et al. 1987). Based on this observation the "size-reactivity continuum model" was proposed by Amon and Benner (1996). The model hypothesized that aquatic organisms are arranged in an ordered continuum based on the selective degradation of DOC from terrestrial sources, regularly decreasing the MW of DOC at every step. In support of this model, between 12 and 44% of the DOC released from forest floors is degraded microbially (Yano, McDowell et al. 1998). Heterotrophic bacteria preferentially consume carbohydrates, proteins and other nutrient rich sources leaving behind aromatic structures (Marschner and Kalbitz 2003). While conceptually

relevant, this model is questionable for some surface water systems (Winterbourn, Rounick et al. 1981). Rather than a stepwise degradation, multiple biological pathways are more likely, including biotic production of DOC from autotrophs (Pomeroy 1974). Another important biota are algae, which produce DOC high in MW and low in aromaticity (McKnight, Andrews et al. 1994). DOC from terrestrial sources and algae serves as a primary food sources for heterotrophic microorganisms (McCallister, Bauer et al. 2006).

DOC biodegradability is related to DOC elemental composition (Sun, Perdue et al. 1997) and aromatic structure (Gilbert 1988; Zoungrana, Desjardins et al. 1998). Aromaticity, molecular weight and hydrophobicity are all indictors of bioavailability and previous transformation processes (Cabaniss, Zhou et al. 2000; Marschner and Kalbitz 2003; Wickland, Neff et al. 2007). Hydrophobic fractions of DOC are less accessible to microorganisms and are assumed to be less susceptible to microbial decomposition than hydrophilic components (Qualls and Haines 1992; Piccolo 1998).

There are four main physico-chemical processes of importance to DOC in surface water systems: adsorption, precipitation, oxidation-reduction reactions and complexation (Thurman 1985). Adsorption of DOC by iron and aluminum oxides present in immobile or settle able solids plays a significant role in removing DOC from the water column (Dahm 1981). This is also likely one of the main processes responsible for the sequestration of DOC in soil regimes (McDowell 1985). As a result, the concentration of DOC and iron and aluminum in upstream segments of surface waters are related (Johnsen, Martinsen et al. 1987). DOC precipitates from solution when the pH of water decreases or the ionic strength of solution increases, particularly for polyvalent cations

(Hope, Billett et al. 1994). While this may occur at the confluence of freshwater streams (Thurman 1985), estuaries are more significant (Sholkovitz 1976; Sholkovitz, Boyle et al. 1978; Mantoura and Woodward 1983). Oxidation of DOC can be induced photochemically by solar radiation (Dafher and Wangersky 2002; Waiser and Robarts 2004; Lou and Xie 2006; Shiller, Duan et al. 2006) or chemically (Richey, Brock et al. 1980; Thurman 1985). While it is possible the mobility of DOC can be altered via metal complexation (McDowell 1985; Moore, Desouza et al. 1992; Wang, Chen et al. 1997), complexation greatly alters the fate and transport of many trace metals (Florence 1982).

The influence of DOC on the behavior of trace metals is significant (Florence 1982). Partitioning coefficients between dissolved trace metals and colloids have been found to be greater than for particles (Wen, Santschi et al. 1999; Shafer, Hoffmann et al. 2004). DOC has been found to be the dominant factor in determining the speciation of metals (Mn, Cu, Zn, Pb, Cr, Cd) in some surface waters (Linnik 2003) and complexation greatly alters their mobility (Drever 1997). Irving-Williams Order describes the adsorption tendency of divalent transition metals by hydrous oxides (Cu>Zn~Ni>Co>Fe>Mn>Ca) and overall adsorption tendencies are suggested Pb>Cu>Cd>Zn>Ni>Ca (Langmuir 1997). Similar orders have been described for metal complexation with humic acid (Kerndorff and Schnitzer 1980). However, humic substances are, by definition, more diverse than specific binding sites, such as hydrous oxides. Understanding of DOC-trace metal complexation would be greatly enhanced by simultaneously measuring metal complexation and assessing the characteristics of the organic ligand.

Land Use and Seasonal Influences

The influence of land use on surface water chemistry is well documented. (Rhodes, Newton et al. 2001; Wayland, Long et al. 2003; Xie, Norra et al. 2005). Urban and agricultural land uses are known to produce non-point source pollutants, such as nitrogen and phosphorus which result in significant eutrophication of rivers, lakes, and coastal waters (Carpenter, Caraco et al. 1998). In northern climates, road salt and other de-icing chemicals alter surface water chemistry (Amrhein, Mosher et al. 1993; Buttle and Labadia 1999; Mason, Norton et al. 1999). The loading rates to surface waters of many trace metals and organic contaminants, such as pesticides, to surface waters are dependent on land use (Stangroom, Collins et al. 1998; Gardner and Carey 2004; Leu, Singer et al. 2004). While DOC itself is not a pollutant, through complexation, ion exchange and sorption reactions, it can alter the fate and transport of many pollutants of interest (Cho, Park et al. 2002; Merritt and Erich 2003; Yamamoto and Liljestrand 2003).

The concentration of DOC in surface water is found to vary spatially depending on the flux from terrestrial sources (Eckard, Hernes et al. 2007). Alterations to land use are also known to modify the amount of DOC exported from the watershed to surface waters (Bernardes, Martinelli et al. 2004; Stedmon, Markager et al. 2006). The amount of wetlands correlates highly with the flux of DOC from watersheds (Canham, Pace et al. 2004; Frost, Larson et al. 2006). However, most investigations into the influence of land use, primarily agricultural and forested (Kalbitz, Solinger et al. 2000), on DOC tend to focus on production, rather than DOC composition or quality (Richey, Brock et al. 1980). Due to the close relationship between DOC and biotic processes, Hedges (1980) proposed unique chemical differences exist for DOC from different landscapes.

Variations in parameters indicative of aromaticity have been related to watershed metrics (Larson, Frost et al. 2007) and physico-chemical characteristics of DOC are found to vary (Thacker, Tipping et al. 2005). This is important because functional and structural characteristics of DOC (1) alter the transport of pollutants (Chiou 2002) and (2) impact aquatic food webs (Qualls and Haines 1992; Lennon and Pfaff 2005).

Part of the difficulty in assessing the relationship between land use and DOC is separating this influence from other processes, especially in larger systems where competing processes are inevitable. Additionally, in large watersheds where the transport of DOC is slow and flow paths are long, degradation weakens relationships between DOC composition and terrestrial processes (Frost, Larson et al. 2006). However, using small scale watersheds it is possible to show differences in DOC quality that are not visible in larger watersheds (Dalzell, Filley et al. 2007). Samples collected from lower order stream segments, the upstream sections of streams that are close to the stream headwater, show progressively stronger correlations to changes in land use (Bernardes, Martinelli et al. 2004).

When attempting to isolate land use at the micro-watershed scale, anthropogenic alterations to hydrology must be considered. In agricultural areas, drain tiles alter traditional flow paths and can enhance the transport of organic contaminants to surface waters (Leu, Singer et al. 2004). In urban areas, impervious surfaces and sewer networks greatly alter watershed hydrology by increasing the amount and speed at which stormwater is transported to surface water systems (Sheeder, Ross et al. 2002). As a result, connections formed between urban catchments and surface waters through storm sewer networks are strongly correlated to pollutant and DOC loading rates (Hatt, Fletcher

et al. 2004). Furthermore, the delineation of micro-watersheds in urban areas is not based solely on topography because inlets to storm sewers can artificially extend watershed boundaries. Taking these factors into consideration and collecting DOC samples directly from micro-watersheds with unique land uses are required to effectively evaluate the influence of land use on DOC characteristics.

In addition to variations attributed to land use, seasonal variations in DOC characteristics have also been observed (Tipping and Woof 1983; Lindell, Graneli et al. 2000; Saliot, Derieux et al. 2002; Meier, Chin et al. 2004; Porcal, Hejzlar et al. 2004; Yano, Lajtha et al. 2004; Guo and Macdonald 2006; Minor, Simjouw et al. 2006). Seasonal variations in DOC characteristics from terrestrial sources influence degradability (Porcal, Hejzlar et al. 2004).The amount of aromatic moieties present in DOC has been found to be seasonally dependent (Kortelainen 1993; Molot and Dillon 1997; Larson, Frost et al. 2007; Rodríguez-Zúñiga, Milori et al. 2008). Some of the seasonal variations are also reported to be dependent on land use (Aitkenhead-Peterson, Smart et al. 2007).

Despite some connections between land use and seasonal influences, factors influencing DOC characteristics, utilization and microbial transformation are poorly understood (Christian and Lind 2007). The amount of precipitation and the extent of solar radiation are two factors that influence the amount of light absorbed by DOC (Curtis and Schindler 1997; Molot and Dillon 1997; Lindell, Graneli et al. 2000; Reche and Pace 2002). Despite the variability observed in terrestrial sources of DOC, the factors controlling the chemical characteristics of DOC remains largely unknown (Canham, Pace et al. 2004).

Dissolved Organic Carbon Characterization

Early efforts to characterize DOC were based on procedures developed for the soil organic matter (Stevenson 1982). The humic fractions of soil organic matter consist of: humic acids (HAs), which are the fraction of organic matter soluble under alkaline conditions but not acidic conditions (generally pH < 2); fulvic acids (FAs), the fraction soluble under all pH conditions; and humin, the insoluble fraction of humic substances. Consistent with soil chemistry, methods were developed using XAD resins to isolate HA and FA fractions of dissolved organic matter (Thurman and Malcolm 1981). While HA and FA fractions do represent isolates with similar chemical and physical properties, these properties are selected by the method of isolation and do not represent distinct types of organic molecules (Hayes, MacCarthy et al. 1989; Sutton and Sposito 2005).

An array of analytical techniques have been used to characterize DOC based on chemical structure, molecular weight, and fractions isolated using different SPE media (McDonald, Bishop et al. 2004). Excitation-emission matrix (McKnight, Boyer et al. 2001; Chen, Westerhoff et al. 2003; Cory and McKnight 2005) and nuclear magnetic resonance spectrometry (Gauthier, Seitz et al. 1987; Grasso, Chin et al. 1990; Swift, Leonard et al. 1992; Christl and Kretzschmar 2001; Kaiser, Simpson et al. 2003) have also been used to determine structural features of DOC. While NMR based techniques can elucidate complex chemical structures, the application of this technology is difficult and expensive (Leenheer and Croue 2003). Other less costly and intensive techniques have been shown to describe structurally unique forms of DOC (Hayes and Swift 1978; Swift, Leonard et al. 1992; Chin, Aiken et al. 1994; Peuravuori and Pihlaja 1997; Everett,

Chin et al. 1999; Pelekani, Newcombe et al. 1999; Christl, Knicker et al. 2000; Muller, Schmitt et al. 2000; Croue, Benedetti et al. 2003).

Protocols have been developed using SPE to separate bulk DOC into fractions with significantly different chemical structures and similar chemical properties (Croue, Benedetti et al. 2003; Janos 2003). One of the more extensive fractionation methods derives six groups of DOC (Aiken, McKnight et al. 1992) that have been shown to differ significantly depending on the sample origin (Imai, Fukushima et al. 2001).

One of the most common methods used to fraction DOC into hydrophobic and hydrophilic fractions employs the use of XAD-8 and XAD-4 resins (Thurman and Malcolm 1981). Like all analytical techniques, there are some negative attributes for these methods. Isolation of DOC by XAD resins typically requires a significant change in pH. Changes in pH may result in hydrolysis and alter the molecular configuration of DOC through bond breakage (Mace, Lin et al. 2001). Large humic molecules (>30kDa) are not absorbed by XAD resins (Town and Powell 1993). Additionally, XAD-8 resins are primarily marketed for industrial purposes. As a result, production of these resins are performed with low quality solvents resulting in significant impurities (Thurman, Ferrer et al. 2001). In order to remove impurities present in XAD-8 resins, extensive purification procedures are required (Thurman and Malcolm 1981; Louchouarn, Opsahl et al. 2000).

Recently, methods utilizing C_{18} SPE media to isolate DOC have received increased use. C_{18} preferentially retains aromatic moieties and the chemical composition of DOC retained differs significantly from DOC isolated using ultra-filtration (Schwede-Thomas, Chin et al. 2005). DOC retained by C_{18} appears enriched in aromatic compounds

while ultra-filtration produces DOC enriched in short chained polysaccharides along with amino sugars (Simjouw, Minor et al. 2005).

Most SPE reactions are rapid, with the limiting factor being diffusion (Wu and Gschwend 1986), allowing SPE media to behave similar to partitioning phases. Theoretically, SPE media provides chemical binding sites in which individual compounds can achieve equilibrium at a lower thermodynamic energy state. In reality, there are two primary factors which prevent true equilibrium from being achieved: competition reactions and mass transfer limitations. In order to ensure competing reactions do not interfere with thermodynamic equilibrium, a large ratio of potential binding sites to chemical constituents should be maintained. This is achieved by using a high surface area media and utilizing appropriate quantities of SPE media relative to the amount of analyte to be retained. Styrene-divinylbenzene (SDVB) cartridges are capable of providing high surface areas; potentially double that of common C_{18} media. Mass transfer limitations are combated by using a SPE media with high number of theoretical plates (high surface area and low particle size) and keeping flow rates sufficiently low to allow diffusion. With extremely high surface area and low particle size of modern SPE material, DOC extraction efficiencies for some SPE cartridges are reported to be nearly independent of flow rates.

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CHAPTER 3

SIMULTANEOUS QUANTIFICATION OF DISSOLVED ORGANIC CARBON FRACTIONS AND COPPER COMPLEXATION USING SOLID-PHASE EXTRACTION

Abstract

Trace metal cycling in natural waters is highly influenced by the amount and type of dissolved organic carbon (DOC). Although determining individual species of DOC is unrealistic, there has been success in classifying DOC by determining operationally defined fractions. However, current fractionation schemes do not allow for the simultaneous quantification of associated trace metals. Using operational classifications, a scheme was developed to fractionate DOC based on a set of seven solid-phase extraction (SPE) cartridges. The cartridges isolated fractions based on a range of specific mechanisms thought to be responsible for DOC aggregation in solution, as well as molecular weight. The method was evaluated to determine if it can identify differences in DOC characteristics, including differences in Cu-DOC complexation. Results are that: (1) cartridge blanks were low for both DOC and Cu, (2) differences are observed in the distribution of DOC amongst the fractions from various sources that are consistent with what is known about the DOC materials and the mechanisms operative for each cartridge, (3) when present as a free cation, Cu was not retained by non-cationic cartridges allowing the method to be used to assess Cu binding, (4) the capability of the method to provide quantitative assessment of Cu-DOC complexation was demonstrated for a variety of DOC standards, (5) Cu was found to preferentially complex with high molecular weight

fractions of DOC, and (6) estimated partitioning coefficients and conditional binding constants for Cu were similar to those reported elsewhere. The method developed describes DOC characteristics based on specific bonding mechanisms (hydrogen, donor-acceptor, London dispersion, and ionic bonding) while simultaneously quantifying Cu-DOC complexation. The method provides researchers a means of describing not only the extent of DOC complexation but also how that complex will be behave in natural waters.

Introduction

Dissolved organic carbon (DOC) is known to significantly influence aquatic biology and chemistry (Ghabbour and Davies 2004). Biologically, DOC serves as one of the primary food sources for microbes and can be considered the foundation of aquatic food webs in surface water systems (Perga, Kainz et al. 2006). Chemically, DOC alters the fate and transport of both organic (Chin, Aiken et al. 1997; Raber, Kogel-Knabner et al. 1998; Burkhard 2000; Lee, Gan et al. 2003) and inorganic pollutants (Kimball, Callender et al. 1995; Santschi, Lenhart et al. 1997; Icopini and Long 2002). DOC is often the dominant factor in determining the speciation and partitioning behavior of trace metals in surface waters (Wen, Santschi et al. 1999; Linnik 2003). Trace metal binding by DOC derived from various sources, or by broadly isolated fractions of DOC (e.g. humic or fulvic acid fractions), has been investigated and general orders of binding affinities have been documented (Kerndorff and Schnitzer 1980; Langmuir 1997).

DOC is best described as heterogeneous aggregates of low-molecular weight organic molecules, including recognizable biomolecules, held together by hydrophobic and hydrogen bonding (Sutton and Sposito 2005). An assortment of analytical techniques has been developed to characterize DOC for a variety of purposes. Of particular interest related to environmental mobility are techniques based on retention by an immobile solid phase, such as liquid chromatography (LC) and solid-phase extraction (SPE), as these provide information that can be directly related to the physical-chemical behavior and chemical structure (Janos 2003; McDonald, Bishop et al. 2004). The most commonly used fractionation techniques involve XAD resins which can be used to separate DOC fractions based principally on polarity (Aiken, McKnight et al. 1992). Previous

investigations have evaluated the extent to which various fractions produced using XAD resins bind trace metals (Croue, Benedetti et al. 2003). There are two primary limitations to using this approach to assess the binding that occurs under natural conditions. First, it has not been established that binding to broadly isolated fractions of DOC are representative of a larger mixture (Hayes, MacCarthy et al. 1989). Second, many of these techniques alter the nature of the DOC, and it is likely this affects binding. For example, the XAD fractionation methods rely on substantial changes in pH, which may result in hydrolysis of the DOC (Mace, Lin et al. 2001).

A limited number of studies have attempted to evaluate trace metals bound to different DOC components (Groschner and Appriou 1994; Appelblad, Baxter et al. 1999; Yamini and Tamaddon 1999; Abollino, Aceto et al. 2000; Icopini and Long 2002; Gardner and van Veen 2004; Turner, Le Roux et al. 2004). Most of these methods utilize a single, and often unspecified, mechanism for retaining DOC-trace metal complexes. A few utilize octadecyl-bonded silica, or C_{18}, solid phases which may contain open silanols that provide uncomplexed trace metals the possibility of bonding directly to the support phase (Donat, Statham et al. 1986). Because of these limitations, previous methods offer only a limited characterization of the DOC responsible for complexation.

The aim of this study was to develop a technique to quantify DOC fractions based on multiple characteristics that would likely affect environmental transport, and to simultaneously quantify the amount of trace metal that had been bound by each fraction. This method attempts to address some of the major limitations of previous LC and SPE techniques which could not be employed for one or more of the following reasons a) they required pH adjustment or chemical changes that would alter binding, b) the leaching of

trace metals or DOC from the solid phase was either excessive or required extensive cleanup procedures, and c) the solid phase retained free metals which would preclude the quantification of binding.

Materials and Methods

The isolation of DOC and trace metals was achieved by passing aqueous samples through a set of seven SPE cartridges (Table 3-1). Cartridges were run in parallel and utilized a range of retention mechanisms: four were prepared to isolate fractions based on ionic interactions, two primarily on the basis of hydrophobic mechanisms, and one that utilized hydrogen bonding. DOC and trace metal concentrations were determined before and after samples were passed through the cartridges to identify the amount retained on the solid phase, allowing the quantification of binding.

Solid phases which utilize styrene-divinylbenzene (SDVB) copolymer structure were employed to avoid problems reported for other bonded phases media (e.g. direct interaction between trace metals and unreacted silanols on C_{18} media). SDVB is particularly well suited for the retention of hydrophobic organic compounds but, unlike C_{18}, does not contain silica that may retain uncomplexed trace metals. Additionally, the capacity of SDVB media is typically greater than C_{18} due to increased surface area (Thurman and Mills 1998). However, because of the heterogeneous composition of DOC it is nearly impossible to calculate a single capacity coefficient for each type of cartridge. By identifying fractions of DOC retained by each cartridge, this method essentially evaluates the extent of organic constituents with a range of affinities (capacity factors) for each type of cartridge media. The Argonaut Isolute 101 (200 mg) SPE cartridge was one

of the cartridges selected to retain DOC primarily through hydrophobic mechanisms and is referred to as the *hydrophobic* cartridge. This cartridge is a highly cross-linked SDVB copolymer that facilitates strong pi-bonding with aromatic organic compounds. The other cartridge selected to retain analytes through hydrophobic mechanisms was the Waters Oasis HLB (200 mg), identified as the *extended hydrophobic* cartridge. While this cartridge is similar to the Isolute 101 cartridge, it is expected to retain more hydrophilic compounds (i.e. some semi-polar compounds) due to additional N-vinylpyrrolidone functionality (Waters 2003). The third type of non-ionic SPE cartridge used was the the *H-bonding* cartridge, Supelco Discovery DPA-6S (250 mg). The Discovery DPA-6S media consists of a polyamide resin designed to participate in hydrogen bonding. It is reported that the Discovery DPA-6S media extracts tannins, chlorophyll, humic acid and other compounds with hydroxyl groups, especially aromatic carboxylic acids (Supelco 2005).

In addition to the non-ionic mechanisms, four cartridges utilizing ion-exchange mechanisms were also employed. Only one of these four cartridges was designed to isolate cations, the Bio-Rad Chelex 100 (50-100 mesh, Na^+ counterion). This resin consists of SDVB copolymers containing paired iminodiacetate ions that act as chelating groups in binding polyvalent metal ions (Bio-Rad 1998). Two basic types of anion-exchange cartridges were used, both retaining negatively charged species through bonding with quaternary ammonium functional groups. One type of anion-exchange cartridge utilized Bio-Rad AG MP-1 (50-100 mesh) resin, the other Bio-Rad AG-X8 resin. The AG MP-1 resin is designed to retain a wide size range of constituents within a large effective surface area (~23 m^2 g^{-1}) and porosity (20%), while the AG-X8 resin

contains a 1kDa molecular weight (MW) cutoff, a copolymer lattice with an 8% cross linkage limiting the size of analytes that can access the anion-exchange sites to those with a MW of less than approximately 1 kDa (Bio-Rad 1998).

One cartridge utilizing the AG-X8 resin (*anion-1kD*) and two cartridges utilizing the AG MP-1 are employed in this method. One of the two AG MP-1 cartridges (*anion-F*) used a F^- counter ion while the other (*anion-I*) had an I^- counterion. By using different counterions it is possible to gauge the ionic affinity of negatively charged analytes (DOC and trace metal complexes) that are retained by the resins. The F^- counterion has a very low affinity for the anion-exchange sites, second only to OH^- ions, while an I^- counterion has a high affinity (Bio-Rad 1998). Fluoride counterions were used for all anion-1kD cartridges.

By comparing the retention of DOC on each of the seven cartridges it is possible to characterize DOC generally, as hydrophobic and hydrophilic, and specifically, on the basis of ionic bonding, donor-acceptor interactions, hydrogen bonding and London dispersion forces. The hydrophilic fraction of DOC can be defined as the additional amount of DOC retained by the extended hydrophobic cartridge due to the more hydrophilic functionality (i.e. DOC retained by the extended hydrophobic cartridge minus DOC retained by the hydrophobic cartridge). Specifically, the amount of DOC retained by ionic bonding is approximated by the amount of DOC retained by the anion-F cartridge. Hydrophobic bonding accounts for both donor-acceptor interactions and London-dispersion forces. Based on the dominate retention mechanism of the cartridges used, it is possible to determine the amount of DOC retained through hydrophobic

bonding, as well as, a relative assessment of donor-acceptor interactions and London dispersion forces. Retention by the H-bonding cartridge describes the propensity of DOC to undergo hydrogen bonding, while retention by the hydrophobic cartridge estimate the extent of donor-acceptor interactions. The proportion of DOC retention due to London dispersion forces is approximated by dividing the amount of DOC retained on the extended hydrophobic cartridge, which facilitates donor-acceptor, London dispersion, and hydrogen bonding interactions, by the retention of the hydrophobic and H-bonding cartridges.

The anion and cation-exchange cartridges used to isolate DOC fractions were prepared in the laboratory. Resins were slurried (1.005 ± 0.005 g for anion exchange, 2.005± 0.005 g for cation exchange) into 8mL Ultra-Clean™ polypropylene cartridge tubes (Alltech) with a 20 μm Extract-Clean™ polyethylene frit (Alltech). The terms Ultra-Clean and Extract-Clean referred to are product names and should not be confused with the *ultra-clean* acid-washing procedure described later. Once cartridges were filled with resin, they were tapped to eliminate air bubbles and sonicated for 30 minutes. Another 20 μm frit was then placed on top of the resin. Care was taken to prevent resins from becoming dry at any time during assembly and cartridges were stored with a small amount of water remaining above the top frit. Cartridges were prepared with the desired counter ion (F^- or I^-) by flushing with appropriate amounts of 1N NaOH and NaF or NaI solutions, as recommended by the manufacturer (Bio-Rad 1998). Before use, all cartridges were conditioned. First, non-ionic manufactured cartridges received 1mL of methanol which was allowed to soak for 2 minutes. Second, all cartridges, with the exception of the cation-exchange cartridges, were rinsed with 1 mL of 0.02% Trace

SELECT^{TM} hydrofluoric acid (HF) (Fluka). Finally, all cartridges were rinsed with 80 mL of ultra-pure water (>18 MΩ).

All laboratory experiments were conducted by passing samples through SPE cartridges (in parallel) using ultra-clean 60 mL syringes arranged on a syringe pump (Harvard Apparatus) to control the sample flow rate. Syringes were connected to SPE tubes with the use of cartridge adapters (Alltech). With the bottom of the SPE tube capped, syringes were filled with 60 mL of sample from the top. Once filled, the SPE tube cap was removed, the syringe plunger was inserted and ~10 mL of sample was forced through the SPE cartridge and discarded (without the plunger contacting the aqueous sample). Flushing 10 mL of sample effectively replaced the ~2 mL of ultra-pure water remaining from cartridge conditioning and allowed the SPE media to equilibrate with the sample solution. Eluent from cartridges was collected in 40 mL borosilicate glass vials for DOC analysis. After the DOC samples were collected, SPE cartridges were temporarily removed from the syringes to be refilled. At all times the SPE media remained fully saturated with excess sample. Syringes were then reloaded with sample solution to 40 mL. Again plungers were inserted and placed back into the syringe pump. Eluent was collected in ultra-clean 30 mL polypropylene bottles for trace metals analysis.

DOC samples were capped with TFE/silicone liners and refrigerated until analysis, typically within 1-2 days. The concentration of DOC was determined by automated analysis based on the Heated-Persulfate Oxidation Method (Clesceri, Greenberg et al. 1998) using an OI Analytical Model 1010 Wet Oxidation Total Organic Carbon Analyzer. Prior to conducting experiments, all glassware used during DOC analysis was acid (18% HCl) washed, rinsed with ultra-pure water and placed in a 550 °F

oven for more than 2 hours to ensure cleanliness. Trace metal samples were preserved by adding 180 μL of Optima nitric acid (Fisher Scientific) and refrigerated until analysis. Concentrations of Cu were determined by inductively coupled plasma/mass spectrometry (ICP/MS) as outlined by Standard Methods method 3125 B (Clesceri, Greenberg et al. 1998). Indium and bismuth internal standards (20 $\mu g\ L^{-1}$) were used and samples were analyzed using a Micromass Platform ICP/MS with a hexapole collision cell. All calibration standards were within 15% of the calibrated value. Syringes and sample bottles were subjected to ultra-clean procedures to eliminate the possibility of trace metal contamination, with the exception of the rubber caps on the syringe plungers which were soaked in ultra-pure water for >24hrs. Special care was taken (maintaining an air gap) during experiments to prevent possible contamination from the rinsed rubber caps. The ultra-clean process consisted of soaking syringes and bottles in 18% hydrochloric acid, 35% nitric acid at 20 °C, 35% nitric acid at 45 °C and ultra-pure water for ~24 hours. All acids were trace element grade (Fisher Scientific) and cleaning was done inside an EPA class 100 clean room. All experiments were conducted within a clean hood at 25°C. Major anions and cations in solutions were measured by ion chromatography and flame atomic adsorption (Clesceri, Greenberg et al. 1998).

Aldrich humic acid (HA) (cat. no. H1,675-2; lot no. MV01816HH), a water sample containing natural DOC from the Red Cedar River (RCR) in East Lansing, Michigan, and seven reference standards from the International Humic Substances Society (IHSS) were used to evaluate DOC fractionation and behavior. The IHSS standards used were: Nordic Reservoir natural organic matter (NOM) (cat. no. 1R108N); Suwannee River NOM (cat. no. 1R101N), fulvic acid (FA) (cat. no. 2S101F) and HA

(cat. no. 2S101H); Waskish Peat FA (cat. no. 1R107F) and HA (cat. no. 1R107H); and Pahokee Peat HA (cat. no. 1S103H). It should be noted that NOM samples are isolated by reverse osmosis while HAs and FAs are isolated using XAD resins. These reference standards were included because they are well characterized and widely used. Differences in DOC composition and structure have been reported for the reference standards and are available elsewhere (IHSS, 2007). Dissolved organic matter (DOM) from the RCR was prepared by passing the natural water sample through an acid washed 0.45 μm glass-fiber filter. Samples containing copper were spiked using 1000 mg Cu L^{-1} aqueous standards of copper nitrate in dilute nitric acid (Fischer Scientific).

To simulate complexation and ionic interactions likely to occur in natural surface waters, an artificial solution was made using the solution chemistry of a sample from the RCR as a template (Table 3-2). The artificial solution was constructed by dissolving the salts $CaCO_3$, $CaSO_4$, $MgCl_2$, KCl, KNO_3, $NaHCO_3$, NaCl and Na_2HPO_4 in 20 L of ultra-pure water. The resulting artificial solution was similar to the RCR solution used as a except for a lower Ca concentration. While the RCR sample was supersaturated with respect to calcite, the artificial solution was in equilibrium with calcite. The chemistry of the solution remained stable for the duration of experimentation (one week) and, in fact, maintained stability for more than one year.

Solution chemistry was modeled in PHREEQCi (Parkhurst and Appelo 2005). For solutions without DOC present, the MINTEQ.v4 database was used. However, to study Cu-DOC complexation, a database containing a PHREEQCi compatible version of Tipping and Hurley's WHAM model was used (Appelo and Postma 2005). Solutions containing DOC were modeled in two steps and in each step Na was allowed to vary to

achieve charge balance. While Na was not expected to vary significantly during experiments, it was used to attain charge balance for two reasons: (1) changes in Na concentration have a relatively minor impact on complexation and (2) the error associated with its measurement is typically larger than the error observed in the charge balance. Allowing other parameters to vary, such as pH, could greatly alter solution chemistry and not accurately represent conditions observed. The first step modeled solutions without organic complexation. For solutions containing reference DOC standards, the initial pH was set to 8.0 (measured pH of the artificial solution), carbonate equilibrium was established (pCO_2 = -2.84). This resulted in a 2.7% decrease in Na, a pH of 8.00, and a pE of 11.56. For the RCR sample, the initial pH was set to the pH measured (7.8), equilibrium carbonate was included (pCO_2 = -2.32). This resulted in a 0.3% increase in Na, a pH of 7.78, and a pE of 13.23. The second modeling step described organic complexation by equilibrating a surface layer with the solution and gas phase from the first step; resulting in a pH of 7.77 to 7.99 and pE of 12.73 to 13.28. The surface layer approximated site specific binding to 4 carboxylic-like, 4 phenolic-like and 12 bidentate sites according to the model developed by Tipping and Hurley (1992). The fraction of bidentate sites (0.4) remained constant for all types of DOC modeled.

For solutions containing DOC, PHREEQCi model variables based on those reported in the literature (Table 3-3) were adjusted in order to effectively model observed complexation, based on conditional stability constants. First, estimates for the number of binding sites were derived from the literature. For Suwannee River HA, FA and NOM, the total number of binding sites (nHA + nHB) was based on the sum of the maximum charge density of carboxylic and phenolic groups, assuming a single charge per binding

site (1 eq mol^{-1}) (IHSS 2007). This resulted in total binding-site densities (mmol gC^{-1}) for Suwannee River samples of 9.81 for HA, 11.58 for FA, and 3.82 for NOM. For both NOM samples, the total number of binding sites was set equal to the total number of Cu binding sites observed for DOM isolated by reverse osmosis, 4.55 mmol gC^{-1} (Lu and Allen 2002). A charge density of 3.8 mmol gC^{-1} was estimated for Aldrich HA, based on data from Saito et al. (2005) who used a purified form of the acid at pH~8. Because information was not available on the composition of RCR DOM, it was assumed that 25% of DOC was composed of HA and FA fractions. Using an average charge density of HA (8.0 meq gC^{-1}) and FA (5.2 meq gC^{-1}) at a pH~8 (Higgo and Rees 1986), an overall charge density of 3.3 mmol gC^{-1} was estimated.

Second, the distribution of carboxylic-like (type A) to phenolic-like (type B) sites was estimated in two ways. For DOM samples with the amount of carboxylic and phenolic groups reported (IHSS 2007), the same ratio was used for type A and B sites. For DOM samples lacking compositional data, the amount of type A binding sites were assumed to be double the amount of type B binding sites (Thurman 1985).

Partitioning coefficients (K_{DOC}) and conditional stability constants (K_C) were calculated using a short Basic program within the USER_PUNCH feature of PHREEQCi. The PHREEQCi model calculated K_{DOC} as follows:

$$K_{DOC}(\text{MODEL}) = \frac{\frac{[M_{bound}]}{[M_{total}]}}{[OC_{surface}]} \qquad \text{(Equation 3-1)}$$

where $[M_{bound}]$ is the amount of metal (moles) associated with surface binding sites and $[M_{total}]$ is the total amount of metal (moles) present in solution, and the ratio is normalized to the concentration of organic carbon (kgC L^{-1}) modeled as a surface layer $[OC_{surface}]$. Ions calculated to be present within the double layer were assumed to be retained by the SPE cartridge and therefore are accounted for in $[M_{bound}]$. The PHREEQCi model calculated K_{C_MODEL} as follows:

$$K_{C\_MODEL} = \frac{[M_{org\_complex}]}{[M_{non\_org}][OC]} \qquad \text{(Equation 3-2)}$$

where $[M_{org\_complex}]$ is the amount of metal (moles) complexed with DOC, $[M_{non\_org}]$ is the amount of metal (moles) not complexed with DOC, and [OC] is the concentration of organic carbon (gC L^{-1}). Ions calculated to be present within the double layer were assumed to be retained by the SPE cartridge and are included for in $[M_{org\_complex}]$.

Results and Discussion

Five basic experiments were conducted: (1) the levels of Cu and DOC that leached from conditioned cartridges were established, (2) the retention of a model DOC (Aldrich HA) was measured under different flow rates, (3) the retention of different types of DOC (i.e. HA, FA, NOM, and a non-purified surface water sample) obtained from

multiple sources (Suwannee River, Waskish Peat, Nordic Reservoir, etc.) was assessed, (4) the removal of uncomplexed Cu by cartridges was evaluated, and (5) the retention of Cu in aqueous solutions containing different forms of DOC was determined.

The first experiment conducted was to determine the cleanliness of the SPE cartridges and sampling procedure. With this aim, ultra-pure water was run through each cartridge according to the method procedure described above. Cartridge eluent was analyzed to determine the extent of Cu and organic carbon leaching (Table 3-4). After conditioning, cartridge blanks were below the limit of quantification (standard deviation + 10 sigma, n=3) for Cu (0.5 $\mu g\ L^{-1}$) with the exception of one cartridge. The anion-I cartridge produced a blank eluent concentration of $2.5 \pm 0.6\ \mu gCu\ L^{-1}$. DOC concentrations observed in the eluent of conditioned cartridges ranged from 0.06 ± 0.11 $mg\ L^{-1}$ for the anion-I cartridge to $0.6 \pm 0.1\ mg\ L^{-1}$ for the anion-1kD cartridge.

While other methods tend to focus on either trace metal or DOC retention, this method is one of only a few that is designed to simultaneously produce both, requiring clean blanks for Cu and DOC. Cartridge blanks for Cu were typically less than a few $\mu g\ L^{-1}$ and DOC concentrations were well below those found in natural samples, establishing these cartridges to be relatively free of contaminants. Once cartridges were made, sample preparation and cartridge conditioning required less than ten minutes per sample using a multi-port vacuum manifold. This is favorable compared to other methods which isolate DOC fractions and require up to 120 hr of resin clean-up prior to analysis (Louchouarn, Opsahl et al. 2000). Overall, the method proved to be simple to implement and required very little pre-cleaning.

The second experiment was designed to determine the optimum flow rate to isolate DOC while minimizing sample processing time. Water samples for this experiment were prepared by dissolving Aldrich HA in unsoftened groundwater (tap water; Table 3-2), and passing the samples through cartridges at a series of five flow rates ranging from 0.5 to 10 mL min^{-1}. For all cartridges studied, the retention of DOC was found to decrease with increasing flow rate (Figure 3-1). Retention by anion exchange was relatively constant up to 2.0 mL min^{-1}, regardless of the counter ion, with the anion-I and anion-F resins showing only a slight decrease at 4 mL min^{-1}. Non-ionic cartridges showed similar retention for flow rates up to 1.0 mL min^{-1}.

In order to decrease sample processing time while maximizing DOC retention, a flow rate of 1.0 mL min^{-1} was selected as the most appropriate to obtain DOC fractions. This flow rate is lower than has been reported by other investigators for the isolation of lignin and humic substance by C_{18} and trace metals by cation exchange (Abollino, Aceto et al. 2000; Louchouarn, Opsahl et al. 2000; Gardner and van Veen 2004; Shafer, Hoffmann et al. 2004). The flow rate selected was also deemed prudent to assess the dissociation of Cu-DOC complexes toward the competing iminodiacetic acid ligand immobilized on SDVB polymers (cation cartridge) (Shafer, Hoffmann et al. 2004).

The amount of Cu retained by cation exchange approximates the portion of "free" species, or labile Cu, that can quickly dissociate from natural ligands (Shafer, Hoffmann et al. 2004). Differentiating between labile and non-labile fractions of Cu using SPE depends on (1) the ability of the cation-exchange resin to compete with other ligands in

solution and (2) the amount of time Cu-DOC complexes are exposed to competing iminodiacetic acid ligands. The flow rate used in this study results in a retention time of greater than 3 minutes for the cation-exchange cartridge. This retention time is greater than the retention time previously used to determine labile Cu, ~8 seconds (Shafer, Hoffmann et al. 2004), and quantify "free" Cu species, 2 minutes (Gardner and van Veen 2004), using similar cation cartridges with smaller mesh sizes. While a smaller mesh size may increase the retention of Cu by cation exchange, it is unclear if information obtained using a smaller mesh size resin provides a better assessment of Cu mobility or toxicity. Two factors support the use of a larger mesh size resin: (1) the amount of Cu retained by cation exchange has been found to vary depending on DOC ligands (Shafer, Hoffmann et al. 2004); therefore, a resin that is capable of allowing a wider range of ligands to access iminodiacetic binding sites may provide a more comprehensive assessment of metal-ligand interaction, and (2) labile fractions determined using cartridges with smaller mesh-size have been found to overestimate toxicity from free Cu (Florence, Morrison et al. 1992). Ultimately, any method using cation exchange to measure the labile fractions of a trace metal relies on an arbitrary definition of labile and these measurements provide only a relative approximation of kinetic limitations.

The ability of the selected SPE cartridges to isolate fractions of DOC based on different mechanisms was tested using a variety of DOC standards and one natural water sample. Seven solutions were made by spiking approximately one liter of artificial solution with a single humic sample. The natural water sample was filtered through an acid-washed Type A/E glass-fiber filter (Pall) and otherwise untreated. These eight solutions were passed through the selected cartridges as described previously. Given the

known mechanisms of retention for each cartridge, the amount retained by each provides a powerful tool for assessing the functional characteristics of DOC. For example, the propensity of DOC to undergo ionic versus hydrophobic interactions can be assessed by comparing the ratio of DOC retained by the anion-F cartridge versus the extended hydrophobic cartridge. Another DOC characteristic, the fraction of DOC less than 1kDa in molecular size, can be obtained by comparing the amount of DOC retained on the anion-1kDa cartridge versus the anion-F cartridge. Additionally, comparing the ratio of binding-site densities for Cu retained by the anion-F cartridge relative to the anion-1kD cartridge provides a measure of the tendency of Cu to bind with high versus low MW ligands. The MW binding-site density (BSD) ratio is described as

$$\text{MW BSD ratio} = \frac{\left[\frac{M_F - M_X}{C_F - C_X}\right]}{(M_X / C_X)} \qquad \text{(Equation 3-3)}$$

where M_F is the amount of metal retained by the anion-F cartridge, M_X is the amount of metal retained by the anion-1kD cartridge, and C_F and C_X are the amounts of carbon retained by each of the cartridges. A MW BSD ratio equal to one means binding constants are the same for high and low MW DOC. As this value increases, so does the affinity of that metal to complex with higher MW fractions of DOC.

Generally, it was expected that DOC standards isolated by reverse osmosis (NOM) would contain HA and FA constituents and therefore would show greater retention on all cartridges. It was also expected that DOC retention by individual

cartridges would follow the order of bond strength: hydrogen bonding < hydrophobic bonding < ionic bonding. Based on the pH of the artificial solution (~8), limited quantities of these constituents were expected to be retained by non-anion exchange mechanism (Wells, Smith et al. 2000). No DOC was expected to be isolated by cation exchange, with the possible exception of a small, likely negligible, amount retained through cation bridge formation (Sutton and Sposito 2006). Additionally, it was expected that little HA would be retained on the anion-exchange cartridge containing a MW cutoff (Anion-1kD) since the MW range of HA is reported to be 1 to 85 kDa (Appelo and Postma 2005).

Significant differences were observed in the ability of DOC samples to participate in various types of bonding (Figures 3-2 to 3-7). For example, when comparing Waskish Peat and Suwannee River samples, HAs were more likely than FAs to participate in donor-acceptor and hydrogen bonding interactions (Figures 3-2 and 3-5), while FAs were retained more effectively by London dispersion forces than HAs (Figure 3-4). Generally, HAs were more likely than other types of DOC to participate in donor-acceptor interactions (Figure 3-2) and hydrogen bonding (Figure 3-5), which results in hydrophobic interactions being relatively more important than ionic interactions for HAs when compared to other forms of DOC (Figure 3-3). As expected, FA had a greater amount of DOC less than 1 kDa in size than HA, with the possible exception of Pahokee Peat HA (Figure 3-6). DOC from the RCR and DOC isolated by reverse osmosis (NOM) showed the greatest fraction of low MW components (Figure 3-6). The cation exchanger did not retain quantifiable amounts of DOC, regardless of the type (i.e. HA, FA, and reverse osmosis isolate).

Differences observed in the retention of various types of DOC from the same source material are likely attributed to the techniques initially used to isolate and purify the standardized material. Suwannee River HA samples were obtained by precipitation of the hydrophobic fraction initially isolated by XAD-8 resins and extracted under alkaline conditions (IHSS 2007). As a result Suwannee River HA is enriched in hydrophobic constituents. NOM samples are obtained by reverse osmosis. Suwannee River NOM (RO isolate) consists of greater amounts of hydrogen, nitrogen and oxygen functional groups than other forms Suwannee River standards isolated by IHSS resulting in a more polar aliphatic substance (Serkiz and Perdue 1990). Aliphatic structure with high functionality does not offer good surfaces for hydrophobic interactions; therefore, NOM is expected to be less hydrophobic than DOC present in natural water samples. The biases of each isolation technique are consistent with observations of the ionic to hydrophobic ratio (% DOC retained by the anion-F cartridge versus the extended hydrophobic) which show Suwannee River NOM being greater than Suwannee River HA (Figure 3-3).

Of the samples tested, the proportion of DOC less than 1 kDa in size is larger for FA than for HAs (Figure 3-6). This is consistent with known properties of HAs and FAs. HAs typically consist of large molecules (1-85 kDa), while FAs are typically composed of much smaller constituents (0.5-1.5 kDa) (Benedetti, Van Riemsdijk et al. 1996; Appelo and Postma 2005). Of the three types of DOC tested, NOM is more highly retained by Anion-1kD cartridges, which can be attributed to a large assortment of small molecules preferentially isolated by reverse osmosis.

The fourth experiment was designed to determine if uncomplexed Cu present in solution would be retained by the SPE media used. This was achieved by passing an

ultra-pure water solution spiked with 50 μgCu L^{-1} through each of the cartridges as described previously. In a solution of ultrapure water, the only complexation available to "free" ions is through hydroxides and carbonates. The unbuffered nature of ultra-pure water results in a solution pH that rapidly decreases when exposed to air due to CO_2 dissolution. Both the lack of complexing ligands and low pH were expected to result in primarily free Cu^{2+} ions and positively charged complexes. Therefore, it was expected that positively charged ions would be effectively retained by cation exchange, not by anion exchange or through non-ionic mechanisms.

When present in the ultrapure water little Cu was retained by non-cation exchange cartridges (Figure 3-8). As anticipated, most Cu was found to exist as primarily free species (e.g. Cu^{2+}) in ultra-pure water, also supported by PHREEQCi modeling. The amount of Cu retained by most cartridges was within the range of analytical error. Retention was greatest (12%) on the anion-F cartridge. Retention by cation exchange approached unity (91%). Surface complexation between Cu in solution and the F^- counter ion attached to the anion-F cartridge may be responsible for retention of Cu by these cartridges. Cu is known to complex with F^- (NIST 1997). However, when PHREEQCi modeling included a hypothetical addition of fluoride, the resulting aqueous complexation did not help to explain retention and there is no clear mechanism for this attachment.

The next objective was to determine the amount of Cu that was bound to each DOC fraction for various types of DOC (i.e. HA, FA, reverse osmosis isolate, and non-purified). Using the artificial solution as the solvent to simulate complexation likely to

occur in natural surface waters, a group of aqueous samples were prepared with a single type of DOC and spiked to achieve a total concentration of ~100 μgCu L^{-1}. One artificial solution was prepared without DOC to assess the impact of inorganic complexation. Once made, samples were stirred for greater than 2 hr to ensure Cu-binding equilibrium (Kerndorff and Schnitzer 1980), and subjected to the fractionation scheme described previously. It was expected that inorganic complexation alone, mainly carbonate and hydroxide complexes, would not result in significant retention, while organic complexation would dominate resulting in Cu being removed as a Cu-DOC complex when DOC was present.

In the artificial solution without DOC present, the retention of Cu by non-ion exchange mechanisms remained low (less than 8%), similar to Cu retention in ultra-pure water (Figure 3-8). However, under these conditions Cu was retained by anion-exchange cartridges. Retention ranged from 73% by the anion-F cartridge to 97% by the anion-1kD cartridge. Speciation modeling in PHREEQCi predicted 55-98% of the Cu present in the artificial solution existed as a neutral species, mainly as hydroxide and carbonate complexes (Figure 3-9a). The lack of negatively charged complexes (<0.4%), does not account for this behavior. While it is unclear why Cu was retained in these instances, one possible explanation could be a minor mechanism of retention; cation-pi bonding through aromatic moieties present in SDVB (Dougherty 1996) or a kinetic separation process related to the aqueous diffusion of the metals, the hydrated ionic radius of the metals, and the porosity/geometry of the SPE media. Additionally, inorganically complexed Cu was retained by both anion and cation exchange (both greater than 75%). The seemingly simultaneous presence of anion and cation species can be explained by the following

possibilities: (1) the complex formed is amphoteric allowing for both positive and negatively charged regions of the molecule to interact with the ion-exchange cartridges, (2) the microenvironment at the resin surface changed in pH which resulted in different complexation conditions within each cartridge, or (3) the inorganic complexation was relatively flexible and the speciation shifted depending on the resin environment. Multiple factors may have been involved and they suggest limitations of the current model.

When DOC was present in solution, increased removal of Cu by SPE was observed for all cartridges except the cation and anion-1kD cartridges, which showed very little retention (Figure 3-8). The decrease in Cu retention by these cartridges when DOC was present signifies the dominance of organic complexation. For example, HA is known to have a MW greater than the MW cutoff of the anion-1kD cartridge and when DOC was present in solution, Cu retention by this cartridge was less than 6%. The decrease in Cu retention by this cartridge suggests that Cu-DOC complexes were not able to access the anion exchange sites. Similarly, based on the limited removal of Cu by cation exchange from solutions containing DOC, Cu appears to form stronger organic complexes than inorganic complexes, with HA forming the strongest complex. Based on differences in Cu retention by the anion-exchange cartridge with a MW cutoff and cation-exchange cartridge, the primary form of retention for Cu by all cartridges is through Cu-DOC complexation when sufficient amounts of DOC are present. Like the complexation observed with inorganic species, there are multiple reasons for why Cu complexes may be retained as neutral, negative and positive charged species. Unlike inorganic complexes, organic ligands commonly form amphoteric molecules, especially amines.

For Nordic Reservoir NOM and all forms of Suwannee River DOC, HA, FA and NOM (reverse osmosis isolate), the following equation described the relationship between DOC and Cu retained by SPE (R^2=0.96):

$$\text{Complexed } Cu_{(\mu mol)} = 1.30\ DOC_{(mmol)} + 0.18 \qquad \text{(Equation 3-4)}$$

A similar correlation was observed for Adrich HA, but with a different slope (R^2=0.99):

$$\text{Complexed } Cu_{(\mu mol)} = 0.63\ DOC_{(mmol)} + 0.02 \qquad \text{(Equation 3-5)}$$

Binding ratios ranged from 0.63 μmol Cu $mmol^{-1}$ OC for Aldrich HA to 1.6 μmol Cu $mmol^{-1}$ OC for Suwannee River FA.

High MW DOC was found to preferentially complex Cu (Figure 3-7). All DOC isolates tested showed a MW BSD ratio greater than two. The only sample which showed similar complexation for high and low MW DOC was the natural water solution. This was the only DOC not subjected to the isolation or purification techniques used by IHSS (2007) necessary to create standards.

Assuming all Cu retained by SPE in the presence of DOC was complexed with DOC; partitioning coefficients for DOC (K_{DOC}) can be calculated for each organic fraction:

$$K_{DOC}(SPE) = \frac{\frac{[M_{ret}]}{[M_{total}]}}{[OC_{ret}]} \qquad \text{(Equation 3-6)}$$

where $[M_{ret}]$ and $[M_{total}]$ are the mass (μg) of metal retained and the total mass in solution, respectively, which is normalized to the concentration (kgC L^{-1}) of the organic carbon fraction simultaneously retained $[OC_{ret}]$. Partitioning coefficients for Cu ranged from 1.4×10^4 L kgC^{-1}, for the organic fraction of SRHA retained by the anion-1kD cartridge, to 5.3×10^5 L kgC^{-1} for the hydrophilic fraction of NRN.

Partitioning coefficients and conditional binding constants determined during this study (Table 3-5) are similar to values reported by others. For example, Shafer et al. (2004) computed Cu partition coefficients for colloids (K_D) in samples from estuarine, oceanic and riverine environments by taking amount of Cu associated with DOC in 1kDa permeate and dividing it by the total amount of dissolved Cu (<0.4 μm) and reported log values ranging from 5.3 to 6.4 L kg^{-1} DOC. Similar to the K_D calculated by Shafer et al. (2004), K_{DOC} was calculated by taking the ratio of Cu and DOC retained by SPE and dividing it by the total amount of dissolved Cu (<0.45 μm) ($[M_{total}]$, Equation 3-6). Using this analogous method, similar partitioning coefficients were observed in this study: log K_{DOC} = 4.2-5.7 L kg^{-1} DOC. Thacker et al. (2005) reported a conditional binding constant (log K_C) of 4.0 L gC^{-1} for Suwannee River FA at a lower pH (6.3) by

directly measuring free Cu^{2+} and assuming hydrolysis and inorganic complexation were negligible. Similarly, we are able to calculate an observed conditional stability constant based on the observed retention of Cu and DOC by SPE (K_{C_SPE}):

$$K_{C\_SPE} = \frac{[M_{ret}]}{[M_{non\_org}][OC_{ret}]} \qquad \text{(Equation 3-7)}$$

where $[M_{ret}]$ is the mass (μg) of metal retained as an organic complex, $[M_{non\_org}]$ is the concentration (μg L^{-1}) of metal in solution that is not complexed with DOC, and $[OC_{ret}]$ is the mass (g) of the organic carbon fraction simultaneously retained. Using Equation 3-7 we produced a log K_{C_SPE} of 2.72 L gC^{-1} for the same DOC sample using the anion-F cartridge, which is less than the 4.0 L gC^{-1} reported by Thacker et al (2005). However, Thacker et al (2005) assumed that hydrolysis and inorganic complexation was negligible. While nearly all DOC was removed from solution using this cartridge (98.7%), the assumption that all Cu not retained during these experiments was in the form of free Cu^{2+} likely overestimates the true concentration of Cu^{2+} due to inorganic complexation. A hybrid conditional stability constant, K_{C_HYBRID} (L gC^{-1}), is produced by substituting $[M_{non\_org}]$ in Equation 3-7 with the concentration (μg L^{-1}) of uncomplexed Cu^{2+} predicted by PHREEQCi as $[M_{free}]$.

$$K_{C\_HYBRID} = \frac{[M_{ret}]}{[M_{free}][OC_{ret}]} \qquad \text{(Equation 3-8)}$$

The anion-F fraction of Suwannee River FA was found to have a log K_{C_HYBRID} of 6.06 L gC^{-1}, which is two orders of magnitude larger than the K_C reported Thacker et al.(2005). There are two possibilities which explain the why the K_{C_HYBRID} values reported here do not match those reported by Thacker et al. (2005): (1) uncomplexed Cu^{2+} is not the only Cu species capable of complexing with DOC, and (2) it is not valid to assume inorganic complexation is negligible. Regardless of the assumptions used, the K_C values reported within this paper are found to bracket the value reported by Thacker et al. (2005).

Based on previously published data, the estimated number of binding sites resulted in theoretical K_{C_MODEL} values that were generally within an order of magnitude of those observed (optimized values), with the exception of Suwannee River FA which resulted in K_{C_MODEL} values that deviated significantly from those observed (Table 3-6). Theoretical K_{C_MODEL} values produced using parameters reported in the literature (i.e. the number and distribution of binding sites) showed greater variability than K_{C_SPE} values observed experimentally amongst the different types of DOC employed in this study (Table 3-5). Additionally; it was not possible to account for variations in complexation observed for NOM from different sources. Even though DOC samples used in this study are extremely well characterized and models describing their

complexation exist, this information alone was not sufficient to accurately describe complexation. To accurately describe observed behavior, the number and distribution of binding sites were adjusted until model stability constants (Equation 3-2) matched average stability constants observed (Equation 3-7) for cartridge fractions (with the exception of anion-1kD and cation cartridges). After optimizing binding-site densities, the amount of Cu bound by DOM ranged from 68.7%, for the solution containing Aldrich HA, to 93.2%, for the solution containing Nordic Reservoir NOM (Figure 3-9).

Conclusions

Results show the method described in this paper offers a simple and rapid approach to characterizing DOC fractions based on functional behavior and Cu binding by these fractions. The method produces a greater number of DOC fractions than previous fractionation techniques and retention is based on specific binding mechanisms and molecular size. The mechanisms of retention used to isolate fractions include those that are responsible for stabilizing humic substances (i.e. hydrogen and hydrophobic bonding). Using this method, differences are observed in fractions of DOC from various sources (e.g. Suwannee River and Nordic Reservoir). DOC fractions were also found to differ based on type (i.e. HA, FA, reverse osmosis isolate, and non-purified), possibly as a result of the techniques used to isolate sample material.

This method employs SPE cartridges which require little preparation and do not leach significant amounts of Cu or DOC. When Cu was present in ultra-pure water as a free species it was not retained effectively by SPE. DOC was effectively retained and the observed retention is consistent with characteristics known for each type of DOC used. In

aqueous solutions designed to mimic natural waters Cu was shown to preferentially complex with DOC. Complexation constants were derived for individual fractions and these values are similar to those reported elsewhere for bulk DOC using more intensive analytical techniques.

This method provides researchers a means of rapidly describing DOC characteristics based on specific bonding mechanisms while simultaneously quantifying Cu-DOC complexation for each fraction. Because aqueous samples do not require chemical treatment before analysis, complexation is expected to be representative of that found under natural conditions. Complexation observed using this method can be directly compared to chemical models and provides researchers a way to test theoretical predictions with in-situ behavior.

Table 3-1. Properties of SPE cartridges used to isolate fractions of DOC and Cu complexed with DOC.

Cartridge ID	Cartridge Type	SPE Media	Retention Mechanisms	Molecular Weight Cutoff
Cation	BioRad Chelex 100	Iminodiacetic acid exchange - styrene divinylbenzene copolymer	Cation Exchange	none
H-bonding	Supelco DPA-6S	Polyamide Resin	Hydrogen Bonding	none
Hydrophobic	Biotage Isolute 101	Polystyrene-divinylbenzene copolymer	Hydrophobic	none
Extended Hydrophobic	Waters Oasis HLB	Poly(divinylbenzene-co-N-vinylpyrrolidone)	Hydrophobic	none
Anion-F	BioRad AG MP-1	Quaternary ammonium - styrene divinylbenzene copolymer (fluoride counterion)	Anion Exchange	none
Anion-I	BioRad AG MP-1	Quaternary ammonium - styrene divinylbenzene copolymer (iodide counterion)	Anion Exchange	none
Anion-1kD	BioRad AG-1 X8	Quaternary ammonium - styrene divinylbenzene copolymer (fluoride counterion)	Anion Exchange	1kDa

Table 3-2. General water chemistry parameter for Michigan State University tap water, artificial solution, and natural Red Cedar River (RCR) water.

Parameter	Tap Water	Artificial Solution	RCR	Units
pH	7.2-7.4	7.99	7.78	
Conductivity	0.5-0.7	0.967	0.618	$mS\ cm^{-1}$
Hardness	404	186.1	337.2	$mg\ L^{-1}$ as $CaCO_3$
Alkalinity		136.9	273.5	$mg\ L^{-1}$ as $CaCO_3$
Ca		25.5	86.0	$mg\ L^{-1}$
Cl	14	93.7	53.2	$mg\ L^{-1}$
Mg		29.8	29.8	$mg\ L^{-1}$
NO_3^-	<0.4	0.48	2.5	$mg\ N\ L^{-1}$
PO_4^{3-}		5.69	1.0	$mg\ L^{-1}$
K		4.6	4.7	$mg\ L^{-1}$
Na	12	62.7	33.2	$mg\ L^{-1}$
SO_4^{2-}	76	49.0	55.5	$mg\ L^{-1}$

Table 3-3. Parameters used in PHREEQCi to model Cu-DOC complexation based on values reported in the literature and found to describe experimental observations (optimized).

Sample ID:	AHA	NRN	RCR	SRF	SRH	SRN
			Literature Values			
nHA (moles)	5.69×10^{-5} [a]	4.23×10^{-5} [b]	2.20×10^{-5} [c]	8.93×10^{-5} [d,g,h,i] 7.95×10^{-5} [e,i] 1.06×10^{-4} [f]	7.43×10^{-5} [d,i] 7.24×10^{-5} [e,i]	4.24×10^{-5} [b] 3.56×10^{-5} [e,i] 3.72×10^{-5} [d,i]
nHB (moles)	2.84×10^{-5} [a]	2.12×10^{-5} [b]	1.10×10^{-5} [c]	2.27×10^{-5} [d,g,h,i] 3.98×10^{-5} [e,i] 2.70×10^{-4} [f]	3.29×10^{-5} [d,i] 3.62×10^{-5} [e,i]	2.12×10^{-5} [b] 1.78×10^{-5} [e,i] 1.49×10^{-5} [d,i]
pK_{HA}	0.63 [t]	0.63 [t]	0.63 [t]	0.63 [t] 1.09 [f] 1.19 [g] 0.9 [h]	0.63 [t]	0.63 [t]
pK_{HB}	3.75 [t]	3.75 [t]	3.75 [t]	3.75 [t] 3.03 [f] 1.17 [g] 3.80 [h]	3.75 [t]	3.75 [t]
			Optimized Values			
nHA (moles)	3.13×10^{-5}	7.78×10^{-5}	7.15×10^{-5}	4.62×10^{-5}	4.96×10^{-5}	7.06×10^{-5}
nHB (moles)	1.56×10^{-5}	3.89×10^{-5}	3.58×10^{-5}	2.31×10^{-5}	2.48×10^{-5}	3.53×10^{-5}
pK_{HA} [t]	0.63	0.63	0.63	0.63	0.63	0.63
pK_{HB} [t]	3.75	3.75	3.75	3.75	3.75	3.75

(a) Koopal et al. (2005)
(b) Lu and Allen (2002)
(c) Higgo and Rees (1986)
(d) Ratio of binding sites based on ratio of carboxylic and phenolic content (IHSS, 2007)
(e) Ratio of carboxylic to phenolic sites based on Thurman (1985)
(f) Cabaniss and Shuman (1988)
(g) Beneditti et al. (1996)
(h) McKnight et al. (1983)
(i) Number of binding sites derived from IHSS (2007)
(t) Tipping and Hurley (1992)

Table 3-4. Concentrations of Cu and DOC found to leach from SPE cartridges used to isolate DOC fractions.

Cartridge	Cu ug L^{-1}	DOC mg L^{-1}
None (ultra pure water blank)	4.8	0.01
Cation	2.3	0.11
H-bonding	2.5	0.57
Hydrophobic	2.5	0.28
Extended Hydrophobic	5.0	0.34
Anion-I	2.4	0.07
Anion-F	3.1	0.06
Anion-1kD	3.9	0.63

Table 3-5. Conditional binding constants and partitioning coefficients for Cu complexation with DOC fractions (NA–not available).

Sample ID:	**AHA**	**NRN**	**RCR**	**SRF**	**SRH**	**SRN**
DOC Source:	Aldrich	Nordic Res.	Red Cedar R.	Suwannee R.	Suwannee R.	Suwannee R.
DOC Type:	Humic Acid	NOM	DOM	Fulvic Acid	Humic Acid	NOM
Metal:	Cu	Cu	Cu	Cu	Cu	Cu
DOC (mg L^{-1})	20.20	12.56	8.99	9.28	9.96	12.57
Total Cu (μg L^{-1})	95.4	95.0	65.6	95.0	93.0	95.2
Fraction	SPE Conditional Binding Constant (log K_{C_SPE}, L gC^{-1})					
Anion-F	2.04	2.80	3.02	2.72	2.72	2.80
Anion-I	2.03	2.82	3.09	2.77	2.76	2.84
H-bonding	2.09	3.17	NA	3.02	3.08	3.13
Extended Hydrophobic	2.03	2.93	3.16	2.84	2.78	2.99
Hydrophobic	2.03	2.87	3.13	2.81	2.71	2.98
Hydrophilic	2.03	3.65	3.41	2.93	3.08	3.06
Anion-1kD	1.67	2.49	3.03	2.26	1.94	2.30
Fraction	Hybrid Conditional Binding Constant (log K_{C_HYBRID} , L gC^{-1})					
Anion-F	5.01	6.44	6.18	6.06	6.10	6.38
Anion-I	5.00	6.47	6.25	6.11	6.14	6.41
H-bonding	5.05	6.81	NA	6.36	6.46	6.71
Extended Hydrophobic	4.99	6.57	6.32	6.18	6.16	6.57
Hydrophobic	4.99	6.51	6.28	6.15	6.09	6.55
Hydrophilic	4.99	7.30	6.56	6.27	6.46	6.64
Anion-1kD	4.64	6.13	6.18	5.59	5.31	5.88
Fraction	Partitioning Coefficient (log K_{DOC}, L kgC^{-1})					
Anion-F	4.55	4.87	5.03	4.96	4.93	4.86
Anion-I	4.54	4.89	5.10	5.01	4.97	4.90
H-bonding	4.60	5.24	NA	5.25	5.30	5.19
Extended Hydrophobic	4.54	5.00	5.17	5.08	4.99	5.05
Hydrophobic	4.54	4.94	5.13	5.05	4.92	5.04
Hydrophilic	4.54	5.72	5.42	5.17	5.29	5.12
Anion-1kD	4.19	4.56	5.03	4.49	4.15	4.36

Table 3-6. Theoretical (based on values reported in the literature) and observed (optimized) conditional stability constants produced by PHREEQCi modeling results for Cu-DOC complexation.

Sample ID:	AHA	NRN	RCR	SRF	SRH	SRN
Theoretical $\log K_{C\_MODEL}$ ($L\ gC^{-1}$)	2.50	2.59	3.79	3.32 [d,i,t] 3.24 [a,e,t] 4.99 [d,f,i] 6.75 [d,g,i] 4.42 [d,h,i]	3.15 [d,i,t] 3.13 [e,i,t]	2.59 [b,e,t] 2.45 [e,i,t] 2.45 [d,i,t]
Observed $\log K_{C\_MODEL}$ ($L\ gC^{-1}$)	2.04	3.04	3.16	2.85	2.86	2.97

(a) Koopal et al. (2005)
(b) Lu and Allen (2002)
(c) Higgo and Rees (1986)
(d) Ratio of binding sites based on ratio of carboxylic and phenolic content (IHSS, 2007)
(e) Ratio of carboxylic to phenolic sites based on Thurman (1985)
(f) Cabaniss and Shuman (1988)
(g) Beneditti et al. (1996)
(h) McKnight et al. (1983)
(i) Number of binding sites derived from IHSS (2007)
(t) Tipping and Hurley (1992)

Figure 3-1. The retention of Aldrich Humic Acid in MSU tap water at different flow rates for each of the SPE cartridges used to fraction DOC: Anion-F (▲), Anion-I (□), Anion-1kD (△), Extended Hydrophobic (■), Hydrophobic (●), and H-bonding (◆).

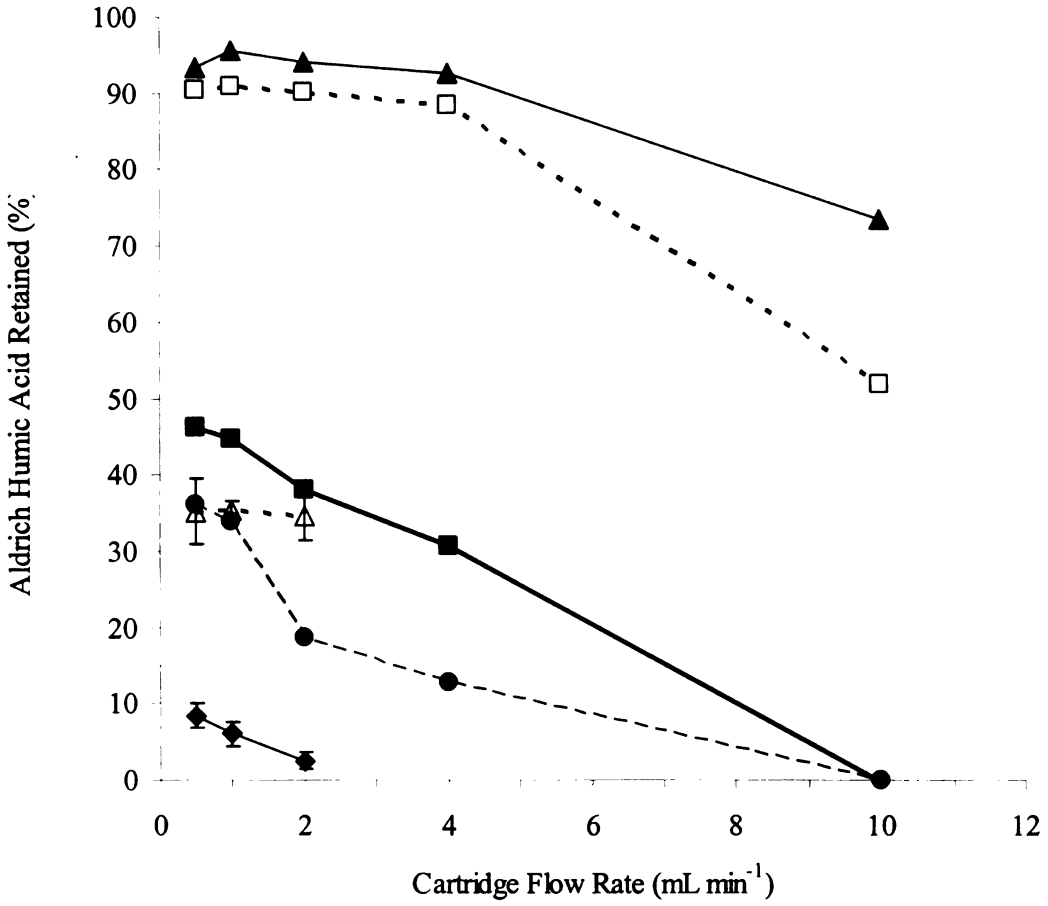


Figure 3-2. Relative amount of donor-acceptor interactions based on the fraction of DOC retained.

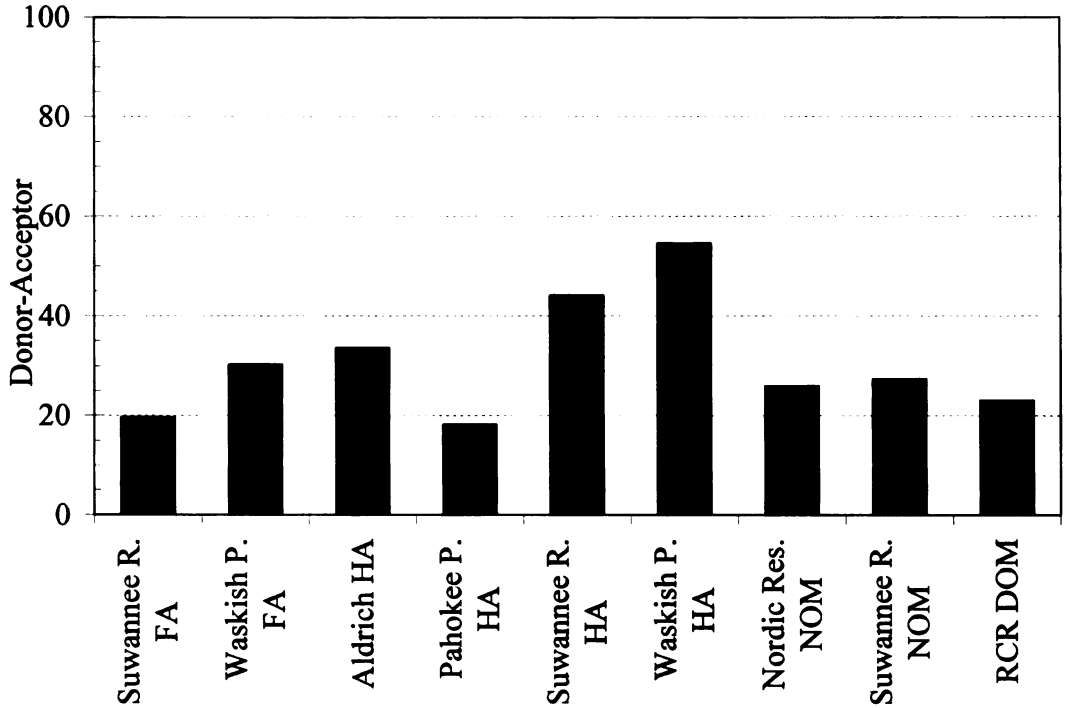


Figure 3-3. Ratio of DOC retained by ionic/hydrophobic mechanisms.

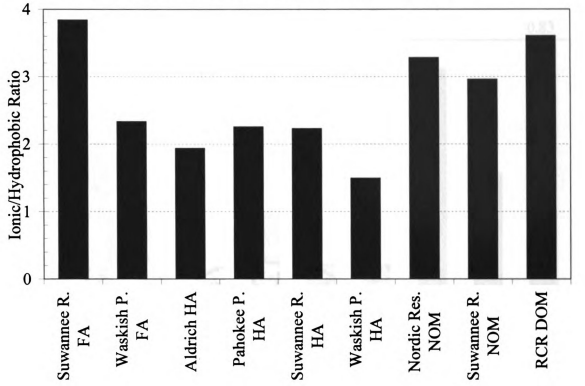


Figure 3-4. Relative amount of London dispersion forces based on the fraction of DOC retained.

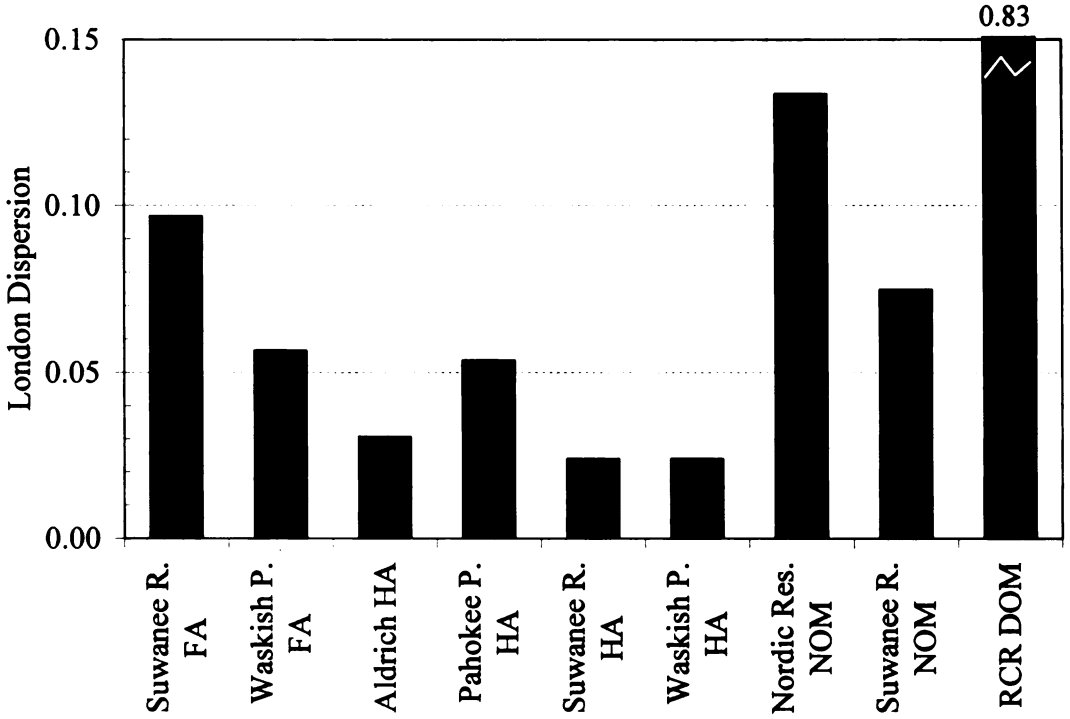


Figure 3-5. Relative amount of hydrogen bonding based on the fraction of DOC retained.

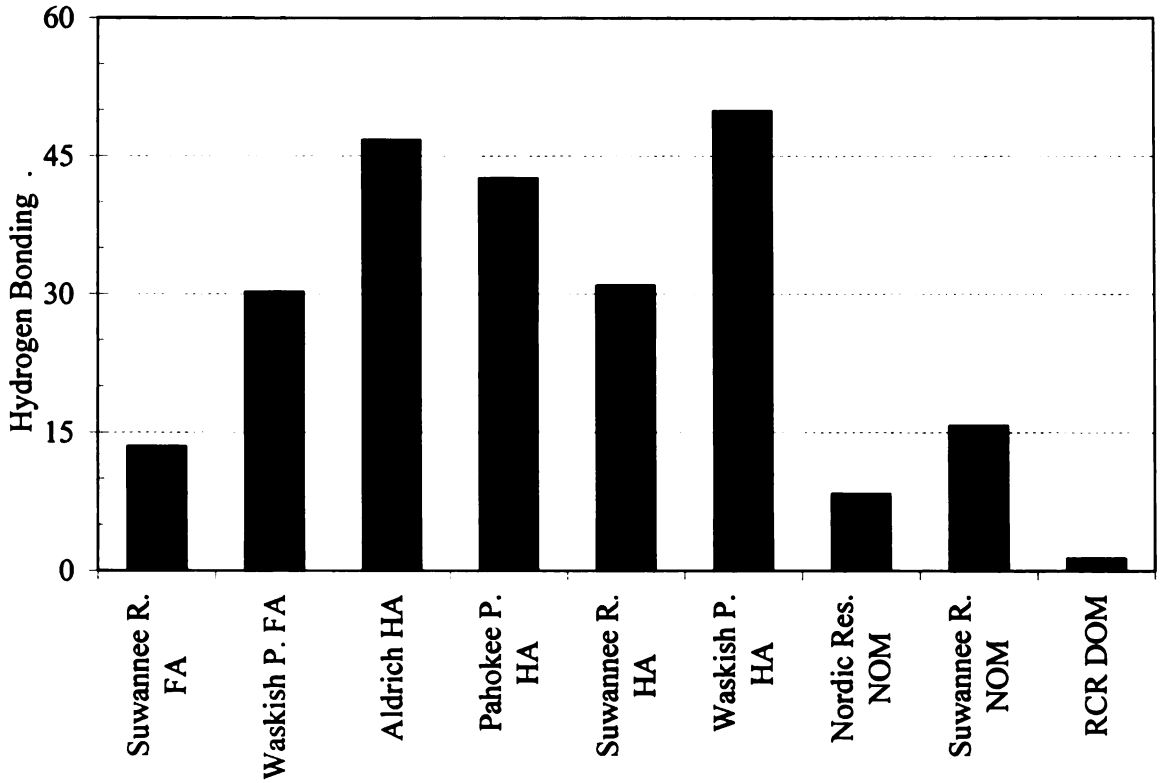


Figure 3-6. The fraction of DOC less than 1 kDa in molecular weight.

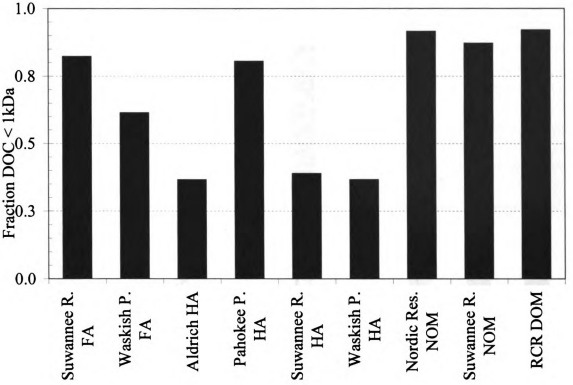


Figure 3-7. The ratio of molecular weight binding site densities.

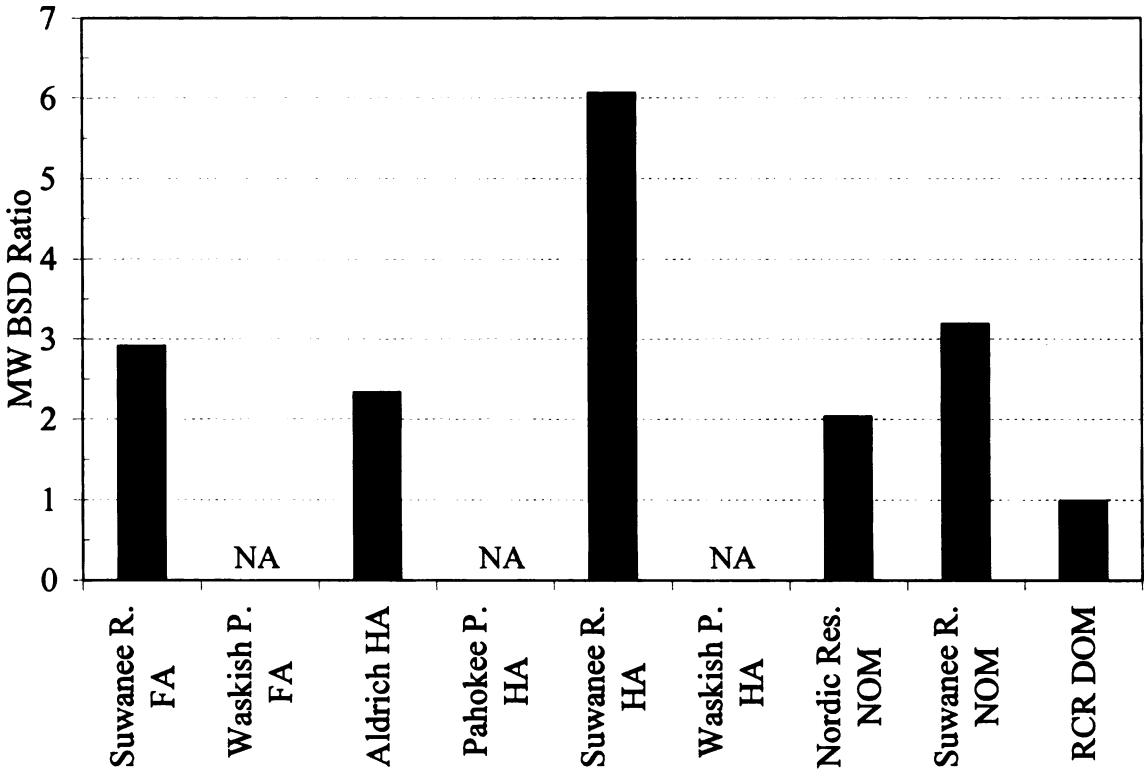


Figure 3-8. The relative amount of Cu retained by SPE cartridges in different types of solutions: artificial solution with Suwannee River FA (■), artificial solution with Suwannee River HA (▨), artificial solution with Suwannee River NOM (▧), artificial solution without DOC (□), and ultra-pure water without DOC (⊠).

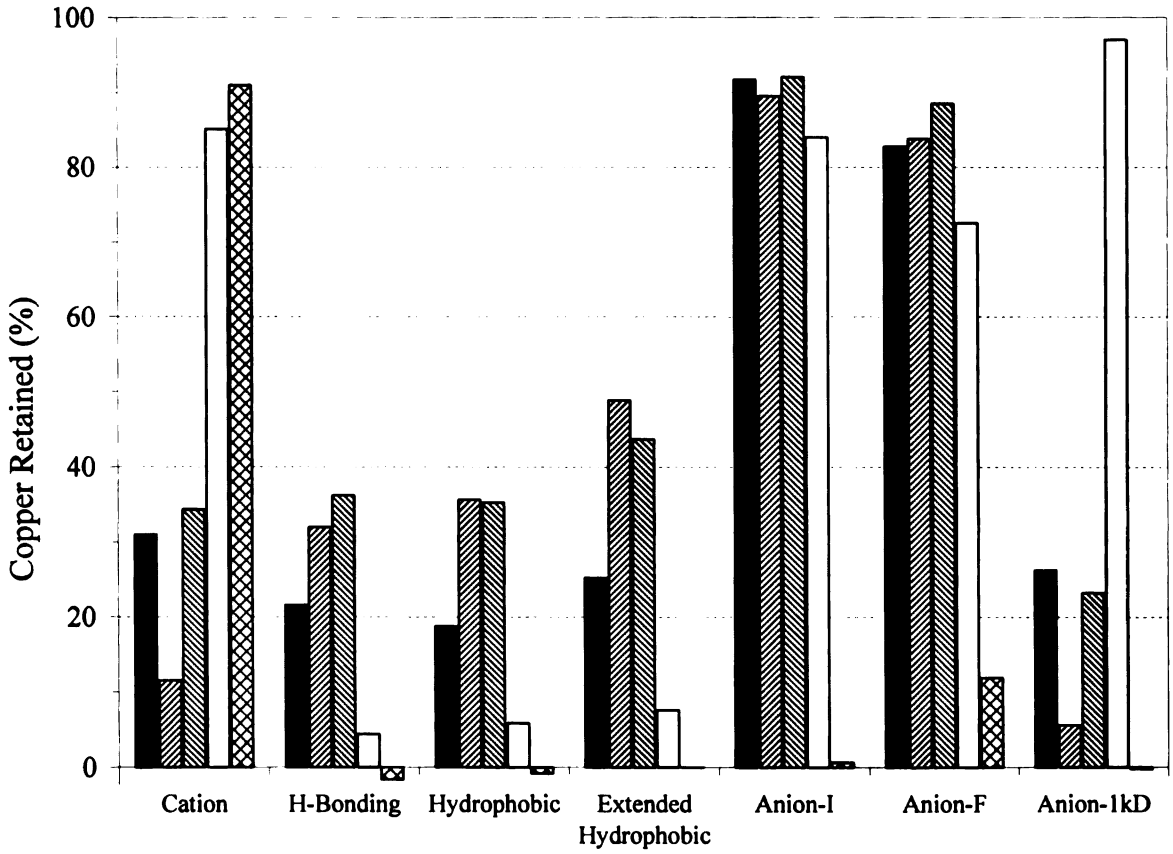
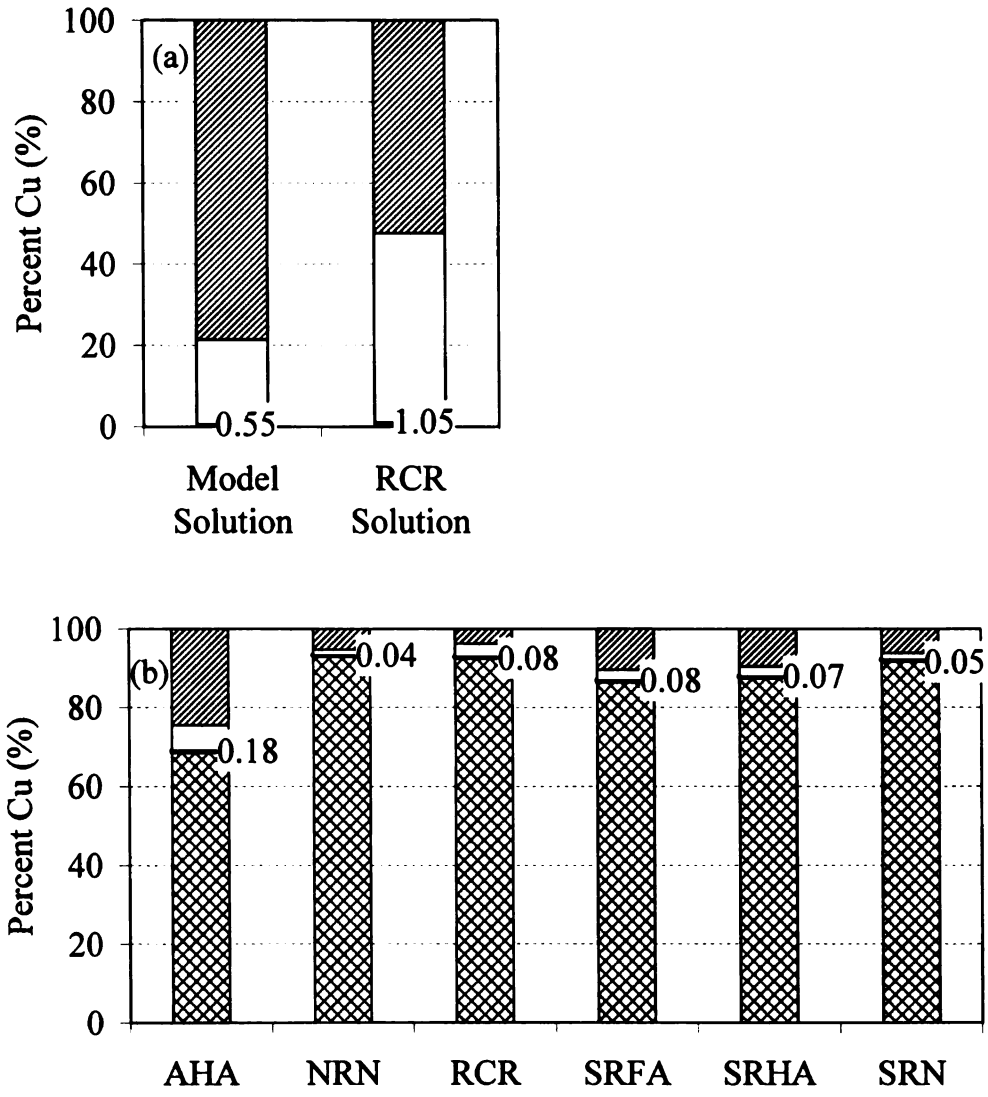


Figure 3-9. The fraction of free Cu^{2+} (■, percentage shown), Cu-hydroxide complexes (▨), Cu-carbonate complexes (□), and Cu-organic complexes (⊠) following (a) the first modeling step (equilibrium with CO_2, without organic complexation) and (b) the second modeling step (with optimized organic complexation) using PHREEQCi.



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CHAPTER 4

INFLUENCE OF AROMATICITY ON COPPER COMPLEXATION BY DISSOLVED ORGANIC CARBON

Abstract

Dissolved organic carbon (DOC) plays a significant role in cycling of copper in surface water systems. Previous investigations into how the molecular weight (MW) and molecular structure of DOC influence complexation have yielded inconclusive and contradictory results. Experiments were conducted to determine what role molecular structure had in influencing copper complexation with high (>1 kDa) or low MW DOC. Potential differences in how molecular structure influenced DOC verses soil organic carbon (SOC) were also explored. The hypotheses that copper complexation with low MW fractions of DOC is dependent on oxygen functional groups and that aromatic structures provide strong binding sites for copper complexation were tested. An assortment of humic standards, with known structural composition, were used to complex copper. Copper-DOC complexes were then isolated by solid phase extraction (SPE) to quantify complexation with high and low MW DOC. The relative strengths of complexes were evaluated by subjecting samples to cation exchange to determining the amount of liable. High MW fractions of DOC were found to have greater binding site densities (BSDs) than low MW fractions. The BSD of high MW DOC was found to increase with increasing aromaticity, while the BSD of low MW DOC was found to increase with oxygen content. The strength of Cu-DOC complexes was found to increase with aromaticity. Significant differences were observed in the structural composition between

low MW fractions of DOC and SOC. Results demonstrate the importance of aromatic structure in Cu-DOC complexation and suggest mechanisms for the preferential complexation of copper by selected size fractions.

Introduction

In surface water systems, copper is greatly influenced by complexation with dissolved organic carbon (DOC) (Linnik 2003). Complexation by DOC can reduce copper toxicity (Di Toro, Allen et al. 2001) and, through the sorption of Cu-DOC complexes to solid surfaces, can influences copper mobility (O'Day, Carroll et al. 1998). In surface waters, up to 99.99% of copper is complexed with strong binding sites present in DOC (Hoffmann, Shafer et al. 2007). Investigations into the DOC ligands responsible for Cu-DOC complexation have primarily focused on marine systems, where DOC concentrations are low and solution ionic strength is high; surface water systems can contain relatively high concentrations DOC and solution ionic strength is low (Hoffmann, Shafer et al. 2007). In surface water systems DOC has been found to vary in molecular weight (MW) and molecular structure depending on the source of DOC and season (Leenheer 1994; Perminova, Frimmel et al. 2003; Mash, Westerhoff et al. 2004). An assessment of how MW and molecular structure influence Cu-DOC complexation would greatly enhance understanding of Cu cycling in surface water systems.

In general, aquatic sources of DOC are reported to have greater binding affinities for copper than terrestrial sources (Mantoura and Riley 1975; Bresnahan, Grant et al. 1978; Mantoura, Dickson et al. 1978). DOC from aquatic sources are reported to contain greater heteroaliphatic structure (Jackson 1975). Functional groups containing nitrogen, oxygen, phosphorous and sulfur have all been suggested as possible heteroaliphatic binding sites (Tang, Warnken et al. 2001; Croue, Benedetti et al. 2003; Vachet and Callaway 2003; Karlsson, Persson et al. 2006). Oxygen and nitrogen functional groups are suspected because of their prevalence in DOC and evidence showing they form strong

bonds with copper inner-sphere electrons through multi-dentate bonding arrangements (Korshin, Frenkel et al. 1998; Karlsson, Persson et al. 2006).

In freshwater systems, attempts to quantify the size fraction of organic ligands responsible for copper complexation have produced mixed of results. Due to differences in sampling techniques (e.g. membrane separation, dialysis and high pressure-size exclusion chromatography), directly comparing data describing the MW of DOC ligands is difficult. However, the majority of copper in surface water samples is generally found in the colloidal phase (1-10 kDa in size)(Wen, Santschi et al. 1999), with the average MW of DOC associated with copper reported to be around 1.3 kDa (Wu, Evans et al. 2003). The prevalence of copper in the colloidal fraction may be due to a preponderance of DOC ligands within this size range (Sigg, Xue et al. 2000; Hoffmann, Shafer et al. 2007). The ability of specific binding sites present within fractions of DOC to complex copper may also be responsible (i.e. binding site affinities for specific size fractions). Generally, DOC fractions with MWs less than a few thousand Daltons are reported to have strong binding affinities for copper. Superior binding has been reported for freshwater DOC ligands less than 3 kDa (Hoffmann, Shafer et al. 2007), 1 kDa (Vulkan, Mingelgrin et al. 2002; Merritt and Erich 2003) and 0.5 kDa in size (Wu, Evans et al. 2003).

In Chapter 3, binding site densities for low MW (LMW) DOC (<1 kDa) were found to be greater than high MW (HMW) DOC (>1 kDa) for a natural water sample; while binding site densities (BSDs) for HMW DOC were greater than LMW DOC for purified standards of fulvic acid (FA), humic acid (HA) and DOC isolated by reverse osmosis, referred to as natural organic matter (NOM). The ability of specific size

fractions of DOC to bind copper appear to be related to the molecular structure. As MW increases, the percentage of aromatic structure present in aquatic DOC has been found to increase (Chin, Aiken et al. 1994) and LMW DOC is reported to contain high amounts of aliphatic constituents (Her, Amy et al. 2003). Additionally, with decreasing apparent molecular size, the proportion of phenols in humic fractions tends to increase (Christl, Knicker et al. 2000; Scheinost, Kretzschmar et al. 2001).

Since LMW fractions of aquatic DOC appear to preferentially complex copper and the presence of aliphatic structure is associated with LMW fractions (Chin, Aiken et al. 1994; Her, Amy et al. 2003), it is reasonable to infer: (1) aliphatic portions of DOC with Cu binding functional groups (i.e. heteroaliphatic structure) are primarily responsible for the preferential complexation observed, and (2) as the amount of heteroaliphatic structure increases, so too will Cu complexation. However, DOC containing different amounts of heteroaliphatic structure (e.g. Suwannee River HA and FA) had similar abilities to complex copper and HMW fractions of several types of DOC have been shown to preferentially complex copper (Chapter 3; (Kogut and Voelker 2001). In the absence of clear evidence identifying the cause of the reported preferential complexation, others must be considered. Likewise, multiple mechanisms for Cu complexation are also possible. In addition to complexation with heteroaliphatic binding sites, such as oxygen and nitrogen, aromatic constituents have also been found to complex cations through strong cation-π bonding (Dougherty 1996). Complexation via aromatic moieties is consistent with observations that binding site densities for strong ligands are found to degrade when exposed to UV light, while weaker ligands are unaffected (Moffett, Zika et al. 1990; Gordon 1992). Base solely on these observations it

would be reasonable to suggest that not only do UV absorbing aromatic moieties complex copper, they also offer stronger binding sites. To clarify possible mechanisms of Cu complexation and to better understand how MW may impact Cu complexation, there is a need to investigate the influence of DOC structure on Cu complexation.

To determine the influence of DOC structure on Cu complexation, Cu was allowed to complex with humic standards of known elemental and structural composition. LMW and HMW fractions of Cu-DOC complexes were then quantified. Hypotheses tested were: (1) as the amount of oxygen present in LMW fractions of DOC increases, so will Cu complexation, and (2) aromatic moieties in DOC provide stronger binding for Cu complexation than functional groups containing oxygen. Additionally, differences in the aqueous behavior of humic samples derived from aqueous (i.e. DOC) and terrestrial sources (i.e. soil organic carbon) were also evaluated.

Materials and Methods

A variety of humic samples were characterized by the same SPE method presented in Chapter 3. Concentrations of copper and DOC were measured before and after SPE to determine the amount, or fraction, retained by each type of SPE media. This method is unique because uncomplexed copper (free Cu^{2+}) is not retained on the SPE cartridges used to fraction DOC and specific retention mechanisms can be identified for each fraction (e.g. hydrogen bonding), effectively characterizing DOC behavior. Two of the SPE cartridges used utilize anion exchange resins. Nearly 100% of DOC, including DOC that is complexed with copper, is retained by anion exchange (Chapter 3). Additionally, one of the two anion exchange cartridges employed by this method utilizes

a 1 kDa molecular cutoff, essentially making it possible to determine the amount of HMW (0.45 μm-1 kDa) and LMW (<1 kDa) DOC. This MW cutoff is appropriate for studying copper complexation because it is near the average MW of organic ligands most associated with copper in natural water solutions (Wu, Evans et al. 2003).

The strength of Cu-DOC complexes was evaluated by passing samples through a SPE cartridge consisting of a strong cation exchanger (BioRad Chelex 100). When no DOC is present in solution, nearly 100% of copper present in solution is retained (Chapter 3). The strong iminodiacetate bonding site present in the cation exchange cartridge offer a strong ionic potential capable of liberating copper from weak complexes, effectively discriminating between labile and nonlabile copper species (Shafer, Hoffmann et al. 2004). Due to the strong ionic bonding potential of the cation exchange resin, it can be assumed that copper not retained by cation exchange would be less likely to participate in other geochemical reactions, due to thermodynamic or kinetic limitations, when complexed by the DOC ligand. Based on this, in solutions with DOC available for complexation, if the amount of copper retained by the Chelex 100 resin is high, then Cu-DOC complexation is relatively weak. Similarly, if the amount of copper retained by the Chelex 100 resin is low, then Cu-DOC complexation is relatively strong. It should be stressed that this assessment technique is only relative and similar methods using nearly the same SPE media have been found to over-estimate toxicity (Florence, Morrison et al. 1992).

In order to maintain consistent experimental conditions that were representative of natural waters while investigating DOC with an array of composition and structure, samples were prepared using of a variety of well characterized DOC standards in aliquots

of a single artificial solution with inorganic chemistry similar to a freshwater sample (Table 4-1). To study Cu-DOC complexation, appropriate amounts of 1000 mg L^{-1} aqueous copper standard (Fischer Scientific) were added to five of the eight aqueous samples to achieving a final concentration of approximately 95 μgCu L^{-1}. Once made, samples were stirred for more than two hours to ensure copper-binding equilibrium (Kerndorff and Schnitzer 1980).

Aldrich HA (cat. no. H1,675-2; lot no. MV01816HH) and standards from the International Humic Substances Society (IHSS) and were selected as DOC samples because they are well characterized and offer a range of elemental and structural composition. The IHSS standards used were: Nordic Reservoir natural organic matter (NOM) (cat. no. 1R108N); Suwannee River NOM (cat. no. 1R101N), fulvic acid (FA) (cat. no. 2S101F) and HA (cat. no. 2S101H); Waskish Peat FA (cat. no. 1R107F) and HA (cat. no. 1R107H); and Pahokee Peat HA (cat. no. 1S103H). NOM samples are isolated by reverse osmosis (RO) while HAs and FAs are isolated using XAD resins (IHSS 2006). Rigorously, Aldrich HA, Pahokee Peat HA, and Wakish Peat FA and HA are soil organic carbon standards that have been dissolved in the aqueous phase, therefore becoming DOC. Throughout this paper all standards will be regarded as DOC, since they are being evaluated in the aqueous phase, unless distinction is required to improve understanding.

The percentage of aliphatic (60-0 ppm), heteroaliphatic (90-60 ppm) and aromatic (165-110 ppm) structure for IHSS standards was determined by others by integrating peak areas for selected ranges of chemical shifts using solution state ^{13}C and ^{1}H nuclear magnetic resonance spectrometry (Aiken, McKnight et al. 1992). The composition (%O,

%N, %P, %S) of IHSS standards were determined by others and are reported on the IHSS website (http://www.ihss.gatech.edu). The chemical structure of Aldrich HA used in this study was assumed to be the same as a sample used in a previous study (Lot no. LE3601KE) which was characterized using methods similar to those used to characterize IHSS standards (Malcolm and MacCarthy 1986).

Concentrations of DOC were determined by automated analysis based on the Heated-Persulfate Oxidation Method (Clesceri, Greenberg et al. 1998) using an OI Analytical Model 1010 Wet Oxidation Total Organic Carbon Analyzer. Concentrations of copper were determined by inductively coupled plasma/mass spectrometry (ICP/MS) using a Micromass Platform ICP/MS with a hexapole collision cell and an In internal standard (20 $\mu g\ L^{-1}$) as outlined by Standard Methods method 3125 B (Clesceri, Greenberg et al. 1998).

Results and Discussion

The ratio of copper (as Cu-DOC) retained on anion exchange cartridges with and without a 1 kDa MW cutoff was used to identify the fraction of DOC, HMW (0.45 μm^{-1} kDa) or LMW (<1 kDa), that was predominately associated with copper. The DOC ligand size (LS) ratio was calculated by the following equation:

$$\text{DOC LS ratio} = \frac{(M_F - M_X)}{M_F} \qquad \text{(Equation 4-1)}$$

where, M_F is the amount of metal, Cu (as Cu-DOC), retained by anion exchange on a SPE cartridge without a MW cutoff (Anion-F) and M_X is the amount of metal, copper (as Cu-DOC), retained by anion exchange on a SPE cartridge with a 1 kDa MW cutoff (Anion-1kD). A ratio equal to one would indicate that copper is distributed evenly between high and low MW fractions of DOC. If this ratio is above one, more copper is associated with the HMW fraction of DOC. If this ratio is below one, copper is associated with the LMW fraction of DOC.

Another indicator of copper complexation with different size fractions of DOC is the ratio of binding site densities for HMW (0.45 μm-1 kDa) and LMW (<1 kDa) DOC. The MW Binding Site Density (BSD) ratio is described as

$$\text{MW BSD ratio} = \frac{\left[\frac{M_F - M_X}{C_F - C_X}\right]}{(M_X / C_X)} \qquad \text{(Equation 4-2)}$$

where M_F is the amount of metal retained by the Anion-F cartridge, M_X is the amount of metal retained by the Anion-1kD cartridge, and C_F and C_X are the amounts of carbon retained by each of the cartridges. If the MW BSD ratio equals one, then binding is equal in each fraction of DOC. As this ratio increases, BSDs become greater for HMW DOC. As this ratio decreases, copper binding site densities become greater for LMWDOC.

Of the five solutions prepared to examine Cu-DOC complexation, all samples showed more copper was complexed by HMW fractions of DOC than LMW fractions of DOC (Figure 4-1). MW BSD ratios were found to be greater than one for all samples,

indicating the ability of HMW DOC ligands to complex copper was greater than for LMW DOC. Nordic Reservoir NOM was found to have only slightly more copper complexed with HMW DOC, suggesting the MW cutoff used was near the average MW of DOC ligands for this type of DOC. A nearly equal distribution of copper between the two MW fractions of DOC was observed despite BSDs being 138 times greater for HMW DOC than LMW DOC. This indicated preferential complexation by HMW fractions of DOC while the sample contained a greater proportion of LMW DOC (Chapter 3). The importance of the quantity of DOC ligands and the ability of those ligands to complex copper is highlighted by HA and FA samples. Both HA samples had the largest ratio of copper complexed by HMW versus LMW DOC, despite the smallest MW BSD ratios. The predominance of Cu in the HMW fraction of DOC for HA is not surprising since HA is larger in MW than FA (Appelo and Postma 2005). However, it would be inaccurate to assume that copper complexation by FA is more strongly influenced by the LMW fraction of DOC; rather, the small portion of HMW DOC present in FA was found to have binding site densities nearly 300 times greater than the LMW fraction (Figure 4-1). One reason for this may be the presence of oxygen functional groups, since FAs tend to contain slightly more phenolic groups than HAs (Christl, Knicker et al. 2000). Overall, both the quantity and quality of DOC ligands were found to be important in identifying the MW fraction of DOC that complexes copper.

MW BSD ratios were found to increase with the aromatic content of DOC (Figure 4-2). This produces a relatively strong (r^2=0.70) correlation described by the following equation:

MW BSD ratio = 0.30 (%Aromaticity) - 3.9 (Equation 4-3)

When Aldrich HA, which is derived from terrestrial a source, was removed, leaving only DOC obtained from aquatic sources, the trend was even stronger (r^2=0.997). Because BSDs increase for higher MW fractions of DOC when DOC aromaticity increases, and aromaticity is correlated with MW (Chin, Aiken et al. 1994), these observations suggest that the aromatic structure is responsible for increased Cu binding. This is consistent with previous reports of strong cation-π bonding capable of outcompeting inorganic complexation in aqueous solutions (Dougherty 1996). Based on the observed linear regression for aromaticity, the MW BSD ratio appear to be equal when the aromatic structure of DOC is around 16%. Although untested, this may suggest that when aromaticity falls below 16%, other structural components within the DOC molecule, such as oxygen and nitrogen functional groups, may provide the dominant mechanism of binding.

As the amount of oxygen present in DOC increased, the MW BSD ratio was found to decrease (Figure 4-3). This offers further evidence that oxygen functional groups play a significant role in complexation for the LMW fractions of DOC. A similar trend was not observed for nitrogen, or any of the other elementals previously cited as important to copper complexation (Tang, Warnken et al. 2001; Croue, Benedetti et al. 2003; Vachet and Callaway 2003; Karlsson, Persson et al. 2006). While nitrogenous groups may have a higher capacity to complex copper than those of oxygen (Merritt and Erich 2003), dramatically greater amounts of oxygen functional groups are likely responsible for oxygen's dominance. The strong complexation offered in multi-dentate

arrangements, such as a Jahn-Teller distorted octahedron, requires a large number of binding sites (Korshin, Frenkel et al. 1998; Karlsson, Persson et al. 2006); binding sites that are most likely to come from oxygen (42-44% of DOC mass) rather than nitrogen species (<2% of DOC mass).

Based on the observed importance of aromaticity and oxygen composition, the following equation was found to describe the MW fraction of DOC that preferentially complexes copper (r^2=0.99):

$$\text{MW BSD ratio} = 0.3(\%\text{Aromaticity}) - 2.7(\%\text{Oxygen}) + 57.6 \qquad \text{(Equation 4-4)}$$

This equation was developed using by combining the slopes of linear regression lines used to describe the relationship between the MW BSD ratios and aromaticity (Figure 4-2) and oxygen content (Figure 4-3a).

As the aromatic content of DOC increased the amount of copper retained by the Chelex 100 resin was found to decrease (Figure 4-4). No similar trend was observed for changes in the amount of oxygen present in the same DOC samples. This suggests bonding between copper and the π-orbitals present in aromatic moieties is stronger than the bonding between cooper and oxygen functional groups. It also suggests the aromatic content of DOC may be more important in determining copper availability and toxicity than oxygen functional groups.

The influence of aromaticity and heteroaliphaticity on the amount of organic carbon (OC) retained by ionic mechanisms from solution was different for DOC and SOC samples (Figure 4-5). As the heteroaliphatic content of SOC increased, the

percentage of SOC greater than 1 kDa in MW increased. As the heteroaliphatic content for truly aquatic DOC increased, the percentage of DOC in solution greater than 1 kDa in MW decreased. As the aromatic content increased for bulk DOC, the percentage of DOC greater than 1 kDa increased. As the aromatic content increased for SOC, the percentage of SOC greater than 1 kDa decreased. The trend in aromatic content and the percentage of DOC greater than 1 kDa is consistent with previous reports of increasing aromaticity with increasing MW for DOC from natural waters (Chin, Aiken et al. 1994). The amount of DOC less than 1 kDa that contained a high percentage of heteroaliphatic structure is also consistent with reports of increased aliphatic content of LMWDOC from aquatic sources (Her, Amy et al. 2003). Opposite trends for DOC and SOC are unambiguous. It appears as though HMW fractions of DOC and SOC are similar in molecular structure, while the LMW fractions of differ considerably.

The amount of DOC removed from solution by hydrophobic mechanisms was found to increase with aromaticity for aquatic DOC (Figure 4-6). The same trend was not observed for SOC. Donor-acceptor interactions, largely responsible for hydrophobic bonding, are greatly enhanced through the presence of π-orbitals in aromatic rings. The aromatic structure present within SOC molecules may not be available for external bonding based on fact that the removal of SOC from aqueous solutions did not increase with aromatic structure. However, other factors contributing to hydrophobic bonding, such as the molar volume of the DOC molecule, may also be responsible.

Aromaticity was found to have a significant influence on DOC. As the amount of aromatic structure increased: (1) copper BSDs were greater for HMW DOC than for LMW DOC (Figure 4-2); (2) the ability to remove copper from solutions when

complexed with DOC decreased (Figure 4-4a); (3) HMW fractions of DOC increased (Figure 4-5b), which favors increased removal of Cu-DOC complexes from solution through coagulation and settling; and (4) more DOC is able to be removed from solution through hydrophobic mechanisms (Figure 4-6), which increases the removal of Cu-DOC complexes from solution through sorption to immobile or settling solids. When the relationships between the aromatic structures of DOC and (a) copper complexation and (b) DOC characteristics are taken together, results suggest that copper removal from aquatic systems will increase with increasing aromatic structure of DOC.

Conclusions

Using an assortment of standardized humic samples in solutions of the same chemical composition, the influence of molecular structure on DOC characteristics was investigated. Both the quantity of DOC ligands present in and the BSD of HMW and LMW fractions of DOC influenced which MW fraction of DOC copper associated with. Aromatic moieties and oxygen functional groups were found to be important for Cu-DOC complexation, with the bonding between copper and aromatic moieties (cation-π orbital bonding) proving to be stronger than bonding with oxygen functional groups. Aromaticity was found to be the most important structural characteristic for copper complexation with HMW fractions of DOC, while the oxygen content appeared to be more important for LMW fractions of DOC. Based on the aromatic and oxygen content of bulk DOC, it was possible to describe which fraction of DOC, the HMW fraction or the LMW fraction, would have greater BSDs for copper.

The percentage of heteroaliphatic and aromatic structure found in bulk DOC was also found to correlate with the fraction of DOC greater than 1 kDa, and opposing correlations were found for SOC versus DOC. This suggests that there are substantial differences in molecular structure for LMW fractions of humic substance from terrestrial and aquatic sources. As the percentage of aromatic content present in aquatic DOC increased, the amount of DOC retained by SPE media by hydrophobic mechanisms also increased. Overall, the aromatic structure of DOC was found to play a critical role in copper complexation and likely influences Cu-DOC mobility.

Table 4-1. General chemical composition of the artificial river water solutions investigated.

Artificial River Water Solution Chemistry		
pH	7.99	
Conductivity	0.967	mS cm^{-1}
Hardness	186.1	mg L^{-1} as $CaCO_3$
Alkalinity	136.9	mg L^{-1} as $CaCO_3$
Ca	25.5	mg L^{-1}
Cl	93.7	mg L^{-1}
Mg	29.8	mg L^{-1}
NO_3^-	0.48	mg N L^{-1}
PO_4^{3-}	5.69	mg L^{-1}
K	4.6	mg L^{-1}
Na	62.7	mg L^{-1}
SO_4^{2-}	49.0	mg L^{-1}

Solution	Source of DOC	Type of DOC	Concentration DOC mg L^{-1}	Concentration Cu ug L^{-1}
1	Nordic Reservoir	NOM	12.6	95.0
2	Suwannee River	Fulvic Acid	9.28	95.0
3	Suwannee River	Humic Acid	10.0	93.0
4	Suwannee River	NOM	12.6	95.2
5	Aldrich	Humic Acid	20.2	95.4
6	Pahokee Peat	Humic Acid	4.23	
7	Waskish Peat	Fulvic Acid	15.9	
8	Waskish Peat	Humic Acid	13.6	

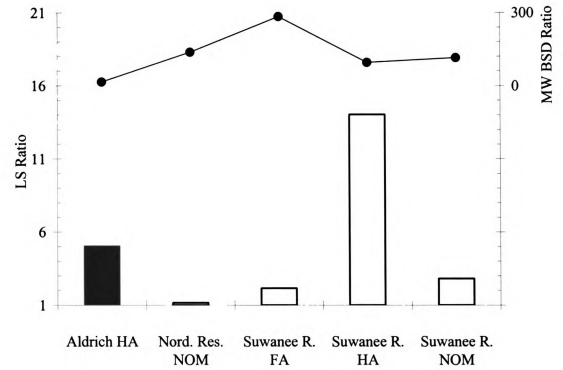

Figure 4-1. The LS ratio and the MW BSD ratio for Cu and a set of standardized DOC samples.

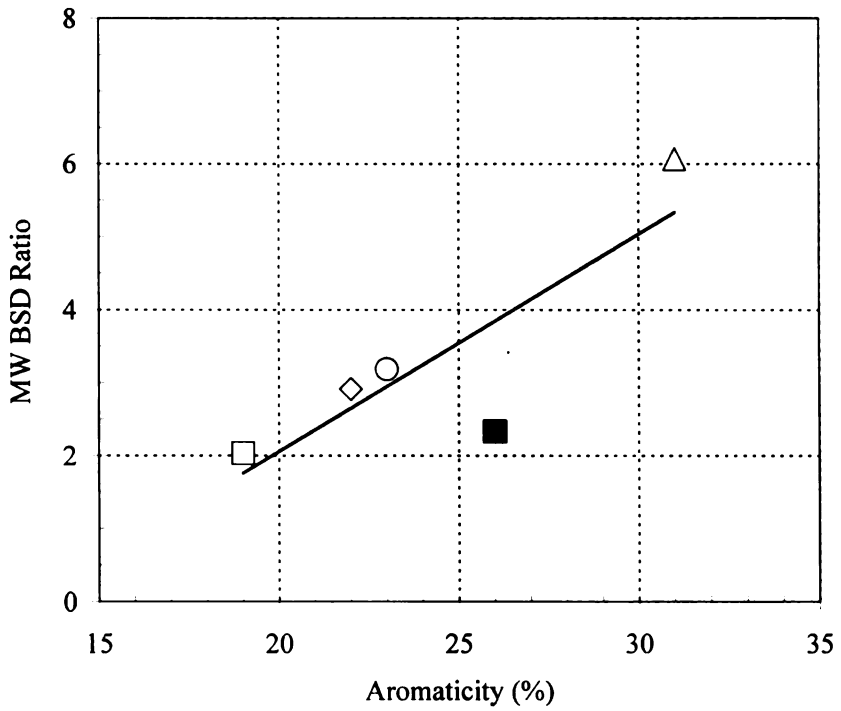

Figure 4-2. The correlation between MW BSD ratio for Cu and aromaticity of Nordic Reservoir NOM (□), Suwannee River FA (◇), Suwannee River HA (△), and Suwannee R NOM (○), and Aldrich HA (■).

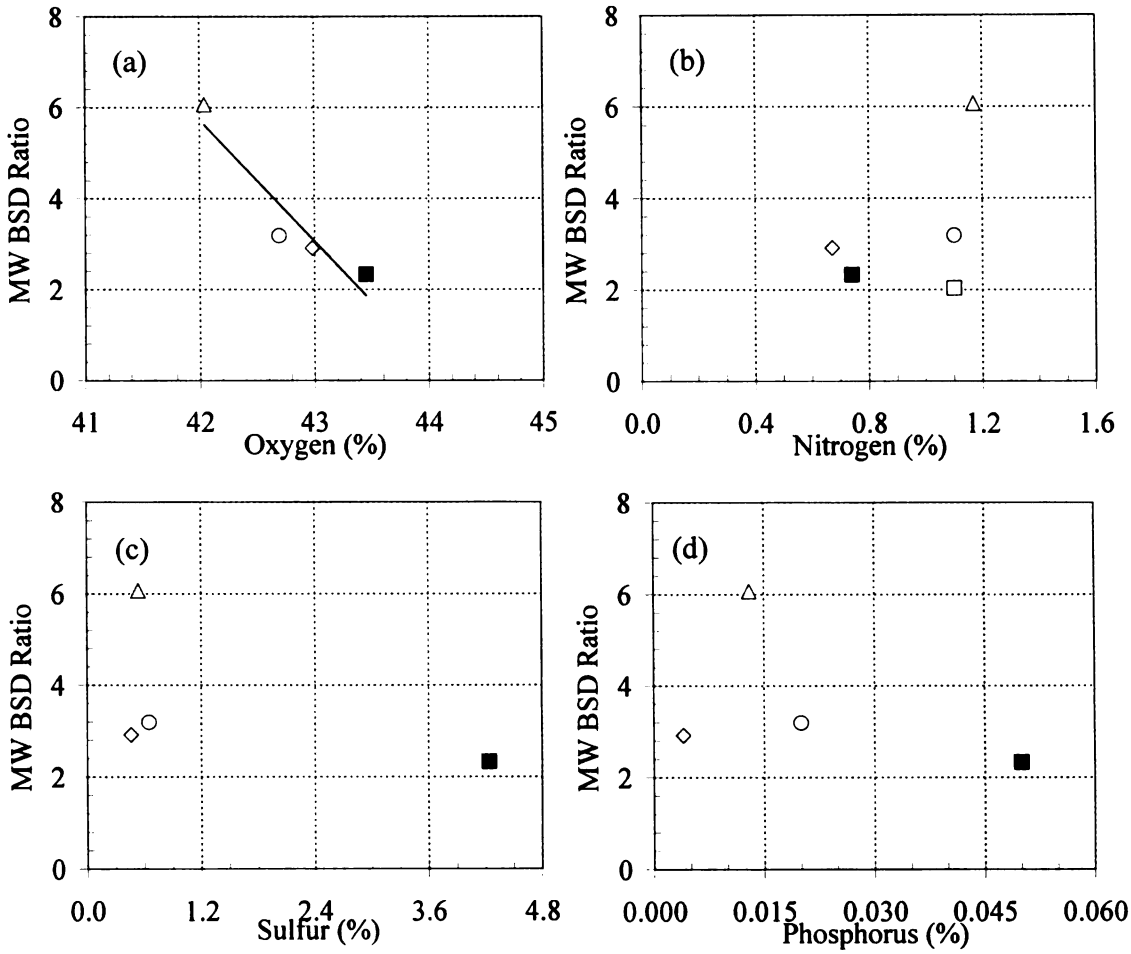

Figure 4-3. The MW BSD ratio as a function of the concentration of various elements: (a) oxygen, (b) nitrogen, (c) sulfur and (d) phosphorus. DOC samples include: Nordic Reservoir NOM (□), Suwannee River FA (◇), Suwannee River HA (△), and Suwannee River NOM (○), and Aldrich HA (■).

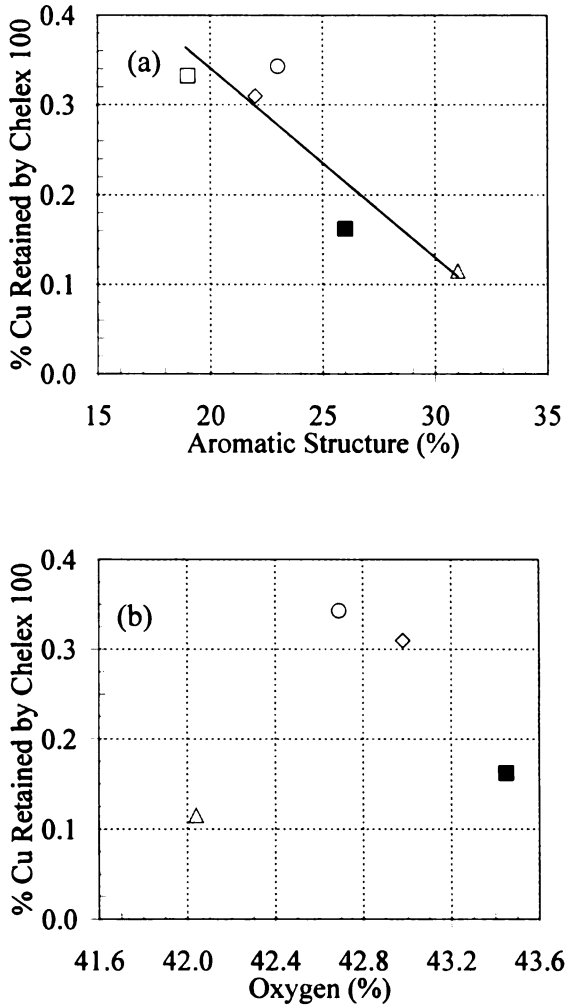

Figure 4-4. Copper removed from Cu-DOC complexes by a Chelex 100 resin as a function (a) aromatic structure and (b) oxygen content of DOC. DOC samples include: Nordic Reservoir NOM (□), Suwannee River FA (◇) Suwannee River HA (△), and Suwannee River NOM (○), and Aldrich HA (■).

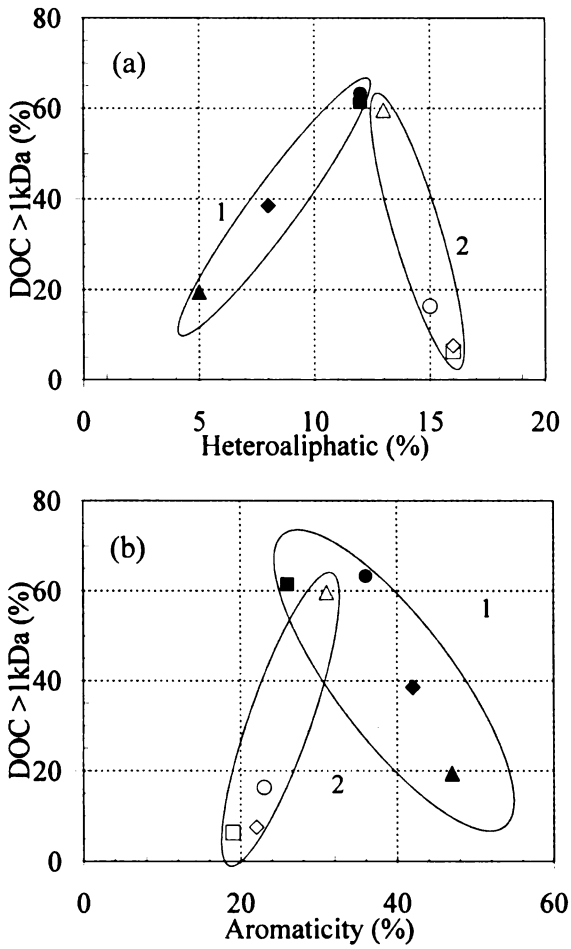

Figure 4-5. Organic carbon retained by ionic mechanisms greater than 1 kDa in size versus the amount of (a) heteroaliphatic and (b) aromatic structure in SOC (black, oval 1) and DOC (white, oval 2). DOC samples include: Aldrich HA (■), Nordic Reservoir NOM (□), Pahokee Peat HA (▲), Suwannee River FA (◇), Suwannee River HA (△), Suwannee River NOM (○), Waskish Peat FA (◆) and Waskish Peat HA (●).

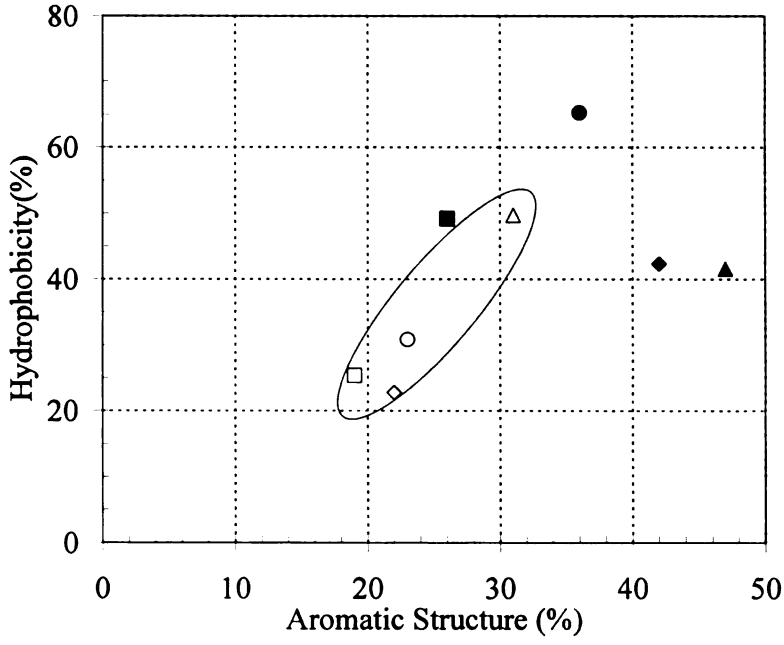

Figure 4-6. The influence of aromatic structure on hydrophobicity, measured as the percentage of organic carbon retained by hydrophobic mechanisms, DOC (white, oval) and SOC (black). DOC samples include: Aldrich HA (■), Nordic Reservoir NOM (□), Pahokee Peat HA (▲), Suwannee River FA (◇), Suwannee River HA (△), Suwannee River NOM (○), Waskish Peat FA (◆) and Waskish Peat HA (●).

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CHAPTER 5

VARIATIONS IN DISSOLVED ORGANIC CARBON CHARACTERSITICS BASED ON LAND USE

Abstract

Despite the importance of DOC in surface water systems, little is known about the factors responsible for determining its composition and character. Due to large fluxes of terrestrial DOC and the presence of plant biomolecules in DOC, land use/cover is suspected of being a significant factor. To investigate this connection, two hypotheses were evaluated: (1) DOC from forested and agricultural land uses are larger in molecular weight and greater in aromaticity than DOC from other sources which contained less vegetative cover (i.e. urban land uses), and (2) DOC produced from watersheds dominated by impervious surfaces contain greater amounts of hydrophobic constituents since landscapes with vegetation preferentially retain hydrophobic fractions of DOC; DOC produced in watersheds with flow paths over impervious surfaces (urban) produce hydrophobic DOC. Aromaticity (measured as normalized ultraviolet absorbance at 280 nm, NUVA), molecular weight, polydispersity and the fraction of DOC retained by hydrophobic and H-bonding mechanisms were used to evaluate DOC characteristics. The influence of individual land uses was assessed by sampling from sub-watersheds with only a single type of land use (>95%) present within the catchment. Multiple sub-watersheds containing an array of urban (e.g. industrial, high and low density residential) or agricultural (e.g. different types of row crop, sod) land uses were sampled. Results show (1) molecular characteristics of DOC differ as a function of land use, (2) DOC characteristics produced by forested land uses were consistently different from other

types of DOC, and (3) agricultural and forest land uses appear to preferentially retain hydrophobic fractions. Finding indicated that DOC derived from watershed with dissimilar land uses will likely have different characteristics.

Introduction

In surface water systems dissolved organic carbon (DOC) constitutes the base of aquatic food chains (Lennon and Pfaff 2005) and plays a critical role in the transport of many organic and inorganic molecules (Chin, Aiken et al. 1997; Santschi, Lenhart et al. 1997; Ghabbour and Davies 2004). Despite the importance of DOC in surface water systems, the processes responsible for the production of DOC as well as its behavior and characteristics are not well defined. This limited understanding of DOC formation and transformation make it difficult to accurately predict the role of DOC across a variety of surface water systems. To resolve this lack of understanding, there is a need to assess the environmental processes responsible for controlling DOC quantity and quality, especially within a range of land use influences (Kalbitz, Solinger et al. 2000).

Based upon differences in composition, DOC can be broadly classified as derived from terrestrial and aquatic processes. The production of DOC by aquatic biota are the result of the exudation and excretion of biomolecules and the decay of organisms from all trophic levels (Tranvik 1993). Terrestrial sources of DOC include exudates from vegetation, litter decomposition, soil leachates, microbial enzymes and biomass (Wickland, Neff et al. 2007). Because peat lands provide all of these processes, they have long been recognized as a significant source of DOC to surface waters and much of the information concerning terrestrial DOC is based on this source (Hemond 1990, Mullholland et al. 1990, Sachse et al. 2000).

Due to the influence of algae and bacteria present in surface water ecosystems, aquatic DOC can be expected to contain larger amounts of carboxyl functional groups and are more aliphatic than terrestrial sources (Zumstein and Buffle 1989; Croue,

Benedetti et al. 2003). While DOC from terrestrial sources is generally lower in molecular weight (MW) than that from aquatic sources (Sachse, Henrion et al. 2005), very high MW (>100 kDa) DOC has been attributed to production of organic molecules by algal during blooms freshwater lakes (Cole, McDowell et al. 1984). Algal-derived DOC is also reported to be low in aromaticity (McKnight, Andrews et al. 1994). Compared to aquatic sources, DOC from terrestrial sources contain a greater amount of aromatic structure with phenolic functional groups, and generally higher in MW (Jackson 1975; Zumstein and Buffle 1989; Croue, Benedetti et al. 2003; Linnik 2003). Similarly, soil pore water DOC is known to be highly aromatic in structure, have a high in MW and can be characterized as having a large hydrophobic fraction (Chin, Traina et al. 1998; Wickland, Neff et al. 2007). Additionally, a correlation between the aromatic content, approximated by measuring the absorbance of ultraviolet light at 280nm normalized to the concentration of DOC, and the average molecular weight (MW) of DOC has been found for multiple samples from surface water bodies (Sachse, Henrion et al. 2005).

The concentration and quality of DOC derived from terrestrial sources are influenced by hydrologic factors, sorption reactions and microbial processes (Kawahigashi, Kaiser et al. 2004). The fluxes of terrestrial DOC from soils are highly dependent on source strength and the amount of water moving through soils to surface water (Hope, Billett et al. 1994). Microorganisms selectively degrade carbohydrates, organic acids and proteins (Marschner and Kalbitz 2003). Due to microbial metabolism, relatively hydrophilic (aliphatic) DOC is transformed to more hydrophobic (aromatic) DOC. The hydrophobic fraction of DOC is selectively sorbed by lignin degradation products (Guggenberger, Zech et al. 1994; Kaiser, Arscott et al. 2004) and microbial

processes generally enhance the removal of DOC from the aqueous phase. As a result DOC present in peat has been found to increase in aromaticity with long-term agricultural practices (Kalbitz, Geyer et al. 1999). Overall, adsorption is thought to inhibit the transport of DOC from terrestrial sources more than biodegradation (Qualls and Haines 1992).

Due to the integral relationship between biological and geochemical processes and DOC production, the type of land cover, or land use, present within watershed catchments is likely to control DOC quantity and quality. Surface water chemistry is dependent on the catchment characteristics (Molot and Dillon 1997) and the concentration of both DOC and lignin have been found to vary significantly depending watershed characteristics (Eckard, Hernes et al. 2007). The chemical composition of DOC derived from Boreal forests has been found to vary significantly depending on the type of vegetation present (Wickland, Neff et al. 2007). Furthermore, DOC characteristics, measured by the isolation of DOC fractions and UV absorbance at various wavelengths, have been shown to vary depending on the origin of DOC samples, whether from forested or agricultural land, or raw or treated sewage (Imai, Fukushima et al. 2001).

Previous studies by others on the influence of land use tend to focus on DOC production, not DOC composition or quality (e.g. the ability to bind trace-metals and serve as a substrate for microbial growth) (Richey, Brock et al. 1980), most of which have focused on agricultural or forestry practices rather than the influence of urbanization (Kalbitz, Solinger et al. 2000). Part of the difficulty in assessing the impact of land use and biological processes on DOC formation and transformation is identifying unique

signals characteristic for each in large watersheds where their influence is mixed. Additionally, in surface waters with large watersheds, degradation processes weaken relationships observed between DOC composition and terrestrial processes (Frost, Larson et al. 2006). However, it is possible to show differences in DOC from small scale watersheds that are not visible in larger watersheds (Dalzell, Filley et al. 2007).

Hedges (1980) originally proposed that unique chemical differences exist for DOC from different landscapes. To our knowledge, no researcher has evaluated this hypothesis across a wide range of agricultural and urban landscapes. To further test this hypothesis, surface water runoff was collected from sub-watersheds which contained unique land use signatures and DOC quality was assessed through a variety of analytical techniques. In addition to the broad hypothesis proposed by Hedges (1980), two additional hypotheses were tested: (1) sub-watershed with agricultural and forested land uses, which are dominated by vegetation, produce DOC with larger molecular weight and with greater aromaticity than sub-watersheds with urban land use, and (2) sub-watersheds with indirect flow paths through soil and leaf litter (agriculture, forested, etc.) preferentially remove hydrophobic fractions of DOC; sub-watersheds with direct flow paths over impervious surfaces (urban) produce hydrophobic DOC.

Materials and Methods

The concentration of DOC was determined by automated analysis based on the Heated-Persulfate Oxidation Method (Clesceri et al., 1998) using an OI Analytical Model 1010 Wet Oxidation Total Organic Carbon Analyzer after passing through a 0.45μm glass fiber filter that was double acid washed. Prior to conducting experiments, all glassware used during DOC analysis was acid (18% HCl) washed, rinsed with DDI water

and placed in a 550°F oven for more than 2 hours to ensure cleanliness. DOC characteristics were measured by assessing the amount of aromaticity, the molecular weight, polydispersity and the amount of DOC retained by hydrophobic and hydrogen bonding (H-bonding). The aromaticity of DOC was approximated by normalized ultraviolet (UV) absorbance at 280 nm, NUVA (Chin, Aiken et al. 1994). The extent of UV absorbance was determined using a Shimadzu UV-160 spectrophotometer by measuring the amount of UV light at a wavelength of 280nm absorbed and normalizing it by the concentration of DOC resulting in units of L gC^{-1} cm^{-1}. A wavelength of 280nm is a more effective surrogate for measuring the aromatic content of DOC than 254nm for two reasons: (1) the transfer of electrons between overlapping π-orbitals occurs at this wavelength for phenolic and other humic like organ substances(Traina, Novak et al. 1990), and (2) nitrate, which also absorbs UV light and is ubiquitous in natural waters, does not absorb UV light at 280nm (Chin, Aiken et al. 1994).

The molecular weight (MW) and polydispersity of DOC was determined by size exclusion chromatography (HPSEC) (Chin, Aiken et al. 1994; Zhou, Cabaniss et al. 2000). The number-averaged MW (M_n) and weight-averaged MW (M_w) were calculated by the following equations:

$$M_n = \sum_{i=1}^{N} h_i / \sum_{i=1}^{N} h_i (M_i) \qquad \text{(Equation 5-1)}$$

and

$$M_w = \sum_{i=1}^{N} h_i (M_i) / \sum_{i=1}^{N} h_i \qquad \text{(Equation 5-2)}$$

where h_i is the height and M_i is the molecular mass of the sample HPSEC eluted at volume *i*. The M_w is commonly referenced as the average MW and this custom will be maintained throughout the rest of the paper. Polydisperity (ρ) is the ratio of the weight-averaged MW and the number-averaged MW:

$$\rho = \frac{M_w}{M_n} \qquad \text{(Equation 5-3)}$$

Low polydispersity indicates a DOC with a relatively narrow range of molecular weights. The HPSEC system employed utilized a Gilson Model 303 pump (Middleton, WI), a Waters Protein-Pak 125 modified silica column (Milford, MA) and UV detection at 254 nm on a Dionex Variable Wavelength Detector (Sunnyvale, CA) (Chin, Aiken et al. 1994; Zhou, Cabaniss et al. 2000). The mobile phase consisted of 0.1 M NaCl, 0.002 M KH_2PO_4 and 0.002 M Na_2HPO_4 solutions buffered to an approximate pH of 7 and calibration was performed using random coil sodium polystyrene sulfonates (Polysciences, Inc.) (1.8, 5.4, 8 and 18 kDa) and acetone (58 Da) (Zhou, Cabaniss et al. 2000). The amount of DOC retained by hydrophobic and H-bonding was determined by passing filtered DOC samples through solid phase extraction (SPE) cartridges and measuring the concentration before and after to obtain the fraction, or percentage, of DOC retained. Two types of hydrophobic cartridges, one designed to retained organic constituents primarily through donor-acceptor binding (identified as hydrophobic) and another with a more hydrophilic SPE media (identified as extended hydrophobic) were

used to asses the hydrophobicity of DOC. These fractions have been shown to relate to DOC structure (Chapter 4) and details of the method used to isolate these fractions can be found in Chapter 3.

Samples were collected from 48 different locations within the Grand River watershed in central Michigan. Of the 48 locations, 29 were from sub-watersheds comprised of only one type of land use (>95% of area) present within the tributaries of the Red Cedar and Looking Glass Rivers. For these 29 sub-watersheds, land use was identified based on the Michigan Land Cover/Use Classification System (MDNR 2001) (Table 5-1). Potential sampling locations were initially identified with the aid of geographic information systems (GIS) by overlaying hydrologic information on land use classifications to determine rough boundaries of sub-watersheds that would contain one type of land use in ArcMAP 9.1 (ESRI 2003). Sub-watersheds with unique land use characteristics were identified by conceptually moving hypothetical sampling locations from the farthest point upstream (i.e. water source or headwater) of the smallest hydrologic units (intermittent streams) down stream until just before the catchment would contain more than one land use. This point, where the sub-watershed still consisted of a single type of land use, was identified as a potential sampling location. Potential sub-watersheds were then inspected to determine if it was logistically possible to collect samples. Ideally, sample locations were selected where stormwater runoff could be collected from outfalls or in open channels where water was free flowing so that no backwater effects would result in mixing from downstream sources. Once sampling locations were confirmed, sub-watersheds were manually delineated based on topography in GIS to ascertain the true sub-watershed boundaries. County digital elevation maps

were used to determine watershed topography (MDEQ 2005). For urban systems where storm sewer networks alter natural watershed boundaries, sewer maps from the City of East Lansing and Michigan State University were used to accurately describe catchment storm sewer networks. Land use data was based on the IFMAP/GAP Lower Peninsula Land Cover raster data set (MDNR 2001). Land use within the Ramey Chandler drain in Ingham County was updated to include the type of land use present at the time of sampling. Details of the method used to delineate sub-watersheds are available in the appendices.

The influence of terrestrial processes on DOC was isolated by collecting samples directly from storm sewer outlets and from ephemeral streams and ponds within 24 hours of the start of runoff events. While significant effort was made to eliminate the influence of aquatic process on DOC, it is impossible to completely isolate only terrestrial processes. Because samples were collected from surface water at golf courses and ephemeral ponds that formed after heavy rains in forested areas, the DOC collected from these two locations are undoubtedly influenced in someway by aquatic processes (i.e. rapid microbial and algal growth). Both sample locations contained significant algal mats which remained present at the sampling location even during the longest stretches of dry weather. Runoff generated from agricultural fields also requires some pooling before generating overland flow. As a result of these limitations in sampling logistics, the influence of aquatic processes on DOC characteristics is assumed to follow the order urban << agricultural < forested ~ recreational golf course for samples collected within unique sub-watersheds. The 19 samples collected from the Grand and Red Cedar rivers, where multiple land use types are present upstream from the sampling location, aquatic

processes were also assumed to be a major factor in determining DOC quantity and quality.

SYSTAT (version 12.02.00; San Jose, CA) was used for all statistical analysis. Unless otherwise noted, an α-level of 0.05 was used to determine significance. Questionable data was not included in statistical analysis if it was deemed an outlier by Dixon's Q-test at the 95% confidence level (Rorabacher 1991). The standard analysis of variance (ANOVA) was used to evaluate if differences existed in sample groups and Tukey's Honestly Significant Difference (HSD) test was used to evaluate if differences existed between specific land uses. For the purpose of statistical analysis, similar samples were grouped according their primary land use classification: urban (URB), agricultural (AG), forested (FOR) or mixed (MIX). Samples collected from Michigan State University storm sewer outfalls (MSU), automobile parking lot (PL) and recreational golf course (GC) were analyzed separately from all other types of urban samples because of their sample size and shared characteristics (i.e. MSU has parking lots and manicured lawns that are similar to PL and GC). An ANOVA was conducted on the replicate samples from sub-watersheds with urban land uses other than those specifically from MSU campus, parking lots and golf courses and no statistically significant difference was observed in DOC concentration and the parameters used to assess DOC characteristics for each sampling location. As a result, these samples were grouped as urban (Table 5-1). Similar to urban samples, an ANOVA was performed on all samples from sub-watersheds composed of only agricultural land uses. Samples from sub-classes of agricultural land use were found to differ based on the concentration of DOC (p-value = 0.041) and the amount DOC retained by H-bonding (p-value = 0.038). A difference in

DOC concentration was found between sub-watersheds planted with corn versus sugar beets (p-value = 0.027) and a difference in the amount of DOC retained by H-bonding was found between sub-watersheds with corn versus sod (p-value = 0.032). However, samples from all agricultural land uses were grouped together as AG because: (1) MW, polydispersity, hydrophobicity were not found to be significantly different and (2) the number of samples were too small to obtain a representative population for individual types of agricultural land use; there as only a maximum of four replicates for specific subgroups of agricultural land use.

Based on land use classifications, sets of data for each land use type were further grouped for statistical analysis to determine if there were statistically significant differences that could be attributed to land use characteristics or aquatic transformation processes. Group 1 (URB, FOR, MSU and AG) was intended to determine if there was a significant difference in DOC characteristics between land uses. Group 2 (GC, PL and URB) was evaluated to determine which of the primary components of URB landscapes influence DOC characteristics. Group 3 (AG, FOR, GC and MIX) was used to determine if there was a significant difference between samples likely influenced by aquatic processes. To provide context for solution chemistry, the general water chemistry for each of the grouped land uses is described in Table 5.2. Alkalinity was measured by Gran titration and the pH, conductivity and DO were measured using a Horiba U-10 water quality analyzer.

Results and Discussion

The greatest concentrations of DOC found in surface water samples were from sub-watersheds with forested and golf course land uses, around 22 mg L^{-1} (Figure 5-1). A high amount of variability in DOC concentrations was observed at each sampling location, with the exception of DOC derived from AG and MIX samples. Among the four primary types of land use, samples from forested sub-watersheds were found to have a DOC concentration greater (p-values < 0.001) than from agricultural, MSU and urban land uses. The concentration of DOC from GC samples was found to be greater than from PL (p-value = 0.002) and URB (p-value < 0.001). Among the four types of samples collected from sub-watersheds where terrestrial processes were suspected, DOC concentrations from forests and golf courses were found to be significantly different from agricultural and mixed land use (p-values < 0.001).

The MW of DOC from forests was found to be around 2 kDa in size, while all other sample were found to have MWs less than 1.5 kDa, which was statistically significant for all contrasts evaluated (Figure 5-2). The average MW of DOC from primary land uses followed the order forested > agricultural > urban ~ MSU, with only urban and MSU samples not being statistically different. The average MW of DOC from urban sub-watershed, 1.22 kDa, was found to be significantly different from, although between, the MW of DOC from both golf courses, 1.43 kDa (p-value = 0.011), and parking lots, 0.98 kDa (p-value = 0.004). No statistically significant difference was observed between the average MW of DOC from agricultural, golf course or mixed land uses.

Based on a mean polydispersity of 2.0, samples from forested sub-watersheds were significantly different from all other sources of DOC; with the exception of parking lots which had a polydispersity of 1.6 (Figure 5-2). There was considerable variation in polydispesity observed in both forested and parking lot samples. No statistical difference was observed between DOC from agricultural, MSU or urban land uses and between DOC from urban and golf course or parking lot samples.

Sub-watersheds assumed to have the highest amount of vegetation (AG, FOR, CG) were found to contain DOC with higher values of NUVA (Figure 5-3). The NUVA was found to follow the order forested > agricultural > urban > MSU, and all differences were found to be statistically significant with the exception of differences between AG, 2.22 L gC^{-1} cm^{-1}, and MSU, 1.58 L gC^{-1} cm^{-1}. No statistically significant difference was observed between samples collected from golf course, parking lot and urban sub-watersheds and the only significant difference between samples potentially influenced by aquatic processes was between samples from FOR and mixed land uses (p-value = 0.003).

A strong correlation ($r^2 = 0.89$) was observed between the average MW and NUVA for DOC from sub-watersheds with forested land cover (Figure 5-4). This relationship can be described by the equation:

$$NUVA = 0.0015 \times M_W - 0.035 \qquad \text{(Equation 5-4)}$$

Although DOC from other sampling locations appears to also cluster around this line, a clear trend was not observed.

For the primary land use types, DOC hydrophobicity followed the general order MSU > urban >> agricultural > forested, with DOC from MSU and urban samples being significantly (p-values < 0.02) greater than agricultural and forested samples (Figure 5). Differences in hydrophobicity between sample types were approximately the same for both types of hydrophobic cartridges used. The amount of DOC retained through H-bonding tended to follow the same trend as hydrophobicity, with the exception of DOC from forested sub-watersheds. Based on H-bonding, forest samples were significantly (p-values < 0.015) greater the other primary types of land use (URB, MSU and AG) as well as MIX samples (p-value = 0.001). For both measures of hydrophobicity and the extent of DOC retained by H-bonding, no statistical difference was observed between DOC from urban and parking lot samples.

A strong correlation (r^2=0.89) was observed between the NUVA and the hydrophobicity of DOC from sub-watersheds with urban land use (highlighted by oval 1 in Figure 5-6). This trend can be described by the equation:

$$\text{Hydrophobic DOC} = 22.7 \times (\text{NUVA}) - 2.3.\ \text{DOC} \qquad \text{(Equation 5-5)}$$

DOC collected from sub-watersheds intended to account for two of the primary types of land cover, manicured lawns (GC) and paved surfaces (PL), present in urban landscapes do not show similar trends, although DOC from parking lot runoff does plot within the

same region as urban samples. DOC samples from golf course (highlighted by oval 2 in Figure 5-6) did not increase in hydrophobicity despite increasing in NUVA.

The high concentration of DOC from forested watersheds (Figure 5-1) is consistent with previous reports of DOC generated from deciduous forests (Moore and Jackson 1989; Park and Matzner 2003). The high concentration of DOC in both forests and golf courses may be due to the influence of aquatic processes since samples collected from these locations were collected from pooling surface water rather than directly from stormwater runoff. However, according to the size-reactivity continuum model proposed by Amon and Benner (1996), DOC altered by heterotrophic bacteria are expected to decrease in MW. Samples from sub-watersheds with forested land were found to have the highest MW of all locations sampled (Figure 5-2). Furthermore, fulvic acids derived from algae are marked by low aromaticity (McKnight, Andrews et al. 1994) and DOC from forested sub-watersheds were found to have the highest molar absorbitivity, which is directly related to the amount of aromatic structure in DOC (Chin, Aiken et al. 1994). This molecular characterization does not support the supposition that aquatic processes are responsible for the high DOC concentration observed in water samples from sub-watersheds with forests and golf courses. Regardless of the cause, the concentration of DOC in surface water runoff was found to vary depending on land use and the concentration was generally consistent with the assumed amount of vegetative cover: forested >agricultural > urban.

Differences in the MW and aromaticity of DOC were also observed between the primary types of land use, supporting the hypothesis originally proposed by Hedges (1980) that unique chemical differences exist for DOC from different landscapes. Sub-

watersheds with land uses comprised primarily of vegetative cover produced DOC higher in molecular weight than urban and parking lot runoff (Figure 5-2). The MW of DOC observed from other locations was within the range observed in other surface water systems (Chin, Traina et al. 1998; Sachse, Babenzien et al. 2001; Maurice, Pullin et al. 2002; Frost, Larson et al. 2006). The distinctly larger MW of DOC from FOR sub-watersheds is consistent with an expectedly large input of plant exudates, such as phytosterols and triterpenoids (Jaffe, Rushdi et al. 2006). The relatively high retention of DOC via H-bonding from sub-watersheds with forested land cover (Figure 5-4) is consistent with large amounts of plant exudates, such as terpenoids and flavonoids which can be effectively isolated by the H-bonding cartridge (Supelco 2005), are known to be biomakers for higher plants (Jaffe, Rushdi et al. 2006) and are major precursors of Suwannee River fulvic acids (Leenheer and Rostad 2004). Like the concentration of DOC observed in surface water samples, the MW was found to follow the presumed order of vegetative cover: FOR>AG>URB.

The larger polydispersity values observed for DOC from sub-watersheds with forested land cover indicates a more diverse MW assemblage than for the other landscapes evaluated (Figure 5-2). The majority of polydispersity values observed are similar to those reported for surface waters in other studies (Chin, Traina et al. 1998; Zhou, Cabaniss et al. 2000). While one might expect low polydispersity for samples from parking lot runoff, given the low diversity of land cover, and larger polydispersity for samples from mixed watersheds, given the heterogeneous watershed characteristics, the opposite was observed. One explanation could be due to the wavelength used for DOC detection using HPSEC. At a wavelength of 254nm, -C=C- bonds are preferentially

detected and non-chromophoric DOC components (e.g., polysaccharides) are not included in the molecular weight determination and thus are not reflected in the calculated polydispersity values. (O'Loughlin and Chin 2004). This may be particularly relevant for this study because the sample collection scheme was designed to minimize the influence of aquatic processes and the preferential degradation of polysaccharides and other non-saturated components of biomolecules. The high polydispersity observed for parking lot runoff may be attributed to an anthropogenic source of DOC, rather than plant derived biomolecules.

The aromaticity of DOC, measured as NUVA, generally appeared to increase with MW for all samples, although DOC from sub-watersheds with forested land uses were found to have a strong correlation (Figure 5-4). This trend is consistent with the observations reported by Sachse et al. (2005) for specific ultraviolet absorbance at 254 nm ($SUVA_{254}$). It is important to note that the trend observed by Sachse et al. (2005) was for DOC collected from surface water bodies where aquatic processes were likely. Additionally, the MW of DOC was greater (3.5-5.5 kDa) and a greater amount of C-bonds absorb the ultraviolet light with a wavelength of 254 nm than 280 nm used to determine the NUVA in this study, hence a larger UV absorbance ($SUVA_{254}$ = 2.3-4.3 L mgC^{-1} m^{-1}) was observed by Sachse et al (2005).

The amount of hydrophobic DOC present in samples from locations believed to be influenced only by terrestrial processes was greater than from locations where some aquatic processes were likely (Figure 5-5). The linear relationship (highlighted by oval)

between average molecular weight and NUVA for DOC from sub-watersheds with forested land use can be described by the equation (r^2=0.89):

$$NUVA = 0.0015 \times MW - 0.035. \quad \text{(Equation 5-6)}$$

Observed differences in hydrophobicity are not explained by the influence of pH on DOC protonation (Table 5-2). One plausible explanation for the observed difference in hydrophobicity is due to the hydraulic connectivity present within urban land uses. Samples collected from MSU, parking lots and urban land uses were transported over impervious surfaces and through sewer networks, whereas the flow path for other types of land use included extensive contact soil, vegetation and detritus. Forested soils have been shown to remove hydrophobic fraction of DOC from aqueous the phase (Meier, Chin et al. 2004) and low amounts of hydrophobic DOC are retained by soils with low microbial activity result (Kawahigashi, Kaiser et al. 2004). Further evidence to support the hypothesis that sub-watersheds with indirect flow paths preferentially retain hydrophobic fractions of DOC is the lack of a connection between aromaticity, measured as NUVA, and the hydrophobic content of DOC (Figure 5-6). The presence of aromatic structure generally increases hydrophobicity (Chapter 4). Hydrophobicity was only found to increase with NUVA for urban and parking lot samples. However, the hydrophobic fraction remained relatively constant for DOC from other types of land use (e.g. GC) despite a wide range of aromaticity.

Statistical analysis performed on data when grouped to determine the influence of land use and aquatic processes revealed: (1) DOC characteristics were different among

the main types of land use investigated (URB, FOR, MSU and AG), (2) DOC characteristics from urban land use more closely resembled that from parking lots rather than golf course, and (3) there were significant differences between samples that may have been influenced by aquatic processes. DOC from sub-watersheds with the four main types of land use were found to be statistically different (p-values ≤ 0.001) for each of the parameters used to measure DOC characteristics. As a result, it is important to note that DOC from forested sub-watersheds is not representative of DOC from other types of land use. No statistical difference ($\alpha = 0.05$) was observed between DOC from urban and parking lot samples in the total concentration of DOC concentration, NUVA, hydrophobicity (based on the retention on two types of hydrophobic SPE cartridges) and the amount of DOC retained by H-bonding. Similarities in DOC characteristics between urban and parking lot runoff suggest the influence of paved surfaces dominate urban processes. Unlike other studies where the signal produced from terrestrial process was muted by aquatic process (Frost, Larson et al. 2006), all measures (NUVA, MW, polydispersity, total concentration, percent hydrophobic and percent H-bonding) showed a statistically significant difference between urban, forested, golf course and mixed land uses, with the exception of the amount of DOC retained via the extended hydrophobic cartridge (F-ratio=2.78, p=0.052). The consistent differences observed were undoubtedly due in part to a sampling scheme which isolated sub-watershed with unique land uses and obtained samples directly from overland flow before reaching surface water bodies where aquatic transformation processes were likely.

Conclusion

Statistically significant differences in DOC concentrations and characteristics (MW, polydispersity, aromaticity, and fractions of DOC retained by H-bonding and hydrophobic mechanisms) were observed based on land use. These differences were identified by isolating sub-watersheds with unique land uses and sampling stormwater runoff to minimize the impact of aquatic transformation processes (i.e. microbial and physicochemical). Sub-watersheds dominated by vegetation, such as agricultural and forested land uses, were found to produce DOC higher in MW and aromaticity than sub-watersheds with urban land uses. Variations in the hydrophobicity observed support the hypothesis that hydrophobic fractions of DOC are preferentially removed during the transport of surface water runoff from sub-watersheds with vegetative cover, such as agricultural and forested land. Results of this study support the hypothesis proposed by Hedges (1980) that unique chemical differences exist for DOC from different land uses.

Table 5-1. Number of samples collected from each type of land use. Samples were grouped according to the land use identification (LUID). All samples were classified based on their MLCUC - Michigan Land Cover/Use Classification (MDNR 2001).

LUID	MLCUC	Land Use Description	DOC	NUVA	Molecular Weight	Polydispersity	Extended Hydrophobic	Hydrophobic	H-bonding
URB	**1**	**Urban (total)**	**22 (2)**	**21**	**10**	**10**	**18**	**19**	**18 (1)**
	1	Urban & Build Up	1	1	1	1	0	1	1
	11	Residential	1	1	0	0	1	1	1 (1)
	1121	Apartments	9 (1)	8	4	4	9	9	9
	112	Multi-Family, Low-Rise	1	1	0	0	1	1	0
	113	Single Family/Duplex	4 (1)	4	2	2	3	3	3
	115	Manufactured Home Park	1	1	0	0	1	1	1
	122	Shopping Center/Malls/Retail	3	3	2	2	3	3	3
	124	Secondary/Neighborhood	2	2	1	1	0	0	0
MSU	**12644**	**Michigan State University**	**56**	**43**	**39**	**39 (2)**	**47 (1)**	**47**	**39**
PL	**1449**	**Automobile Parking Lot**	**13**	**10 (2)**	**7**	**7**	**13**	**11 (1)**	**7**
GC	**19331**	**Recreational Golf Course**	**15**	**12**	**7**	**7**	**15**	**15**	**10 (1)**
AG	**2**	**Agricultural (total)**	**14 (2)**	**8**	**8**	**8 (1)**	**13**	**14 (2)**	**10 (1)**
	2113	Agricultural Row Crop, Corn	3	3	3	3	3	3	3
	2111	Agricultural Row Crop, Sugar Beets	6 (2)	3	3	3 (1)	5	6 (2)	2 (1)
	22	Ornamental Horticulture	1	0	0	0	1	1	1
	2231	Sod Farm	4	2	2	2	4	4	4
FOR	**4**	**Forested (total)**	**10 (2)**	**9 (1)**	**6**	**6**	**10**	**10**	**7**
	411	Northern Hardwoods	1	1	1	1	1	1	1
	431	Upland Hardwoods and Pine	9 (2)	8 (1)	5	5	9	9	6
MIX		**Mixed**	**16 (2)**	**13 (1)**	**18**	**18 (1)**	**14 (1)**	**15 (2)**	**7**

Table 5-2. Average water chemistry for different land use (grouped) sampling locations.

LUID	Land Use Description		pH	Conductivity $mS\ cm^{-1}$	DO $mgO\ L^{-1}$	Alkalinity $mgCaCO_3\ L^{-1}$
URB	Urban	Mean	7.1	0.72	5.7	90.3
		Std. Dev.	1.2	0.91	3.3	65.6
		n	22	22	17	11
MSU	Michigan State University	Mean	7.9	0.69	6.7	121.0
		Std. Dev.	0.4	0.53	2.6	118.0
		n	44	8	7	15
PL	Automobile Parking Lot	Mean	8.4	1.34	4.3	137.6
		Std. Dev.	1.5	1.67	3.3	197.5
		n	10	10	8	9
GC	Recreational Golf Course	Mean	7.4	0.54	7.7	276.0
		Std. Dev.	0.3	0.14	1.9	55.4
		n	13	13	11	11
AG	Agricultural	Mean	7.0	1.70	3.3	337.2
		Std. Dev.	0.3	1.55	3.4	203.5
		n	13	12	10	8
FOR	Forested	Mean	5.8	0.10	2.3	41.5
		Std. Dev.	1.0	0.12	1.1	51.9
		n	6	6	6	6
MIX	Mixed	Mean	8.0	0.55	7.9	84.6
		Std. Dev.	0.5	0.19	2.6	136.2
		n	19	19	11	10

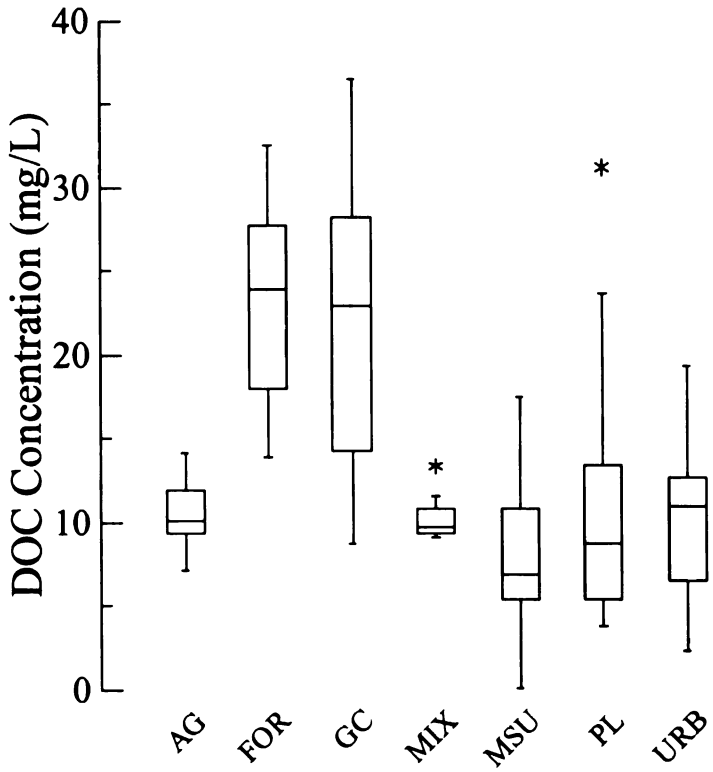

Figure 5-1. Box plots of the total concentration of DOC (mg L^{-1}) in surface water runoff from sub-watersheds with specific types of land cover: agricultural (AG), forested (FOR), golf course (GC), mixed (MIX), Michigan State University (MSU), automobile parking lot (PL) and urban (URB). Values plotted as asterisks are 1.5 times beyond the range where the central 50% of the observations fall.

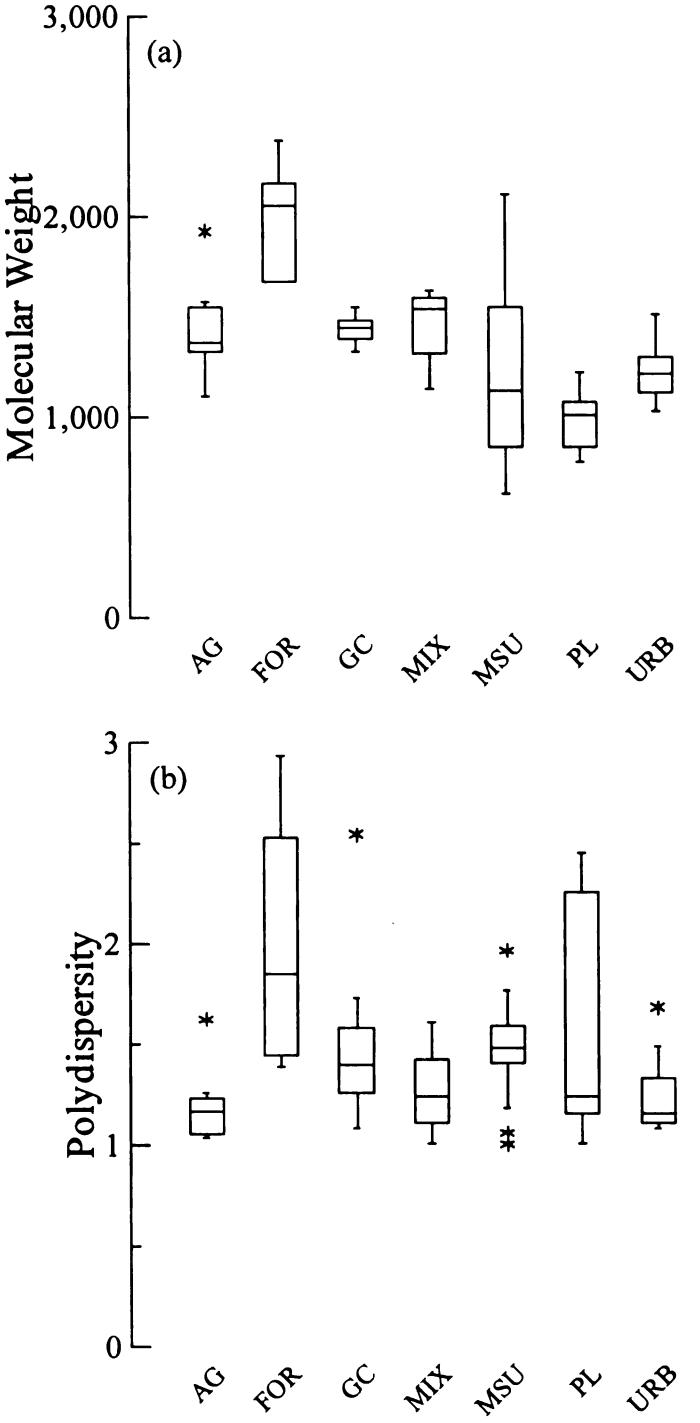

Figure 5-2. The average molecular weight (a) and polydispersity (b) of DOC in surface water runoff from sub-watersheds with specific types of land cover: agricultural (AG), forested (FOR), golf course (GC), mixed (MIX), Michigan State University (MSU), automobile parking lot (PL) and urban (URB). Values plotted as asterisks are 1.5 times beyond the range where the central 50% of the observations fall.

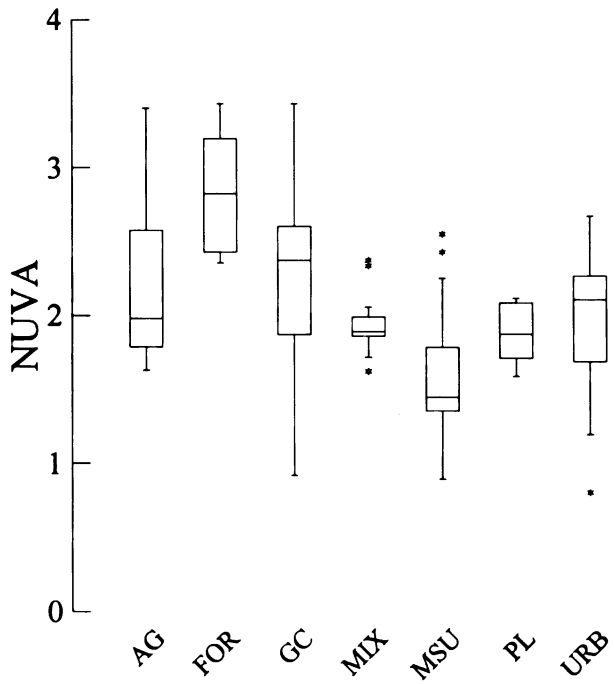

Figure 5-3. Box plots of the NUVA (L mgC m^{-1}) in surface water runoff from sub-watersheds with specific types of land cover: agricultural (AG), forested (FOR), golf course (GC), mixed (MIX), Michigan State University (MSU), automobile parking lot (PL) and urban (URB). Values plotted as asterisks are 1.5 times beyond the range where the central 50% of the observations fall.

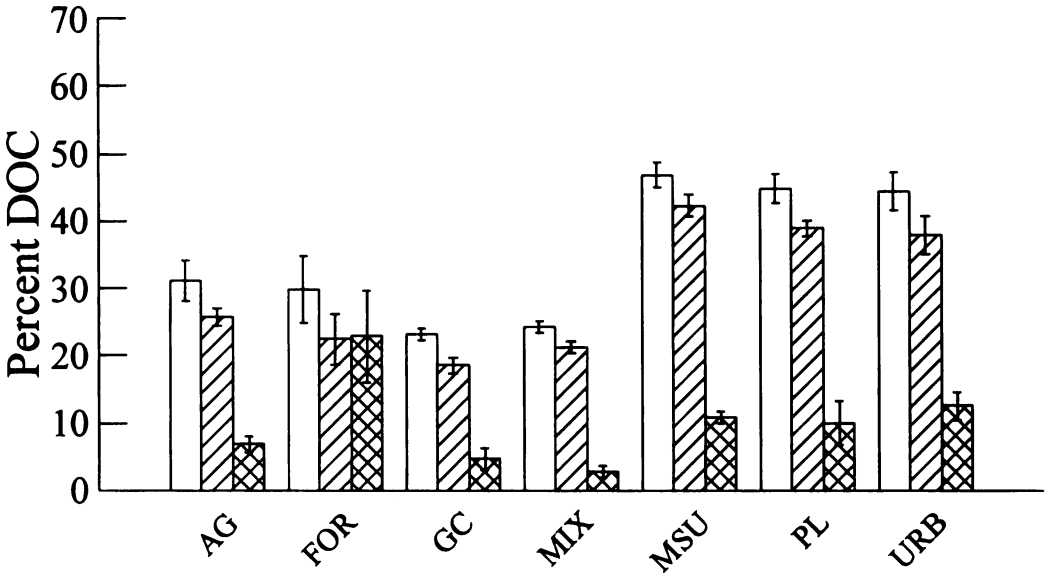

Figure 5-4. The percentage of DOC retained on extended hydrophobic (□), hydrophobic (▨) and H-bonding (⊠) cartridges for samples from sub-watersheds with specific types of land cover: agricultural (AG), forested (FOR), golf course (GC), mixed (MIX), Michigan State University (MSU), automobile parking lot (PL) and urban (URB). The standard error is plotted as error bars from the mean.

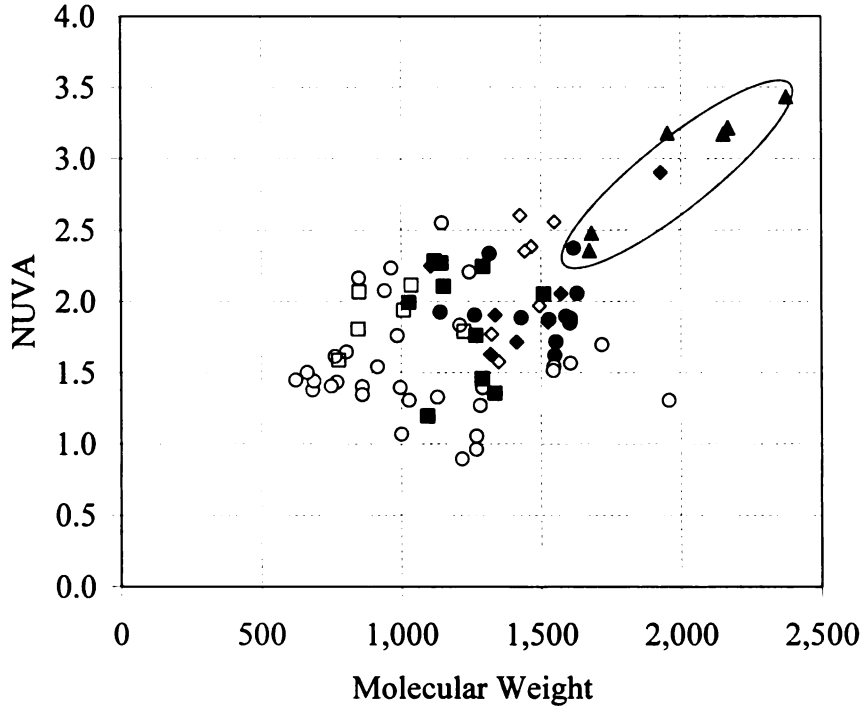

Figure 5-5. NUVA (L mgC m^{-1}) versus molecular weight (Da) for samples collected from sub-watersheds with specific and mixed land uses: agricultural (◆), forested (▲), golf course (◇), mixed (●), Michigan State University (○), automobile parking lot (□) and urban (■).

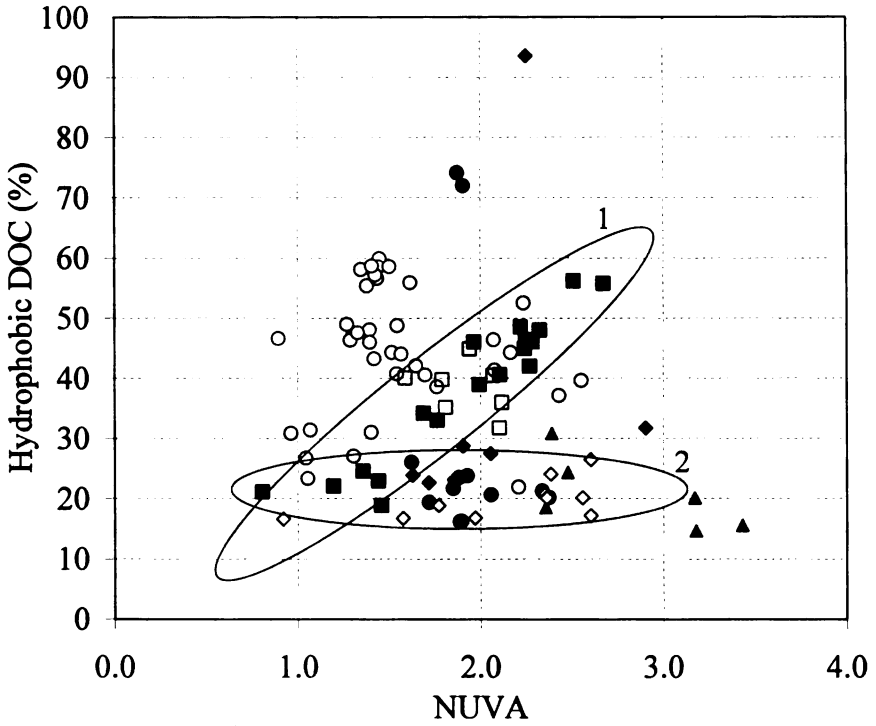

Figure 5-6. The amount of DOC retained on the hydrophobic cartridge versus NUVA (L mgC m^{-1}) for DOC collected from sub-watersheds with specific and mixed land uses: agricultural (◆), forested (▲), golf course (◇), mixed (●), Michigan State University (○), automobile parking lot (□) and urban (■).

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CHAPTER 6

INFLUENCE OF ENVIRONMENTAL FACTORS ON DISSOLVED ORGANIC CARBON CHARACTERISTICS

Abstract

Significant temporal variability has been observed in DOC characteristics, even when from a constant source. Seasonal variations are known to alter the type of DOC found in many watersheds; however, specific factors responsible for these differences in DOC characteristics remain unclear. Environmental factors (e.g. solar radiation, water temperature) that may influence the production and transformation of organic carbon from terrestrial sources are investigated to determine if they are related to DOC characteristics. Samples were collected in a manor to minimize the influence of aquatic processes, such as microbial and algal growth, on DOC characteristics, however, results suggest photochemical and biological alteration of DOC may have occurred. General linear models (GLMs) incorporating precipitation, solar radiation and some aqueous chemical parameters were found to successfully explain variations observed in DOC aromaticity (measured as normalized UV absorbance at 280nm), molecular weight and hydrophobicity. GLMs incorporating multiple environmental factors and land use accounted for up to 86% of the variability observed in DOC characteristics. Interactions between land use - solar radiation, water temperature and water conductivity were found to be statistically significant (p-values < 0.05). Of all DOC characteristics investigated, hydrophobicity was found to be the most difficult to correlate with a specific environmental parameter; however, the hydrophobicity of DOC from sub-watersheds

with forested land use did appear to oscillate during the year. This work demonstrates strong correlations between land use, environmental factors and DOC characteristics which can be explored to further elucidate cause and effect relationships responsible for observed variations in DOC characteristics.

Introduction

Dissolved organic carbon (DOC) is composed of a heterogeneous mixture of biological organic compounds at various stages of microbial and physico-chemical transformation (Ghabbour and Davies 2004; Wickland, Neff et al. 2007). In surface water systems, DOC represents a critical ecological component by influencing chemical and biological reactions (Qualls and Haines 1992; Chin, Aiken et al. 1997; Santschi, Lenhart et al. 1997; Lennon and Pfaff 2005). Due to the important role of DOC in food webs and chemical cycles, there is a need to determine the factors responsible for DOC characteristics and production (Kalbitz, Solinger et al. 2000).

Previous investigations by others into possible variations in DOC quality have revealed inconclusive results, while Chapter 5 identified correlations between land use and DOC characteristics. Little variation in DOC characteristics has been observed between watersheds with similar types of land use (Schumacher, Christl et al. 2006). The likely cause of the ambiguity observed by others are sampling schemes which do not collected DOC samples immediately from the terrestrial environment before aquatic processes influence DOC characteristics. In large surface water systems where aquatic processes are dominant and residence times for DOC are high - oligotrophic lakes, large rivers or oceans – DOC characteristics consist of relatively stable pools of aliphatic molecules with low abilities to attenuate light (Tipping, Hilton et al. 1988; McKnight, Andrews et al. 1994; Sun, Perdue et al. 1997). However, the characteristics of DOC found in stable surface water environments are different from the characteristics of DOC entering them from terrestrial landscapes (Cole, McDowell et al. 1984; Loh, Bauer et al. 2006). DOC produced from different types of vegetation are also found to vary in

biodegradability (Wickland, Neff et al. 2007). Chapter 5 described statistically significant differences in aromaticity, molecular weight, polydispersity and the fraction of DOC retained by hydrophobic and H-bonding mechanisms based on land use; however, considerable variability still existed among some types of samples.

In order to address the variability observed between samples collected from sub-watersheds with the same land use, environmental factors must also be considered. The cause for variability observed in DOC characteristics from terrestrial sources remains largely unknown (Canham, Pace et al. 2004). Some DOC characteristics, such as the aromatic content and photo-degradation of DOC are thought to be seasonally dependent (Kortelainen 1993; Molot and Dillon 1997; Larson, Frost et al. 2007; Rodríguez-Zúñiga, Milori et al. 2008). For instance, the rate at which DOC from terrestrial sources is chemically altered has been found to vary seasonally (Porcal, Hejzlar et al. 2004). Additionally, clear seasonal trends have been observed in the structural composition (i.e. aromatic content) of DOC (Clair and Sayer 1997). Environmental factors that are seasonally dependent, precipitation and solar radiation, have been found to influence the amount of light absorbed by DOC (Curtis and Schindler 1997; Molot and Dillon 1997; Lindell, Graneli et al. 2000; Reche and Pace 2002). Due to these limited observations, environmental factors responsible may be responsible for variations in DOC characteristics and requires further investigation (Christian and Lind 2007).

In order to determine the processes responsible for DOC variation, it is necessary to collect and evaluate DOC immediately downstream from its source. By carefully isolating DOC produced from unique catchments, a range of DOC characteristic have been shown to vary depending on the land cover present in watersheds (Imai, Fukushima

et al. 2001; Page, van Leeuwen et al. 2001). As a result, the farther upstream DOC samples are collected, or closer to the terrestrial source of organic carbon, the smaller the influence of aquatic processes, such as chemical transformations from UV light, and microbial and algal growth (Vannote, Minshall et al. 1980).

DOC aromaticity, molecular weight (MW) and hydrophobicity offer effective measures DOC characteristics since they have been suggested as indictors of bioavailability and transformation processes (Cabaniss, Zhou et al. 2000; Marschner and Kalbitz 2003; Wickland, Neff et al. 2007). The aromaticity of DOC can be approximated by measuring the extent of ultraviolet (UV) light absorbed at a wavelength of 280nm when normalized by the concentration of DOC (Chin, Aiken et al. 1994). Differences in the UV absorption of DOC have been found to depend on watershed characteristics (Larson, Frost et al. 2007). It has been proposed that the MW of DOC indicates the extent to which it is susceptible to microbial degradation (Vannote, Minshall et al. 1980; Amon and Benner 1996). Initially, it was suggested that microorganisms in the headwater of riverine systems preferentially degrade smaller organic molecules (Vannote, Minshall et al. 1980). As DOC moved downstream the low MW fractions of DOC would be preferentially utilized producing recalcitrant pools of high MW DOC in oceans, lakes and other receiving waters. However, a new size-reactivity continuum model has challenged this assumption and suggests that high MW DOC may be more reactive and a better indicator of microbial diagenesis (Amon and Benner 1996; Fischer, Sachse et al. 2002). Additionally, the hydrophobicity of DOC may also be an indicator of microbial utility. Polysaccharides and other high energy constituents are rapidly consumed by microorganisms (Fischer, Sachse et al. 2002). As these energy rich constituents, which

are more hydrophilic than hydrophobic, are consumed by microorganism DOC becomes increasingly hydrophobic. Hence, hydrophobicity may serve as an indicator of the extent that DOC has been processed by microorganisms.

The focus of this chapter is to investigate environmental variables associated with variations in DOC characteristics. These factors are in addition to the influence of land use on DOC characteristics described in Chapter 5. Based on seasonal fluctuations in DOC characteristics and the presences of plant biomarkers present in DOC, variations in DOC characteristics from catchments composed of the same land uses are hypothesized to be due to the growth of terrestrial plants. The amount of solar radiation was used as a surrogate for vegetative growth. Possible correlations between multiple environmental factors and the aromaticity, MW and hydrophobicity of DOC from catchments with one of seven types of land use were used to evaluate this hypothesis. Other weather related and water chemistry parameters were investigated with the use of General Linear Models (GLMs) to determine if they were responsible for variations observed in DOC characteristics from similar land uses (Chapter 5).

Methods and Materials

Samples were collected from 48 different locations within the Grand River watershed in central Michigan. Of the 48 locations, 29 were from catchments with only one type of land use present within the Red Cedar and Looking Glass River tributaries. Based in part on the Michigan Land Cover/Use Classification System (MDNR 2001), samples were classified into one of seven land use types: urban (URB), agricultural (AG), forested (FOR), Michigan State University's campus (MSU), automobile parking lot

(PL), recreational golf course (GC) or mixed (MIX). Samples classified as MIX were collected from the Grand and Red Cedar Rivers at points where it would be impossible to differentiate the influence of the different land uses responsible for DOC characteristics. General chemistry and a more detailed description of sampling locations can be found in the previous chapter (Chapter 5) and the appendix.

The concentration of DOC was determined by automated analysis based on the Heated-Persulfate Oxidation Method (Clesceri et al., 1998) using an OI Analytical Model 1010 Wet Oxidation Total Organic Carbon Analyzer after passing though a 0.45μm glass fiber filter that was double acid washed. Prior to conducting experiments, all glassware used during DOC analysis was acid (18% HCl) washed, rinsed with DDI water and placed in a 550°F oven for more than 2 hours to ensure cleanliness. DOC characteristics were measured by assessing the amount of aromaticity, the molecular weight, polydispersity and DOC hydrophobicity.

The aromaticity of DOC was approximated by the normalized ultraviolet absorbance at a wavelength of 280 nm (NUVA) (Chin, Aiken et al. 1994). The NUVA was determined using a Shimadzu UV-160 spectrophotometer and normalizing by the concentration of DOC, resulting in units of L gC^{-1} cm^{-1}. A wavelength of 280 nm is a more effective surrogate for measuring the aromatic content of DOC than 254 nm for two reasons: (1) the transfer of electrons between overlapping π-orbitals occurs at this wavelength for phenolic and other humic like organ substances (Traina, Novak et al. 1990), and (2) nitrate, which also absorbs UV light and is ubiquitous in natural waters, does not absorb UV light at 280 nm (Chin, Aiken et al. 1994).

The molecular weight (MW) and polydispersity of DOC was determined by size exclusion chromatography (HPSEC). The number-averaged MW (M_n) and weight-averaged MW (M_w) were calculated by the following equations:

$$M_n = \sum_{i=1}^{N} h_i / \sum_{i=1}^{N} h_i(M_i) \qquad \text{(Equation 6-1)}$$

and

$$M_w = \sum_{i=1}^{N} h_i(M_i) / \sum_{i=1}^{N} h_i \qquad \text{(Equation 6-2)}$$

where h_i is the height and M_i is the molecular mass of the sample HPSEC eluted at volume *i*. The M_w is commonly referenced as the average MW and this custom will be maintained throughout the remainder of this paper. Polydisperity (ρ) is the ratio of the weight-averaged MW and the number-averaged MW:

$$\rho = \frac{M_w}{M_n} \qquad \text{(Equation 6-3)}$$

Low polydispersity indicates a DOC with a relatively narrow range of molecular weights. The HPSEC system employed utilized a Gilson Model 303 pump (Middleton, WI), a Waters Protein-Pak 125 modified silica column (Milford, MA) and UV detection at 254 nm on a Dionex Variable Wavelength Detector (Sunnyvale, CA) (Chin, Aiken et al. 1994; Zhou, Cabaniss et al. 2000). The mobile phase consisted of 0.1 m NaCl, 0.002 m KH_2PO_4 and 0.002 m Na_2HPO_4 solutions buffered to an approximate pH of 7 and calibration was performed using random coil sodium polystyrene sulfonates

(Polysciences, Inc.) (1.8, 5.4, 8 and 18 kDa) and acetone (58 Da) (Zhou, Cabaniss et al. 2000).

DOC hydrophobicity was determined by the amount of DOC retained on a hydrophobic solid phase extraction (SPE) media. Briefly, surface water samples were filtered through a 0.45 μm filter, samples were passed through a styrene-divinyl benzene SPE cartridge (Biotage, Isolute 101). The concentration of DOC was measured before and after to obtain the fraction, or percentage, of DOC retained. The hydrophobicity of DOC is related to its structure (Chapter 4) and details of the method used to isolate these fractions can be found in an earlier chapter (Chapter 3).

Questionable data was not included in statistical analysis if it was deemed an outlier by Dixon's Q-test at the 95% confidence level (Rorabacher 1991). SYSTAT (version 12.02.00; San Jose, CA) was used for all statistical analysis. Unless otherwise noted, and α-level of 0.05 was used to determine significance. Due to the influence of land use in determining the characteristics of DOC (Chapter 5), GLMs were used to evaluate the influence of weather on DOC characteristics. GLMs are statistical models used to investigate many environmental problems (El-Shaarawi and Piegorsch 2001), and have been used successfully to identify environmental factors responsible for seasonal variations in the production of organic acids by plants (Burkey, Neufeld et al. 2006). GLMs utilize multiple regression coefficients, each for different factors, which allows for the influence of numerous factors to be assessed. The GLM for a single independent variable can be described as:

$$Y = X_1ß_1 + X_2ß_2 \ldots X_nß_n + e \qquad \text{(Equation 6-4)}$$

where Y is a vector of the dependent variable, X are vectors of the independent variables, ß are vectors of regression coefficients, and e is a vector of random errors. For each combination of independent variables there is a linear regression coefficient. A simplified version of Equation 6-4 can be written excluding the regression coefficient showing simply the dependent and independent variables, for one independent variable:

$$A = B + C \qquad \text{(Equation 6-5)}$$

where A represents the dependent variable, B represents the independent variable and C is a constant representing the random error. All models were evaluated to determine the data was normally distributed, the errors had constant variance and the linear model generally described all samples evenly. Once an appropriate GLM was established, Tukey's Honestly Significant Difference (HSD) Test was used to evaluate differences between factors responsible for DOC characteristics (Tukey 1949).

Two methods were used to determine the effectiveness of statistical models in describing variations in DOC characteristics. The first method used was to evaluate the r-squared value of the resulting linear regression. The value of r-squared effectively determines the accuracy of model predictions. GLMs unable to explain two-thirds of observed variation were considered as highly suspect at best, and most likely to be an indication that other factors were responsible for the observed variation. Because it is possible to add and infinite number of model parameters to effectively describe any variation, regardless of the true meaning behind model parameters, another method was

required to evaluate the amount of bias and uncertainty present in suspected models. The second method used to evaluate the effectiveness of statistical models was the Akaike Information Criterion (AIC) (Akaike 1974). The AIC is based on the principle of parsimony; the ideal model is one with the optimal combination of bias and variability. Effectively, the AIC adds a penalty for the increased likelihood of bias and uncertainty. A corrected AIC (AIC_c) was used in this study due to the limited sample size for some comparisons (Hurvich and Tsai 1989).

Results and Discussion

For catchments dominated by vegetation (e.g. agricultural, golf course) a possible negative correlation between NUVA and the mean weekly solar flux density was observed (Figure 6-1). Some of the sites dominated by vegetation that did not show a decrease in NUVA with increased solar radiation were forested catchments. Using NUVA as a surrogate for the aromaticity (Chin, Aiken et al. 1994), the observed decrease in aromaticity with increased solar radiation suggests the breakdown of aromatic moieties. Solar induced breakdown of DOC chromophores, sometimes call photo-bleaching, has been reported for DOC in wetlands (Osburn, Morris et al. 2001; Waiser and Robarts 2004) and marine waters (Del Vecchio and Blough 2004). A lack of photo-degradation of DOC from forested catchments is likely due to the shielding from the forest canopy (Frost, Larson et al. 2005).

After evaluating multiple other environmental parameters (e.g. pH, precipitation) for correlations indicating factors which influence NUVA, the following simplified GLM was found to be most effective in explaining NUVA variability (Figure 6-2a):

$$NUVA = LU + (LU \times TEMP_w) + (LU \times SOLAR_w) + C \quad \text{(Equation 6-6)}$$

where NUVA is the normalized UV absorbance at 280nm, LU is land use, $TEMP_w$ is the water temperature (°C), $SOLAR_w$ is the mean maximum solar flux density ($W\ m^{-2}$) for the week prior to sampling, and C is a constant representing the random errors. As described previously (Equation 6-4), for each combination of independent variables there is a regression coefficient determined by SYSTAT, these coefficients are described in Table 6-1. This GLM (Equation 6-6) accounts for 67% of the variation in NUVA observed with an AIC_c of 93.8. While other parameters are likely to have major roles in determining NUVA, evidenced by the poor model fit, all variables were found to be statistically significant (p-values < 0.001). Based on Tukey's HSD Test, statistically significant differences in NUVA were found for contrasts between four types of land (Table 6-2). These differences were between catchments containing storm sewer infrastructure (MSU, PL and URB) and catchments where the possibility of aquatic processes were likely (FOR and GC).

While a large portion (33%) of the variation in NUVA remains unexplained, the influence of water temperature and solar radiation was still found to be signficant. Microbial and photo-chemical processes may be responsible for changes in NUVA. Heterotrophic microorganisms preferentially consume carbohydrates, proteins and other nutrient rich sources leaving behind aromatic structures (Marschner and Kalbitz 2003). As water temperature increases, microbial growth can be expected to increase. Therefore, as water temperature increased, the influence of microbial processes would be expected to increase aromaticity, relative to the concentration of DOC (i.e. NUVA). The other

process that could be responsible for changes in aromatic content is UV degradation of aromatic moieties (Figure 6-1). Distinguishing between microbial and photo-chemical processes within this set of samples is difficult because water temperature is likely to rise with mean weekly solar flux. However, a decrease in the amount of photo-degradation in forested sub-watersheds due to the forest canopy and increased microbial growth from rising water temperatures may both contribute to a greater aromaticity for DOC from forested areas.

The following simplified GLM accounted for 86% of the observed variation in DOC MW (Figure 6-2b):

$$MW = LU + PRECIP_w + (LU \times SOLAR_m) + (LU \times COND) + C \qquad \text{(Equation 6-7)}$$

where MW is the weight-averaged molecular weight (kDa), LU is land use, $PRECIP_w$ is the total precipitation (mm) for the week prior to sampling, $SOLAR_m$ is the mean maximum solar flux density ($W\ m^{-2}$) for the month prior to sampling, COND is the conductivity ($mS\ cm^{-1}$) of the sample solution, and C is a constant representing the random errors. Again, regression coefficients determined by SYSTAT are presented in Table 6-1. In addition for this model accounting for nearly all the variability observed in DOC MW, it was also found to have a relatively low AIC_c (-17.2). With the exception of the interaction between land use and solution conductivity (p-value = 0.08), all variable were found to be statistically significant (p-values < 0.009). Similar to the equation used

to describe the variability observed in MW, variations in polydispersity were explained by the following equation (Figure 6-2c):

$$POLYD = LU + (LU \times SOLAR_m) + (LU \times COND) + C \quad \text{(Equation 6-8)}$$

where POLYD is the polydispersity of DOC, LU is land use, $PRECIP_w$ is the total precipitation (mm) for the month prior to sampling, $SOLAR_m$ is the mean maximum solar flux density (W m^{-2}) for the month prior to sampling, COND is the conductivity (mS cm^{-1}) of the sample solution, and C is a constant representing the random errors. Equation 6-8 explains 79% of the variation observed in polydispersity with an AIC_c of 16.0 (Figure 6-2). Using the same model for polydispersity as the model used to explain MW resulted in a similar r-squared value but a higher likelihood of bias and uncertainty (AIC_c = 45.8). Variations observed in polydispersity were statistically significant for all the factors included in Equation 6-8: land use (p-value = 0.011), the interaction between land use and the mean monthly solar flux density (p-value = 0.002), and the interaction between land use and conductivity (p-value < 0.001).

The influence of land use and solar flux density on MW and polydispersity may be attributed to plant growth by specific types of land use (i.e. golf course, forested) (Lawlor 1995). The production of leaf soluble proteins has been shown to increase with solar radiation (Liu, Xu et al. 2005). The production of proteins and other biological compounds, such as terpenoids and flavonoids (Jaffe, Rushdi et al. 2006), by terrestrial plants can result in the production of high MW organic compounds that are likely responsible for the increases in DOC MW. And unlike the GLM for NUVA, the GLMs

for MW and polydispersity were found to account for variability based on the monthly mean solar flux density rather than the weekly. This suggests the influence of solar radiation on MW and polydispersity are more dependent on longer seasonal trends, such as growing seasons.

There are multiple plausible explanations for the significant influence of land use dependent conductivity on DOC MW and polydispersity: (1) conductivity is simply a seasonal indicator for urban landscapes where road salt is applied during the winter months, (2) conductivity is indicative of where in the hydrograph samples were collected (Johnsen, Martinsen et al. 1987), (3) increased ionic strength, related to conductivity, results in increased thermodynamic driving force for intermolecular assembly, and (4) the ionic strength of surface water bodies influences microbial populations which produce or transform DOC. Unfortunately, no clear trend between conductivity and MW is evident (Figure 6-4), and we therefore cannot eliminate any of these possibilities.

Variations in hydrophobicity did not appear to be influenced by weather. Based on the parameters investigated, the following GLM best described hydrophobicity (Figure 6-2d):

$$\%Ho = C + LU + ALK \qquad \text{(Equation 6-9)}$$

where %Ho is the percent DOC retained by hydrophobic mechanisms by SPE, LU is land use, ALK is the sample alkalinity (mg L^{-1} as $CaCO_3$), and C is a constant representing the random errors. This model was only able to account for 64% of the variability in hydrophobicity (Figure 6-2) and had a high AIC_c of 410.8, however, variations in land

use (p-value < 0.001) and alkalinity (p-value = 0.032) were found to be a significant source of variation.

Alkalinity may indicate the flow path experienced by DOC during stormwater runoff events. Rain water is low in alkalinity. Water which passes through soils and leaf litter (e.g. interflow), where organic acids are likely to dissolve, are likely to have a low alkalinity. Alternatively, ground water that has slowly seeped in to storm sewers and surface water that has been trapped within storm sewers for extended periods of time will contain a high amount of carbonates, silicates and other constituents which will increase alkalinity. As these different water masses elute during the hydrograph, it is reasonable to infer that the alkalinity will vary. Likewise, the production of DOC during stormwater runoff events has been found to vary over the course of hydrographs based on catchment hydrology (Schlesinger and Melack 1981; McGlynn and McDonnell 2003). DOC with indirect flow paths through leaf litter and soil may be stripped of hydrophobic DOC (Kawahigashi, Kaiser et al. 2006). Furthermore, soils also demonstrate chromatographic tendency to release predominantly hydrophilic DOC when flushed (Kaiser and Zech 1998). As a result, alkalinity may correlate to DOC hydrophobicity because it serves as an indicator of when in the hydrograph the samples were collected.

The hydrophobicity of DOC did exhibit some seasonal variability for forested catchments (Figure 6-3). During the summer months, hydrophobic fractions of DOC were found to decrease. This is contrary to what would be expected when aromaticity and MW increase, due to the growth of terrestrial plants. Additionally, as water temperatures increase, so does microbial growth, resulting in the formation of more aromatic DOC, which also increases hydrophobicity. In forested catchments the canopy prevents solar

radiation from reaching surface waters and causing photo-degradation. All of these factors can be expected to result in DOC that is more hydrophobic during summer months. It is suggested that the observed decrease in hydrophobicity may be due to the preferential retention of hydrophobic fractions of DOC during transport to the sampling location.

Conclusions

In addition to land use, variations in DOC aromaticity were found to correlate with changes in solar radiation and water temperature. For sub-watersheds that contained pooling water susceptible to high amounts of UV light exposure (i.e. golf course, agricultural fields, and large rivers) a slight decrease in aromaticity suggests photo-degradation did occur (Figure 6-1). For land uses with little direct solar radiation (i.e. forested), increased microbial activity due to rising water temperatures may have been more of a factor in determining the aromatic content of DOC, measured as NUVA (Table 6-2a). The interaction between solar radiation and land use was found to correlate with the MW and polydispersity of DOC. GLMs using monthly mean solar flux, conductivity and land use were effective in explaining approximately 80% or more of the observed variability (Figure 6-2). Environmental parameters were ineffective in reducing the observed variability in DOC hydrophobicity (Figure 6-2d). The hydrophobicity of DOC from forested sub-watersheds was found to be seasonally dependent (Figure 6-3). Alkalinity was the most effective parameter found to reduce the variability observed in DOC hydrophobicity. Results suggest hydrophobicity is related to the flow path experienced by DOC and watershed hydrologic characteristics. Overall, variations in DOC characteristics from land uses with primarily vegetative cover were found to be

influenced by environmental factors that vary seasonally: water temperature, precipitation and solar radiation.

Table 6-1. Regression coefficients (ß) for the GLMs describing DOC characteristics.

	NUVA	MW	Polydispersity	Hydrophibicity
constant	2.185	1.198	1.445	33.20
$ß_1$	3.37E+00	5.76E-03	-2.43E-01	-1.49E+00
$ß_2$	-5.35E-01	5.06E-01	-5.68E+00	-8.10E+00
$ß_3$	6.37E-01	1.83E+00	1.84E+00	-7.76E+00
$ß_4$	1.96E+00	-4.12E-02	1.21E+00	-1.12E+01
$ß_5$	-2.15E+00	-3.28E+00	5.52E+00	1.67E+01
$ß_6$	2.19E-01	7.78E-01	-3.62E+00	8.01E+00
$ß_7$	1.24E-01	7.16E-01	-3.34E-05	-2.14E-02
$ß_8$	-3.12E-02	-4.10E-04	7.44E-03	
$ß_9$	-7.44E-02	-1.25E-03	-2.71E-03	
$ß_{10}$	6.91E-04	-3.19E-04	-1.46E-03	
$ß_{11}$	5.47E-02	3.28E-03	-5.31E-03	
$ß_{12}$	-2.61E-03	-8.62E-04	3.15E-03	
$ß_{13}$	-6.22E-03	-8.45E-04	-2.58E-02	
$ß_{14}$	2.07E-03	-4.11E-03	-2.24E+00	
$ß_{15}$	1.01E-03	-9.80E-01	1.85E+00	
$ß_{16}$	-2.09E-03	1.04E+00	2.15E-01	
$ß_{17}$	8.25E-04	1.15E-01	-1.44E-01	
$ß_{18}$	-4.38E-04	-6.28E-02	3.82E-01	
$ß_{19}$		-6.26E-02		

For NUVA the regression coefficients represent the following variables: $ß_{1-6}$, land use; $ß_{7-12}$, land use × water temperature (°C) ; and $ß_{13-18}$, land use × mean weekly solar flux density (W m^{-2}).

For MW: the regression coefficients represent the following variables: $ß_{1-6}$, land use; $ß_7$, total weekly precipitation (mm), $ß_{8-13}$, land use × mean monthly solar flux density (W m^{-2}); and $ß_{14-19}$, land use × conductivity (mS cm^{-1}).

For polydisperity: the regression coefficients represent the following variables: $ß_{1-6}$, land use; $ß_{7-12}$, land use × mean monthly solar flux density (W m^{-2}); and $ß_{13-18}$, land use × conductivity (mS cm^{-1}).

For hydrophobicity: the regression coefficients represent the following variables: $ß_{1-6}$, land use; and $ß_7$, alkalinity (mg L^{-1} as $CaCO_3$).

Table 6-2. Differences determined by Tukey's HSD Test (Tukey 1949) in NUVA, MW and polydispersity resulting from the interaction between land use and parameters describing weather and water chemistry.

DOC Characteristics			
Land Use Contrasted		*Difference*	*p-value*
	NUVA		
FOR	MSU	1.077	0.003
FOR	PL	0.982	0.023
FOR	URB	1.196	0.001
GC	URB	0.560	0.025
	MW		
GC	MSU	0.659	0.001
GC	PL	0.705	<0.001
GC	URB	0.644	0.001
	Polydispersity		
AG	GC	-0.899	0.025
GC	URB	0.753	0.034

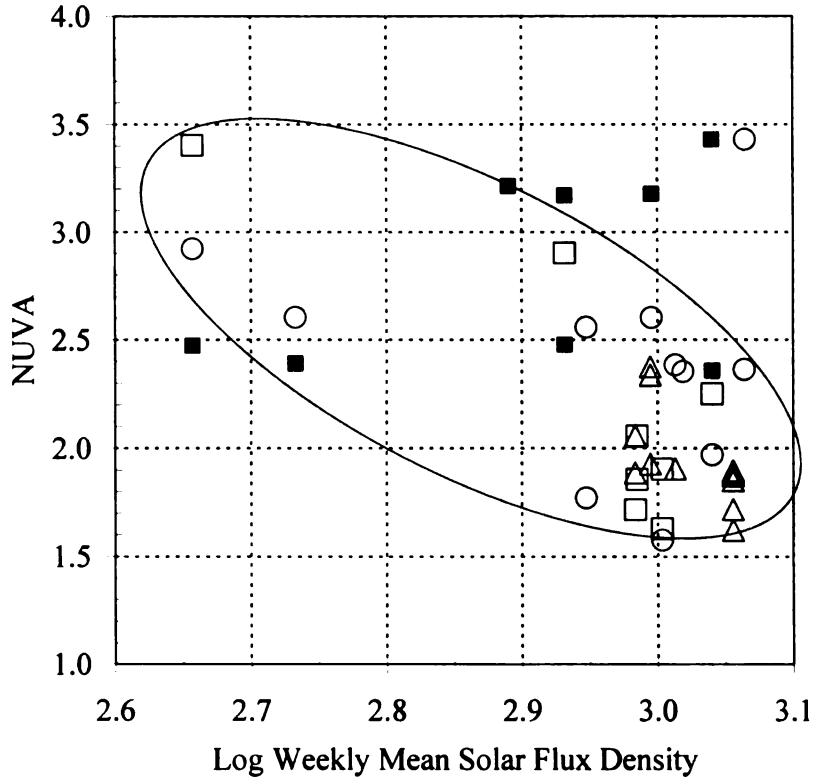

Figure 6-1. The influence of solar radiation, measured as $\log_{10}$ weekly mean solar flux density (W m^{-2}), on the aromaticity, measured as NUVA (L mgC m^{-1}) for samples from catchments with the agricultural (□), forested (■), recreational golf course (○), and mixed (△) land use.

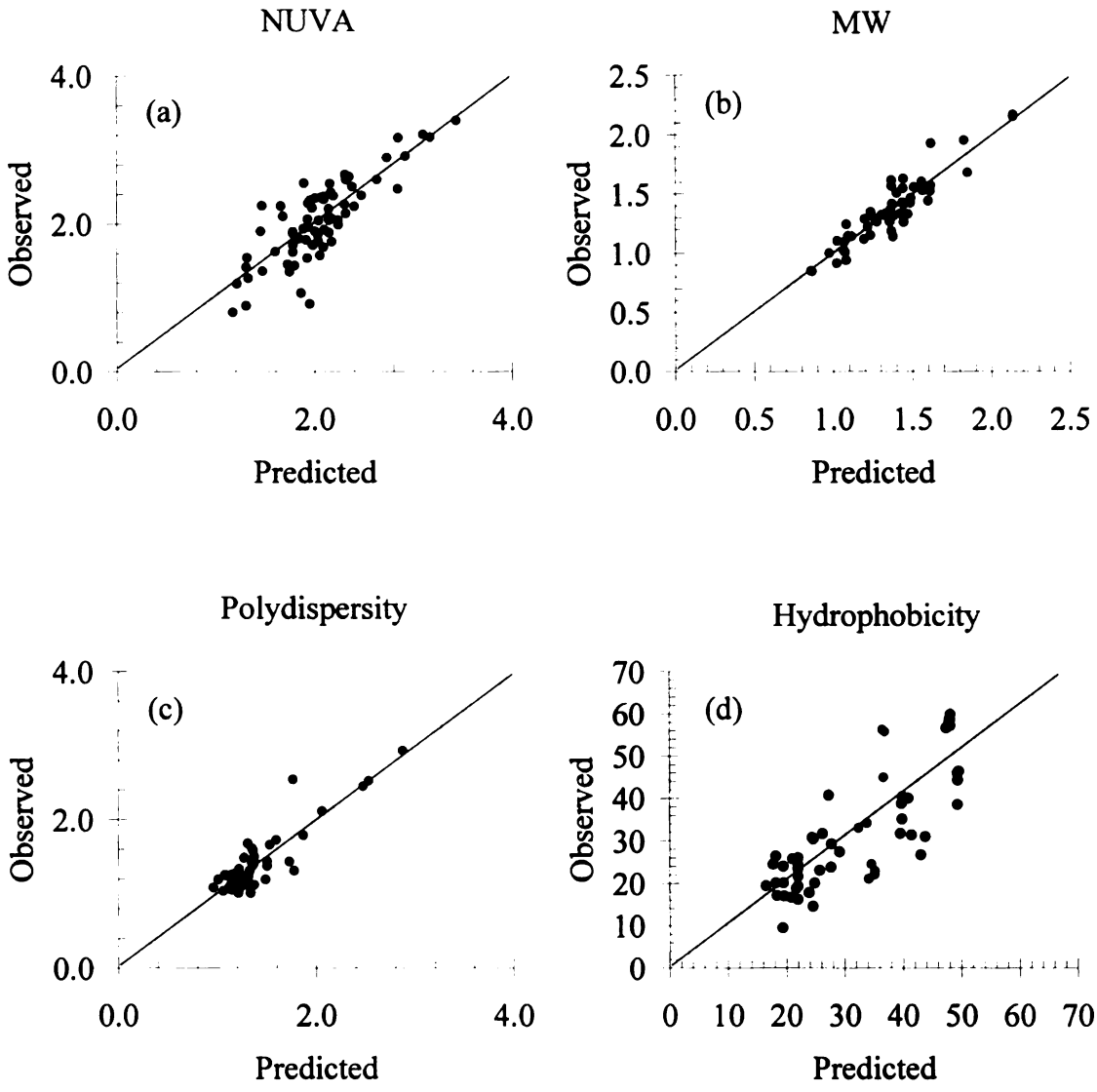

Figure 6-2. Ability of generalized linear models (predicted) used to describe observed DOC characteristics: (a) NUVA, (b) weight-averaged molecular weight, (c) polydispersity, and (d) hydrophobicity.

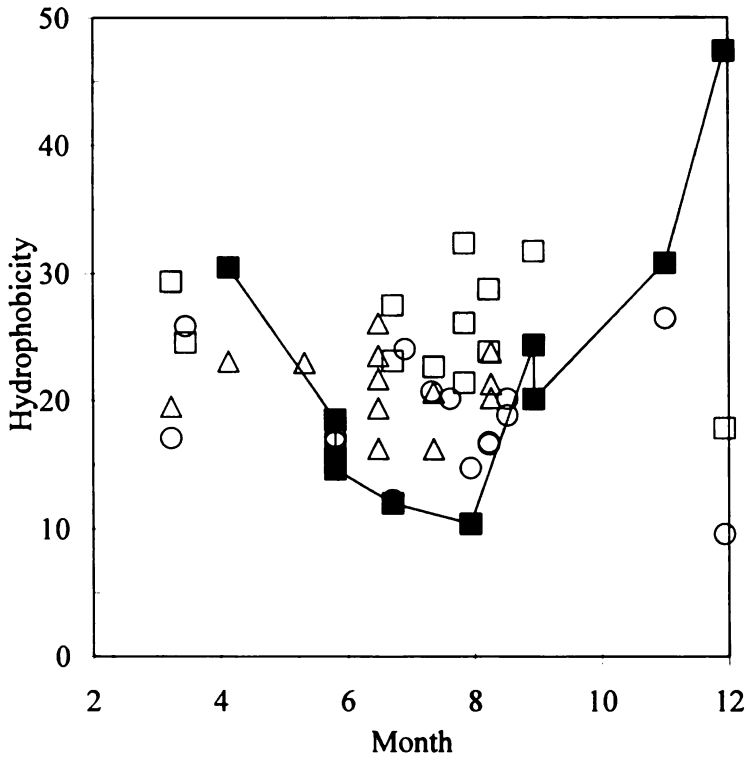

Figure 6-3. Seasonal influence on the hydrophobicity of DOC (%) for samples from catchments with the agricultural (□), forested (■), recreational golf course (○), and mixed (△) land use.

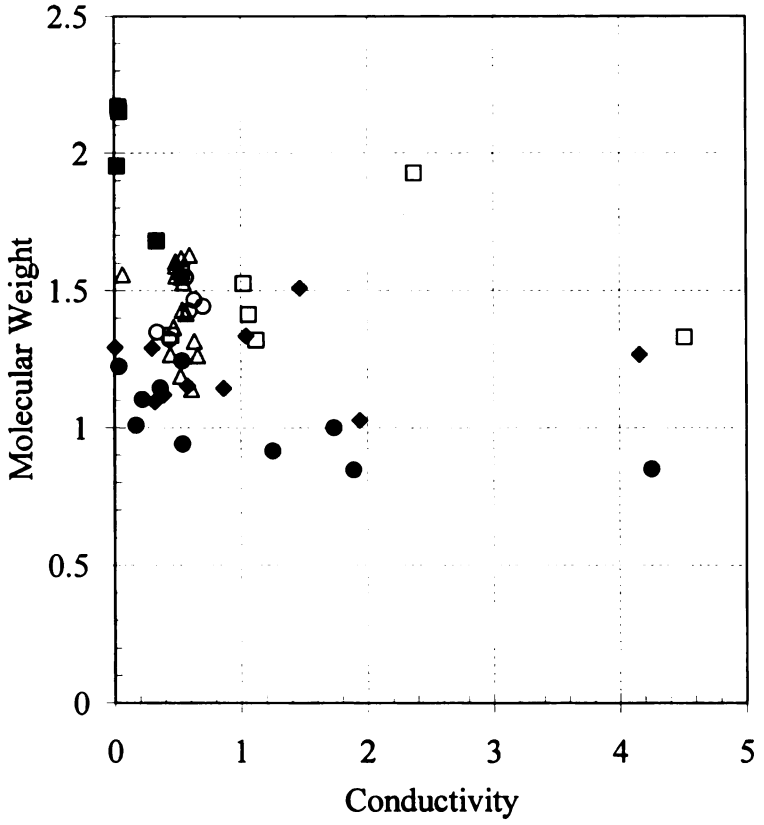

Figure 6-4. The influence of conductivity on the MW of DOC for sub-watersheds with agricultural (□), forested (■), urban (◆), MSU (●), recreational golf course (○), and mixed (△) land use.

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CHAPTER 7

CONCLUDING REMARKS

The work presented here demonstrates DOC characteristics vary depending on land use and other environmental factors, and this variability likely influences Cu complexation. In Chapter 3, variations in DOC characteristics were identified using a new analytical technique that retains fractions of DOC based on specific bonding interactions thought to be responsible for stabilizing humic substances. These variations were found to be a function of the type of DOC evaluated. Additionally, Cu was found to preferentially complex with DOC. The influence of DOC structure on Cu complexation was explored further in Chapter 4. Cu complexation was dependent upon both the quantity and quality of DOC ligands present in solution. Aromatic structure and oxygen functional groups influenced Cu-DOC complexation. Overall, stronger complexation was observed with aromatic moieties. Aromaticity played a significant role in determining Cu complexation with the high molecular weight fractions of DOC, while the oxygen content influenced Cu complexation with low molecular weight fractions. DOC from terrestrial and aquatic sources showed difference in the molecular structure of the low molecular weight fraction of humic substances. The influence of land use on DOC characteristics was demonstrated in Chapter 5. Catchments dominated by vegetation, such as agricultural and forested land uses, were found to produce DOC higher in molecular weight and aromatic structure than catchments with urban land uses. Results suggest the preferential removal of hydrophobic fractions of DOC during transport from catchments with indirect flow paths. In Chapter 6, generalized linear models (GLMs) were used to

assess possible correlations between DOC characteristics and environmental factors in an attempt to explain some of the variability observed in DOC characteristics. Results show changes in solar radiation and water temperature, in addition to land use, correlate to variations in DOC aromaticity. Solar radiation also correlated with DOC molecular weight and polydispersity. By taking into account the influence of land use and environmental factors (water temperature, precipitation and solar radiation), GLMs were able to account for 80% or more of the variability observed in DOC characteristics.

Observed variations in DOC characteristics have a wide range of implications. First, variations in DOC characteristics likely influence the microbial ecology present in surface waters since DOC constitutes the base of aquatic food webs. As DOC characteristics change, microbial communities will also likely change. While the dynamic nature of these interactions are unknown, it is clear that not all DOC derived from terrestrial sources is the same. This alone implies microbial communities, which constitute the base of the aquatic food webs, likely differ in surface waters fed by watersheds with dissimilar land uses. Second, variations in DOC characteristics also influence the fate and transport of pollutants within surface water systems. As demonstrated in Chapter 4, increases in DOC aromaticity will likely increase the amount of Cu removed from solution. Cu is just one of the many trace-metals known to complex with DOC. Furthermore, the solubility and reactivity of many organic constituents are also influenced by DOC. With differences in characteristics observed for DOC derived from catchments composed of various land uses, it can be expected that the transport of pollutants within these systems will differ. Third, the connection between land use and DOC characteristics offers another factor to consider when assessing the impact of land

use alterations. For example, based on the results discussed in chapter 5, if forested landscapes are developed into residential properties there will likely be a change in DOC characteristics which will impact surface water ecology and the transport of pollutants. Given that human activity will continue to alter watershed surfaces, appropriate precautions and treatment systems are required to ensure surface water quality. The results of this work suggest stormwater treatment systems that utilize indirect flow paths and vegetative cover (i.e. rain gardens) will likely alter DOC characteristics and may produce DOC similar to that produced from natural landscapes.

Future research is required to determine the impact variations in DOC characteristics have on microbial ecology and the transport of trace metals, other than Cu, and organic constituents. More extensive experiments are required to determine if the correlations observed between molecular structure and Cu complexation hold for a wider range of DOC, not just isolated humic substance. Additionally, the environmental factors identified through the use of GLMs can serve as a guide for further research aimed to describe the mechanisms responsible for DOC formation and transformation. For example, the proposed removal of hydrophobic fractions during transport through indirect flow paths should be specifically tested. Research is required to design stormwater treatment systems that not only remove pollutants of interest but also produces DOC with characteristics similar to those found in natural environments.

Ultimately, a better understanding of the chemical and biological roles of DOC in aquatic systems is required to accurately describe the dynamic processes within surface water systems.

APPENDICES

APPENDIX A

METHODS

Alkalinity Analysis

The Gran titration method was used to determine sample alkalinity (Gran, 1950; Gran, 1952). Details of this method can be found elsewhere (Stumm and Morgan, 1981).

Protocol for determining alkalinity

1. Weigh 50mL disposable beaker (Fisher Scientific, part number 01-291-10). Record weight (in grams) of empty beaker (W_1).
2. Pour ~15mL into dry pre-weighted disposable beaker for alkalinity titration.
3. Re-weigh beaker to determine exact volume of sample titrated. Record weight (in grams) of beaker with sample (W_2). The volume (in milliliters) of sample (V_S) titrated is the difference between W_2 and W_1.

   $V_S = W_2 - W_1$ (Equation A-1)

   Record volume of sample titrated.
4. Add clean micro-stir bar (Fisher Scientific, part number 14-511-97) to beaker.
5. Place beaker on stir plate.
6. Insert calibrated pH probe so that probe tip is above stir bar.
7. Turn on stir plate briefly (~5 sec) to ensure solution is completely mixed.
8. With stir plate turned off, measure and record solution pH and temperature (°C).
9. Turn on stir plate and add 100μL of normalized 0.02N H_2SO_4 to sample. After solution is fully mixed (~5sec) turn off stir plate and measure pH. Record volume of acid added and resulting pH.

10. Continue to add normalized 0.02N H_2SO_4 to sample solution until pH drops below 3.5, using the stir plate as before to ensure solution is fully mixed. Record volume of acid added and resulting pH for each addition.

    It should be noted that the volume of acid added depends on the solution. Four or more data points between pH 4.5 and 8.3 and below 4.5 (total of 8 data points) are necessary to obtain a good estimate of alkalinity. If the solution has relatively high alkalinity (>250mg L^{-1} as $CaCO_3$), the a few 1mL additions of acid may be appropriate. If the solution has relatively low alkalinity (<50mg L^{-1} as $CaCO_3$), the total amount of acid added during titration may only be a few milliliters. As the solution approaches the theoretical equivalence points for carbonate dissociation (pH = 4.5 and 8.3) reduce the volume of acid added to accurately capture large changes in pH.

11. Titration is complete when 4 data points (pH and volume of acid added) have been recorded for solution with pH below 4.5. Titrated solution can be disposed of and beaker and stir bar can be washed for reuse.
12. Calculate the total volume of acid (mL) added for each pH measurement.
13. Enter the data into online alkalinity calculator (http://or.water.usgs.gov/alk/):
    a. Enter *sample temperature* (°C) – temperature at beginning of titration
    b. Enter *specific conductance* (µS cm^{-1}) - use conductivity from field sampling multi-probe
    c. Select *other* from dropdown menu for acid concentration and specify other acid concentration as 0.02N.
    d. Enter *sample volume* (mL) - V_S

e. Select *yes* for filtered sample.

f. Select *buret titration* for titration type.

g. Past delimited data in *titration data* box and determine the *order* of data points (pH in first column, total volume of acid used for during titration in second column or total volume of acid used for during titration in first column, pH in second column).

h. Select *Gran function plot method* for analysis method

i. Select *advanced speciation method* for speciation method

j. Click *Calculate!*

Table A-1. Example data for Gran Titration.

Vol. Acid Added (μL)	Total Acid (mL)	pH
0	0.000	7.76
500	0.500	7.43
1000	1.500	6.84
1000	2.500	6.58
1000	3.500	6.37
1000	4.500	6.15
1000	5.500	5.78
1000	6.500	5.09
500	7.000	4.01
100	7.100	3.82
100	7.200	3.69
100	7.300	3.59
100	7.400	3.51
100	7.500	3.44

14. Inspect Gran plot to make sure there were no problems with titration or data entry.

15. From the Alkalinity Calculator output, record the Gran F_1 bicarbonate alkalinity (mg L^{-1} as $CaCO_3$).

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Cartridge Preparation

Preparation of anion and cation extraction cartridges

1. Place Extract-Clean™ 20μm polyethylene frit (Alltech, part number 211408) into bottom of 8.0ml Ultra-Clean™ treated polypropylene reservoir (Alltech, part number 70218) using large end (end pipette bulb would be placed) of clean class pipette.
2. Sequentially label cartridge with prefix to identify cation exchange (C-), anion exchange without a molecular weight cutoff (A-) and anion exchange with a 1 kDa molecular weight cutoff (X-) (e.g. A-005 means fifth anion exchange cartridge prepared).
3. Cap cartridges (Alltech, part number 220600 and 220710).
4. Weigh out appropriate mass of resin material for each cartridge into a clean 100mL weigh boat.
    a. Anion exchange: 1.0000 – 1.0100g of AG-MP1 or AG-1 X8 resin.
    b. Cation exchange: 2.0000 – 2.0100g of Chelex 100 resin
5. Using approximately 4ml of ultra-clean water (>18MΩ), rinse resin from weigh boat into appropriate cartridge.
    a. If more water is necessary to rinse all of the resin from weigh boat, carefully draw down water from cartridge using vacuum suction first, then rinse remaining resin into cartridge.
    b. There should be approximately 2cm between top of tube and water in cartridge when done.

6. Cap both ends of cartridge and swirl tube to remove large air bubbles.
7. Open top cap and rinse remaining resin from sidewalls into slurry at bottom.
    a. If more water is necessary to rinse resin down, carefully draw down water from cartridge using vacuum suction first, then rinse remaining resin to bottom.
    b. The cartridge should be approximately half-full.
8. Place 10 new cartridges (capped) into an acid washed 400mL glass beaker and cover beaker with Parafilm.
9. Fill bottom of glass beaker with ultra-clean water to prevent beaker from floating in sonicator bath (water level should be just above slurry depth in tubes).
10. Place beaker with new cartridges in sonicator bath for 20min to remove small bubbles in bottom of cartridge.
11. After sonicating, remove caps from top of cartridge and place Extract-Clean 20μm polyethylene frit on top of resin. Compress resin slightly by the using large end (end pipette bulb would be placed) of a clean class pipette with frit.
12. Recap cartridge to prevent drying; always keep water above resin.
13. Store upright in vial rack with vial rack sealed in side plastic bag.

Counter-ion conversion for ion-exchange cartridge

Converting the counter-ion on ion-exchange cartridges requires polytetrafluoroethylene (PTFE) tubing (Supelco, part number 57276) and solid phase extraction (SPE) tube adapter (Supelco, part number 57020-U) be preassembled and

ultra-cleaned before use. To assemble, pull tubing through adapter so that flange in tubing creates a seal on the narrow end of the adapter, as shown below.

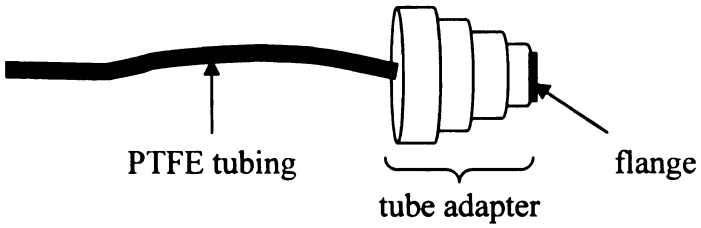

Figure A-1. Attachment of adapter to PTFE tubing.

Procedure to convert chloride to fluoride counter-ion (for AG MP-1 resin)

1. Prepare 1L of 1N NaOH by dissolving 17.01g of NaOH in 1L beaker and filling to 1L with ultra-pure (>18MΩ) water. Make sure NaOH completely dissolves before using; this typically requires the use of a stir bar and stir plate.
2. Prepare 1L of 1N NaF by dissolving 19.00g of NaF in beaker and fill to 1L with nano-pure water. Make sure NaF completely dissolves before using; this typically requires the use of a stir bar and stir plate.
3. Record cartridge identification (ID) in lab notebook that will be converted and write "F" on SPE tubes with sharpie to denote fluoride counter ion.
4. Place cartridges on vacuum manifold.
5. Remove tops and draw water within cartridge down so that meniscus touches top frit.
6. Rinse ultra-clean preassembled PTFE tubing and SPE tube adapter with ultra-pure water.
7. Fill tubing with ultra-pure water (to ensure siphon) and block end of tubing at SPE adapter with finger. Place open end of tubing in 1N NaOH solution.

Quickly, to avoid loosing siphon, attach tubing to top of SPE cartridge with SPE tube adapter.

8. Repeat steps 6-7 until all cartridges have tubing with siphon to 1N NaOH solution. It may be necessary to tape tubing together to prevent it from popping out of beaker.
9. Turn on vacuum manifold and pass 40mL of 1N NaOH through cartridges. Keep valve on glass block open so that flow rate is approximately 5mL min^{-1}.
10. Discard rinse eluent collected in 40mL glass vials. Return vials to glass block. Do not disconnect tubing to SPE cartridges.
11. Rinse cartridges with another 40mL of 1N NaOH (step 9). Do not allow cartridges to run dry!
12. Discard rinse eluent collected in 40mL glass vials. Return vials to glass block. Do not disconnect tubing to SPE cartridges.
13. Replace beaker containing 1N NaOH solution with beaker containing 1N NaF solution. Remove open end of tubing from 1N NaOH solution and place in 1N NaF solution.
14. Rinse cartridges with 20mL of 1N NaF.
15. After rinsing with NaF, remove tubing from solution and cartridges. Draw down solution within cartridges until the bottom of meniscus touches upper frit.
16. Cartridges are now converted. Cap and store up right in tray with bag taped shut. Write "converted" and the date on bag.

Procedure to convert chloride to iodide counter-ion (for AG MP-1 resin)

1. Prepare 1L of 1N NaI by dissolving 126.9g of NaI in beaker and fill to 1L with nano-pure water. Make sure NaI completely dissolves before using; this typically requires the use of a stir bar and stir plate.
2. Record cartridge IDs in lab notebook that will be converted and write "I" on SPE tubes with sharpie to denote iodide counter ion.
3. Place cartridges on vacuum manifold.
4. Remove tops and draw water within cartridge down so that meniscus touches top frit.
5. Rinse ultra-clean preassembled PTFE tubing and SPE tube adapter with ultra-pure water.
6. Fill tubing with ultra-pure water (to ensure siphon) and block end of tubing at SPE adapter with finger. Place open end of tubing in 1N NaI solution. Quickly, to avoid loosing siphon, attach tubing to top of SPE cartridge with SPE tube adapter.
7. Repeat steps 5-6 until all cartridges have tubing with siphon to 1N NaI solution. It may be necessary to tape tubing together to prevent it from popping out of beaker.
8. Turn on vacuum manifold and pass 40mL of 1N NaI through cartridges. Keep valve on glass block open - so that flow rate is approximately 5mL min^{-1}.
9. Discard rinse eluent collected in 40mL glass vials. Return vials to glass block.
10. Cartridges are now converted. Cap and store up right in tray with bag taped shut. Write "converted" and the date on bag.

Procedure to convert hydroxide to fluoride counter-ion (for AG-1 X8 resin)

1. Prepare 1L of 1N NaF by dissolving 19.00g of NaF in beaker and fill to 1L with nano-pure water. Make sure NaF completely dissolves before using; this typically requires the use of a stir bar and stir plate.
2. Record cartridge IDs in lab notebook that will be converted and write "F" on SPE tubes with sharpie to denote fluoride counter ion.
3. Place cartridges on vacuum manifold.
4. Remove tops and draw water within cartridge down so that meniscus touches top frit.
5. Rinse ultra-clean preassembled PTFE tubing and SPE tube adapter with ultra-pure water.
6. Fill tubing with ultra-pure water (to ensure siphon) and block end of tubing at SPE adapter with finger. Place open end of tubing in 1N NaF solution. Quickly, to avoid loosing siphon, attach tubing to top of SPE cartridge with SPE tube adapter.
7. Repeat steps 5-6 until all cartridges have tubing with siphon to 1N NaF solution. It may be necessary to tape tubing together to prevent it from popping out of beaker.
8. Turn on vacuum manifold and pass 20mL of 1N NaF through cartridges.
9. After rinsing with NaF, remove tubing from solution and cartridges. Draw down solution within cartridges until the bottom of meniscus touches upper frit.

10. Cartridges are now converted. Cap and store up right in tray with bag taped shut. Write "converted" and the date on bag.

Counter-ion conversion notes:

- Conversion procedures are for 2 batches of 10 cartridges (20 cartridges total)
- It is possible to reuse tubing after conversions, only if conversions follow the order OH, F, then I.
- Sodium iodide is expensive and cannot be stored for more than 1 week. If only 10 cartridges will be made, reduce amount of conversion solution to 500mL.

Procedure to condition anion exchange cartridges

1. Place cartridges on vacuum manifold.
2. Remove tops and draw water within cartridge down so that meniscus touches top frit.
3. Rinse ultra-clean preassembled PTFE tubing and SPE tube adapter with ultra-pure water.
4. Fill tubing with ultra-pure water (to ensure siphon) and block end of tubing at SPE adapter with finger. Place open end of tubing in ultra-pure water solution. Quickly, to avoid loosing siphon, attach tubing to top of SPE cartridge with SPE tube adapter.
5. Repeat steps 3-4 until all cartridges have tubing with siphon to ultra-pure water solution. It may be necessary to tape tubing together to prevent it from popping out of beaker.

6. Draw down solution in cartridge so that bottom of meniscus touches the top frit. Do not allow cartridge to run dry!
7. Add 1mL of dilute (0.02%) hydrofluoric acid (HF).
8. Connect ultra-pure water tubing.
9. Draw through cartridge ~10mL of ultra-pure water into plastic falcon tubes (VWR, product number 21008-931).
10. Discard HF solution in hazardous waste container.
11. Remove plastic falcon tubes and replace with 40mL silica TOC vials.
12. Rinse cartridges with 40mL of ultra-pure water twice (80mL total).
13. Draw water level down to approximately 1cm from top frit.
14. Cartridge is now conditioned. Place red check mark on cartridge to denote conditioned.
15. Cap and store up right in tray with bag taped shut. Write "conditioned" and the date on bag.

Procedure to prepare and condition non-ionic cartridges

1. Mark cartridges with date and sequential number (ex: 020701, 020702, etc.)
2. Place cartridges on vacuum manifold.
3. Add 1mL methanol.
4. Open manifold valve to allow methanol to soak entire media.
5. Once soaked, draw down methanol in cartridge so that bottom of meniscus touches the top frit. Do not allow cartridge to run dry!
6. Add 1mL of dilute (0.02%) HF.

7. Remove tops and draw water within cartridge down so that meniscus touches top frit.
8. Rinse ultra-clean preassembled PTFE tubing and SPE tube adapter with ultra-pure water.
9. Fill tubing with ultra-pure water (to ensure siphon) and block end of tubing at SPE adapter with finger. Place open end of tubing in ultra-pure water solution. Quickly, to avoid loosing siphon, attach tubing to top of SPE cartridge with SPE tube adapter.
10. Repeat steps 8-9 until all cartridges have tubing with siphon to ultra-pure water solution. It may be necessary to tape tubing together to prevent it from popping out of beaker.
11. Draw ~10mL of ultra-pure water through cartridge into plastic falcon tubes.
12. Discard methanol-HF solution in hazardous waste container.
13. Remove plastic falcon tubes and replace with 40mL silica TOC vials.
14. Rinse cartridges with 40mL of ultra-pure water twice (80mL total).
15. Draw water level down to approximately 1cm from top frit.
16. Cartridge is now conditioned. Place red check mark on cartridge to denote conditioned.
17. Cap and store up right in tray with bag taped shut. Write "conditioned" and the date on bag.

Procedure to condition cation exchange cartridges

1. Place cartridges on vacuum manifold.

2. Remove tops and draw water within cartridge down so that meniscus touches top frit.
3. Rinse ultra-clean preassembled PTFE tubing and SPE tube adapter with ultra-pure water.
4. Fill tubing with ultra-pure water (to ensure siphon) and block end of tubing at SPE adapter with finger. Place open end of tubing in ultra-pure water solution. Quickly, to avoid loosing siphon, attach tubing to top of SPE cartridge with SPE tube adapter.
5. Repeat steps 3-4 until all cartridges have tubing with siphon to ultra-pure water solution. It may be necessary to tape tubing together to prevent it from popping out of beaker.
6. Rinse cartridges with 40mL of ultra-pure water twice (80mL total).
7. Draw water level down to approximately 1cm from top frit.
8. Cartridge is now conditioned. Place red check mark on cartridge to denote conditioned.
9. Cap and store up right in tray with bag taped shut. Write "conditioned" and the date on bag.

DOC-Trace Metal Fractionation

Dissolved organic carbon (DOC) and trace metal fractions were collected by passing samples through solid-phase extraction (SPE) cartridges (in parallel) using trace element clean 60 mL syringes arranged on a syringe pump (Harvard Apparatus) to control the sample flow rate. Syringes were connected to SPE tubes with the use of cartridge adapters (Alltech). With the bottom of the SPE tube capped, syringes were filled with 60 mL of sample from the top. Once filled, the SPE tube cap was removed, the syringe plunger was inserted and ~10 mL of sample was forced through the SPE cartridge and discarded (without the plunger contacting the aqueous sample). Flushing 10 mL of sample effectively replaced the ~2 mL of ultra-pure water remaining from cartridge conditioning and allowed the SPE media to equilibrate with the sample solution. Eluent from cartridges was collected in 40 mL borosilicate glass vials for DOC analysis. After the DOC samples were collected, SPE cartridges were temporarily removed from the syringes to be refilled. At all times the SPE media remained fully saturated with excess sample. Syringes were then reloaded with sample solution to 40 mL. Again plungers were inserted and placed back into the syringe pump. Eluent was collected in ultra-clean 30 mL polypropylene bottles for trace metals analysis. Used cartridges were then frozen for the possible future extraction of retained compounds.

DOC-Trace Metal Fractionation Procedure

1. Place syringe pump (Harvard Apparatus) so that syringes and cartridges are vertical and adequate space is available below to collect eluent (setup shown below).

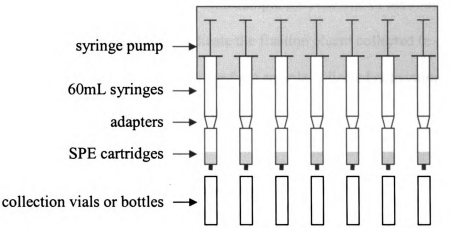

Figure A-2. Arrangement of syringes and cartridges on for sample collection.

2. Set flow rate to 1 mL min^{-1}.
3. Place ultra-clean cartridge adapter on end of ultra-clean 60ml syringe.
4. Remove plunger from syringe tube.
5. Remove top cap from SPE cartridge and connect to syringe tube via cartridge adapter. The Oasis HLB cartridges requires the use of Teflon tape (DuPont, part number T-27730A) to ensure a good seal.
6. Fill open syringe tube from top with 60 mL of sample.
7. Remove bottom cap from cartridge.
8. Insert syringe plunger and push 10 mL through SPE cartridge into waste beaker (can be disposed of down drain). Be sure that rubber cap on plunger does not contact sample!
9. Place syringe-SPE assembly on syringe pump.
10. Repeat steps 3-8 until each type of SPE cartridge required for fractionation are loaded with sample solution.

11. Record cartridge identification numbers (IDs) and label clean 40 mL borosilicate glass vials for DOC analysis with sample ID. Add one of the following suffixes to end of base sample ID to indicate the fraction eluent collected (e.g. the eluent collected from anion-F cartridge for a sample collected at location AG on August 8, 2007 would be labeled AG080807-F).

Table A-2. Cartridge ID key.

Suffix	Cartridge
C	cation
F	anion-F
H	H-bonding
Hi	extended hydrophobic
Ho	hydrophobic
I	anion-I
X	anion-1kDa

12. Arrange vials under appropriate SPE cartridges and turn on syringe pump.
13. After vials are filled (~40 min), turn off syringe pump, replace bottom cap on SPE cartridge and cap DOC vials. Fraction samples should form an inverted meniscus above top of the vial so that no air is trapped when vials are capped.
14. Immediately after collecting DOC samples, place vials in dark fridge (4 °C).
15. While keeping syringe-SPE cartridge assemblies' vertical remove from syringe pump and place in vial tray.
16. Separate syringe from cartridge adapter (leaving adapter attached to SPE tube).
17. Dispense excess sample present in syringe (~10 mL) back into bulk solution of filtered sample (1 L glass beaker).
18. Remove plunger from syringe tube. Make sure plunger (with rubber cap) is not contaminated by setting it down on unclean surface or touching anything.

19. Reconnect syringe tube to SPE cartridge via adapter.
20. Fill open syringe tube from top with 40 mL of sample.
21. Remove bottom cap from cartridge.
22. Insert syringe plunger and push 10 mL through SPE cartridge into waste beaker (can be disposed of down drain). Be sure that rubber cap on plunger does not contact sample!
23. Place syringe-SPE assembly back on syringe pump.
24. Repeat steps 15-23 until each type of SPE cartridge required for fractionation are re-loaded with sample solution.
25. Label 30mL ultra-clean HDPE bottles for trace metal analysis with sample ID (as was done for DOC vials in step 11).
26. Arrange bottles under appropriate SPE cartridges and turn on syringe pump.
27. After vials are filled (~30 min, during this step cartridges can be run dry), turn off syringe pump, replace bottom cap on SPE cartridge and cap DOC vials. Samples for trace metal analysis should be filled to the base of the bottle neck, not completely full like DOC samples.
28. Add 180 μL of Optima nitric acid to each trace metal fraction, cap bottles and store in fridge (4 °C) until analysis.
29. Remove syringe-SPE cartridge assembly from syringe pump.
30. If sample remains in cartridge or syringe, push solution through cartridge until SPE media is dry.
31. Disconnect syringe-SPE cartridge assembly.
32. Place adapter in bin to be washed.

33. Discard used syringe in sharps box for disposal.
34. Cap both ends of SPE cartridge and place in Ziplock bag. Place all cartridges used to fraction a sample in the same bag. On outside of bag write base sample name, the date and your initials. Place bag in freezer.
35. Repeat steps 29-34 for all cartridges used.

Identifying Unique Land Use Micro-Watersheds

Catchments for sampling were initially identified using geographic information systems (GIS) by overlaying hydrologic data on land use classifications to determine rough boundaries of micro-watersheds that contained unique land use characteristics in ArcMAP 9.1 (ESRI, 2003). Land use was identified based on the Michigan Land Cover/Use Classification System (MDNR, 2001b). Micro-watersheds with unique land use characteristics were identified by conceptually moving sampling locations from the farthest point upstream (i.e. water source or headwater) of the smallest hydrologic units (intermittent streams) down stream until just before the catchment would contain more than one land use. For example, the traditional method for determining watershed boundaries, based solely on topography, often produces catchments with multiple land uses, sample location A.

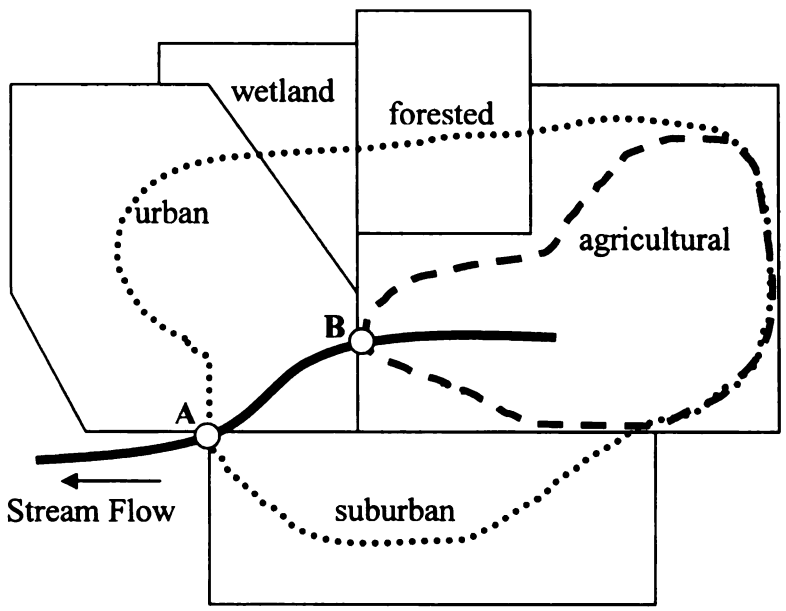

Figure A-3. Differences in land use for traditional and new watershed boundaries.

By starting at the farthest point upstream and moving downstream until just before the catchment would contain multiple land uses (i.e. moving the sampling location farther upstream) a sampling location is identified for a micro-watershed that contains only one type of land use, sample location B. After identifying potential micro-watersheds were then inspected to determine if sampling locations existed where overland flow could be collected. Micro-watersheds were manually delineated based on topography. For urban systems and agricultural fields where storm sewers and drain tiles fields alter natural watershed boundaries, maps containing drainage networks were used to accurately describe catchments boundaries. Land use data was based on the IFMAP/GAP Lower Peninsula Land Cover raster data set (MDNR, 2001a). Land use within the Ramey Chandler drain in Ingham County was updated to include the type of land use present at the time of sampling. Topography was determined by county digital elevation maps (MDEQ, 2005).

Procedure used to identify unique land use micro-watersheds within the Remey-Chandler Drainage

1. The following datasets were downloaded from the Michigan Geographic Data Library (www.michigan.gov/cgi/):
    a. *Lower Peninsula Land Cover 2001* (IFMAP/GAP Lower Peninsula Land Cover)
    b. *Clinton Digital Elevation Model*
    c. *Ingham Digital Elevation Model*
    d. 14-Grand Watersheds

e. *Clinton MI Geographic Framework Hydrography*

f. *Ingham MI Geographic Framework Hydrography*

2. A Personal Geodatabase (*RemyChandler.mdb*) was set up for the Remey Chandler drain using the following coordinate system:

   AD_1983_Hotine_Oblique_Mercator_Azimuth_Natural_Origin
   Hotine_Oblique_Mercator_Azimuth_Natural_Origin
   False_Easting: 2546731.496000
   False_Northing: -4354009.816000
   Scale_Factor: 0.999600
   Azimuth: 337.255560
   Longitude_Of_Center: -86.000000
   Latitude_Of_Center: 45.309167

3. All files previously downloaded were then imported into the RemyChandler personal geodatabase and projections were adjusted to align all layers.

4. AutoCAD files containing drainage maps for storm sewer networks and tile drain fields for Clinton and Ingham counties were obtained from the Clinton County Drain Commissioner's Office and Michigan State University. These files were then imported into the RemyChandler personal geodatabase.

5. Blueprints containing the storm sewer networks (Section 18) from the City of East Lansing were scanned and converted to digital images by Capital Imaging (Lansing, MI). Images were rectified to GCS_North_American_1983 coordinate system.

   Table A-3. Master conversion file used to rectify data and the resulting bounding coordinates.

17.313583	13.054817	626894.263280	246147.222737
8.171042	14.276063	622325.134476	246668.000578
14.957873	3.434961	625801.770873	241316.418030
9.832593	3.389521	623199.756292	241255.200115

Bounding coordinates

Horizontal
In decimal degrees
West: -84.495625
East: -84.481747
North: 42.750289
South: 42.742617
In projected or local coordinates
Left: 623002.406532
Right: 624123.153837
Top: 245521.961176
Bottom: 244690.416466

6. From the Grand River watershed shape file (*14-Grand Watersheds*), the Remey Chandler Drain sub-watershed was selected (EPA 14-digit Hydrologic Unit Code: 4050004060120) and exported to its own shape file. This shape file was called *Watershed_Boundary* and temporarily contained only one feature.
7. The raster dataset used for land cover/use (IFMAP/GAP Lower Peninsula Land Cover) was cut and converted to a shape file for further editing.
    a. The IFMAP/GAP Lower Peninsula Land Cover raster datasets was clipped by Remey Chandler drainage watershed boundary using Hawth's Analysis tools for ArcGIS (www.spatialecology.com/htools/cliprasterbypolys.php).
    b. The newly clipped raster data file was converted to a shape file using the *Export to Shapefile* feature of ET Geowizards 9.3 (www.ian-ko.com). The new shapefile was named *Old_landuse*.

c. Using the *SampleShedCleanup* script written in python, similar polygons with the same land uses were grouped together.

8. The *Old_landuse* shapefile was manually updated using current land cover/use obtained visually from a MrSID raster database of the study area obtained from AirPhotoUSA (www.airphotousa.com) and renamed *Updated_Landuse.*

9. By overlaying multiple layers containing the hydrology, digital elevation models (DEMs), and relevant drainage networks (i.e. tile drains and storm sewer networks), potential sampling locations were identified. As explained previously, potential sampling locations with unique land use characteristics were identified by conceptually moving the farthest point upstream (i.e. water source or headwater) of the smallest hydrologic units (intermittent streams) down stream until just before a catchment would contain more than one land use. From a potential sampling location, the DEM was used to manually delineate a micro-watershed. Using the *Watershed_Boundary* shape file as a template, micro-watersheds were created by sketching a new polygon and using the Cut Polygon Features option in ArcEditor. The new shape file was called *sample_sheds.*

10. After multiple sampling locations were identified and potential micro-watersheds were created in the *sample_sheds* shape file, potential sampling locations were field investigated to ensure the digital information reflect real world characteristics. The following characteristics were required to confirm the validity of all sampling locations:

    a. current land cover/use did not deviate from that described by the *Updated_Landuse* shape file,

b. it was possible to access the sampling location (e.g. there were no fences restricting access),

c. Surface water runoff would be free flowing stormwater runoff event (i.e. no backwater effects would create mixed water samples),

d. the topography did not visually deviate from that described in the DEM,

e. and, no additional drainage networks (i.e. storm sewers or tile drain fields) were present in catchments that were not described in GIS databases.

Molecular Weight Characterization

The molecular weight (MW) and polydispersity of DOC was determined by size exclusion chromatography (HPSEC). The HPSEC system employed utilized a Gilson Model 303 pump (Middleton, WI) with a 20μL loop, a Waters Protein-Pak (125 angstrom, 7.8 x 300mm, part number WAT084601) modified silica column (Milford, MA) and UV detection at 254nm on a Dionex Variable Wavelength Detector (Sunnyvale, CA) (Chin et al., 1994; Zhou et al., 2000). The mobile phase consisted of 0.1M NaCl, 0.002M KH_2PO_4 and 0.002M Na_2HPO_4 solution buffered to an approximate pH of 6.8 (Meier et al., 2004) and the flow rate was 0.5 mL min^{-1}. Calibration was performed using 1.8, 5.4, 8, 18 and 35kDa average MW random coil sodium polystyrene sulfonate standards (Polysciences, Inc.) and acetone (58Da) (Zhou et al., 2000). To prevent biofouling, the column was rinsed with and stored in 0.05% sodium azide solution before and after running samples.

The number-averaged MW (M_n) and weight-averaged MW (M_w) were calculated by the following equations:

$$M_n = \sum_{i=1}^{N} h_i / \sum_{i=1}^{N} h_i(M_i) \qquad \text{(Equation A-2)}$$

and

$$M_w = \sum_{i=1}^{N} h_i(M_i) / \sum_{i=1}^{N} h_i \qquad \text{(Equation A-3)}$$

where h_i is the height and M_i is the molecular mass of the sample HPSEC eluted at volume i. Polydisperity (ρ) is the ratio of the weight-averaged MW and the number-averaged MW:

$$\rho = \frac{M_w}{M_n} \qquad \text{(Equation A-4)}$$

The extent of symmetrical (Λ) and asymmetrical (sk) band-broadening were calculated for the 5.4kDa standard in every calibration to ensure the primary mechanism of separation was size exclusion. The extent of symmetrical band-broadening was calculated using the following equation:

$$\Lambda = \left(\frac{M_{n\_reported}}{M_{n\_observed}} + \frac{M_{w\_observed}}{M_{w\_reported}} \right) \qquad \text{(Equation A-5)}$$

where $M_{n\_reported}$ and $M_{w\_reported}$ are the known average molecular number and average molecular weight for the standard evaluated, and $M_{n\_observed}$ and $M_{w\_observed}$ are the average molecular number and average molecular weight for the standard evaluated using HPSEC. The extent of asymmetrical band-broadening was calculated using the following equation:

$$sk = \left(\frac{\Omega - 1}{\Omega + 1} \right) \qquad \text{(Equation A-7)}$$

where

$$\Omega = \left(\frac{M_{n\_reported}}{M_{n\_observed}} \times \frac{M_{w\_observed}}{M_{w\_reported}} \right) \qquad \text{(Equation A-8)}$$

No additional corrections were deemed necessary in order to calculate MW characteristics when $\Lambda < 1.05$ and $sk < 0.05$ (Yau et al., 1979).

Sample preparation

1. Immediately after sampling, filter water sample through an acid washed (18% HCl and deionized-distilled water for 24hrs) 0.45μm glass fiber filter into acid

washed (18% HCl and deionized-distilled water for 24hrs) 30mL polypropylene bottle.

2. Once filled, bottle is capped and stored in the dark at 4°C.
3. Open new 5mL syringe and Millex-GV 0.22μm filter (Millipore; Billerica, MA). Without touching bottom of syringe or removing filter from packaging, remove plunger from syringe and attached syringe to filter unit. Fill syringe with 4mL of sample. Taking care not to touch rubber syringe cap to sample solution, insert plunger and discard 1mL of filter eluent.
4. Fill Alcott 8x35mm autosampler vial (Grace; Deerfield, IL) with 900μL of filtered sample and insert cap.

Standard preparation

1. The following solutions were prepared in acid washed (18% HCl and deionized-distilled water for 24hrs) glass bottles with Teflon caps using ultra-pure water (>18mΩ).

Table A-4. MW standards for calibration.

MW Standard	Concentration	Standard	Average MW (Da)
1	3 mL L^{-1}	acetone	58
2	275 mg L^{-1}	polystyrene sulfonate	1,800
3	225 mg L^{-1}	polystyrene sulfonate	4,600
4	210 mg L^{-1}	polystyrene sulfonate	8,000
5	200 mg L^{-1}	polystyrene sulfonate	18,000
6	140 mg L^{-1}	polystyrene sulfonate	35,000

2. For each set of calibration standards, a blank standard was prepared using ultra-pure water (>18mΩ).
3. For each sample run, a Suwannee River fulvic acid check standard (~5mg L^{-1}) was also prepared.

Instrument start-up

1. Connect mobile phase solution (0.1M NaCl, 0.002M KH_2PO_4 and 0.002M Na_2HPO_4 buffered to an approximate pH of 6.8) and purge with helium (~2min). After purging mobile phase, close valve and pressurize system.
2. Turn on UV detector. Set wavelength to 254nm and output range to 0.01au (sampling rate should be once every second).
3. Turn on pump at a flow rate of 0.2mL min^{-1}. Every 20 minutes, increase flow rate 0.1mL min^{-1}. Backpressure for clean system should remain below 300psi. If backpressure is higher the frit most likely needs to be changed.
4. After 1hr warm up, the flow rate should be 0.5 mL min^{-1} and the instrument is ready for use.

Calibration and sample analysis

1. Each run should start with the following sequence described in Table A-5. where n is the number of vials run during analysis.
2. Run up to 20 samples between calibration curves with the Suwannee River FA sample placed somewhere in the middle.
3. After standards have been run, plot log MW of known standards versus the retention time of peak maximums and perform linear regression. Linear regression can be used to calculate the molecular mass M_i for unknown samples.
4. Before calculating M_i for samples, select one standard or sample (typically the DDI water blank) with similar chemistry without DOC for baseline correction. Subtract this baseline from each sample to correct for water dip and baseline shift observed during the course of the run.

Table A-5. Typical arrangement of calibration standards.

Vial #	Description
1	mobile phase
2	DDI water
3	MW standard 1
4	MW standard 2
5	MW standard 3
6	MW standard 4
7	MW standard 5
8	MW standard 6
...	...
n-6	DDI water
n-5	MW standard 1
n-4	MW standard 2
n-3	MW standard 3
n-2	MW standard 4
n-1	MW standard 5
n	MW standard 6

5. Using a minimum peak height of 3 times the standard deviation of the signal produced by the blank baseline, integrate and evaluate unknown DOC samples using equations 1-3.
6. After the run is complete, use equations 4-6 to calculate the amount of symmetrical (Λ) and asymmetrical (sk) band-broadening for standard #3 (5.4 kDa). If $\Lambda > 1.05$ or $sk > 0.05$, samples should be rerun.

Instrument shutdown

1. After last sample, flush column with mobile phase turning flow rate down 0.1mL min^{-1} every 20 minutes.
2. After 1hr of flushing system with mobile phase (flow rate should be 0.2mL min^{-1}), switch the mobile phase to 0.5mM sodium azide solution (pH ~ 6.2). At a flow rate of 0.2mL min^{-1}) the system should be flushed for 2hrs. After 2hrs, the system

can be turned off. The column should always be stored in 0.5mM sodium azide solution.

Additional notes

1. Never allow backpressure to exceed 3,000psi. Once backpressure exceeds 1,000psi, complete run and change frit. Backpressure should be <300psi for system with new frit, guard-column and main column.
2. Never allow column to run dry. If column runs dry, flow paths will collapse and the column is ruined.
3. If samples are from relatively pristine waters, samples should be adjusted to approximate pH and ionic strength of mobile phase before filtering into autosampler vials.

References

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Meier, M., Chin, Y.P. and Maurice, P., 2004. Variations in the composition and adsorption behavior of dissolved organic matter at a small, forested watershed. Biogeochemistry, 67(1): 39-56.

Yau, W.W., Kirkland, J.J. and Bly, D.D., 1979. Modern Size-Exclusion Liquid Chromatography: Practic of Gel Permeation and Gel Filtration Chromatography. Wiley-Interscience, New York, 315-326 pp.

Zhou, Q.H., Cabaniss, S.E. and Maurice, P.A., 2000. Considerations in the use of high-pressure size exclusion chromatography (HPSEC) for determining molecular weights of aquatic humic substances. Water Research, 34(14): 3505-3514.

Sample Collection Procedure

Sample preparation

Before leaving the laboratory to collect field samples, a field kit is required for each sample to be collected. Field kits consist of the following items:

- 1 – pair of powder-free nitrile gloves (Kimberly-Clark, part number 50603)
- 1 – 3" x 1" strip of Parafilm (VWR, part number 52858-000)
- 1 – 1L ultra-clean wedge shaped polypropylene bottle (ISCO, part number 68-3700-001). Sample bottles should be labeled with unique identification (ID) numbers using permanent ink.

In addition to field kits, a multi-parameter water quality meter (Horiba U-10 or YSI 556) is used to measure dissolved oxygen (DO), conductivity, pH, temperature, salinity (calculated by instrument based on conductivity) and turbidity at each sampling location. The multi-parameter water quality meters should be calibrated according to the instrument instruction manual before each sampling trip. A cooler with ice is also helpful to store samples when collecting more than a few (4) samples during hot weather. Using a cooler prevents samples from being exposed to UV light and helps to maintain natural sample temperature. Samples should not be allowed to exceed 25°C.

Once samples are collected from the field, they are further processed into additional sample bottles. Laboratory analysis requires the following items to determine alkalinity, total suspended solids (TSS), anions, major cations, molecular weight (MW), total nitrogen and total phosphorus (TN/TP), trace metal and dissolved organic carbon

(DOC) analysis (including 7 fractions typically run for DOC and trace metal analysis) for each sample:

- 8 – 30mL ultra-clean high-density polyethylene (HDPE) bottles for cation analysis. Bottles should be wrapped twice with ¾" orange laboratory tape (TimeMed, part number T-3460-6) and marked "Cations". Note: One bottle is used to collect an unfractioned sample for measurement of the total amount of major cations and trace metals present in solution; the other seven samples are for fractions. If more fractions are collected, additional bottles are necessary.
- 1 – 30mL ultra-clean HDPE bottle for anion analysis. Bottle should be wrapped twice with ¾" blue laboratory tape (TimeMed, part number T-3460-7) and 250µL of ACS grade 37% formaldehyde (Mallinckrodt, part number 5016-04) should be added. Bottle should be labeled for "Anions" analysis and "+250µL of formaldehyde" to denote the added preservative.
- 1 – 30mL acid washed HDPE bottle (Fisher Scientific, part number 01-288-33) for MW analysis. Bottle should be soaked in 18% HCl bath for ~24hrs, rinsed with 3 times with ultra-pure (>18 MΩ) water, soaked in ultra-pure water for ~24hrs and dried. Once dry, bottles should be wrapped twice with ¾" yellow laboratory tape (TimeMed, part number T-3460-2) and labeled "MW".
- 1 – 30mL acid washed HDPE bottle (Fisher Scientific, part number 01-288-33) for TN/TP analysis. Bottle should be soaked in 18% HCl bath for ~24hrs, rinsed 3 times with ultra-pure water, soaked in ultra-pure water for ~24hrs and

dried. Once dry, bottles should be wrapped twice with ¾" green laboratory tape (TimeMed, part number T-3460-3) and labeled "TN/TP".

- 8 – 40mL clean amber colored, borosilicate glass vials (I-Chem, part number S346-0040) capped with polytetrafluorethylene (PTFE)/silicone septa (0.010/0.050in; Alltech, part number 95322) and open top screw caps (20-400; Alltech, part number 95321). Vials should be acid washed in 18% HCl bath for ~24hrs, rinsed 3 times with ultra-pure water, soaked in ultra-pure water for ~24hrs and dried in oven at 550°C for 1hr. Septa should be soaked in ultra-pure water for ~24hrs and dried on clean counter paper (VWR, part number 52857-120). Clean and capped vial should be wrapped with ¾" white laboratory tape (TimeMed, part number T-3460-1) and labeled "TOC". Note: One bottle is used to collect an unfractioned sample for the total amount of major cations and trace metals present in solution; the other seven are for fractions. If more fractions are collected, additional bottles are necessary.
- 1-6 (depending on sample turbidity) – acid washed 47mm type A/E glass fiber filters (Pall Corporation, part number 61631). Filters should be acid washed in 18% HCl bath for ~24hrs, rinsed 3 times with ultra-pure water, soaked in ultra-pure water for ~24hrs, and dried in clean hood.
- 1 – ultra-clean filter assembly: filter flask, fritted glass support and Buchner funnel (Kontes, part number 953845-0000). Aultra-puretionally, a clamp for filter assembly, vacuum tubing and a vacuum pump are required to filter samples.
- Optima Nitric Acid (Fisher Scientific, part number A467-250)

Sample collection

Collecting samples required two people, one person who handles only ultra-clean equipment (clean hands) and one who handles other sampling equipment (dirty hands). Below describes the procedure for each person by identifying them as *clean hands* and *dirty hands*.

1. Both clean hands and dirty hands begin sampling by putting on a new pair of powder-free nitrile gloves (Kimberly-Clark, part number 50603). A new pair is required for each sample collected.
2. Dirty hands – open field kit bag and hold for the clean hands
3. Clean hands – remove second pair of nitrile gloves from inside field kit and places on top of first pair (double gloving).
4. Dirty hands – record bottle ID, sampling location, time, and weather conditions on sampling sheet.
5. Clean hands – remove 1L sample bottle from field kit. At sampling location clean hands should remove cap from 1L sample bottle and rinse both 3 times with sample water.
6. Dirty hands – while clean hands is collecting sample, collect any additional data or samples (i.e. multi-parameter water quality meter measurements).
7. Clean hands – collect sample and cap bottle. If possible, submerge the sample bottle completely and cap while underwater. Sample should be collected to minimize the amount of air trapped in sample bottle (the goal is not to have an air bubble).

8. Clean hands – remove Parafilm from field kit and wrap around sample cap to ensure seal.
9. Clean hands – place full sample bottle back in field kit bag.
10. Dirty hands – close field kit bag and seal with tape or by tying knot.
11. If collecting more than one sample, place used field kits upright (to minimize the possibility of sample leakage) in a cooler.

Sample processing

After collecting samples, field kits are taken back to the laboratory to filter and further processes aliquots for specific analyses. Directions below are for isolating and preserving samples intended for the measurement of alkalinity, TSS, anions, major cations, MW, TN/TP, trace metal and DOC (including 7 fractions typically run for DOC and trace metal analysis). Analyst should wear a new pair of nitrile gloves for each sample.

1. Remove sample bottles from field kit bags and allow them to reach room temperature (usually take 30-60 minutes).
2. Collect TSS and filter sample
    a. Remove ultra-clean type A/E filter from pre-weighed, pre-numbered zip-lock bag and place in a new ultra-clean filter assembly (Kontes, part number 953845-0000).
    b. Clamp filter paper between the fritted glass support and funnel.
    c. Attach vacuum tubing to vacuum pump.
    d. Shake sample vigorously to ensure fully mixed.

e. Turn on vacuum pump and filter 100mL of sample at a time, collecting all eluent in the 1L ultra-clean flask.

f. If filter becomes clogged (takes longer than 60 sec to pass 100mL through filter):

    i. Completely filter the last 100mL aliquot of the sample and allow the sample to dry by passing air through the filter by vacuum suction.

    ii. Turn off vacuum pump

    iii. Remove filter paper and place in pre-weighed ultra-clean zip-lock bag.

    iv. Place a new ultra-clean type A/E filter in the filter assembly currently being used and restart at step b. Repeat as necessary

g. If filter does not clog, continue adding 100mL aliquots until the full 1L of sample has been filtered.

h. Turn off vacuum pump

i. Without breaking the seal on the glass fiber filter, remove fritted glass support and Buchner funnel (with clamp) and place on a dirty 1L filter flask.

j. Cover original flask containing filtered sample with Parafilm.

k. Turn on vacuum pump.

l. Rinse filter and Buchner funnel with ultra-pure water so that all particles are removed from glassware onto filter surface.

m. Continue to pull air through filter paper using vacuum suction until filter paper is dry.

n. Turn off vacuum pump

o. Remove filter paper and place in pre-weighed ultra-clean zip-lock bag.

p. All filter papers and their numbered zip-lock bags used for each sample should be sealed in a larger zip-lock bag. The larger zip-lock bag should be labeled with the sample ID, the date and time of filtration and the analyst's initials.

q. Immediately place TSS samples in freezer for storage.

r. The fritted glass support and Buchner funnel can be placed in the dirty glassware bin for washing. The spare filter flask containing ultra-pure water rinse can also be emptied and placed in the dirty glassware bin for washing; however it can still be used for filtering additional samples if necessary.

s. Filtered samples in glass flasks can be stored cover with Parafilm in dark at 4°C for up to a week. If samples are refrigerated, they should be allowed to reach room temperature before further processing.

3. Divide filtered sample into appropriate containers for further analysis. From 1L glass flask:

   a. Pour ~15mL into pre-weighted disposable beaker for alkalinity titration.

b. Fill 40mL clean amber colored, borosilicate glass vials and cap with PTFE/silicone septa for TOC analysis (seal sample without an creating an air bubble). Label vial with sample ID.

c. Fill 30mL acid washed HDPE bottle for MW analysis. Label bottle with sample ID.

d. Fill 30mL acid washed HDPE bottle for TN/TP analysis. Label bottle with sample ID.

e. Fill 30mL ultra-clean HDPE bottle (containing 250μL of 37% formaldehyde) for anion analysis. Label bottle with sample ID.

f. Fill 30mL ultra-clean HDPE bottles for major cation and trace metal analysis. Add 180μL of Optima nitric acid to prevent precipitation. Label bottle with sample ID.

g. Perform DOC fractionation.

h. Place vials for TOC analysis and bottles for molecular weight, anion and cation analysis in 4°C fridge for storage.

i. Place bottles for TN/TP analysis in freezer for storage.

4. Complete necessary analyses.

a. Alkalinity should be run immediately (same day the sample was collected).

b. Anion, TN/TP and DOC samples should be run within 1 week of collecting the sample.

c. Major cations and trace metal analysis should be run within 6 months of collecting the sample.

5. After all samples and fractions have been collected the glass flasks can be washed according the ultra-clean procedure.

Additional notes

- If additional sample remains after all samples have been collected, duplicate samples can be collected. A duplicate sample for unfractioned DOC is extremely helpful for identifying any possible dilutions that may be necessary for running fractioned and unfractioned DOC samples.

Sample Watershed Cleanup Script

```
## ---------------------------------------------------------------------------
## sshedclean.py
## Created on: Monday December 05, 2005 10:30AM
## Created by: Shawn P. McElmurry and Saradhi Balla
## Usage: sshedclean <Input_Shapefile> <Diss_Field>
## Description: This program consolidates and recalculates landuse areas within micro-
watersheds.
## Landuse area is calculated first and then summed based on the dissolve field.
## Individual landuse codes will create individual features. This will
## delete all fields except landuse levels and labels, and shape area.
## MAKE BACKUP BEFORE RUNNING SCRIPT!
##---------------------------------------------------------------------------
# Import system modules
import sys, string, os, win32com.client
# Create the Geoprocessor object
gp = win32com.client.Dispatch("esriGeoprocessing.GpDispatch.1")
# Load required toolboxes...
gp.AddToolbox("C:/Program Files/ArcGIS/ArcToolbox/Toolboxes/Spatial Statistics
Tools.tbx")
gp.AddToolbox("C:/Program Files/ArcGIS/ArcToolbox/Toolboxes/Data Management
Tools.tbx")
gp.AddToolbox("C:/Documents and Settings/Shawn/Application
Data/ESRI/ArcToolbox/My Toolboxes/Shawn's Tools.tbx")
#Set the input shapefile to clean
Input_Shapefile = sys.argv[1]
Dis_Field = sys.argv[2]
Output_shed = Input_Shapefile
# Process: Calculate Areas...
try:
   # Output of calculate area creates temporary shapefile that will later be deleted...
   temp_shed = os.path.dirname(Input_Shapefile) + "\\temp_" +
os.path.basename(Input_Shapefile)
   gp.CalculateAreas_stats(Input_Shapefile, temp_shed)
except:
   gp.addMessage("ERROR-Failed to calculate areas.")
   del gp
   sys.exit
# Process: Dissolve Landuses...
try:
   gp.delete(Input_Shapefile)
except:
   gp.addMessage("ERROR-Failed to delete input shapefile.")
   del gp
   sys.exit
```

```
try:
   # Dis_Field = "CODE"
   Stat_Field = "LABEL FIRST;LEVEL1 FIRST;LABEL1 FIRST;LEVEL2
FIRST;LABEL2 FIRST;LEVEL3 FIRST;LABEL3 FIRST;F_AREA SUM"
   gp.Dissolve_management(temp_shed, Output_shed, Dis_Field, Stat_Field)
except:
   gp.addMessage("ERROR-Failed to dissolve landuses.")
   del gp
   sys.exit
try:
   gp.delete(temp_shed)
except:
   gp.addMessage("ERROR-Failed to delete temp file.")
   del gp
   sys.exit
gp.addMessage("-----Calculate and dissolve successfull!")
##------------------RENAME FIELD-------------------
rename_file = Output_shed
# Need to cycle through fields that have been created by dissolve to rename...
try:
   fields = gp.ListFields(rename_file)
   fields.reset()
   field = fields.next()
   while field:
      if field.Name == "FIRST_LABE":
         old_field = field.Name
         new_field = "LABEL"
         gp.Rename_Field(rename_file, old_field, new_field)
      elif field.Name == "FIRST_LEVE":
         old_field = field.Name
         new_field = "LEVEL1"
         gp.Rename_Field(rename_file, old_field, new_field)
      elif field.Name == "FIRST_LA_1":
         old_field = field.Name
         new_field = "LABEL1"
         gp.Rename_Field(rename_file, old_field, new_field)
      elif field.Name == "FIRST_LE_1":
         old_field = field.Name
         new_field = "LEVEL2"
         gp.Rename_Field(rename_file, old_field, new_field)
      elif field.Name == "FIRST_LA_2":
         old_field = field.Name
         new_field = "LABEL2"
         gp.Rename_Field(rename_file, old_field, new_field)
      elif field.Name == "FIRST_LE_2":
```

```
            old_field = field.Name
            new_field = "LEVEL3"
            gp.Rename_Field(rename_file, old_field, new_field)
        elif field.Name == "FIRST_LA_3":
            old_field = field.Name
            new_field = "LABEL3"
            gp.Rename_Field(rename_file, old_field, new_field)
        elif field.Name == "SUM_F_AREA":
            old_field = field.Name
            new_field = "SHED_AREA"
            gp.Rename_Field(rename_file, old_field, new_field)
            break
        field = fields.next()
    gp.addMessage("-----Rename field successfull!")
except:
    gp.addMessage("ERROR-Did Not Rename Field!")
    del gp
    sys.exit
gp.addMessage("==========================================================
")
gp.addMessage("CONGRATULATIONS," + rename_file + " has been cleaned!")
gp.addMessage("==========================================================
")
```

Total Organic Carbon Analysis

Dissolved organic carbon (DOC) was determined by total organic carbon analysis (TOC) using an OI Analytical Model 1010 Wet Oxidation Total Organic Carbon Analyzer (College Station, TX). The automated analysis utilizes heated-persulfate oxidation to measure the total amount of organic carbon present in aqueous samples (1998). Generally, samples were filtered through a 0.45μm filter into acid washed borosilicate glass vials that were capped with TFE/silicone liners and refrigerated until analysis, typically within 1-2 days. Clean gloves and counter paper should be used at all times when performing procedure described below.

Sample preparation

1. Soak 40mL amber colored, borosilicate glass vials (I-Chem, part number S346-0040) in 18% HCl acid bath for 24hrs.
2. Soak polytetrafluoroethylene (PTFE)/silicone septa (0.010/0.050in; Alltech, part number 95322) and open top screw caps (20-400; Alltech, part number 95321) in ultra-pure water for 24hrs.
3. Remove vials from acid bath after 24hrs, rinse in ultra-pure water and bake in oven 550°C for 1hr.
4. Remove septa from ultra-pure water after 24hrs and dry on counter paper in hood (usually ~12hrs).
5. After baking for 1hr, remove vials from oven, cover with aluminum foil and allow vials to come back to room temperature (usually ~2hrs).

Sample collection and preservation

1. Fill sample vials until meniscus becomes inverted above the vial top. When capping with septa, attempt to minimize the amount of air trapped. If sample vial is full, it should be possible to seal vial without creating an air bubble.
2. Immediately after sampling, place sample vials in dark fridge (4°C) for up to one week. Although no limit on storage time is given by Standard Methods (1998), run all samples with in one week of sample collection.

Operating the OI Analytical 1010 TOC Analyzer

1. Turn on ultra-pure nitrogen (>99.99% or better) gas supply to a line pressure of 60 psi.
2. Turn on TOC analyzer and auto sample tray. Allow analyzer to fully boot.
3. Check to make sure rinse bottle is empty. Empty any rinse water down drain with copious amounts of tap water.
4. Fill water reservoir with ultra-pure water (>18MΩ). If this jug is needs to be filled, carefully remove the cap (do not twist lines) and make sure the submerged tubing remains clean when filling bottle. This can often be accomplished by placing the lines on clean counter paper. After rinsing the tubing with clean water, place lines back into reservoir and make sure nitrogen is bubbling.
5. Make sure acid and oxidant reagents for TOC machine are full (>250mL for 59 vial run) and fresh (< 3 months old).

a. To make acid, fill 1L bottle with approximately 500mL of ultra-pure water (>18MΩ), add 59mL of ACS grade (85%) phosphoric acid and fill to with ultra-pure water.

b. To make oxidant, add 100gm of low organic carbon sodium persulfate (OI Analytical, part number 178848) to acid washed 1L volumetric flask, fill to 1L with ultra-pure water(>18MΩ), add acid washed stir bar and stir until fully dissolved.

6. Make sure there is a sufficient amount of unused desiccant. For a 24 hr run, ¾ of the tube should be unused (blue). Every time the desiccant tube is replaced a leak check should be performed.
7. Turn on computer and click the winTOC 1010 icon.
8. Select *System 1*.
9. Make sure there is an IR signal (red number in lower left of window). If there is no signal an error warning will be displayed in message window. Allow system to warm up until IR signal stabilizes around 6,000-10,000.
10. Remove the cover to observe the pumps and injection loops.
11. Flush system with reagents:

    a. Click *instrument* on the toolbar; click *diagnostics*; type in *99* for oxidant pump and *99* for acid pump. Click *pump*.

    b. This should make both pumps perform *99* strokes.

    c. Watch for leak.

    d. Notice glass reaction chamber filling with solution

e. Without closing Diagnostics window; in the miscellaneous block, click *drain*. After all the liquid has drained from reaction vessel, click *drain* again to turn it off (keep the *diagnostics* window open).

12. Check the alignment of autosampler.

a. With a vial in sample tray position #1, place the sample tray on metal platform making sure to line up center hole and guide pine.

b. Once the sample tray is positioned securely- click *go to first vial* in the autosampler block. This will cause the tray to rotate the first sample vial directly under the needle.

c. Again in the Diagnostics window, click *calibrate* in the Autosampler block.

d. Manually align the center of the vial (while the autosampler tray is still positioned securely on metal platform) with the sample needle with the center of vial by moving metal platform in any direction. Click *done*.

e. While still in the diagnostics window and without adjusting the tray or vial after the previous calibration, click *needle down*. The auto-sampling needle should go into the center of the septa of vial #1. When finished, click *needle up*.

f. Click *home* and the tray should adjust so that the tray is positioned with the needle slightly ahead vial #1.

g. Close diagnostics window.

13. Setup output files.

a. Click *setup* on the toolbar and then *Win TOC Output* on pop-down menu.

b. Enter subdirectory (typically your name, up to 6 characters long)

c. Enter log file name (typically the date in MMDDYY format)

d. Set the output file prefix (typically the month and day in MMDD format). The prefix and counter number are used to create result files.

e. Reset output file counter to desired number.

Sample analysis

1. Under the instrument menu, select sequence.
2. Load standards and samples in the following order:

Table A-6. Sequence of TOC standards.

Position #	Sample Name	Run Type
1	DDI Water	Sample
2	TOC STD #1	Std 1
3	TOC STD #2	Std 2
4	TOC STD #3	Std 3
5	TOC STD #4	Std 4
6	TOC STD #5	Std 5
7	Sample 1	Sample
etc.		

Before the run is started and any calibration is performed, reagent blanks should be performed. For an instrument that has not been turned off since the last time samples were run and no changes have been made to reagents, 3 reagent blanks should be run. For an instrument that has run samples recently (7 days) and no changes have been made to reagent, or reagents have been changed since the last calibration, 6 reagent blanks should be run. Any time the instrument has been idle for longer than 7 days, 9 reagent blanks have been run.

3. Every 6-20 samples, check standards should be run. For these standards, the run type should be changed to the appropriate check standard in the drop down list.

References

Standard Methods, 1998. Method 5310 C-Total Organic Carbon, Heated-Persulfate Oxidation Method. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC, 5-22 pp.

Ultra-Clean Procedure

Procedures described below should be performed in an EPA class 100 clean room. The procedure employs strong acids and requires the use of safety goggles, acid apron, closed toed shoes, long sleeves, long pants and appropriate gloves. While thick rubber gloves are typically used when handling strong acids, the rubber gloves initially used were found to leach metals. Due to the potential for contamination, two pairs of powder-free nitrile gloves (Kimberly-Clark, part number 50603) were used instead. Because nitrile gloves are not designed to withstand the strong acid solutions used they should be changed frequently, especially when the gloves begin to change color (from purple to blue). Other gloves may be more appropriate (e.g. Kimberly-Clark Kim Tech Pure G3 Cleanroom Acid Gloves, part number 40222), however the extent these gloves leach trace metals has not been investigated and should be verified before routine use.

For all cleaning procedures described below, the following acid baths should be prepared in appropriate size containers (heavy duty plastic containers designed to withstand strong acids).

- 18% HCl solution prepared in ultra-pure (>18MΩ) water using trace metal grade acid (Fisher Scientific, part number A508-212)
- 35% HNO_3 solution prepared in ultra-pure water using trace metal grade acid (Fisher Scientific, part number A509-212)

Cleaning procedure for 30mL bottles and 60mL syringes

1. The following acid baths should be prepared in appropriate size containers (heavy duty plastic containers designed to withstand strong acids).

a. 18% HCl solution prepared in ultra-pure water using trace metal grade acid (Fisher Scientific, part number A508-212)
b. 35% HNO_3 solution prepared in ultra-pure water using trace metal grade acid (Fisher Scientific, part number A509-212)

2. While wearing powder-free nitrile gloves, open 60mL syringes (Becton Dickinson, part number 309620) and place rubber caps in ultra-clean volumetric flask.
3. Place new 30mL high-density polyethylene (HDPE) bottles and caps (narrow mouth bottles are available from Fisher Scientific, part number 01-288-33), and 60mL syringes (plungers and tubes) in 18% HCl bath. Batches of 21 syringes and ~60 bottles works well. Soak for ~ 24hrs.
4. Rinse rubber caps 3 times with ultra-pure water. Fill volumetric flask containing rubber caps with ultra-pure water, cover with Parafilm (VWR, part number 52858-000) and soak for greater than 24hrs. Do not allow Parafilm to contact solution.
5. Carefully transfer bottles and syringes (plungers and tubes) from 18% HCl bath to 35% HNO_3 bath. Soak for ~24hrs at 20 °C.
6. Remove rubber caps from ultra-pure water solution, shake/tap caps to help remove water and place in tubs lined with counter paper (VWR, part number 52857-120), paper side up. Allow to dry, typically 2-3 days.
7. Fill bottles with 35% HNO_3 and cap. Place capped bottles in square polycarbonate bin suitable for hot acid bath. Fill bin with ultra-pure water to submerge bottles. Transfer syringes (plungers and tubes) in square polycarbonate bin suitable for

acid bath. Fill bin with syringes with 35% HNO_3. Place sealed bins in hot water bath at 45 °C for ~24hrs.

8. Remove bins from water bath. Pour HNO_3 from square polycarbonate bin filled with syringes back into HNO_3 bath. Rinse syringes 3 times with ultra-pure water and soak for ~24hrs. For the square polycarbonate bin filled with capped bottles, pour ultra-pure water down drain. With plenty of water from tap (neutralize if necessary), uncap acid washed bottles and dispose of used acid. Rinse bottles and caps 3 times with ultra-pure water and soak for ~24hrs. Be careful not to allow tap water to contaminate acid washed labware.
9. Cut 3 new 3' x 3' pieces of counter paper (VWR, part number 52857-120). Place one, paper side up, on counter and line a drying tub with another. The third piece of counter paper will be used to cover tub during drying. Remove syringes and bottles from ultra-pure water soak, tap labware on counter paper to remove as much water as possible and place in tub lined with counter paper. Allow to dry, typically 2-3 days.
10. Once dry, cap sample bottles, replace syringe cap on plunger head and insert plunger into syringe barrel. Place bottles and syringes in plastic bags and seal bags with laboratory tape (TimeMed, part number T-1260-1). On tap mark the number of bottles or syringes in bag. Double bag each set of ultra-cleaned items and seal with lab tape. Label outside of bag with: "ULTRA-CLEAN", quantity, your name and date.

Cleaning procedure for 1L sample bottles and glassware

The following procedure should be used for 1L wedge shaped polypropylene bottles (ISCO, part number 68-3700-001), or similar size sample bottles, and any glassware that comes in contact with water samples intended for trace metal analysis. For example, the removal of total suspended solids (TSS) required a filtration assembly to be ultra-clean for each sample: filter flask, fritted glass support and Buchner funnel (Kontes, part number 953845-0000).

1. Wash bottles and glassware using liquid soap solution of diluted Liqui-Nox detergent (VWR, part number 21837-027) and rinse 3 times with ultra-pure water.
2. Place in 18% HCl bath. Soak for ~ 24hrs.
3. Carefully transfer from 18% HCl bath to 35% HNO_3 bath. Soak for ~24hrs at 20 °C.
4. Remove from acid bath, rinse 3 times with ultra-pure water and soak for ~24hrs.
5. Cut 3 new 3' x 3' pieces of counter paper (VWR, part number 52857-120). Place one, paper side up, on counter and line a drying tub with another. The third piece of counter paper will be used to cover tub during drying. Remove bottles and glassware from ultra-pure water soak, tap on counter paper to remove as much water as possible and place in tub lined with counter paper. Allow to dry, typically 2-3 days.
6. Once dry, place bottles in plastic bags and seal with laboratory tape (TimeMed, part number T-1260-1). On tap mark the number of bottles in bag. Double bag each set of ultra-cleaned items and seal with lab tape. Label outside of bag with: "ULTRA-CLEAN", quantity, your name and date. Glassware should be covered

with Parafilm (VWR, part number 52858-000), when appropriate, and stored separately from other glassware.

Additional notes

- When working with labware at multiple stages in the ultra-clean process, begin by moving labware at the clean end of the process forward (i.e. from ultra-pure water rinse to drying tubs) and work backwards to the dirty end, the start of the acid washing procedure (i.e. placing labware in 18% HCl acid bath).
- To decrease drying time, tubs can be left uncovered in hood. Additionally, periodically tapping labware on counter paper to remove water droplets will greatly decrease drying time. Drying time can be reduced to as little as 1-2 days.

APPENDIX B

SAMPLE LOCATIONS

Table B-1. Sample location key.

Location ID	Latitude	Longitude
8	42.793732	-84.499154
9	42.791795	-84.496150
14	42.782244	-84.483383
17	42.773184	-84.488345
18	42.782102	-84.472273
28	42.782299	-84.453063
37	42.813222	-84.538336
64	42.789039	-84.515634
84	42.819833	-84.549150
87	42.765698	-84.512780
88	42.791685	-84.476050
95	42.753488	-84.505666
96	42.757112	-84.503660
98	42.763575	-84.486242
99	42.746776	-84.502062
100	42.758727	-84.493629
101	42.798424	-84.495828
A	42.685137	-84.483903
AG	42.685137	-84.483903
W	42.718248	-84.476216
F	42.718248	-84.476216
BW	42.718248	-84.476216
CR	42.942920	-85.804550
CS	42.773208	-84.488388
CSO	42.930600	-85.743680
DC	43.010620	-85.936450
DLM	42.976680	-85.876080
EE	42.790740	-84.485250
ERC	42.716124	-84.469627
FL	42.727458	-84.477729
G	42.710925	-84.488876
GC	42.710925	-84.488876
GRER	42.534722	-84.623056
GRFU	42.964230	-85.676600
GRGR	42.968137	-85.676360
GRIO	42.971944	-85.069167
GRJA	42.281476	-84.409214
GRLA	42.750321	-84.555266

Table B-1 (cont'd)

Location ID	Latitude	Longitude
H131	42.948830	-85.706170
HHS	42.800029	-84.464392
LGEA	42.828056	-84.759444
OF22	42.729692	-84.483753
OF37	42.729554	-84.494557
OF38	42.730193	-84.491759
OF52	42.728088	-84.471678
OF53	42.728277	-84.462343
PL	42.712558	-84.482343
RCMSU	42.728569	-84.481242
RCR	42.728951	-84.482149
RCWI	42.683056	-84.219167
RRRO	43.072203	-85.597486
SW	42.728534	-84.463888
U28	42.920780	-85.764750

Table B-2. Samples collected.

Sample ID	Date Sampled	Sample Source	Landuse ID
37-0425	April 25, 2007	Creek	11
14-0726	July 26, 2006	Creek	22
99-0816	August 16, 2006	Pond	112
100-0710	July 10, 2006	Creek	113
100-0711	July 11, 2006	Creek	113
100-080707	August 7, 2007	Creek	113
84-0425	April 25, 2007	Creek	113
64-0623	June 23, 2006	Pond	115
87-0425	April 25, 2007	Pond	122
87-0623	June 23, 2006	Pond	122
87-0711	July 11, 2006	Pond	122
96-0425	April 25, 2007	Creek	124
96-0622	June 22, 2006	Creek	124
SW-0829	August 29, 2006	Pond	411
BW-0622	June 22, 2005	Pond	431
BW-0729-T	July 29, 2005	Pond	431
BW-1031	October 31, 2006	Pond	431
BW-112905	November 29, 2005	Pond	431
BW-52506	May 25, 2006	Pond	431
BW-040407	April 4, 2007	Pond	431
BW-052507	May 25, 2007	Pond	431
BW-0829	August 29, 2006	Pond	431
BW-112905	November 29, 2005	Pond	431
BW-52506	May 25, 2006	Pond	431
17-0726	July 26, 2006	Creek	1121
17-080707	August 7, 2007	Creek	1121
95-0816	August 16, 2006	Pond	1121
98-0623	June 23, 2006	Pond	1121
CS-080707	August 7, 2007	Outfall	1121
HHS-080707	August 7, 2007	Pond	1
HR-0425	April 25, 2007	Pond	1121
HR-0623	June 23, 2006	Pond	1121
HR-0710	July 10, 2006	Pond	1121
HR-080707	August 7, 2007	Pond	1121
ERC-071907	July 19, 2007	Runoff	1449
PL-031407	March 14, 2007	Pond	1449
PL-040407	April 4, 2007	Pond	1449
PL-052507	May 25, 2007	Pond	1449

Table B-2 (cont'd)

Sample ID	Date Sampled	Sample Source	Landuse ID
PL-071907	July 19, 2007	Pond	1449
PL-52506	May 25, 2006	Pond	1449
PL-0307	March 7, 2006	Pond	1449
PL-0622	June 22, 2005	Pond	1449
PL-0628	June 28, 2006	Pond	1449
PL-0710	July 10, 2006	Pond	1449
PL-0711	July 11, 2006	Pond	1449
PL-0729-T	July 29, 2005	Pond	1449
PL-1031	October 31, 2006	Pond	1449
PL-112905	November 29, 2005	Pond	1449
AG-031407	March 14, 2007	Pooling	2111
AG-040407	April 4, 2007	Pooling	2111
AG-0829	August 29, 2006	Pooling	2111
AG-52506	May 25, 2006	Pooling	2111
AG-0307	March 7, 2006	Pooling	2111
AG-0622	June 22, 2005	Pooling	2111
AG-112905	November 29, 2005	Pooling	2111
08-0622	June 22, 2006	Creek	2113
09-0622	June 22, 2006	Creek	2113
101-0711	July 11, 2006	Creek	2113
18-0726	July 26, 2006	Creek	2231
18-080707	August 7, 2007	Creek	2231
28-0726	July 26, 2006	Creek	2231
28-080707	August 7, 2007	Creek	2231
OF38-1107-1	October 30, 2006	Outfall	12644
OF38-1107-2	November 7, 2006	Outfall	12644
OF38-1107-3	November 7, 2006	Outfall	12644
OF38-1107-4	November 7, 2006	Outfall	12644
OF38-1107-5	November 7, 2006	Outfall	12644
OF38-1107-6	November 7, 2006	Outfall	12644
OF38-1107-7	November 7, 2006	Outfall	12644
OF38-1107-8	November 7, 2006	Outfall	12644
OF38-1107-9	November 7, 2006	Outfall	12644
OF38-0425-1	April 25, 2007	Outfall	12644
OF38-0425-2	April 25, 2007	Outfall	12644
OF38-0628	June 28, 2006	Outfall	12644
OF38-0724-12	July 24, 2006	Outfall	12644
OF38-0725	July 25, 2006	Outfall	12644
OF38-0725-5	July 25, 2006	Outfall	12644

Table B-2 (cont'd)

Sample ID	Date Sampled	Sample Source	Landuse ID
OF38-0725-A1	July 25, 2006	Outfall	12644
OF-22-0726	July 26, 2006	Outfall	12644
OF22-0803-01	August 3, 2006	Outfall	12644
OF37-0726	July 26, 2006	Outfall	12644
OF38-0802-14	August 2, 2006	Outfall	12644
OF38-0803-01	August 3, 2006	Outfall	12644
OF38-0803-A1	August 3, 2006	Outfall	12644
OF38-0803-A2	August 3, 2006	Outfall	12644
OF38-1130-1	November 30, 2006	Outfall	12644
OF38-1130-10	November 30, 2006	Outfall	12644
OF38-1130-11	November 30, 2006	Outfall	12644
OF38-1130-12	November 30, 2006	Outfall	12644
OF38-1130-13	November 30, 2006	Outfall	12644
OF38-1130-13	November 30, 2006	Outfall	12644
OF38-1130-15	November 30, 2006	Outfall	12644
OF38-1130-16	November 30, 2006	Outfall	12644
OF38-1130-17	November 30, 2006	Outfall	12644
OF38-1130-2	November 30, 2006	Outfall	12644
OF38-1130-4	November 30, 2006	Outfall	12644
OF38-1130-5	November 30, 2006	Outfall	12644
OF38-1130-9	November 30, 2006	Outfall	12644
OF38-1130-A10	November 30, 2006	Outfall	12644
OF38-1130-A11	November 30, 2006	Outfall	12644
OF38-1130-A9	November 30, 2006	Outfall	12644
OF38-1201-12-D	December 1, 2006	Outfall	12644
OF38-1201-14	December 1, 2006	Outfall	12644
OF38-1201-16	December 1, 2006	Outfall	12644
OF38-1201-17	December 1, 2006	Outfall	12644
OF38-1201-19	December 1, 2006	Outfall	12644
OF38-1201-21	December 1, 2006	Outfall	12644
OF38-1201-22	December 1, 2006	Outfall	12644
OF38-1201-23	December 1, 2006	Outfall	12644
OF38-1201-24	December 1, 2006	Outfall	12644
OF38-1206	December 6, 2006	Outfall	12644
OF38-1208-2	December 8, 2006	Outfall	12644
OF38-1208-3	December 8, 2006	Outfall	12644
OF38-1212-1	December 12, 2006	Outfall	12644
OF38-1212-2	December 12, 2006	Outfall	12644
OF38-619-0	June 20, 2007	Outfall	12644

Table B-2 (cont'd)

Sample ID	Date Sampled	Sample Source	Landuse ID
OF38-619-1	June 19, 2007	Outfall	12644
OF38-619-13	June 20, 2007	Outfall	12644
OF38-619-14	June 20, 2007	Outfall	12644
OF38-619-15	June 19, 2007	Outfall	12644
OF38-619-16	June 19, 2007	Outfall	12644
OF38-619-17	June 19, 2007	Outfall	12644
OF38-619-18	June 19, 2007	Outfall	12644
OF38-619-19	June 19, 2007	Outfall	12644
OF38-619-2	June 19, 2007	Outfall	12644
OF38-619-20	June 19, 2007	Outfall	12644
OF38-619-25	June 19, 2007	Outfall	12644
OF38-619-5	June 19, 2007	Outfall	12644
OF38-619-6	June 19, 2007	Outfall	12644
OF38-619-8	June 20, 2007	Outfall	12644
OF38-619-9	June 19, 2007	Outfall	12644
OF52-0726	July 26, 2006	Outfall	12644
OF53-0726	July 26, 2006	Outfall	12644
88-080707	August 7, 2007	Pond	19331
EE-080707	August 7, 2007	Pond	19331
EE-0816	August 16, 2006	Pond	19331
GC-031407	March 14, 2007	Pond	19331
GC-040407	April 4, 2007	Pond	19331
GC-052507	May 25, 2007	Pond	19331
GC-071907	July 19, 2007	Pond	19331
GC-52506	May 25, 2006	Pond	19331
GC-0307	March 7, 2006	Pond	19331
GC-0622	June 22, 2005	Pond	19331
GC-0628	June 28, 2006	Pond	19331
GC-0710	July 10, 2006	Pond	19331
GC-0729-T	July 29, 2005	Pond	19331
GC-0816	August 16, 2006	Pond	19331
GC-1031	October 31, 2006	Pond	19331
GC-112905	November 29, 2005	Pond	19331
CR-0615	June 15, 2006	River	mixed
CSO-0615	June 15, 2006	River	mixed
DC-0615	June 15, 2006	River	mixed
DLM-0615	June 15, 2006	River	mixed
FL-0307	March 7, 2006	River	mixed
FL-0628	June 28, 2006	River	mixed

Table B-2 (cont'd)

Sample ID	Date Sampled	Sample Source	Landuse ID
GR-0615	June 15, 2006	River	mixed
GRER-0711	July 11, 2006	River	mixed
GRFU-0615	June 15, 2006	River	mixed
GRGR-0808	August 8, 2006	River	mixed
GRIO-0808	August 8, 2006	River	mixed
GRJA-0711	July 11, 2006	River	mixed
GRLA-0808	August 8, 2006	River	mixed
GRPO-0808	August 8, 2006	River	mixed
H131-0615	June 15, 2006	River	mixed
LGEA-0808	August 8, 2006	River	mixed
RCMSU-0808	August 8, 2006	River	mixed
RCR 0404	April 4, 2006	River	mixed
RCR-0226	February 26, 2007	River	mixed
RCR-1005	October 5, 2006	River	mixed
RCWI-0808	August 8, 2006	River	mixed
RRRO-0808	August 8, 2006	River	mixed
U28-0615	June 15, 2006	River	mixed

APPENDIX C

DATA

Table C-1. Molecular characterization data.

Sample ID	NUVA ($L\ gC^{-1}\ cm^{-1}$)	Average Molecular Number (Da)	Average Molecular Weight (Da)	Polydispersity
08-0622	2.06	1349.2	1571.9	1.17
09-0622	1.86	1212.8	1525.3	1.26
100-0710	1.99	921.0	1027.0	1.12
100-0711	2.11	684.5	1151.7	1.68
100-080707	2.22			
101-0711	1.72	1171.9	1412.6	1.21
14-0726				
17-0726				
17-080707	1.96			
18-0726				
18-080707	1.63	1241.8	1319.1	1.06
28-0726				
28-080707	1.90	1266.5	1336.0	1.05
37-0425	2.67			
64-0623	1.44			
84-0425	2.65			
87-0425	2.24			
87-0623	1.76	1171.3	1266.9	1.08
87-0711	2.25	981.9	1292.4	1.32
88-080707	0.92			
95-0816	1.19	974.1	1094.7	1.12
96-0425	2.15			
96-0622	2.05	1129.8	1509.1	1.34
98-0623	1.36	1150.2	1334.1	1.16
99-0816	0.81			
AG-0307				
AG-031407				
AG-040407		1279.2	1330.3	1.04
AG-0622				
AG-0829	2.90	754.3	1927.4	2.56
AG-112905	3.40			
AG-52506	2.25	681.2	1106.3	1.62
BW-040407	3.21	1562.5	2168.1	1.39
BW-052507	3.18	665.6	1952.6	2.93

Table C-1. (cont'd)

Sample ID	NUVA ($L\ gC^{-1}\ cm^{-1}$)	Average Molecular Number (Da)	Average Molecular Weight (Da)	Polydispersity
BW-0622				
BW-0729-T	4.49			
BW-0829	3.17	852.5	2151.9	2.52
BW-1031	2.39			
BW-112905				
BW-112905	2.47			
BW-52506	2.36	1160.4	1673.1	1.44
BW-52506	3.43	1243.1	2375.4	1.91
CR-0615	1.874	1201.0	1528.1	1.27
CS-080707	2.28	974.8	1119.4	1.15
CSO-0615	1.622	607.6	1549.3	2.55
DC-0615	1.717	1400.5	1552.9	1.11
DLM-0615	11.410	1241.3	1557.7	1.25
EE-080707	1.58	1241.6	1348.8	1.09
EE-0816	1.77	1105.6	1323.0	1.20
ERC-071907	1.588	324.0	776.0	2.40
FL-0307				
FL-0628	1.905	1026.8	1262.4	1.23
GC-0307				
GC-031407				
GC-040407				
GC-052507	2.60	560.2	1425.4	2.54
GC-0622				
GC-0628	2.38	846.9	1465.5	1.73
GC-0710	2.35	1097.9	1442.5	1.31
GC-071907	2.36			
GC-0729-T	3.43			
GC-0816	2.56	1075.5	1548.0	1.44
GC-1031	2.60			
GC-112905	2.92			
GC-52506	1.97	1065.8	1494.0	1.40
GR-0615				
GRER-0711	2.057	1314.3	1628.6	1.24
GRFU-0615	1.882	1486.3	1604.7	1.08

Table C-1. (cont'd)

Sample ID	NUVA ($L\ gC^{-1}\ cm^{-1}$)	Average Molecular Number (Da)	Average Molecular Weight (Da)	Polydispersity
GRGR-0808		1171.4	1266.5	1.08
GRIO-0808		1077.9	1567.9	1.45
GRJA-0711	1.886	1245.0	1428.7	1.15
GRLA-0808	1.927	809.2	1139.7	1.41
GRPO-0808		836.1	1188.1	1.42
H131-0615	1.850	1490.8	1603.4	1.08
HHS-080707	1.46	1176.6	1290.0	1.10
HR-0425	2.51			
HR-0623	1.69			
HR-0710	2.27	767.7	1143.0	1.49
HR-080707	2.32			
LGEA-0808	2.375	1000.8	1616.5	1.62
OF-22-0726	2.08	651.7	940.7	1.44
OF22-0803-01		585.3	852.6	1.46
OF37-0726	2.43			
OF38-0425-1	2.25			
OF38-0425-2	1.36			
OF38-0628	1.07	942.3	1000.5	1.06
OF38-0724-12	1.30	863.8	1027.8	1.19
OF38-0725	1.54	607.3	914.9	1.51
OF38-0725-5				
OF38-0725-A1	1.65	545.7	804.0	1.47
OF38-0802-14	2.15			
OF38-0803-01	1.81			
OF38-0803-A1				
OF38-0803-A2	1.83	1023.3	1209.2	1.18
OF38-1107-1				
OF38-1107-2				
OF38-1107-3				
OF38-1107-4				
OF38-1107-5				
OF38-1107-6				
OF38-1107-7				
OF38-1107-8				

Table C-1. (cont'd)

Sample ID	NUVA ($L\ gC^{-1}\ cm^{-1}$)	Average Molecular Number (Da)	Average Molecular Weight (Da)	Polydispersity
OF38-1107-9				
OF38-1130-1	0.96	893.5	1267.7	1.42
OF38-1130-10	1.42			
OF38-1130-11	1.39	793.7	1290.6	1.63
OF38-1130-12				
OF38-1130-13	0.89	713.2	1216.7	1.71
OF38-1130-13	1.57			
OF38-1130-15	1.30	806.2	1956.2	2.43
OF38-1130-16	1.70	1209.7	1716.6	1.42
OF38-1130-17	1.29			
OF38-1130-2	1.05	902.8	1268.7	1.41
OF38-1130-4	1.54	1001.6	1546.0	1.54
OF38-1130-5	1.33	683.4	1129.0	1.65
OF38-1130-9	1.27	770.0	1282.2	1.67
OF38-1130-A10	1.51	981.8	1543.8	1.57
OF38-1130-A11	1.57	1082.3	1604.8	1.48
OF38-1130-A9				
OF38-1201-12-D				
OF38-1201-14				
OF38-1201-16		1315.5	1740.0	1.32
OF38-1201-17		1330.2	2112.8	1.59
OF38-1201-19		1126.5	1652.8	1.47
OF38-1201-21		1111.5	1672.8	1.50
OF38-1201-22		1016.0	1631.8	1.61
OF38-1201-23				
OF38-1201-24		1159.1	1736.8	1.50
OF38-1206				
OF38-1208-2				
OF38-1208-3				
OF38-1212-1				
OF38-1212-2				
OF38-619-0	1.04			
OF38-619-1	1.40	666.8	860.5	1.29
OF38-619-13	1.62	495.6	763.5	1.54

Table C-1. (cont'd)

Sample ID	NUVA ($L\ gC^{-1}\ cm^{-1}$)	Average Molecular Number (Da)	Average Molecular Weight (Da)	Polydispersity
OF38-619-14	1.50	466.4	663.3	1.42
OF38-619-15	1.38	432.6	682.4	1.58
OF38-619-16	1.45	441.7	622.0	1.41
OF38-619-17	1.44	421.2	687.2	1.63
OF38-619-18	1.43	591.2	769.3	1.30
OF38-619-19	1.42			
OF38-619-2	1.39	563.7	996.4	1.77
OF38-619-20	1.35	306.1	860.3	2.81
OF38-619-25	1.41	381.4	749.8	1.97
OF38-619-5	1.76	665.6	986.0	1.48
OF38-619-6	2.16	598.4	848.3	1.42
OF38-619-8	2.24	958.6	963.8	1.01
OF38-619-9	2.07			
OF52-0726	2.55	688.7	1145.3	1.66
OF53-0726	2.21	902.3	1244.1	1.38
PL-0307				
PL-031407				
PL-040407				
PL-052507	2.069	346.4	849.8	2.45
PL-0622				
PL-0628	6.918	1090.9	1104.0	1.01
PL-0710	1.941	905.5	1009.3	1.11
PL-0711	1.789	1023.2	1223.9	1.20
PL-071907	1.808	400.2	846.4	2.11
PL-0729-T	1.636			
PL-1031	2.104			
PL-112905	3.087			
PL-52506	2.117	838.8	1036.8	1.24
RCMSU-0808		890.0	1416.6	1.59
RCR 0404				
RCR-0226				
RCR-1005				
RCWI-0808	2.337	913.3	1315.2	1.44

Table C-1. (cont'd)

Sample ID	NUVA ($L\ gC^{-1}\ cm^{-1}$)	Average Molecular Number (Da)	Average Molecular Weight (Da)	Polydispersity
RRRO-0808		1348.5	1365.5	1.01
SW-0829	2.48	938.8	1680.8	1.79
U28-0615	1.896	1431.8	1589.2	1.11

Table C-2. DOC fraction data: total carbon in sample (C_T), anion-F eluent (i.e. carbon not retained by cartridge) (C_F), anion-I eluent (C_I), extended hydrophobic eluent (C_{Hi}), hydrophobic eleuent (C_{Ho}), H-bonding eluent (C_H), and anion-1kDa eluent (C_X) (ND – non-detect).

Sample ID	C_T	C_F	C_I	C_{Hi}	C_{Ho}	C_H	C_X
08-0622	12.21	10.23	9.03	5.09	3.36	0.34	9.26
09-0622	14.22	12.52	11.29	2.81	3.28	0.24	11.42
100-0710	29.50	24.81	27.04	13.05	11.50	1.87	24.49
100-0711	19.36	15.09	14.17	9.03	7.87	2.15	14.97
100-080707	12.43	11.10	10.81	6.76	6.04	2.40	10.68
101-0711	11.65	10.91	9.60	3.97	2.64	0.54	10.24
14-0726	13.76	12.97	11.70	3.80	2.94	0.84	12.49
17-0726	7.66	7.02	6.29	2.53	2.26	0.77	6.32
17-080707	12.52	11.36	10.79	6.57	5.77	1.64	10.79
18-0726	7.75	6.57	5.90	2.71	2.51	0.94	6.00
18-080707	9.27	8.52	7.68	2.74	2.21	0.62	8.44
28-0726	11.08	10.37	9.51	2.90	2.89	0.88	9.74
28-080707	9.45	8.33	7.47	3.04	2.72	1.14	7.77
37-0425	2.34	1.81	1.86	1.50	1.30	1.19	1.33
64-0623	12.91	10.31	9.60	3.02	2.96	0.67	9.08
84-0425	6.05						
87-0425	3.12	1.56	1.41	1.59	1.40	0.50	1.14
87-0623	7.09	3.78	6.97	3.10	2.34	0.20	3.41
87-0711	7.48	5.98	5.11	3.71	3.47	1.31	5.39
88-080707	8.80	7.53	6.89	1.88	1.46	ND	6.85
95-0816	7.87	6.95	6.26	2.23	1.74	0.36	6.02
96-0425	12.49						
96-0622	12.13						
98-0623	15.79	11.59	11.65	4.40	3.88	0.54	10.97
99-0816	5.59	4.51	4.01	1.48	1.18	ND	3.78
AG-0307	9.68	5.92	9.29	3.47	2.84		
AG-031407	9.84	5.47	8.46	ND	2.41	ND	5.81
AG-040407							
AG-0622	19.26	15.97		2.27	1.28		
AG-0829	24.87	23.86	23.54	13.71	7.89	1.83	22.89
AG-112905	7.14			2.05	1.27		
AG-52506	10.31	3.29	4.28	2.76	9.64	6.68	7.66
BW-040407	32.57	29.29	29.57	11.61	9.93	15.97	17.14
BW-052507	43.64	37.75		9.71	6.38	8.43	8.39

Table C-2. (cont'd)

Sample ID	C_T	C_F	C_I	C_{Hi}	C_{Ho}	C_H	C_X
BW-0622	19.83	16.84		3.09	2.37		
BW-0729-T	25.65	22.10	18.85	3.74	2.66		
BW-0829	22.33	20.97	20.43	5.83	4.49	6.63	11.57
BW-1031	66.61	59.18	47.84	28.77	20.52	23.64	40.20
BW-112905							
BW-112905	26.53			17.05	12.58		
BW-52506	16.12	14.14	13.86	4.09	2.98	0.02	14.39
BW-52506	28.91	26.25	25.78	6.40	4.49	4.94	13.15
CR-0615	9.50	7.76		2.26	7.05	ND	7.09
CS-080707	13.83	12.22	11.62	7.29	6.37	2.79	11.78
CSO-0615	10.79	8.25		3.26	2.81	ND	7.07
DC-0615	9.84	7.76	6.77	2.44	1.91	ND	6.79
DLM-0615	1.56	ND	ND	ND	ND	ND	ND
EE-080707	11.42	10.40	9.29	2.37	1.91	0.00	9.94
EE-0816	12.93	12.19	11.23	3.22	2.44	0.19	11.56
ERC-071907	23.74	18.94	17.88	11.04	9.52	0.75	18.43
FL-0307	9.34	7.04	7.92	1.67	1.82		
FL-0628	11.39	9.83	ND	2.53	8.20	ND	9.08
GC-0307	15.72	13.28	13.83	3.60	2.68		
GC-031407	12.87	12.22	11.21	4.01	3.32	1.70	11.58
GC-040407							
GC-052507	29.12	27.93	24.21	5.43	5.00	0.26	27.24
GC-0622	19.83	17.69		4.10	2.43		
GC-0628	23.61	18.86	20.02	6.30	5.68	9.39	20.27
GC-0710	19.80	18.60	17.30	4.94	4.10	2.15	17.94
GC-071907	30.21	29.16	26.57	6.44	6.08	1.20	28.30
GC-0729-T	22.97	20.22	22.16	5.41	3.38		
GC-0816	27.40	26.25	24.59	7.15	5.52	1.04	25.10
GC-1031	29.35	28.35	26.84	6.57	7.77	1.60	27.76
GC-112905	36.58			7.57	3.50		
GC-52506	23.25	21.59	20.43	4.68	3.90	0.53	21.34
GR-0615							
GRER-0711	13.42	12.87	11.88	3.07	2.77	0.79	12.20
GRFU-0615	9.67	9.10	7.05	2.24	2.27	ND	7.10

Table C-2. (cont'd)

Sample ID	C_T	C_F	C_I	C_{Hi}	C_{Ho}	C_H	C_X
GRGR-0808							
GRIO-0808							
GRJA-0711	10.81	10.12	9.34	2.51	1.75	0.24	9.61
GRLA-0808	9.34	8.67	7.99	2.48	2.22	0.46	8.11
GRPO-0808							
H131-0615	9.89	8.17	7.24	2.41	2.14	0.03	7.01
HHS-080707	11.04	9.93	9.17	ND	2.08	0.48	9.42
HR-0425	4.30	3.44	3.77	2.31	2.42	1.28	3.33
HR-0623	13.92	11.51	9.81	6.10	4.76	1.15	10.59
HR-0710	33.75	30.24	29.13	16.15	14.19	4.55	29.96
HR-080707	10.93	10.12	9.96	5.96	5.25	2.90	8.60
LGEA-0808	20.92	20.01	18.57		4.23	0.62	19.59
OF-22-0726	6.98	5.65	5.41	ND	2.89	0.90	5.13
OF22-0803-01							
OF37-0726	7.16	5.76	5.20	3.21	2.66	0.75	5.17
OF38-0425-1	5.33						
OF38-0425-2	7.92						
OF38-0628	5.62	3.80	5.55	1.83	1.76	0.15	3.31
OF38-0724-12	4.75	4.16	4.40	1.55	1.29	ND	3.57
OF38-0725	9.02	7.85	8.29	3.93	3.68	0.42	7.33
OF38-0725-5	0.08	0.02	0.03	0.01	ND	ND	ND
OF38-0725-A1	10.08	8.58	9.00	4.62	4.24	0.06	7.95
OF38-0802-14							
OF38-0803-01							
OF38-0803-A1							
OF38-0803-A2							
OF38-1107-1	5.31	4.72	4.73	1.49	1.33	0.12	3.98
OF38-1107-2	5.42	4.59	5.00	1.40	1.42	ND	3.81
OF38-1107-3	13.45	9.43	7.77	3.62	2.99	ND	9.10
OF38-1107-4	16.03	11.71	10.43	6.18	5.80	1.52	10.92
OF38-1107-5	12.24	9.23	5.21	4.49	3.84	0.45	8.32
OF38-1107-6	11.06	8.15	5.89	5.24	4.70	1.70	7.87
OF38-1107-7	12.90	8.70	8.15	6.77	5.61	0.95	8.13
OF38-1107-8	12.18	8.06	7.10	6.04	5.52	1.40	7.81

Table C-2. (cont'd)

Sample ID	C_T	C_F	C_I	C_{Hi}	C_{Ho}	C_H	C_X
OF38-1107-9	5.89	4.79	4.53	3.53	2.97	ND	3.91
OF38-1130-1	5.72	4.82	5.06	2.11	1.77	ND	4.46
OF38-1130-10	4.09	3.45	3.40	2.00	1.77	0.26	2.85
OF38-1130-11	6.89	5.48	5.18	3.62	3.31	1.03	4.86
OF38-1130-12	0.08						
OF38-1130-13	6.04	4.82	4.72	3.13	2.82	1.00	4.27
OF38-1130-13							
OF38-1130-15	6.82	5.28	5.01	3.54	3.23	0.76	4.60
OF38-1130-16	4.42	3.73	3.70	1.99	1.79	0.90	3.21
OF38-1130-17	6.91	5.58	5.15	3.53	3.20	0.77	4.86
OF38-1130-2	5.22	4.39	4.64	1.61	1.22	ND	3.82
OF38-1130-4	4.92	4.27	4.22	2.47	2.40	0.32	3.72
OF38-1130-5	6.93	5.56	5.28	3.59	3.30	0.86	4.89
OF38-1130-9	5.28	4.38	4.35	2.82	2.59	0.40	3.88
OF38-1130-A10	5.81	4.95	4.86	2.85	2.58	0.82	4.32
OF38-1130-A11	5.49	4.80	4.67	2.73	2.42	0.81	4.25
OF38-1130-A9	5.72						
OF38-1201-12-D							
OF38-1201-14							
OF38-1201-16							
OF38-1201-17							
OF38-1201-19							
OF38-1201-21							
OF38-1201-22							
OF38-1201-23							
OF38-1201-24							
OF38-1206	5.81						
OF38-1208-2	7.22						
OF38-1208-3	4.44	3.82	3.82	0.02	0.68	ND	3.17
OF38-1212-1	8.16						
OF38-1212-2	3.81						
OF38-619-0	4.50	3.94	3.76	1.28	1.20	0.20	3.37
OF38-619-1	8.06	6.90	7.02	2.79	2.50	0.34	6.47
OF38-619-13	8.98	7.78		5.11	5.02	0.96	

Table C-2. (cont'd)

Sample ID	C_T	C_F	C_I	C_{Hi}	C_{Ho}	C_H	C_X
OF38-619-14	10.66	9.28	9.06	6.77	6.24	1.37	8.58
OF38-619-15	12.77	11.15	10.89	8.05	7.08	1.59	10.61
OF38-619-16	12.57	11.07	11.08	8.10	7.53	1.63	10.73
OF38-619-17	12.70	11.33	11.11	8.03	7.44	1.58	10.86
OF38-619-18	11.58	10.22	9.99	7.41	6.56	1.52	9.91
OF38-619-19	12.99	11.59	11.27	8.31	7.43	1.55	11.31
OF38-619-2	17.49	11.38	10.49	8.30	8.05	3.57	10.93
OF38-619-20	13.30	11.66	11.59	8.39	7.73	1.74	11.18
OF38-619-25	12.80	11.41	11.30	8.33	7.52	1.57	11.01
OF38-619-5	9.03	6.47	6.17	3.83	3.48	0.93	5.96
OF38-619-6	6.65	4.71	4.69	3.17	2.95	1.10	4.38
OF38-619-8	5.90	4.29		3.34	3.10		
OF38-619-9	4.88	3.58	3.58	2.64	2.26	1.04	3.04
OF52-0726	10.27	8.65	8.17	4.45	4.07	1.43	7.90
OF53-0726	8.24	7.49	6.96	2.15	1.81	0.22	6.81
PL-0307	5.45	3.05	5.43	2.19	2.12		
PL-031407	4.00	3.36	3.19	2.03	1.63	0.85	2.81
PL-040407							
PL-052507	21.65	17.09	15.52	10.12	8.76	0.64	16.55
PL-0622	11.54	9.55		4.98	4.66		
PL-0628	3.83	ND	ND	2.08	ND	ND	ND
PL-0710	31.22	26.54	25.35	15.53	14.02	3.85	25.80
PL-0711	7.49	5.32	5.36	3.86	2.98	1.46	4.97
PL-071907	13.50	12.36	11.75	5.23	4.74	0.62	11.72
PL-0729-T	9.72	6.30	6.28	2.96	2.01		
PL-1031	6.37	5.24	5.09	2.34	2.02	0.38	4.74
PL-112905	5.36			2.85			
PL-52506	8.79	6.67	6.54	3.44	3.16	ND	6.39
RCMSU-0808							
RCR 0404	11.59	10.89	9.86	3.09	2.67		10.09
RCR-0226							
RCR-1005	9.19	8.67	7.78	2.47	2.11	0.11	8.06
RCWI-0808	9.20	8.67	7.63	2.09	1.96	0.20	7.84

Table C-2. (cont'd)

Sample ID	C_T	C_F	C_I	C_{Hi}	C_{Ho}	C_H	C_X
RRRO-0808							
SW-0829	13.88	13.20	12.02	4.11	3.38	1.28	12.53
U28-0615	9.60	8.16	7.06	0.36	1.56	ND	7.12