The Characterization of Dissolved Organic Matter and its Influence on the Photochemical Fate of Antibiotics used in Aquaculture

Dissertation

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Abstract

Antibiotics are a class of pharmaceuticals specifically engineered to kill pathogenic bacteria. In addition to medicinal applications for treating people, antibiotics are also used in a number of agricultural industries to prevent the spread of harmful and costly diseases. However, the increased prevalence of resistant strains of bacteria has raised concern about the environmental fate of such compounds, particularly once in the natural environment. For many antibiotics, little information is known about their breakdown or fate in natural waters.

This work investigated the photochemical transformation of two antibiotics commonly used in aquaculture (catfish), sulfadimethoxine and ormetoprim and the role that dissolved organic matter (DOM) composition plays on the fate of these compounds. Sulfadimethoxine degradation was found to be dependent on DOM source composition, and degraded via triplet excited state pathways. Ormetoprim was found to degrade through indirect pathways that included transformation via two reactive oxygen species (ROS) promoted by DOM - the hydroxyl radical and singlet oxygen.

The effects of source on dissolved organic matter composition were studied, and an intensive investigation was performed to characterize the photoreactivity of a fulvic acid isolated from Pony Lake on Cape Royds, Ross Island, Antarctica, a completely microbially-derived DOM end-member.
Dedication

In loving memory of Blanche Keeney Stephens
Acknowledgments

Many thanks are needed to all of the helpful people without whom this work would not have been possible. First to thank is my advisor, Yo Chin for all of his help, advice, expertise, and friendship. I would also like to thank my committee members, past and present, Diane McKnight, Linda Weavers, Christopher Hadad, and Steven Lower for their guidance and all of their help during the course of this work. Also thanks go to the Chin Research Lab, past and present.

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Fields of Study

Major Field: Environmental Sciences
Area of Emphasis: Aquatic Photochemistry
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Chapter 1: Introduction

1.1. Fate of Antibiotics in the Environment

Antibiotics are a class of pharmaceuticals specifically engineered to kill pathogenic bacteria. In addition to medicinal applications for treating people, antibiotics are also used in a number of agricultural industries in order to prevent the spread of costly and harmful diseases. However, the increased prevalence of resistant strains of bacteria has raised concern about the need to discern the fate of such compounds, particularly once in the natural environment. For many antibiotics, little information is known about their breakdown or fate in receiving waters.

The catfish industry is one of the largest freshwater aquaculture industries in the United States, and antibiotics are an industrial necessity to ensure survival of fish populations. Ponds on average are four to six feet deep, with $5,782 \pm 38$ fish stocked per acre of pond surface area (USDA, 2003). Sulfadimethoxine and ormetoprim are used in combination in a medicated feed mixture called Romet® in order to combat enteric septicemia of catfish (ESC) caused by *Edwardsiella ictaluri*. ESC affects more catfish operations than any other disease according to the USDA report (2003). In 2002, ESC affected 60.6% of all catfish operations, and 28.8% of all food size fish ponds (USDA, 2003). Currently Romet® is one of the main antibiotic feed mixtures approved for use by the FDA for the treatment of ESC. Medicated feed was fed to fish in 11% of food size fish operations, with an increasing proportion of use with larger-sized farms (USDA,
2003). Just under half (48%) of medicated feed use was Romet®, with an average use of six tons per acre when used (USDA, 2003). However, despite the widespread use of these antibiotic compounds, little is known about their fate in these ponds or the environment.

Both of these compounds have chromophoric functional groups that absorb photons present in natural sunlight. Boreen et al. (2005) showed that sulfadimethoxine is susceptible to direct photodegradation through absorption of light and excitation to a triplet state. Even less information is known about the fate or photolytic pathways of ormetoprim. In addition to direct photolysis, however, it may be possible for reactive intermediates promoted by other constituents in natural waters to break down these antibiotics. Such constituents may include nitrate, iron, and dissolved organic matter (DOM) (Figure 1.1). The study by Boreen et al. did not evaluate evidence for indirect photodegradation of sulfadimethoxine in their sample water, Lake Josephine, MN.

Dissolved organic matter is a complex, heterogeneous mixture of organic compounds that are ubiquitous to surface waters and can influence a number of biogeochemical processes in aquatic systems. The abundant chromophoric moieties in DOM are able to initiate a number of photochemical reactions of environmental significance when irradiated by light. These include the production of labile carbon for microbial activity (Moran and Zepp, 1997; Anesio et al., 2005; Kim et al., 2006), screening cell-damaging UV light in the water column (Zepp et al., 1998), and most pertinent to this study, mediating passive and active photochemical processes that can detoxify deleterious substances (Bushmann et al., 2005; Gereke et al., 2001; Lam and Mabury, 2005; Miller and Chin, 2002; Miller and Chin, 2005).
Sunlight irradiated DOM also produces reactive oxygen species (ROS) such as hydrogen peroxide (H$_2$O$_2$), singlet oxygen (\(^{1}\)O$_2$), superoxide (O$_2$•-), and the hydroxyl radical (OH•) (Blough and Zepp, 1995) as well as other phototransients, e.g., carbon centered radicals and excited triplet states of DOM. In many cases, these transients promote the indirect photo-degradation of organic contaminants (Dimou et al., 2005; Gerecke et al., 2001; Lam and Mabury, 2005; Miller and Chin 2005; Werner et al., 2005; Fisher et al., 2006; Halladja et al., 2007; plus many others). To further complicate matters, chromophoric DOM also retards contaminant photodegradation by screening reactive wavelengths of light (e.g., Boreen et al., 2004; Walse et al., 2004), and scavenging ROS (Brezonik and Fulkerson-Brekken, 1998) and other photo-generated reactive species such as triplet DOM (\(^{3}\)DOM) (Canonica and Laubscher, 2008).

### 1.2. Source influence on DOM composition

Chemical analysis of DOM reveals significant compositional variability among DOM of different sources (for examples, see Table 1.1). In aquatic systems, DOM's composition is determined by the relative contribution, transformation and loss processes from its precursor materials. These include both terrestrially and microbially-derived organic matter. Carbon inputs from higher plants containing lignin and tannins dominate the terrestrial DOM chemical signature, whereas microbial DOM is derived from the cellular excretions of phytoplankton and bacteria and the turnover of microbial biomass (McKnight et al., 1997). Because of these source differences, DOM end members bear significantly distinct chemical signatures as determined by a variety of analytical techniques such as \(^{13}\)C-NMR (Dria et al., 2002; Kaiser et al., 2003; Mopper et al., 2007), fluorescence (Coble 1996; Cory and McKnight, 2005; McKnight et al., 2001; Schwede-
Thomas et al., 2005; Stedmon and Markager, 2005) and absorbance spectroscopy (Del Vecchio and Blough, 2002; Green and Blough, 1994; Stedmon and Markager, 2001; Weishaar et al., 2003), high-pressure size exclusion chromatography (HPSEC) (Chin et al., 1994; Her et al., 2003), mass spectrometry (Kim et al., 2006; Reemtsma and These, 2005; Reemtsma et al., 2006) and thermochemolysis (Fimmen et al., 2007). Terrestrial DOM has more aromatic moieties (Chin et al., 1994) and lower nitrogen and sulfur functional groups (McKnight et al., 1991; McKnight et al., 1994; Mash et al., 2004) than autochthonous DOM. Finally, terrestrial DOM has a higher molecular weight (both number and weight average) distribution than microbially-derived DOM (Chin et al., 1994; Chin et al., 1997). With the exception of special aquatic systems, e.g., “black water” environments that are mainly comprised of terrestrial materials, DOM from typical surface waters bears chemical characteristics that fall between these two end members.

Differences in chemical composition invariably affect DOM photochemical function. Compositional effects on photo-physical behavior are well documented. For example, the higher aromatic content in terrestrially-derived DOM allows it to absorb more light per unit carbon resulting in a larger molar absorptivity ($\varepsilon$) or specific UV absorbance (SUVA) compared to microbially-derived DOM (Chin et al., 1994; McKnight et al., 2001). Composition also affects DOM fluorescence. For example, the magnitude of the fluorescence index (FI) (the ratio of corrected emission at 470 nm: 520 nm at an excitation of 370 nm) is inversely correlated to the DOM aromatic content and can be used to delineate whether its organic composition is derived predominantly from phytoplankton and bacteria or higher plants (McKnight et al., 2001). Compositional
differences in DOM may also manifest itself in its photochemical reactivity (Meunier et al., 2005), and thus the fate of a compound in sunlit surface water may be dependent to some extent on the source material of the DOM. For example, White (2000) reported that OH• production rates were consistently higher for terrestrially-derived DOM relative to autochthonous DOM isolated from Antarctica. Such work suggests differences in DOM source contribution may affect its photochemical reactivity in sunlit surface waters however, to date, a systematic study that examines the role of DOM composition on photosensitized reactions is lacking.

1.3. Research Objectives

The research I conducted had three main objectives. The first was to characterize the photoreactivity of dissolved organic matter from an environment devoid of higher plants (Pony Lake at Cape Royds on Ross Island, Antarctica). Most studies to date utilize fulvic acid (FA) derived from predominantly terrestrial sources e.g., Suwannee River FA, because a microbial end-member of fulvic acid had not been previously widely available to scientists in large amounts. The fulvic acid fraction of Pony Lake DOM was isolated and characterized through a variety of analytical methods. Photobleaching experiments in both whole water and isolated DOM solutions were conducted to determine the influence and production of reactive oxygen species, their effect on redox state, as well as the effect of salts on photobleaching of the DOM. These data were then related back to field observations to analyze the biogeochemical importance of the photobleaching characteristics in this unique aquatic system.

The second objective was to investigate the indirect and direct pathways of photodegradation of the two antibiotics sulfadimethoxine (SDM) and ormetoprim (OMP).
Previous work showed that direct photolysis dominates the pathway for sulfadimethoxine (Boreen et al., 2005), but these investigators only studied one water sample. DOM from other waters may be able to enhance SDM photodegradation. Finally, little is known about the photochemical reactivity of ormetoprim or its environmental fate in aquatic systems. I conducted solar simulator experiments using a variety of chemical probes in order to investigate the importance of reactive oxygen species and elucidate possible mechanisms for indirect photolytic degradation in catfish pond and natural waters.

Fulvic acids were used and chosen to represent a spectrum of DOM sources: 1) Suwannee River fulvic acid, as a terrestrial end member; 2) Pony Lake fulvic acid (refer to Objective 1), as an microbial DOM end-member from surface waters devoid of any higher plants in Antarctica; and 3) Old Woman Creek a coastal wetland located on the south shore of Lake Erie in northern Ohio, whose DOM is derived from mostly autochthonous materials (Table 1.1).

The third objective was to characterize the organic matter present within catfish ponds and nearby surface waters, in order to understand how these compounds would behave in a natural setting. Fulvic acid from catfish pond waters and a nearby stream (Deer Creek) at the USDA Delta Research and Extension Center in Stoneville, MS were isolated and characterized. Photodegradation of both sulfadimethoxine and ormetoprim was studied in the presence of solutions containing these fulvic acids to ascertain an environmentally relevant estimate of the photofate of these compounds within these systems.
Evaluating the differences in the contaminants’ susceptibility to direct and indirect photochemical reactions in the presence of these select fulvic acids will provide a basis to posit how source composition and organic matter within catfish ponds mediate contaminant photodegradation.
1.4. Tables

Table 1.1. Elemental analysis and $^{13}$C-NMR data for selected fulvic acid samples. Suwannee River Fulvic Acid (SRFA); Pony Lake Fulvic Acid (PLFA); Old Woman Creek Fulvic Acid (OWCFA).

1The spectral regions for the $^{13}$C-NMR signature defined according to Dria and co-workers (2002). The values listed represent the integral of specified ppm region normalized to the total integrated area and are reported as a percent. 2Solution-state $^{13}$C-NMR data from IHSS (Thorn et al, 1989). 3Solid-state CPMAS $^{13}$C-NMR data from IHSS; Elemental Analysis from R.M. Cory (personal communication). 4 Solid-state $^{13}$C NMR spectra for Old Woman Creek fulvic acid isolate were acquired in our Laboratory by R.L. Fimmen using methods described in Fimmen et al. (2007).

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<td>PLFA$^3$ (Microbial)</td>
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Chapter 2: Kinetics of Photochemical Transformation of an Antarctic Fulvic Acid: 
Mechanism and Biogeochemical Implications

2.1. Introduction

Dissolved organic matter (DOM) is a ubiquitous component of both lacustrine and marine surface waters. In most environments, DOM is a complex heterogeneous mixture of organic material typically derived from a combination of both plant/soil and aquatic microbial sources, particularly in mid-latitude regions. DOM may participate in several aquatic biogeochemical processes in surface waters that can influence a number of factors in the aquatic environment such as a nutrient source for microorganisms (Kaiser et al., 2004; Kim et al., 2006), a screening mechanism of UV light in aquatic systems (Zepp et al., 1998), and a photochemical sensitizer in surface waters (Gao and Zepp, 1998). Ramifications of DOM photochemical processes can profoundly alter the chemical state of redox-active constituents in water such as nitrate, iron and other metals (Zepp et al., 1985; Gabrell et al., 2003; Buschmann et al., 2005; Meunier et al., 2005; McKnight and Duren, 2004). As a photosensitizer, DOM has been implicated in promoting degradation of organic pollutants that have potentially deleterious ecosystem effects (Gerecke et al., 2001; Dimou et al., 2005; Lam and Mabury, 2005; Miller and Chin, 2005; Werner et al., 2005).

Environmental reactivity of aquatic DOM is highly source dependent, broadly encompassing a range of terrestrial to microbial sources. Differences in DOM
contrasted by different original source material have been differentiated through a variety of techniques: NMR (Dria et al., 2002; Kaiser et al., 2003; Mopper et al., 2007), fluorescence (Coble, 1996; McKnight et al., 2001; Cory and McKnight, 2005; Stedmon and Markager, 2005), absorbance (Green and Blough, 1994; Stedmon and Markager, 2001; Del Vecchio and Blough, 2002; Weishaar et al., 2003), high-pressure size exclusion chromatography (HPSEC) (Chin et al., 1994; Her et al., 2003), thermochemolysis (Fimmen et al., 2007) and many others. These differences show that terrestrial DOM derived from higher plants containing lignin and tannins tend to have higher aromaticity and lower amounts of nitrogen and sulfur. Furthermore, terrestrial DOM tends to absorb more light per unit carbon (greater molar absorptivity) compared to microbially-derived DOM (Chin et al., 1994). Conversely, microbial DOM is considered to be derived from cellular excretions and the turnover of microbial biomass, and as a result tends to have lower aromaticity, higher nitrogen and sulfur content (McKnight et al., 1991; McKnight et al., 1994; Mash et al., 2004), and a lower average molecular weight (Chin et al., 1994; Chin et al., 1997).

In sunlit waters, DOM undergoes several molecular-scale transformations as a consequence of photo-transformation. Irradiation of DOM has been shown to alter DOM’s chromophoric character (Bertillon and Tranvik, 2000; Del Vecchio and Blough, 2002; Brinkmann et al., 2003) and size distribution (Brinkmann et al., 2003), and has been shown to affect bioavailability (Wetzel et al., 1995; Moran and Zepp, 1997; Grzybowski, 2002; Scully et al., 2003; Kaiser and Sulzberger, 2004; Rosenstock et al., 2005). The mechanism of photochemical change in DOM can be direct, where the light absorbed breaks bonds to transform DOM, or indirect, where photochemically generated
intermediates such as reactive oxygen species (ROS) can promote DOM transformation. Photo-irradiated DOM is a source of the hydroxyl radical (Vaughan and Blough, 1998; Qian et al., 2001) and other ROS species (e.g., $^1$O$_2$ and H$_2$O$_2$) (Haag and Hoigne, 1986; Latch and McNeill, 2007; Yocis et al., 2000). Furthermore, certain DOM components are susceptible to attack by hydroxyl radical and other ROS species (Goldstone et al., 2002; Molot et al., 2005).

Photochemical reactivity of bulk DOM in surface waters can be experimentally ascertained using isolation methods that intrinsically capture up to 90% of the hydrophobic and chromophoric fraction (Leenheer, 1981; Thurman and Malcolm, 1981). Suwannee River fulvic acid (SRFA), an isolate of DOM from Suwannee River, Georgia, has been used as a terrestrially-derived fulvic acid reference standard, and consequently its chemical properties have been well studied. SRFA is derived from predominantly higher plant precursors and represents a terrestrial end-member of the spectrum of DOM source material. It is characterized by traits associated with organic compounds derived from higher plants, including: high molecular weight (Chin et al., 1994; Chin et al., 1997), high absorbance, lignin content, and aromaticity (McKnight et al., 1994). Not all DOM from surface waters can be approximated using terrestrially-derived material. Indeed, DOM from marine sources is derived from phytoplankton and bacteria. Hence there is a need for a microbial reference standard of fulvic acid that is not influenced by terrestrial sources of DOM. One such place is Pony Lake, a unique hypereutrophic system on Cape Royds, Ross Island, Antarctica, whose DOM is derived solely from microbial sources.
In nearly all ecosystems in temperate regions, DOM of surface waters is composed of a combination of terrestrial and microbial DOM. Understanding the photochemical nature of an exclusively microbially-derived DOM can help us to discern microbial-DOM photochemical function and reactivity and its effects on the biogeochemistry of natural waters. It is important to understand the stability of the photochemically reactive fraction of DOM and how it relates to the overall carbon lability and turnover in this unique ecosystem. Thus, we set out to use a variety of methods and processes to assess the chemical nature of photobleaching of DOM from a purely microbial source. Our objectives were to 1) characterize the kinetics of photobleaching of Pony Lake DOM, 2) assess the influence of reactive oxygen species on the photobleaching of Pony Lake DOM, and 3) connect these results to observations in the field to understand how this microbial DOM can affect the biogeochemistry of Pony Lake.

2.2. Materials and Methods

2.2.1. Study Site and Sample Collection

Pony Lake is a stable hypereutrophic saline lake located at Cape Royds (77° 33’S, 166°E) on Ross Island, and is the site of Shackleton’s historic Nimrod Hut as well as home to an Adelie penguin rookery. The pond has been previously described by Brown et al. (2004) and McKnight et al. (1994), and is a shallow lake devoid of higher plants; thus dissolved organic matter within the lake is derived solely from microbial sources. During most of the year, Pony Lake is frozen; however, during the summer months (Dec-Feb), the lake thaws and the planktonic microbial community becomes extremely active, as evidenced by the high dissolved oxygen levels from photosynthetic processes.
Samples from Pony Lake were collected throughout the summer seasons (2004-2005; 2005-2006) in acid-washed and DI-rinsed plastic carboys and brought back to Crary Laboratory, where they were stored in the dark at 4°C. Samples were filtered with 0.45 µm filter or Balston® filtered to 0.5 µm. Experiments were conducted in two locations – outdoor experiments in Antarctic sunlight were performed in the Crary Laboratory at McMurdo Station on Ross Island, Antarctica (Crary Lab) and solar simulator experiments were performed at the Ohio State University in Columbus, Ohio, USA (OSU).

2.2.2. Chemicals/Reagents

Solutions were prepared in 18 MΩ Milli-Q purified water (Millipore). HPLC grade methanol and acetonitrile and analytical grade hydrochloric acid, acetone, sodium hydroxide, sodium chloride, potassium phosphate (monobasic), reduced glutathione, p-hydroxyphenylacetic acid (POHPAA) which was then recrystallized in our Laboratory, tris(hydroxymethyl)aminomethane (Tris), catalase, and sodium sulfate (dibasic, anhydrous) were obtained from Fisher Scientific (Tustin, CA). Sodium phosphate (dibasic) was obtained from J.T. Baker. 30% Hydrogen peroxide, formaldehyde and sodium nitrate were obtained from Malinckrodt. 99.9%+ Pyridine, 97% p-nitroanisole, mercury (ACS Reagent-grade, 99.9995%), L-cysteine (98%), horseradish peroxidase (Sigma Type IV), and reagent grade (97%) 2,4-dinitrophenylhydrazine (DNPH) were obtained from Sigma Aldrich (Dallas, TX). Polystyrene sulfonate (sodium salt) molecular weight standards of 1.8, 4.6, 8.0, 18.0 kDa were obtained from Polysciences, Inc. (Warrington, PA). Argon gas was obtained from Praxair (North Royalton, OH).
2.2.3. Dissolved Organic Matter Isolation and Characterization

71 g of fulvic acid were isolated from 14,220 L of filtered Pony Lake water using XAD-8 resin (polymethyl methacrylate, originally purchased from Rohm and Haas but has since been discontinued) according to the procedure described by Thurman and Malcolm (1981) and Leenheer (1981). During the multi-day long process during which water was sampled, water chemistries (dissolved organic carbon content, specific UV absorbance (SUVA), pH, conductivity, dissolved oxygen, anions/cations/nutrient analysis, phytoplankton and bacterial counts) were measured on lake water samples to monitor conditions of water and evaluate possible changes in the chemical properties of Pony Lake over the time course of our sampling PLWW. The percent of total DOC captured as XAD-8 isolate was 16.5%. Old Woman Creek fulvic acid (OWCFA) was isolated according to Thurman and Malcolm (1981). Suwannee River fulvic acid (SRFA) was obtained from the International Humic Substances Society (IHSS).

2.2.4. Instrumental Analyses

**DOC:**

Dissolved organic carbon (DOC) was measured on a Shimadzu TOC 5000. Potassium hydrogen phthalate (KHP) was used as the standard for all DOC measurements.

**UV/Vis:**

Absorbance scans were obtained on either a Shimadzu UV-1601PC spectrophotometer at McMurdo Station or a Varian Cary 1 UV-Vis spectrophotometer at OSU. 1 cm path length matched quartz cuvettes were used to hold sample and reference solution (MilliQ) and scans were run from 200-600 nm with a lamp change at 350 nm
and blank subtracted. SUVA values were calculated by normalizing absorbance at 254 nm to concentration of carbon (Chin et al., 1994). Slopes of absorbance curves (S-values) over the range of 290-400 nm were computed using a non-linear least squares regression fitting of a first order model to the absorbance curve as developed by Stedmon and Markager (2001), according to the equation: $a_{\lambda} = a_{\lambda_0}e^{S(\lambda_0-\lambda)}$. 95 percent confidence uncertainty in S-values using triplicate analysis was determined to be 3 percent.

**Fluorescence:**

Fluorescence spectroscopy was conducted on either a Jobin Yvon Fluoromax3 or a Varian Cary Eclipse. Excitation-emission matrices (EEMs) were performed on diluted samples so that absorbance at 254 nm was less than 0.05 (Lackowicz, 1988) using the dimensions $\lambda_{\text{ex}}= 240$-450 nm (5 nm increments) and $\lambda_{\text{em}}= 300$-600 nm (2 nm increments). All EEMs were blank subtracted, corrected using correction files specific to each instrument, and normalized to Raman areas of DI water using a $\lambda_{\text{ex}}=350$ nm. Fluorescence index values are calculated as the ratio of corrected fluorescence values at emission wavelengths 520 nm / 470 nm at $\lambda_{\text{ex}}=370$ nm (McKnight et al., 2001). EEMs run on the Fluoromax 3 were then re-dimensioned to $\lambda_{\text{ex}}=250$-400 nm and $\lambda_{\text{em}}=350$-550 nm and fitted to a 13 component PARAFAC model (Cory and McKnight, 2005) using Matlab (7.0.1) and the nway toolbox. Cory and McKnight (2005) have identified some of these thirteen components, whereby components 2, 11 and 12 were identified as quinone-like, components 5, 7 and 9 were identified as semiquinone-like, and component 4 was identified as hydroquinone-like. In addition, component 8 was identified as tryptophan-like and component 13 was identified as tyrosine-like. These component
descriptions were used in the assessment of fitting the Pony Lake EEMs to the 13-component model. Fluorescence loadings of each component were then normalized to total fluorescence loadings to compute the percent of fluorescence loading in each sample attributed to each of the 13 components, for a total of 100 percent. PARAFAC component analysis of the EEMs run on the Varian Cary Eclipse was not performed because PARAFAC models cannot be appropriately applied across instruments (Cory et al., 2009) and the model was made with EEMs obtained using the Fluoromax.

**Electrochemical Analysis:**

All electrochemical analyses were performed using a Metrohm Computrace 797 interfaced with windows-compatible software (Brinkmann Instruments, software v1.2) with the help and assistance of Dr. Ryan Fimmen. Analyses were conducted with a 3-electrode system, with a hanging mercury drop electrode as the working electrode, an Ag/AgCl reference electrode and a Pt-wire auxiliary electrode. Electrochemical scans of fulvic acid solutions were necessarily acquired in the absence of oxygen using a 10 min initial purge (for approximately 10 ml sample, i.e. 1 min ml⁻¹). Scans were acquired in quiescent solution using a differential pulse waveform, measuring the current generated as a function of applied voltage, using a scan window from -0.1 to -1.9 V. The mercury capillary was pressurized to 15 psi with argon gas. Potentials were applied from −0.1 V to −1.9 V with a 10 s equilibration time, 0.05 V s⁻¹ sweep rate (voltage step = 0.01 V, voltage step time = 0.200 s), pulse amplitude of 0.05 V and pulse time of 0.04 s. All solutions were measured in a cell fitted with an inert air supply that maintained anoxic conditions. Reduced sulfur peak potential was confirmed with scans of L-cysteine and reduced glutathione, and concentrations in the fulvic acid isolate were determined by
preparation of known concentrations of L-cysteine at pH 9.55, and using a linear fit of current vs. concentration.

2.2.5. Photochemical Experiments

**Outdoor Experiments in Antarctic Sunlight:**

The objective of these experiments were to analyze the changes in the quality of Pony Lake DOM (whole water and fulvic acid) with exposure to sunlight. Fulvic acid was reconstituted to form 10 mg-C L$^{-1}$ solutions and brought up to the approximate pH of Pony Lake (9.5) using NaOH. Samples were filled into quartz tubes and placed on a black angled rack on the roof of the Crary Laboratory and was rotated periodically (every 1-2 hours) to maximize exposure to sunlight. Photolyses were attempted as weather permitted, to see the effect of full/mostly full sunlight over 24 hours when outside temperatures were high enough to prevent sample freezing. A temperature probe was placed in a quartz tube filled with MilliQ water to measure temperature of samples during photolysis. Radiometers collecting UV and PAR data were also installed on the photolysis rack to measure the amount of light that the samples were exposed to during photolysis. Actinometry was also conducted over the course of each photolysis using the $p$-nitroanisole/pyridine method as described by Dulin & Mill (1982). At specific time points, samples were collected and analyzed immediately for electrochemistry, and analyzed within a 24-hour period for UV/Vis absorbance, fluorescence, and HPSEC. Between photolysis and analysis, samples were stored at 4 °C in the dark to preserve the content of the sample.
**In Situ Monitoring of Pony Lake:**

Water chemistry in Pony Lake was monitored *in-situ* over a 26-hour period to analyze the diurnal characteristics of the water chemistry and the DOM. Every hour samples were collected for UV/Vis and EEMs, and pH, DO, conductivity, temperature, and salinity were measured. A UV (Kippen and Zonen, CUV3) and PAR (Licor 1000) radiometer recorded readings every minute over the 26-hour period.

**Solar Simulator Experiments:**

Filtered water was acidified to pH 2 with HCl and shipped back under refrigerated conditions to OSU. The pH of the samples was adjusted back up to 9.5 using NaOH and filled into quartz tubes. An Atlas Suntest CPS+ solar simulator was used in place of natural sunlight. A radiometer was used to check stability of UV output during the course of photolysis and photochemical actinometry was performed in order to compare observed rate coefficients between experiments. At specific time points, samples were collected and analyzed immediately for electrochemistry, UV/Vis absorbance, and fluorescence.

**High Oxygen Level Experiments:**

Samples were bubbled with oxygen in a temperature controlled water bath in order to replicate the high DO levels detected in Pony Lake. Since ionic strength is necessary for electrochemical analysis, fulvic acid samples were photolyzed in the presence of ambient Cl\(^-\) and SO\(_4^{2-}\) concentrations as were measured via ion chromatography at Crary Laboratory. Samples were quickly filled into quartz tubes, which were determined to be airtight over 3 days. Immediately after filling and sealing the tubes, 12-hour photolyses were performed.
Low Oxygen Level Experiments:

Photochemical experiments were run in the presence of low oxygen in order to assess the effect of ROS on DOM photobleaching. In the absence of an inert-atmosphere glove box, samples were purged of oxygen by sparging with argon for 1 min mL\(^{-1}\), and transferred by cannulae into quartz tubes. When taking samples for analysis, argon was blown over samples and they were again transferred by cannulae into argon filled 8 mL amber vials and stored with an argon headspace between analyses. These experiments were carried out at Crary Laboratory during the austral summer and then repeated again using a solar simulator and differences in light intensity were corrected using chemical actinometry. Samples stored under argon were analyzed as soon as possible to minimize effects of oxygen.

Measurement of \(^{·}\)OH Production:

Hydroxyl radical production rates and steady state concentrations were measured using the DNPH method outlined by Grannas et al. (2005). DNPH was purified via recrystallization before use. Experiments to measure hydroxyl radical production rates and steady state concentrations were conducted in both Antarctic sunlight and in a solar simulator with whole water and fulvic acid samples.

Measurement of \(\text{H}_2\text{O}_2\) Production:

A fluorescence assay (Miller and Kester, 1988) was adapted to measure hydrogen peroxide production rates in fulvic acid solutions using a standard additions method. Irradiated fulvic acid solutions were spiked with a buffered (0.25 M Tris, pH 8.8) fluorescent reagent containing 0.255 mM recrystallized POHPAA and 0.16 mg mL\(^{-1}\)
horseradish peroxidase. The resultant fluorescent dimer was detected at $\lambda_{\text{excit}}$=313 nm; $\lambda_{\text{emit}}$ = 402 nm using a method of standard addition (Cary Eclipse, Varian Instruments).

**Effect of salts and nitrate on PLFA photobleaching:**

287 µM sodium nitrate was added to fulvic acid solutions to mimic actual nitrate concentrations in Pony Lake. Likewise, a salt solution of 0.047 M NaCl and 0.010 M Na$_2$SO$_4$ was added to fulvic acid solutions to mimic actual salt concentrations in Pony Lake, and one set had both salts and nitrate added. These were conducted in Antarctic sunlight.

**2.3. Results**

Pony Lake has a short active season during which the ice-cover begins to melt, thawing in mid to late December (near Dec. 15, 2004 and Dec. 30, 2005), giving the lake approximately two months of highly active biological productivity. Owing to the proximity of the lake to the Ross Sea, as well as an adjacent penguin colony, Pony Lake is brackish having elevated levels of chloride, nitrate and sulfate relative to most inland fresh-water lakes (Table 2.1). The high primary productivity of the lake is evidenced by the high dissolved oxygen content and high pH measured in the lake.

The fulvic acid isolate comprised 16.5% of the total DOM in Pony Lake water. The remainder of the DOM is hydrophilic and therefore not retained by the non-ionic resin-based isolation technique. While PLFA is just a fraction of the total carbon available, this fraction is the most hydrophobic, containing most of the sp2-hybridized components and therefore the most colored fraction of DOM. The fulvic acid fraction is considered to be largely responsible for the photochemically reactive properties of the bulk DOM. For this reason, I compared photoreactivity and kinetics of photolysis
between whole water samples of Pony Lake whole-water (PLWW) and the fulvic acid isolate (PLFA) to improve our understanding of the dissolved organic matter contribution to the photochemical characteristics of the whole water.

2.3.1. Photobleaching Characterization

Absorbance scans of PLFA and PLWW are similar to previous absorbance profiles of natural water samples and DOM isolates (Del Vecchio and Blough, 2004), showing a nearly exponential decrease in absorbance with increasing wavelength. Absorbance scans show a consistent decrease in absorbance across all wavelengths with increasing time of photolysis (Figure 2.1). A result of decreasing absorbance with increasing exposure to light revealed that the absorbance curves become steeper during photolysis. During photolysis of both PLWW and PLFA, calculated slopes of absorbance curves, S-values, increased with time, indicating decreased overall light absorption at longer wavelengths compared to shorter wavelengths. S-values of PLWW before photolysis was $0.0189 \pm 0.0005$, and over the course of 24 hours the S-value increased to $0.0213 \pm 0.0006$. The S-value of PLFA before photolysis was $0.0177 \pm 0.0005$, and over the course of 24 hours the S-value increased to $0.0223 \pm 0.0007$.

Absorbance values at each wavelength were fitted to a first order model to find observed rate coefficients of absorbance decrease (Figure 2.2). The kinetics of PLWW showed a relatively smooth increase in observed coefficient with increasing wavelength, indicating that chromophores absorbing at higher wavelengths are destroyed at a faster rate than at lower wavelengths. Results of PLFA photobleaching, however, are described by coefficients that do not increase uniformly with increasing wavelength. There is a maximum rate of observed absorbance decrease at approximately 330 nm.
Complete fluorescence scans (excitation-emission matrices, or EEMs) of both PLWW and PLFA were acquired to assess total fluorescence, fluorescence index (FI), and for PARAFAC analysis (Figure 2.3). Whole water had a significantly higher FI value, $\sim 1.77$, where the fulvic acid had a much lower FI value, $\sim 1.45$ (Table 2.1). Total fluorescence of all samples decreased with increasing photolysis time in much the same manner that total absorbance decreased with photolysis time. However, the calculated FI values stayed relatively stable with light exposure. For PLWW, FI changed from 1.75 to 1.79 after 24 hours of photolysis, and for PLFA, FI changed from 1.45 to 1.43 after 24 hours of photolysis. Because FI remained fairly stable during photolysis, this index could not be used to assess any changes in the properties of DOM from exposure to sunlight.

Fitting of EEMs to the 13-component PARAFAC model revealed differences in the relative distribution of fluorophores between fulvic acid and whole water samples (Table 2). Whole water had a lower relative percentage of the hydroquinone (HQ) and semi-quinone (SQ1) components (4 and 5), and higher observed relative abundances of the Tryptophan, quinone (Q3) and Tyrosine components (8, 12, and 13). Differences were also noted for unidentified components 3 and 10, where relative composition in whole water was higher than its fulvic acid for component 3 and lower for component 10. Other component distributions showed no statistical difference between fulvic acid and whole water samples.

Changes in the relative abundance of these components over the course of a photolysis were observed in direct Antarctic sunlight. Although total sample fluorescence diminished in the PLFA photolysis, the relative distributions of fluorescent components remain relatively unchanged (Figure 2.4). In PLWW, a great deal of
variability in distribution of fluorescence components was observed during the first 4 hours of photolysis, after which components remained relatively unchanged during the remainder of the experiment and ended at distributions comparable to initial values.

HPSEC was used to analyze molecular size distribution of PLFA over the course of photolysis (Table 2.3, Figure 2.5). PLFA was measured to have a weighted average molecular size of 2507 Daltons. Over 12 hours of exposure to Antarctic sunlight, number averaged size ($M_n$) decreased by 30 percent, and weighted averaged size ($M_w$) decreased by only 10 percent. Polydispersity, the ratio of $M_w/M_n$, decreased by only 3.6 percent.

2.3.2. Changes in Fulvic Acid Redox Chemistry During Photolysis

The samples of fulvic acid from Pony Lake have a relatively high concentration of organic sulfur (Table 2.1), where approximately 59% of this is known to be in reduced forms (Fimmen et al. 2007). In simple systems containing only the fulvic acid and background electrolyte (to mimic the natural system), rates of organic sulfur destruction were monitored parallel to quantification fluorescence peaks in EEMs of photolysis samples. I chose to focus on peaks where maximum fluorescence occurs, i.e., in the protein region (Peak C), $\lambda_{ex}= 270$ nm $\lambda_{em}= 330$ nm, the humic region (Peak B), $\lambda_{ex}= 330$ nm $\lambda_{em}= 440$ nm, and overall fluorescence in the EEM (Peak A), $\lambda_{ex}= 240$ nm $\lambda_{em}= 450$ nm (Figure 2.6). Correlation with measured reduced-S concentration shows a positive correlation with maximum fluorescence peak A and humic region peak B, with $R^2$ values of 0.88 and 0.91 respectively (Figure 2.6).

2.3.3. Mechanisms of Photobleaching: Importance of ROS

Samples of PLWW and PLFA were photolyzed in the absence and under both ambient concentrations and elevated levels of dissolved oxygen in order to assess overall
effect of photochemically generated reactive oxygen species on DOM photobleaching (Figure 2.7). A plot of the absorbance decay kinetics at each wavelength for samples at low levels of oxygen show differences in the destruction of chromophores compared to photolyses at ambient oxygen levels. Calculated observed decay coefficients at each wavelength were slower in the absence of dissolved oxygen (Table 2.4). This was true for irradiation of both PLFA and PLWW, though with PLWW the difference in $k_{obs}$ was more uniform across all wavelengths, whereas differences in $k_{obs}$ varied with wavelength in PLFA.

By specifically considering photo-bleaching kinetics at one wavelength (320 nm), the influence of ROS can be determined by evaluating degradation kinetics in the presence and absence of oxygen. For PLWW, the percent of photobleaching attributable to ROS is roughly 30% in Antarctic sunlight and 14% for the fulvic acid. Not surprisingly, the role of ROS in photobleaching increased when irradiated with artificial sunlight. The photobleaching that could be attributed to ROS in simulated sunlight for PLWW was 44% and 57% for PLFA. EEMs and FI values were also measured during the course of photolysis, but no significant differences between experiments with ambient or low levels of oxygen were observed.

Samples were also irradiated in oxygen-saturated conditions to mimic oxygen levels found naturally in Pony Lake. At high oxygen conditions, PLFA samples (in a background of salts that replicates levels in the whole water) showed increased absorbance decay during photolysis compared to decay at ambient oxygen levels. The observed rate coefficient ($k_{obs}$) at ambient oxygen levels for PLFA was 0.0551 hr$^{-1}$ and at high oxygen levels was 0.0737 hr$^{-1}$. High oxygen levels appeared to have a different
effect on the photobleaching of PLWW. Observed decay rate coefficients at 320 nm at high oxygen levels were statistically the same as at ambient oxygen levels for PLWW, possibly due to the presence of salts.

To see whether hydroxyl radical had any potential to be an important ROS species in the photochemical transformation of Pony Lake organic matter, nitrate was added to fulvic acid solutions to a level found in the whole water (287 µM) (Figure 2.8). Nitrate produces hydroxyl radical in the presence of UV light (Zepp et al., 1987). Addition of nitrate increased the observed absorbance decay coefficient at nearly all wavelengths, and increased this value by 30%. However, when the other salts (NaCl, Na₂SO₄) also found in PLWW were added to PLFA solutions along with nitrate, observed decay coefficients were the same as without any nitrate addition. The salts had a quenching effect on OH⁻• attack on the organic matter and slowed the decay rate back down to levels without added nitrate.

Hydroxyl radical production rates and steady state concentrations were measured in both PLFA and PLWW during photolysis in a solar simulator using the DNPH method, but had very high uncertainties. The production rate of PLFA was $3.86 \times 10^{-7} \pm 1.53 \times 10^{-6} \text{ M hr}^{-1}$ and the $[\text{OH}^-\text{]ss}$ was $3.94 \times 10^{-16} \pm 1.56 \times 10^{-15} \text{ M}$. The production rate for PLWW was $1.33 \times 10^{-6} \pm 7.55 \times 10^{-6} \text{ M hr}^{-1}$ and the $[\text{OH}^-\text{]ss}$ was $1.35 \times 10^{-15} \pm 7.70 \times 10^{-15} \text{ M}$. These are rather low values compared to those cited in literature, by about an order of magnitude (Grannas et al. 2005). This makes the difference in OH⁻• production statistically similar between PLFA and PLWW. It is worthy to note that error values
were considerably high when running these experiments owing in part to the extremely low steady state concentrations of hydroxyl radical.

\[ \text{H}_2\text{O}_2 \]

production rates in SRFA were higher than those of PLFA or OWCFA, and produced 17 µM of \( \text{H}_2\text{O}_2 \) over the course of six hours (Figure 2.9). The initial production rate of hydrogen peroxide (measured within the first hour) was at least 50 percent larger for SRFA than those for PLFA and OWCFA, which were statistically indistinguishable from each other (Table 2.5).

2.3.4. Field Observations/Results

Prior to thaw, the carbon content of frozen Pony Lake was mainly concentrated in the bottom centimeters of ice and was considerably high, reaching DOC levels as high as 135 mg-C L\(^{-1}\) within the ice cores. As the lake thawed, photosynthetic production in the lake increased, which caused the DOC levels in Pony Lake to gradually increase until it reached a maximum of 29 mg-C L\(^{-1}\) on Jan. 18\(^{th}\) 2006, about 3 weeks after thaw. Subsequently, DOC declined, either as a result of decreasing sunlight intensity leading to diminishing algal growth or further dilution from snow melt runoff entering the lake or a combination of these factors. Given that the lake is fully or partially ice-free for approximately 2 months, this 10-day period captured over 15% of the production season.

SUVA values were calculated using absorbance at 254 nm and DOC data over 10 days of sampling from Pony Lake. SUVA values ranged from 187 – 203 L mol-C\(^{-1}\) cm\(^{-1}\), and over the course of measurements SUVA values (Table 2.6) show remarkable stability, indicating that the chromophoric fraction of DOM is stable during this time period even though photolysis data clearly demonstrate decreases in SUVA values during only 12 hours of photolysis. Fulvic acid samples were isolated at two different times during the
2004-2005 season – Dec. 2004 and Jan. 2005. $^{13}$C CPMAS NMR scans of these isolates show stability in integration of peak areas (Figure 2.10).

*In situ* monitoring of geochemical changes during a complete diurnal cycle illustrate that even though the lake was constantly illuminated under continual sunlight, the measured UV irradiance reached a maximum of around 25 mW cm$^{-2}$ around 15:00 and a minimum of around 4 mW cm$^{-2}$ around 2:00 (Table 2.7). Consequently, temperature also showed diurnal cycling with a slight lag in temporal response relative to total irradiance, with temperature reaching a maximum at 16:30 and a minimum at 7:00. Conductivity measurements demonstrated that salinity remained fairly constant (~5 mS cm$^{-1}$), and that pH also remained relatively constant over the time period, 9.6 ± 0.2. The dissolved oxygen levels continued to increase throughout the time period showing continuous activity of algal community even during times of lower sunlight intensity.

2.4. Discussion

Understanding the photochemical nature of a solely microbially sourced DOM can help us to understand the cycling of the autochthonous organic matter fraction, which will improve our understanding of its effects on the biogeochemistry of natural waters in temperate and polar regions. The results illustrate that microbially-derived DOM: (1) photobleaches faster in whole water than the isolate alone (2) that ROS species play a role in autochthonous DOM photolysis (3) and that *in situ* and seasonal analyses illustrate the stability of this DOM over the long term.

2.4.1. Photobleaching Characterization

Changes in absorbance curves during photolysis show the degradation of chromophores as light breaks the bonds of the chromophores in the DOM, which is seen
in our data of increasing S-values of absorbance curves. As photolysis occurs, fitted S-values increase with time indicating that chromophores are being selectively destroyed with light exposure. The more rapid destruction of chromophores at higher wavelengths causes the slopes of the absorbance curves to increase. One other explanation that could also contribute to the increase in these slopes is that as more conjugated systems that absorb at higher wavelengths degrade, they may be broken apart in to less conjugated systems, thereby causing production of some lower wavelength chromophores and slowing the decrease in absorbance values at these lower wavelengths. Observed photobleaching rate coefficients are not the same at all wavelengths, indicating selective degradation of DOM chromophores. Specifically, I observe apparent increase in $k_{\text{obs}}$ with increasing wavelength. A number of mechanisms could explain this phenomenon. Chromophores absorbing at longer wavelengths (more conjugated systems) could be broken down by light into smaller chromophoric groups that absorb at lower wavelengths. Similarly, the quantum yield for destruction of chromophores that absorb light at the longer wavelengths (>400 nm) could be higher than the chromophores at lower wavelengths, causing them to be more susceptible to photodegradation. Finally, this could simply be an analytical issue whereby absorbance at wavelengths >400 nm are so low that any change in absorbance would be relatively large with equally large given the spectrophotometer’s limits of detection at these wavelengths.

The photobleaching data between the Pony Lake fulvic acid isolate and whole water also differed. The fulvic acid is highly colored and is typically thought to represent the chromophoric fraction of DOM in the carbon pool, and it is surprising to observe that the decrease of absorbance at each wavelength differs from the photolysis of the whole
Increased degradation of chromophores absorbing at higher wavelengths in the whole water compared to the fulvic acid may indicate the presence of non-humic chromophores in PLWW. Instead of showing a uniform increase of $k_{obs}$ with increasing wavelength in the fulvic acid sample, I observed a maximum degradation rate coefficient near 330 nm, which happens to be where a number of the “quinone-like” fluorescent components (Cory and McKnight, 2005) absorb light. This peak represents increased degradation of chromophores that absorb in this region, which can be comprised of phenolics and other $\pi$-conjugated systems. This phenomenon could be unique to the fulvic acid fraction because it is a more concentrated form of the chromophoric moieties of DOM, and this is in effect “masked” when analyzing PLWW photolytic degradation.

Fluorescence analysis shows very different FI values for PLWW (1.77) compared to PLFA (1.45). Higher fluorescence index is usually associated with microbial samples, but the FI for PLFA is surprisingly low. This is because the fluorescence index is made up of a ratio of fluorescence values in the EEM, which correspond to the ratio of components SQ1 to (SQ1 + SQ2) from the 13 component global model (Cory and McKnight, 2005). It could be that SQ1 is preferentially photo-bleached compared to SQ2, which would decrease the FI value in Pony Lake fulvic acid. Other microbially-derived fulvic acids that are not subjected to light such as bottom waters of Lake Fryxell, have higher FI values.

Fluorescence results in general seem to show that photolysis mechanisms are not specific towards a particular fluorescent functional groups in the decay of Pony Lake DOM. This is shown by the PARAFAC data as the component distribution does not change over time for PLWW or PLFA (Figure 2.4), and its FI value does not change
significantly over the course of sunlight exposure (Figure 2.3). The results from the fluorescence analysis differ from the absorbance analysis, which indicates that photolysis selectively transforms components of DOM, and that some chromophores are more susceptible to degradation than others. This is not surprising because fluorescent material (generally aromatics, quinones, phenols and olefins) is comprised of DOM functional groups that do not have the rotational degrees of freedom to relax by means other than emitting photons. Bond breaking is not required to destroy fluorophores, only the destruction of conjugated $\pi$ systems is required.

The fluorescence observations are further corroborated by the molecular weight data, where we see the chromophores bleached by light do not transform into smaller components, since over a 24-hour irradiation period, the weighted average molecular weight decreased by only 10% (Table 2.3). Decrease in $M_w$ would indicate photolysis exposure was breaking DOM into smaller components. But this small change compared to observations in absorbance and fluorescence decay indicate that this cannot completely account for the observed destruction of chromophores in the DOM, unless only the highest molecular weight DOM molecules are comprised of the majority of the chromophores in DOM. This shows that the oxidation of chromophores by the combined action of direct and indirect photolysis (i.e., ROS mediated) is not sufficient to break apart Pony Lake DOM, which is only 13.2% aromatic in character (Fimmen et al., 2007). Measured molecular weight of PLFA seems to be a bit high compared to that of other fulvic acids reported in Chin et al. (1994) and Brown et al. (2004), and in fact there were several issues using the same PSS standards as in that study for calibration and reproducing molecule weight values for SRFA. So the actual molecular weight of this
experiment may not be accurate, though given the extreme similarity in retention times and peak shape, the trends observed are expected to be real.

2.4.2. Photobleaching and Redox: Role of ROS

Photolyses of PLWW and PLFA at ambient and low levels of oxygen show that reactive oxygen species play a role in the photochemical transformation of Pony Lake dissolved organic matter. The fact that $k_{\text{obs}}$ values were slower in the presence of low levels of oxygen implies that the observed differences in decay are due to the photo-oxidation by the collective reactivity of ROS. It is interesting to note that the absorbance of PLFA in argon-sparged photolyses decreased more slowly at wavelengths only up to 350 nm, after which photobleaching rates became statistically identical to those done in ambient oxygen conditions. This implies that ROS are preferentially destroying chromophores that absorb light below 350 nm. While 100% of the oxygen may not have been removed from our system and so it may be that an even higher percentage of photobleaching is due to ROS activity than reported in the results, the data show that ROS does play a role in the photobleaching of DOM.

Fluorescence analysis by EEMs and FI measurements of samples in the presence and near absence of oxygen showed no discernable difference between treatments. Nonetheless, I observed an increase in overall fluorescence intensities in samples with argon treatment. Since oxygen is able to quench fluorescence, this provides evidence that oxygen was removed through the sparging treatment. This does not mean that all oxygen was removed and trace levels of ROS could not have been present during the photolyses. Neither the rate of degradation nor the distribution of fluorophores was altered
significantly during photolysis as a result of ROS-mediated processes as evidenced by the argon sparging treatment.

Experiments comparing chromophore decay at high relative to ambient oxygen levels show Pony Lake fulvic acid’s susceptibility to ROS attack. Higher oxygen levels imply that more reactive oxygen species are able to be generated and thus available to react with DOM during photolysis. The lack of a difference in observed decay coefficients in saturated compared to ambient oxygen conditions for whole water show that PLWW is not as susceptible to ROS attack as the fulvic acid. This is consistent with the observation that fulvic acid is a small fraction of the total DOC pool in Pony Lake water, and its highly colored nature makes it more photochemically reactive. Presence of salts in PLWW would not explain these results as PLFA photolysis was run in a background solution of the same salt levels present in PLWW.

Hydroxyl radical is a very reactive and non-selective ROS, and so its particular involvement in photochemical transformation was investigated. Addition of nitrate to fulvic acid solution was shown to produce more hydroxyl radical in the system, which illustrated that Pony Lake fulvic acid is indeed susceptible to attack by hydroxyl radical (Zepp et al., 1987). However, the addition of salts (NaCl and Na$_2$SO$_4$) to natural PLWW levels quenched this effect back to rates of decay of the fulvic acid with no treatments. The fact that salts did not quench the coefficients of decay below observed levels indicate that hydroxyl radical attack may not be the predominant reactive oxygen species through which Pony Lake organic matter photochemically transforms. Experiments with DNPH show that hydroxyl radical production rates are lower than those reported by Grannas et al. (2005), though comparison of steady state concentrations and production rates
between experiments are not statistically different from each other when taking the large experimental error into account. Likewise DNPH measurements in samples supersaturated with oxygen were also not statistically different.

When measured, the $\text{H}_2\text{O}_2$ production rates in solutions of SRFA were consistently higher than those from PLFA and OWCFA (Figure 2.9). Inner-filter-effects are insufficient to explain the magnitude of these differences, and one may conclude from the evidence that fulvic source composition is important for the generation of ROS within the DOM, and that PLFA is not as capable of producing hydrogen peroxide as more terrestrially-derived organic matter.

Analysis of reduced sulfur moieties with photolysis shows correlations with maximum and humic peaks in fluorescence EEMs. Humic groups have been shown to be redox active and this correlation with the destruction of the reduced sulfur groups further corroborates this result, as well as shows that photolysis processes affect the electron shuttling capacity of DOM.

2.4.3. Relevance to Field Observations

Photolysis lends itself to turnover of DOM, and this is observed in the bleaching of DOM with exposure to irradiation and decrease of reduced sulfur moieties. Despite how photochemically reactive Pony Lake DOM is in simulated sunlight, field measurements do not show a large change in the quality of DOM on a diurnal or seasonal scale. SUVA values remained fairly constant throughout sampling during the field season (only 10% variability), lending evidence to show there is still a very stable fulvic acid fraction in the Pony Lake water throughout the course of the season. This is also
displayed by the NMR data, which show virtually the same functional group composition for fulvic acid fractions isolated at two different points in a single summer season.

2.5. Conclusions

Pony Lake DOM and its fulvic acid component are photochemically reactive, which is observed by absorbance and fluorescence changes and molecular weight changes. It is also able to produce and be susceptible to the very reactive hydroxyl radical. Along with this, a decrease in reduced sulfur moieties was observed during photolysis indicating a shift in redox state of the DOM material, which correlates to changes in humic fluorescent peaks. Despite the photochemical reactivity of both the fulvic acid and whole water, the absolute change in Pony Lake DOM properties is small, rendering it relatively stable. I suspect that the photoreactive fraction of the organic material in Pony Lake is constantly being regenerated by the native microbial consortia to create a stable DOC pool during the course of the productive season.

2.6. Acknowledgments:

A number of people assisted with this research, including Kathy Welch for ion analysis, Tanya Young form the OSU NMR center, Karen Kirchman for assistance with hydrogen peroxide analysis, PHI helo/transport, support staff in Crary Laboratory at McMurdo Station, and the entire B-300 team: Kaelin Cawley, Yu-Ping Chin, Rose Cory, Marcus Dieser, Christine Foreman, Ryan Fimmen, Christopher Jaros, Diane McKnight, Penney Miller, Dena Rosenburger. This work was funded through NSF Grant 0338260.
2.7. Tables

Table 2.1. Measured characterization data of constituents in Pony Lake as well as elemental analysis data of Pony Lake fulvic acid. Elemental analysis values are ash corrected values.

*DOC is maximum measured DOC during ’05-’06 summer season, on Jan. 18, 2006.

**Reported in Fimmen et al. (2007).

Sample Collection Characteristics

<table>
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<tr>
<th></th>
<th>Pony Lake Water</th>
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<th>Pony Lake Fulvic Acid</th>
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<td>Dec. 30, 2005</td>
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<td></td>
<td>Dec. 30, 2005</td>
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<td></td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
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<td>[SO₄²⁻] (mg L⁻¹):</td>
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Table 2.2. Percent loadings of each of the 13 fluorescent components modeled by Cory and McKnight (2005) for PLWW and PLFA, along with descriptions of identifications of fluorescent components as reported in Cory and McKnight (2005).

<table>
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<th>Component</th>
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<td>1</td>
<td>unidentified</td>
<td>7.7%</td>
<td>7.2%</td>
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<td>2</td>
<td>Q2</td>
<td>20.4%</td>
<td>20.4%</td>
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<tr>
<td>3</td>
<td>unidentified</td>
<td>6.8%</td>
<td>8.8%</td>
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<tr>
<td>4</td>
<td>HQ</td>
<td>15.7%</td>
<td>11.1%</td>
</tr>
<tr>
<td>5</td>
<td>SQ1</td>
<td>5.3%</td>
<td>2.0%</td>
</tr>
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<td>6</td>
<td>unidentified</td>
<td>2.8%</td>
<td>2.6%</td>
</tr>
<tr>
<td>7</td>
<td>SQ2</td>
<td>6.2%</td>
<td>6.5%</td>
</tr>
<tr>
<td></td>
<td>Tryptophan-like</td>
<td>4.1%</td>
<td>5.9%</td>
</tr>
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<td>8</td>
<td>SQ3</td>
<td>4.0%</td>
<td>4.3%</td>
</tr>
<tr>
<td>9</td>
<td>unidentified</td>
<td>5.1%</td>
<td>3.2%</td>
</tr>
<tr>
<td>10</td>
<td>Q1</td>
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</tr>
<tr>
<td>11</td>
<td>Q3</td>
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<td>10.7%</td>
</tr>
<tr>
<td>12</td>
<td>Tyrosine-like</td>
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<td>7.4%</td>
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<td>13</td>
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<tr>
<td><strong>Total:</strong></td>
<td></td>
<td><strong>100.00%</strong></td>
<td><strong>100.00%</strong></td>
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Table 2.3. High-pressure size exclusion chromatography data of samples of Pony Lake fulvic acid photolyzed in Antarctic sunlight (Dec. 15, 2005). $M_n$ is number average of molecular size in Daltons, $M_w$ is weighted average of molecular size in Daltons and polydispersity is the ratio of $M_w/M_n$.

<table>
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<tr>
<th>Times (hrs)</th>
<th>$M_n$ (Da)</th>
<th>$M_w$ (Da)</th>
<th>Polydispersity</th>
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<tr>
<td>0</td>
<td>611.38</td>
<td>2507.32</td>
<td>4.1</td>
</tr>
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<td>1</td>
<td>591.23</td>
<td>2433.62</td>
<td>4.12</td>
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<td>2</td>
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</tr>
<tr>
<td>3</td>
<td>683.13</td>
<td>2346.53</td>
<td>4.02</td>
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<td>4.1</td>
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</tr>
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<td>6.5</td>
<td>570.13</td>
<td>2273.32</td>
<td>3.99</td>
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<td>8.2</td>
<td>582.97</td>
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<td>3.94</td>
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<td>12.3</td>
<td>568.83</td>
<td>2249.24</td>
<td>3.95</td>
</tr>
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</table>
Table 2.4. Observed actinometer normalized fitted decay coefficients at 320 nm for PLFA and PLWW in Antarctic sunlight (Ant.) and solar simulator irradiation (Lab) with various treatments. Treatments include control (fulvic acid or whole water alone), argon sparging, nitrate addition, salt addition, nitrate and salt addition. Control solutions were unmodified other than altering pH to bring samples that were acidified.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Control</th>
<th>Argon Sparged</th>
<th>NO$_3^-$ Added</th>
<th>Salt Added</th>
<th>NO$_3^-$ and Salt Added</th>
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</thead>
<tbody>
<tr>
<td>PLWW (Ant.)</td>
<td>0.135</td>
<td>0.096</td>
<td>-------</td>
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<td></td>
</tr>
<tr>
<td>PLFA (Ant.)</td>
<td>0.250</td>
<td>0.216</td>
<td>0.333</td>
<td>0.249</td>
<td>0.257</td>
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<tr>
<td>PLWW(Lab)</td>
<td>0.459</td>
<td>0.258</td>
<td>-------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>PLFA(Lab)</td>
<td>0.504</td>
<td>0.217</td>
<td>-------</td>
<td>-------</td>
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</tbody>
</table>
Table 2.5. Calculated specific UV absorbance (SUVA) values measured from absorbance at 254 nm and dissolved organic carbon (DOC) data for samples obtained from Pony Lake over a 10-day period in mid-summer. DOC is reported in mg L\(^{-1}\). SUVA is reported in absorbance units M\(^{-1}\) carbon (Chin et al. 1994).

<table>
<thead>
<tr>
<th>Date</th>
<th>DOC</th>
<th>SUVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/9/06</td>
<td>23.1</td>
<td>187.9</td>
</tr>
<tr>
<td>1/10/06</td>
<td>21.5</td>
<td>184.0</td>
</tr>
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<td>1/11/06</td>
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<td>178.0</td>
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<td>1/12/06</td>
<td>22.4</td>
<td>173.0</td>
</tr>
<tr>
<td>1/15/06</td>
<td>20.8</td>
<td>207.2</td>
</tr>
<tr>
<td>1/16/06</td>
<td>21.5</td>
<td>174.1</td>
</tr>
<tr>
<td>1/17/06</td>
<td>21.6</td>
<td>207.8</td>
</tr>
<tr>
<td>1/18/06</td>
<td>29.0</td>
<td>184.6</td>
</tr>
</tbody>
</table>
Table 2.6. Description of sample matrices used to reconstitute fulvic acid isolates to study the effect of source composition on the photochemical production of hydrogen peroxide.

$^{1}$ DOC (in mg-C L$^{-1}$) reported ± one standard deviation. $^{2}$Hydrogen peroxide production rates ($R_{H_2O_2}$) were determined from a least squares fit of the initial rates data and normalized to the DOC (in mg-C L$^{-1}$) to account for differences in carbon level. $^{3}$Screening factor for wavelengths 290-320 nm. $^{4}$Compensation for inner filter effects on production rates was performed by dividing production rate by screening factor calculated at a wavelength of 320 nm for these DOM solutions.

<table>
<thead>
<tr>
<th>Fulvic Acid</th>
<th>pH</th>
<th>DOC (mg C L$^{-1}$)</th>
<th>NaCl (M)</th>
<th>Na$_2$SO$_4$ (M)</th>
<th>$R_{H_2O_2}^2$ (µM/hr/mg C L$^{-1}$)</th>
<th>$S_{290-320}^3$</th>
<th>$R_{H_2O_2}^4$ (µM/hr/mg C L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLFA</td>
<td>7.99</td>
<td>8.88 ± .22</td>
<td>4.7 x 10$^{-2}$</td>
<td>1.10 x 10$^{-2}$</td>
<td>0.24 ± 0.01</td>
<td>0.906</td>
<td>0.265</td>
</tr>
<tr>
<td>OWCFCA</td>
<td>8.03</td>
<td>9.54 ± .10</td>
<td>9.31 x 10$^{-4}$</td>
<td>3.12 x 10$^{-4}$</td>
<td>0.18 ± 0.04</td>
<td>0.830</td>
<td>0.217</td>
</tr>
<tr>
<td>SRFA</td>
<td>7.95</td>
<td>10.06 ± .24</td>
<td>9.31 x 10$^{-4}$</td>
<td>3.12 x 10$^{-4}$</td>
<td>0.36 ± 0.03</td>
<td>0.818</td>
<td>0.440</td>
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</table>
Table 2.7. Diurnal monitoring data of Pony Lake, Jan. 16th – 17th, 2006. UV irradiance (mW cm\(^{-2}\)) was measured next to lake surface and represents incoming light onto lake water. FI and S values were measured at Crary Laboratory immediately upon return from the field site. pH, temperature (°C), conductivity (mS cm\(^{-1}\)) and dissolved oxygen (DO) (mg L\(^{-1}\)) were measured in-situ.

<table>
<thead>
<tr>
<th>Time</th>
<th>UV Irradiance</th>
<th>FI Value</th>
<th>S Value</th>
<th>pH</th>
<th>T</th>
<th>Salinity</th>
<th>DO</th>
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</thead>
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<tr>
<td>11:20 AM</td>
<td>18.31</td>
<td></td>
<td></td>
<td>9.56</td>
<td>1.8</td>
<td>4.97</td>
<td>16.5</td>
</tr>
<tr>
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<td>0.0195</td>
<td>9.14</td>
<td>1.3</td>
<td>4.9</td>
<td>15.7</td>
</tr>
<tr>
<td>2:07 PM</td>
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<td>1.8017</td>
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<tr>
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<td>15.5</td>
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<tr>
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<tr>
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2.8. Figures

**Figure 2.1.** Absorbance curves of PLFA and PLWW during irradiation in Antarctic sunlight. UV/Vis scans depicted from 290 – 390 nm.
Figure 2.2. Pony Lake absorbance changes with photolysis. A. Fitted first order observed decay coefficients of absorbance at each wavelength from 290 – 400 nm for PLFA and PLWW through 24 hours of continuous irradiation in Antarctic sunlight. B. Calculated S-values from absorbance curves for PLFA and PLWW photolysis at each time point during irradiation.
Figure 2.3. Pony Lake fluorescence changes with photolysis. A. EEMs of PLFA (1/05/05) at 0 hr, 5 hr, and 24 hr of irradiation in Antarctic sunlight. B. Ratio of fluorescence at $\lambda_{ex} = 300$ nm, $\lambda_{em} = 450$ nm to initial fluorescence at each time point during irradiation. C. Fluorescence Index (FI) values of PLWW (12/28/05) and PLFA (1/05/05) at each time point during irradiation.
**Figure 2.4.** Pony Lake PARAFAC changes with photolysis. Percent of total fluorescence loading of identified components in Pony lake whole water and fulvic acid photolysis as fit to 13 component PARAFAC model developed by Cory and McKnight (2005) at each time point during irradiation.
Figure 2.5. High-pressure size exclusion chromatography of PLFA with exposure to Antarctic sunlight.
Figure 2.6. Fluorescence and reduced sulfur correlations. A. Plot of fluorescence of each EEM peak (A, B, or C) vs. conc. of reduced sulfur as equivalent to mg L\(^{-1}\) of L-cysteine as measured through voltammetry. B. Representative EEM of PLFA with identified peaks A, B, and C used for analysis in part A marked in circles.
Figure 2.7. Effect of ROS on Pony Lake photolysis. A. Fitted first order observed decay coefficients of absorbance at each wavelength from 290 – 400 nm for PLFA in the presence of O₂ and argon sparging during 6 hours of irradiation in Antarctic sunlight. B. Fitted first order observed decay coefficients of absorbance at each wavelength from 290 – 400 nm for PLWW in the presence of O₂ and argon sparging during 24 hours of irradiation in Antarctic sunlight.
Figure 2.8. Fitted first order observed decay coefficients of absorbance at each wavelength from 290 – 400 nm for PLFA, PLFA and NO₃⁻, PLFA and salts, PLFA and salts and NO₃⁻ during 6 hours of irradiation in Antarctic sunlight.
Figure 2.9. Comparison of the photochemical H$_2$O$_2$ production in reconstituted Pony Lake (PLFA), Old Woman Creek (OWCFA) and Suwannee River (SRFA) fulvic acids. For solution conditions please refer to Table 6.
Figure 2.10. Solid state $^{13}$C CPMAS NMR$^1$ scans of Pony Lake fulvic acid from two separate isolations during a single season, December 2004 and January 2005.

$^1$NMR spectra were acquired by Dr. Ryan Fimmen (32,000 scans) using a Bruker Advance DSX 300 MHz spectrometer equipped with a 4 mm magic angle-spinning (MAS) probe. Each sample was packed into a zirconia rotor and spun at 13 kHz during acquisition. Spectra were acquired using a ramped-amplitude cross-polarization (CP) pulse sequence with composite pulse TPPM (two-pulse phase-modulated) proton decoupling, and magic angle spinning (2 s pulse repetition time, 90° $^1$H excitation pulse, 2.0 ms contact time). Spectra were processed with an exponential multiplication with 20 Hz line broadening prior to integration. Spectral regions were divided according to Dria and co-workers (2002).
2.9. References


Chapter 3: Photochemical Fate of Sulfadimethoxine in Aquaculture Waters

3.1. Introduction

The high density of catfish in aquaculture ponds makes the spread of disease a real and costly concern, and antibiotics are crucial to assure the survival of fish. Sulfadimethoxine (SDM) is used in freshwater aquaculture as an antibiotic in a mixture with ormetoprim called Romet 30® to prevent the spread of disease in these environments (Figure 3.1). Catfish ponds are unique relative to natural water bodies due to their design, as they tend to be shallow and lack both shade and a transitory littoral zone. A large portion of the pond’s surface is exposed to sunlight making them ideally suited for photoreactions. Conversely, their highly eutrophic nature limits sunlight penetration in the water column.

Sulfadimethoxine absorbs light in the UV region rendering it susceptible to direct and possibly indirect photodegradation in the presence of photosensitizers such as dissolved organic matter (DOM) (Boreen et al., 2005). In waters where DOM concentrations are high, SDM photodegradation may be enhanced through photosensitized pathways. Conversely, in seawater where DOM levels are low (< 80 μM), degradation has been shown to be very slow (Lunestad et al., 1995). DOM can promote the transformation of many organic contaminants in the presence of sunlight through the formation of reactive oxygen species (ROS), e.g., hydroxyl radical, peroxyl radicals, singlet oxygen, and superoxide (Dimou et al., 2005; Gerecke et al., 2001; Lam and Mabury, 2005; Miller and Chin, 2005; Werner et al., 2005), or by direct oxidation
with triplet-excited state DOM ($^3$DOM). DOM may also retard photodegradation by screening sunlight or quenching ROS and $^3$DOM (Brezonik and Fulkerson-Brekken, 1998; Canonica and Laubscher, 2008; Walse et al., 2004).

DOM composition and its photoreactivity differ as a function of its precursor materials. Terrestrial DOM tends to have higher aromaticity (due to lignin incorporation) and lower nitrogen and sulfur content, whereas microbial DOM tends to have fewer aromatic structures but significantly more nitrogen and sulfur moieties (McKnight et al., 1991; McKnight et al., 1994). These differences in source composition can affect the relative reactivity of DOM in the photosensitized decomposition of organic contaminants (Guerard et al., 2009). To our knowledge, nothing is known about the composition and properties of dissolved organic matter derived from aquaculture ponds.

Few studies have investigated the compositional role of DOM in the photodegradation of SDM. Boreen and co-workers found that water from Lake Josephine in Minnesota did not enhance the photodegradation of SDM (Boreen et al., 2005). Given the unique nature of catfish ponds, it is likely that the DOM in these water bodies differs considerably from other natural waters, and so the exact photochemical fate of SDM in these systems remains unclear.

The objective of this study is to discern the fate and mechanisms behind the DOM promoted (or inhibited) photodegradation of sulfadimethoxine in an aquaculture pond and a nearby stream. I hypothesize that SDM photoreactivity differs significantly between catfish pond water and natural surface waters due to compositional differences in the DOM.
3.2. Materials and Methods

3.2.1. Study Site and Sample Collection:

Samples were collected at the aquaculture facilities located at the Mississippi State University Delta Research and Extension Center, Stoneville, Mississippi, in December 2006 and April 2008. Samples were collected from Catfish Pond 29 (33° 26.6’N, 90° 53.8’W 140’ elevation) and from nearby Deer Creek (33° 25.2’N, 90° 54.5’W 140’ elevation). On site measurements included pH, dissolved oxygen, temperature, and conductivity. pH was measured using a Beckman Φ11 pH meter. Dissolved oxygen and temperature were measured using a YSI Ecosense DO200 probe. Conductivity was measured using an Orion Model 130 conductivity meter. From the 2008 sampling event, samples were collected and prefiltered through 20 and 5 µm prefilters consecutively and stored in 20 L plastic (acid washed) carboys during the trip back to Ohio State University. Samples were acidified to pH 2 before storage in a cold room in the dark at 4° C. 100 L of Deer Creek and 300 L of Pond 29 water were collected during the 2006 sampling event. Samples were filtered with Milli-Q rinsed 0.45 µm in-line groundwater filtration capsules (Pall Gelman) using a Masterflex pump (Cole Parmer Instrument Co.) before use in our experiments. Prior to all experiments the pH of the samples were readjusted using NaOH to values measured in the field.

3.2.2. Chemicals and Reagents

Ammonium acetate (certified ACS), acetonitrile (Optima), ferric chloride hexahydrate anhydrous (certified ACS), hydrochloric acid (certified ACS plus), sodium hydroxide (certified ACS), ferrous ammonium sulfate (certified ACS), ferrozine (certified ACS), and isopropanol were purchased from Fisher Scientific.
Sulfadimethoxine sodium salt (99%) was purchased from MP Biomedicals, Inc., while hydroxylamine HCl (99%) was purchased from Sigma Aldrich. All chemicals were used without further purification. Suwannee River Fulvic Acid (SRFA: reference grade) and Pony Lake Fulvic Acid (PLFA: reference grade) were obtained from the International Humic Substances Society (IHSS). Old Woman Creek Fulvic Acid (OWCFA) was isolated from Old Woman Creek water (Huron, OH) by XAD-8 chromatography in our Laboratory using the methods of Thurman and Malcolm (1981).

3.2.3. Analytical Analysis:

Dissolved organic carbon (DOC) analysis was carried out on a TOC-5000 Shimadzu carbon analyzer using KHP standards. Iron (II) and iron (III) concentrations were analyzed spectrophotometrically using the ferrozine method and calibrated with ferrous ammonium chloride standards (Stookey, 1970). Absorbance scans of natural waters were run on a Varian Cary 1 UV-VIS spectrophotometer from 200 – 600 nm. The SDM peak was resolved using a Restek Ultra IBD column (150 x 4.6 mm), eluted with a mobile phase of acetonitrile and 1 mM ammonium acetate (NH$_4$Oac) (30:70% v/v at 1 mL min$^{-1}$), and detected at a wavelength of 260 nm.

Liquid chromatography (LC) electrospray-ionization (ESI) quadrupole-time of flight (Q-TOF) mass spectrometry was conducted at the U.S. E.P.A. in Cincinnati, OH. Ternary mobile phases were comprised of 0.1% formic acid in water, methanol, and acetonitrile. All experiments were performed on a Bruker UltraTOFQ mass spectrometer interfaced to an Agilent HPLC.
3.2.4. Photolysis Experiments

Solutions of either 0.1 or 1.0 µM SDM dissolved in Milli-Q water, 0.45 µm filtered water from the field site, or 10 mg C/L fulvic acid solutions were placed into 14 mm diameter, 5 cm long quartz reaction tubes that were sealed with a Teflon lined O-ring clamped to a quartz lid. The 0.1 µM SDM experiments were done to elucidate concentration effects on DOM photosensitized pathways and were only conducted in PLFA or SRFA solutions. Samples were irradiated in a Suntest CPS+ (Atlas Devices) solar simulator with a 500 W Xenon lamp over a length of two to three half-lives to determine both the reaction order and rate constants. Actinometer experiments showed that the Suntest operated at ~ 4.5 times the intensity of average sunlight at 40°N at noon in June (Leifer, 1988). A radiometer was used to ensure that no significant changes in irradiance occurred during photolysis, and temperature was maintained at 25° C. Dark controls wrapped in aluminum foil were run concurrently. Quantitative analysis of SDM concentrations by reverse-phase HPLC is detailed in Guerard et al. (2009). Photodegradation was log-linearized and found to fit a pseudo-first order model. Rates were corrected for light screening of fulvic acids and natural waters (see Guerard et al. 2009 for screening calculation methods).

Amendments to the above experiments were conducted to elucidate the reactive pathways. Iron addition (20 µM FeCl₃) in filtered samples and fulvic acid solutions investigated the importance of the photo-Fenton pathway in SDM degradation. The role of the hydroxyl radical (OH•) was assessed in experiments containing 25 mM isopropanol, which efficiently scavenges this radical. The role of ³DOM in the photofate of SDM was investigated by sparging solutions with argon gas (1 min mL⁻¹) and
preparing them in an anoxic glove box to remove dioxygen as a potential triplet scavenger.

3.2.5. Photoproduce Analysis

Solutions of 100 μM SDM in Milli-Q water, PLFA or SRFA were photolyzed for 24 hours in the Suntest solar simulator. Phototubes of solution for each medium were then combined and concentrationed via a Rotovap down from 100 mL to about 10 mL volume and diluted two-fold with methanol. Samples were analyzed by UV/Vis and HPLC before and after concentration on a Rotovap (but before methanol addition) to ensure photolyzed sample was retained an unchanged. Samples were then analyzed via LC-ESI-QTOF-MS/MS conducted in both positive and negative ion mode.

3.3. Results

Pond 29 and Deer Creek had a measured pH of 8.4 and 7.8, and DOC of 11.3 and 6.2 mg-C L⁻¹, respectively (Table 3.1). Both waters had less than 1 μM total iron content. SUVA measurements were 125 L mol⁻¹ cm⁻¹ for Pond 29, and 251 L mol⁻¹ cm⁻¹ for Deer Creek. FI was measured as 1.75 for Pond 29 and 1.58 for Deer Creek. Both the SUVA and FI measurements are consistent with values reported by others (Chin et al., 1994; McKnight et al., 2001) for microbial and terrestrial DOM. Pond 29 is dominated by microbially-derived DOM, while Deer Creek DOM is predominantly derived from terrestrial precursors.

SDM photodegradation obeyed pseudo first-order kinetics with an R² greater than 0.99 for reactions in Milli-Q water (Guerard et al., 2009), fulvic acid solutions (Guerard et al., 2009), and the field site samples (Figure 3.2, Table 3.2). No significant degradation enhancement of SDM was observed in Deer Creek water relative to direct
photolysis. Conversely, significant photo-enhancement was observed in the filtered water from Pond 29 (x2) relative to the direct photolytic pathway. Similar results were observed for the fulvic acids, as SDM photolysis was enhanced in the presence of PLFA, but not SRFA (Figure 3.3, Table 3.2). OWCFA also enhanced the photoreactivity of SDM, but not to the same extent as either PLFA or Pond 29 water. Unlike the other DOM and fulvic acids used in this study, OWCFA is comprised of organic matter derived from a mixed source of precursors (Guerard et al., 2009).

Photolyses run at 0.1 µM in both PLFA and SRFA resulted in an increased observed rate coefficient (x 3) compared to those run at 1.0 µM (Table 3.3). Iron amendment experiments were used to investigate the role of the photo-Fenton pathway in the transformation of SDM in the presence of different DOM isolates. Iron addition to SRFA showed only small enhancements, while in PLFA, the difference in rate constants was more pronounced (Figure 3.4, Table 3.3). Further iron amended PLFA experiments conducted with isopropanol as an OH• scavenger resulted in SDM rate constants that were identical to the ones determined in the absence of iron (Figure 3.5). Surprisingly, addition of Fe (III) in Pond 29 water revealed no additional enhancement of SDM photodegradation.

Experiments to test for SDM photodegradation by 3DOM were done by removing O2, a known quencher for excited triplet states. The argon-sparged photolysis of SDM was carried out in Pond 29 water since the greatest enhancement was observed in this sample. In both solutions, the observed rate coefficient was significantly faster compared to non-sparged experiments (Table 3.3), which strongly suggests oxidation of SDM by 3DOM.
3.3.1. Product Analysis

Mass spectrometry and chromatographic analysis revealed a number of photoproducts resulting from the phototransformation of SDM. Thirty-five distinct peaks with unique mass spectra were observed in positive ion mode (Figure 3.6; Figure 3.7), and 22 peaks were observed in negative ion mode (Figure 3.8) with 9 of those peaks appearing in both modes. Products reflect those observed products that comprised greater than 1% of total integrated area for positive or negative ion mode (refer to Figures 3.6, 3.7 and Table 3.4).

Peak 18 and 13 in respective positive and negative ion modes were identified as the parent SDM compound. Of the resolved peaks with greater than 1% total integrated area, analyses of mass spectra showed that positive mode peaks 2, 3, 13, 16 and negative mode peaks 12, 21, 22 are unlikely to be photoproducts, but rather artifacts of the isolation/analysis (Table 3.4).

Product analysis was also performed on solutions of SDM photodegraded in SRFA or PLFA to look for differences in photoproduct formation. Surprisingly, the SDM photoproducts in both SRFA and PLFA resulted in the same derivatives observed in the direct photolysis experiments (Figure 3.6). Overall, peak areas of photoproducts and residual SDM were smaller in the presence of the fulvic acids, and is most likely due to the greater extent of degradation that occurred. The most abundant photoproduct, peak 6, shows no difference in relative area between DOM solutions. However, peaks 4, 5 and 26 in positive ion mode had higher integrated sensitivities in PLFA relative to SRFA, while peaks 16 and 17 had higher area counts in SRFA.
3.4. Discussion

SDM degrades predominantly through photolytic processes where photosensitization is strongly dependent upon DOM composition. In Lake Josephine water, Boreen and co-investigators observed no enhancement of SDM photodegradation and actually reported a slight decrease in the observed rate coefficient, which they attributed to DOM screening effects (Boreen et al., 2005). Results from the photodegradation of SDM in SRFA (Guerard et al., 2009) and Deer Creek corroborate the observations made by these investigators, as no significant enhancement was observed and the degradation kinetics were slower in the latter sample. While we do not know the physicochemical properties of DOM from Lake Josephine, our results suggest that its composition is similar to both Deer Creek DOM and SRFA. Typically terrestrial DOM has higher SUVA and lower FI values than organic material derived from eutrophic water bodies (Chin et al., 1994; McKnight et al., 2001). SRFA, a terrestrial end-member of DOM samples, has been well characterized with a high percentage of aromatic moieties derived from lignin and tannins (Thorn et al., 1989; Fimmen et al., 2007). Similar to SRFA, Deer Creek has high SUVA values and a low fluorescence index, qualities characteristic of terrestrially-derived DOM (Chin et al., 1994; McKnight et al., 2001). The source material of Deer Creek DOM is likely derived from terrestrial inputs, especially given the abundant cypress tree stands growing within the creek.

In contrast to terrestrial DOM, significant enhancement of photodegradation was observed for SDM in the presence of OWCFA, PLFA (Guerard et al., 2009) and Pond 29 water. PLFA is an algal/microbial end-member of DOM that possesses lower aromaticity with a significant fraction of organic nitrogen and sulfur moieties. Unlike SRFA and
Deer Creek DOM, PLFA was isolated from a hypereutrophic lake in Antarctica with no inputs of terrestrial organic matter. Pond 29 is similar to Pony Lake in that it is also hypereutrophic, but some terrestrial DOM could enter the pond through runoff. Nonetheless, Pond 29 DOM has properties very similar to PLFA, i.e., high FI and low SUVA values, which is indicative of a predominantly autochthonously derived material (Chin et al., 1994; McKnight et al., 2001). As stated earlier OWCFA is of mixed composition and exhibits intermediate reactivity toward SDM. Finally, we have ruled out nitrate as a possible photosensitizer in Pond 29 because a UV/Vis absorbance scan of the pond water from 200 - 600 nm did not reveal the distinctive nitrate spectrum, and appears to be completely dominated by DOM (data not shown).

It has already been reported that SDM is susceptible to degradation by the hydroxyl radical (Boreen et al., 2005). DOM in the presence of iron may be a source of hydroxyl radical whereby Fe (III) is photo-reduced to Fe (II), which subsequently reacts with H₂O₂ via the photo-Fenton pathway to form OH• (Southworth and Voelker, 2003; Voelker and Sulzberger, 1996; Sedlak and Andren, 1991). Our results indicate that PLFA in the presence of added iron (20 µM) is able to facilitate additional SDM degradation by this pathway. The addition of isopropanol to the iron amended experiment reduced photosensitized SDM rate constants to values measured in PLFA alone, which provides further evidence that iron additions resulted in photo-Fenton chemistry. Surprisingly, unlike PLFA, we observed no enhanced SDM degradation in Pond 29 water DOM in the presence of iron despite structural similarities between these two materials. However, because the rate of SDM degradation in PLFA only solutions (Guerard et al., 2009) slowed by 7.7% upon addition of isopropanol, this indicates that the OH• promoted
degradation in the *absence of excess iron* is not the dominant pathway for SDM, and that another mechanism appears to be more important.

Boreen and co-workers demonstrated that SDM reacts via a excited triplet state pathway (2005), and Canonica et al. (1995) predicted that electron transfer between sulfonamides and triplet-excited DOM should be a likely method of interaction based upon a Hammett relationship for the redox potential of anilines and $^3$DOM. We investigated whether this mechanism could occur in our systems. Increases in the analyte rate coefficients were observed in argon-sparged experiments in both Pond 29 water and PLFA solutions and is strong evidence for SDM oxidation by $^3$DOM. A decrease in the rates of photodegradation would have suggested a ROS pathway. Canonica and Freiburghaus (2001) reported similar results for 2,4,6-trimethylphenol (TMP), a highly selective triplet state probe, whereby DOM from more eutrophic water bodies showed higher reactivity toward this compound. They hypothesized that aromatic ketones present in dissolved organic matter could be responsible for their observations (Canonica, 2007; Canonica et al., 2000; Canonica et al., 2006). While PLFA has a much lower percentage of aromatic and phenolic components as determined by $^{13}$C CPMAS NMR (13.2%) (Thorn et al., 1989) compared to SRFA (24%) (McKnight et al., 2001), it is possible that the percentage of aromatic ketones is greater in the former.

Photolyzing SDM at lower and more environmentally relevant concentrations in both PLFA and SRFA resulted in a *significant* increase in the observed rate coefficients and is consistent with observations made by Canonica and Freiburghaus (2001) for the degradation of TMP. These investigators modeled their observations and demonstrated that compounds with electron-donating groups are susceptible to both “short”-lived e.g.,
DOM and “long”-lived photo-oxidants e.g., peroxyl and phenoxy radicals at lower analyte concentrations (≤ 0.1 µM), while they will only react with “short”-lived species at the higher concentrations (Canonica and Freiburghaus, 2001). Thus, our observations suggest that at environmentally relevant levels, SDM is likely to be even more rapidly removed by photolytic pathways, particularly in eutrophic aquaculture ponds.

The direct photolysis of SDM produces many different photoproducts as observed by others (Boreen et al., 2005) and me. The presence of multiple peaks in the LC-QTOF analyses suggests that this compound degrades by complex pathways during direct photolysis. While the predominant direct pathway (through excitation to the triplet state) may be the product identified by Boreen and co-workers (2005) (peak 6 in the positive ion mode chromatograms), it is possible that SDM can act as its own photosensitizer, by excitation to the triplet state and reacting with molecular oxygen to produce ROS, which can subsequently react with SDM and its photo-derivatives to produce even more transformation products. A potential reaction scheme is presented in Figure 3.9, through which SDM might be able to be transformed to this main photoproduct. However, further work is needed to ascertain the exact degradation mechanisms and structures of the other photoproducts.

It is interesting that in the presence of DOM, no new photoproducts were observed compared to its direct photolysis pathway. Nonetheless, the relative abundances of the photo-derivatives differed for each system (Figure 3.5b), which suggest that the pathways are specific to a particular DOM. At this point, future work is needed to determine the exact structures of these products and the mechanisms of SDM degradation that would lead to their formation.
3.5. Acknowledgments

I would like to acknowledge Dr. Patricia Gaunt and the Mississippi State Delta Research and Extension Center for access to ponds and logistical assistance, and Collin Ward for helping us with photolysis experiments. Also much thanks to Heath Mash for running the mass spectrometry and offering assistance in interpretation of photoproducts. This research was funded by EPA STAR FP-91678401-0 awarded to J. J. G., and NSF Grant number CBET 0504434.
3.6. Tables

**Table 3.1.** Water quality measurements of Pond 29 and Deer Creek. Dissolved oxygen is reported in ppm, conductivity is reported in mS cm$^{-1}$, temperature at collection is reported in °C, SUVA is in L mol-C$^{-1}$, FI represents the fluorescence index, and DOC is reported in mg L$^{-1}$.

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<td>Dissolved Oxygen</td>
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<td>FI</td>
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<td>DOC</td>
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Table 3.2. Observed photodegradation rate coefficients for photolysis of SDM. [SDM]₀ is 1.0 µM. $k_{obs}$ is observed rate of degradation. $k_{dp}$ is $k_{obs}$ in MilliQ water times screening factor, to get corrected direct photolysis rate in each sample. $k_{ip}$ is the difference between $k_{obs}$ and $k_{dp}$, and represents the rate coefficient for indirect photolysis in each sample. % DP and % IP represent the percent of observed degradation attributable to direct photolysis and indirect photolysis, respectively.

*indicates reported in Guerard et al. (2009).

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<tr>
<th>Sample</th>
<th>$S_{s290-350}$</th>
<th>$k_{obs} \times 100$ (hr⁻¹)</th>
<th>Half-life (hrs)</th>
<th>$k_{dp} \times 100$ (hr⁻¹)</th>
<th>$k_{ip} \times 100$ (hr⁻¹)</th>
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<th>% IP</th>
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<td>Control*</td>
<td>NA</td>
<td>5.75 ± 0.3</td>
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<td>13.23 ± 0.8</td>
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<td>5.52</td>
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<tr>
<td>Deer Creek</td>
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Table 3.3. Observed photodegradation rate coefficients for photolysis of SDM, normalized by light screening factor. \([SDM]_o = 1.0 \, \mu M\) unless noted otherwise.

<table>
<thead>
<tr>
<th>Sample</th>
<th>(k_{obs} \times 100 , (hr^{-1}))</th>
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<tr>
<td>SRFA</td>
<td>7.37 ± 0.3</td>
<td>9.4</td>
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<tr>
<td>PLFA</td>
<td>15.06 ± 0.9</td>
<td>4.6</td>
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<tr>
<td>Pond 29</td>
<td>11.45 ± 0.5</td>
<td>6.05</td>
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<td></td>
<td>25 (mM) Isopropanol Addition</td>
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<tr>
<td>PLFA</td>
<td>9.49 ± 0.3</td>
<td>7.30</td>
</tr>
<tr>
<td>PLFA + 20(\mu M) Fe(^{3+})</td>
<td>9.62 ± 0.6</td>
<td>7.21</td>
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<td><strong>Anoxic Photolyses</strong></td>
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<tr>
<td>PLFA</td>
<td>16.89 ± 3.4</td>
<td>4.10</td>
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<tr>
<td>P29</td>
<td>22.43 ± 4.7</td>
<td>3.09</td>
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<tr>
<td></td>
<td>0.1 (\mu M) SDM</td>
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<tr>
<td>PLFA</td>
<td>31.95 ± 7.5</td>
<td>2.17</td>
</tr>
<tr>
<td>SRFA</td>
<td>23.09 ± 3.4</td>
<td>3.00</td>
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Table 3.4. Mass spectrometry analysis results for the largest photoproduct peaks in chromatograms of positive and negative ion modes. R.T. is retention time in minutes, area is total sensitivity area in chromatogram, max m/z and largest m/z show m/z values of highest intensity and value.

* peaks observed in both positive and negative mode.

** parent SDM peak.

<table>
<thead>
<tr>
<th>Positive Mode</th>
<th>Negative Mode</th>
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<td>18**</td>
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3.7. Figures

Figure 3.1 Structure of sulfadimethoxine
Figure 3.2. Photodegradation of SDM in natural waters. Black squares show dark controls, blue diamonds show SDM in MilliQ water, gray squares show SDM in filtered Pond 29 water and white triangles show SDM in filtered Deer Creek water.
Figure 3.3. Photodegradation of SDM in different media. SDM photolyzed in collected water samples, Milli-Q, and 10 mg-C L⁻¹ fulvic acid solutions. Error bars show 95% confidence in fitted pseudo-first order observed decay rate coefficients.
Figure 3.4. Effect of Fe$^{3+}$ addition on the photodegradation of sulfadimethoxine in SRFA (A) and PLFA (B). SDM in MilliQ water is shown in black diamonds, SDM in 10 mg-C L$^{-1}$ fulvic acid is shown in gray squares, and SDM in 10 mg-C L$^{-1}$ fulvic acid and 20 μM Fe$^{3+}$ is shown in white triangles.
Figure 3.5. Effect of isopropanol on photodegradation of sulfadimethoxine in PLFA and PLFA with 20 µM Fe$^{3+}$ addition. Solutions of 1 µM SDM were photolyzed at pH 8 in MilliQ, 10 mg-C L$^{-1}$ PLFA, 10 mg-C L$^{-1}$ PLFA and 20 µM Fe$^{3+}$, 10 mg-C L$^{-1}$ PLFA and 25 mM isopropanol, 10 mg-C L$^{-1}$ PLFA, 20 µM Fe$^{3+}$ and 25 mM isopropanol. Error bars show 95% confidence in fitted pseudo-first order observed decay rate coefficients. Bars with isopropanol addition are darkened for comparison.
Figure 3.6. Chromatograms of samples analyzed by LC-ESI-QTOF-MS-MS in positive ion mode where the largest peaks and parent SDM peak are labeled. (A) SDM photolyzed in Milli-Q water. (B) SDM photolyzed in 10 mg-C L⁻¹ SRFA (in black) and PLFA, normalized to the SDM peak in SRFA containing chromatogram (in gray).
Figure 3.7. Mass spectra of positive mode LC-ESI-QTOF-MS chromatograms. Spectra of largest (by peak area count) chromatogram peaks are shown.
Figure 3.8. Mass spectra of negative mode LC-ESI-QTOF-MS chromatograms. Spectra of largest (by peak area count) chromatogram peaks are shown.
Figure 3.9. Possible reaction scheme of SDM to main photoproduct identified by Boreen et al. (2005).
3.8. References


Chapter 4: Photodegradation Pathways of Ormetoprim in Mississippi Dissolved Organic Matter

4.1. Introduction

Ormetoprim (OMP) is an antibiotic used in conjunction with sulfadimethoxine (SDM) in a 5:1 SDM:OMP mixture of medicated feed for aquaculture use called Romet® 30 (Figure 4.1). These antibiotics are used in combination to fight enteric septicemia of catfish (ESC), one of the most common outbreaks of disease in catfish farming (USDA, 2003).

Currently there is very little knowledge of the fate of ormetoprim in waters, let alone its photochemical fate. Ormetoprim has been found at concentrations as high as 12 mg L\(^{-1}\) in fish hatchery waters during Romet® 30 treatment, though these maximum detected levels drop to less than 0.69 mg L\(^{-1}\) during non-treatment periods (Dietze et al., 2005). Although detection was minimal in hatchery effluent waters, there was evidence for the potential of antibiotic transportation to the natural environment (Dietze et al., 2005). In simulated environments however, Bakal et al. (2001) showed that varying salinity, pH, or presence of artificial sediment had little to no effect on ormetoprim concentrations over the course of one year. In natural sunlight experiments containing seawater solutions in Bergen, Norway (60° N), OMP was also found to be stable (Lunestad et al., 1995). To date, no information is currently known about the degradation
mechanisms of ormetoprim, or any interaction (enhanced or impeded) with dissolved organic matter in natural waters in the presence of sunlight.

Dissolved organic matter (DOM) source composition has been shown to affect chemical reactivity of DOM and thus may also affect contaminant fate through the production of reactive oxygen species (ROS) and other reactive transients in the presence of sunlight (Guerard et al., 2009; Dimou et al., 2005; Gerecke et al., 2001; Lam and Mabury, 2005; Miller and Chin 2005; Werner et al., 2005; Fisher et al., 2006; plus many others). I have already demonstrated that the source of DOM can affect the photochemical fate of SDM (Guerard et al., 2009), but little is known about the composition of DOM in these catfish ponds and nearby waters, where these compounds are likely to initially be present in the environment, given their industrial uses. Effective characterization of the organic matter from these sites would help to identify possible pathways of degradation of these antibiotics in an environmentally relevant setting.

The objectives of this study were threefold. The first was to investigate the degradation of OMP and use a series of chemical probe experiments to determine reaction pathways in the presence of light. I used DOM (unaltered and isolated by XAD chromatography) from catfish pond waters and Deer Creek, a nearby stream. The DOM was characterized by spectroscopy and OMP photodegradation was studied in their presence. Finally, this study sought to relate any trends in composition of DOM to the degradation of SDM and OMP, in order to both elucidate the specific pathways and make estimates as to the fate of these compounds in catfish ponds and receiving waters.
4.2. Materials and Methods

4.2.1. Study Site and Sample Collection:

Samples were collected April 2008 at the aquaculture facilities located at the Mississippi State University Delta Research and Extension Center in Stoneville, Mississippi. Details are provided in Chapter 3. Samples were collected and pre-filtered through 20 and 5 µm pre-filters consecutively and stored in 20 L plastic (acid washed) carboys during the trip back to Ohio State University. 160 L of Deer Creek and 400 L of catfish water (a mixture of multiple ponds aggregated together) were collected. Samples were then filtered with Milli-Q rinsed 5 µm (DH) and 0.45 µm (AQ) Balston filters, acidified to pH 2 and kept in a cold room in the dark at 4° C before use in our experiments. Prior to all experiments, the pH of the samples were readjusted using NaOH to values measured in the field.

4.2.2. Chemicals and Reagents:

Pure water (18.2 MΩ) was obtained from a Millipore Mill-Q system. Ormetoprim was purchased from Chem Service, Inc. (West Chester, PA) and sulfadimethoxine (sodium salt) was purchased from MP Biomedicals (Solon, OH). Potassium phosphate (monobasic) (certified ACS), methanol (Optima), ferric chloride hexahydrate anhydrous (certified ACS), hydrochloric acid (certified ACS plus), sodium hydroxide (certified ACS), ferrous ammonium sulfate (certified ACS), Rose Bengal, deuterium oxide, sodium azide, furfuryl alcohol, sodium ascorbate and isopropanol were purchased from Fisher Scientific (Pittsburgh, PA). All chemicals were used without further purification. Suwannee River Fulvic Acid (SRFA: reference grade) and Pony Lake Fulvic Acid (PLFA: reference grade) were obtained from the International Humic
Substances Society (IHSS). Catfish pond fulvic acid (CPFA) and Deer Creek fulvic acid (DCFA) was isolated from catfish pond and Deer Creek water (Stoneville, MS) by XAD-8 chromatography in our Laboratory using the methods of Thurman and Malcolm (1981).

4.2.3. Analytical Analysis:

Dissolved organic carbon (DOC) analysis was carried out on a TOC-5000 Shimadzu carbon analyzer using potassium hydrogen phthalate standards. Absorbance scans of natural waters were run on a Varian Cary 1 UV-VIS spectrophotometer from 200 – 600 nm. Specific UV absorbance (SUVA) was obtained by normalizing absorbance values at 280 nm to the carbon concentration of the sample (Chin et al., 1994).

Fluorescence spectroscopy was run on a Varian Cary Eclipse at an excitation wavelength of 370 nm and an emission scan that ranged from 300 – 600 nm. Solutions were diluted to avoid inner filter effects (absorbance at 254 nm was ≤ 0.05 units) (Lackowicz, 1988). Fluorescence results were blank subtracted, scaled for dilution, and corrected in Matlab R2007a using correction curves provided by the manufacturer. The fluorescence index (FI) as defined by Cory and McKnight (2005) was obtained by taking a 5-point moving average of the emission scan for smoothing purposes, and then taking the ratio of corrected fluorescence intensity at 470 nm to 520 nm.

Quantitative analysis of OMP was performed via reverse phase HPLC using a Restek Ultra IBD column (3.2 µm, 3.5 mm x 150 mm) at 0.5 mL min⁻¹, 70/30% v/v of 25 mM KH₂PO₄ (pH 3)/methanol. UV detection was set at 230 nm. Quantitative analysis of SDM is detailed in Guerard et al. (2009).
4.2.4. Photolysis Experiments

Solutions of either 0.1 or 1.0 µM OMP dissolved in Milli-Q water, 0.45 mm filtered water from the field site, or 10 mg-C L⁻¹ fulvic acid solutions were placed into 14 mm diameter, 5 cm long quartz reaction tubes that were sealed with a Teflon lined O-ring clamped to a quartz lid. The 0.1 µM OMP experiments were done to elucidate concentration effects on DOM photosensitized pathways and were only conducted in PLFA or SRFA solutions. Samples were irradiated in a Suntest CPS+ (Atlas Devices) solar simulator with a 500W Xenon lamp over a length of two to three half-lives to determine both the reaction order and rate constants. Actinometer experiments showed that the Suntest operated at ~ 4.5 times the intensity of average sunlight at 40° N at noon in June (Leifer, 1988). A radiometer was used to ensure that no significant changes in irradiance occurred during photolysis, and temperature was maintained at 25° C. Dark controls wrapped in aluminum foil were run concurrently. Photodegradation was log-linearized and found to fit a pseudo-first order model where applicable. Rates were corrected for light screening of fulvic acids and natural waters (see Guerard et al., 2009 for screening calculation methods).

Amendments to the above experiments were conducted to elucidate the reactive pathways. Iron addition (20 µM FeCl₃) in filtered samples and fulvic acid solutions investigated the importance of the photo-Fenton pathway in OMP degradation. The role of the hydroxyl radical (OH•) was assessed in experiments containing 25 mM methanol or isopropanol, which efficiently scavenges this radical. The role of reactive oxygen species in the photofate of OMP was investigated by sparging solutions with argon gas (1 min mL⁻¹) and preparing them in a glove box filled with 95:5 v/v mixture of
nitrogen/hydrogen gas to remove dioxygen as a potential triplet scavenger. Sodium azide or furfuryl alcohol was used as singlet oxygen quenchers in the presence of fulvic acid solutions. Singlet oxygen production was enhanced through the addition of 40 µM Rose Bengal to determine the susceptibility of OMP to $^1\text{O}_2$. OMP in DCFA or CPFA solutions made up in deuterium oxide ($\text{D}_2\text{O}$) were photolyzed to ascertain more directly the effect of singlet oxygen on OMP reactivity. Singlet oxygen steady state concentrations were conducted in the DOM solutions using furfuryl alcohol in fulvic acid solutions according to Haag and Hoigne (1986).

Competition kinetics experiments were conducted to determine the second order reaction rate of OMP with the hydroxyl radical and singlet oxygen. OH• was produced through a dark Fenton pathway, while $^1\text{O}_2$ was formed in solutions of irradiated Rose Bengal. Competition kinetics were done in the presence of acetophenone (OH•) or sodium ascorbate ($^1\text{O}_2$).

For the dark Fenton competition kinetics experiment, Fenton’s reagent was prepared (Haag and Yao, 1992) and conditions were set up similar to those in Packer et al. (2003). To a 100 mL solution (pH 3) of 100 µM OMP and 100 µM acetophenone was added ferrous ammonium sulfate and a spike of $\text{H}_2\text{O}_2$ for final concentrations of .2 mM FAS and 5 mM $\text{H}_2\text{O}_2$. Once hydrogen peroxide was added a timer was started and samples were taken out every 5 minutes and immediately transferred into HPLC vials containing and equal volume of methanol to stop the reaction (Chen and Pignatello, 1997). Two samples for each time point were taken in order to quantitatively analyze OMP concentrations according to methods stated above or acetophenone as stated in
The entire experiment was carried out in a dark room to prevent light from accelerating the reaction.

The competition kinetics experiment for determining the 2\textsuperscript{nd} order rate constant of OMP with singlet oxygen was carried out using sodium ascorbate as the competitive singlet oxygen quencher ($k_q = 3.2 \times 10^8 \text{ M}^{-1} \text{s}^{-1}$ (Bisby et al., 1999)), and Rose Bengal as the singlet oxygen sensitizer. Solutions of 100 \textmu M OMP, 100 \textmu M ascorbate, and 200 \textmu M Rose Bengal in MilliQ were made up at pH 8 to ensure that AscH\textsuperscript{-} is the dominant species rather than AscH\textsubscript{2} (pKa = 4.2), and photolyzed over the course of 3 hours. In this case, removing the tubes out of the solar simulator and moving them to the dark was sufficient to stop the reaction. Samples were analyzed on the HPLC with UV/Vis detection at 220 nm for the ascorbate (Lloyd et al., 1987), using a 50/50\% v/v phosphate buffer/methanol. Finally, experiments were run with both SDM and OMP together at a 5:1 concentration ratio (to mimic their dosage ratio in aquaculture medicated feed) to determine any competitive effects of these two compounds in the same solution during photolysis.

4.3. Results

Direct ormetoprim photodegradation obeyed pseudo first order kinetics (Table 4.1). There was significant OMP photodegradation beyond direct photolysis in the presence of fulvic acids or natural waters, though the effect was least pronounced in Deer Creek whole water (Table 4.1). In the reference fulvic acid solutions, observed degradation rate coefficients increased by an order of magnitude. Indirect photolysis in these samples accounted for over 90\% of the observed OMP degradation even after light screening effects by DOM were taken into account. Observed rate coefficients of
degradation were similar between Suwannee River and Pony Lake fulvic acid solutions. OMP photodegradation in both catfish pond and Deer Creek fulvic acids (CPFA and DCFA respectively) was only about five times faster than direct photolysis, and rates were statistically indistinguishable between the two sources. Observed OMP rate coefficients in filtered catfish pond water were not statistically different from the fulvic acid solutions. In Deer Creek whole water, however, OMP photodegradation was slower than in DCFA solutions. In all of the fulvic acid solutions and in both surface water samples, degradation of OMP was dominated by indirect processes, which made up over 80% of overall observed phototransformation.

Photolysis of lower concentrations of ormetoprim (0.075 µM) was similar to that of the 1 µM solutions. In SRFA, \( k_{\text{obs}} \) was 0.1614 ± 0.0429 hr\(^{-1} \), and in PLFA, \( k_{\text{obs}} \) was 0.0948 ± 0.019 hr\(^{-1} \). Because of the large experimental error, it is difficult to assess whether there was any enhancement in degradation because I was operating near the limits of detection for OMP. An increase in observed rate coefficients due to a decrease in concentration would indicate a preferential reaction of OMP with long-lived radicals (Canonica and Freiburghaus, 2001). Thus, I concluded that there was no significant concentration effect for OMP in the presence of DOM.

4.3.1. OMP Reaction with Hydroxyl Radical

Iron amendment experiments (adding 20 µM Fe) revealed inconclusive results and so quenching experiments to probe for hydroxyl radical were conducted instead. Methanol and isopropanol were each tested as hydroxyl radical scavengers to compare their effectiveness. Isopropanol and methanol were equally effective as hydroxyl radical scavengers, and the rate of degradation of OMP in Pony Lake fulvic acid in the presence
of either of these scavengers is virtually the same within error (Figure 4.2). Thus, throughout the course of this study, methanol was used as the hydroxyl radical quencher, even though it could participate in side reactions that could interact further with OMP. In the presence of methanol, OMP photolysis in PLFA, SRFA, CPFA, and DCFA solutions slowed by 66.3%, 53.0%, 69.5%, and 58.7% respectively (Figure 4.3). Thus, it appears that OMP degradation by OH• is an important pathway.

Competition kinetics experiments were conducted to determine the reaction rate constant with the hydroxyl radical. Reaction rate of OMP with OH• could be determined using the equation,

$$k^s_{\text{OH}} = \frac{\ln(S/S_0)}{\ln(R/R_0)} k^R_{\text{OH}}$$

where $S$ is the substrate OMP, $R$ is the reference compound acetophenone, and $k^R_{\text{OH}}$ is the second order reaction rate constant for acetophenone with hydroxyl radical ($5.9 \times 10^9$ M$^{-1}$s$^{-1}$: Buxton et al., 1988). Both OMP and acetophenone reaction kinetics, however, did not follow pseudo-first order degradation even though a plot of $\ln(S/S_0)$ vs. $\ln(R/R_0)$ did yield a fairly straight line ($R^2 = 0.98$) from which to determine the reaction rate with hydroxyl radical. Thus, the second order reaction rate constant of OMP with hydroxyl radical was not able to be determined.

4.3.2. OMP Reaction with Triplet DOM and Singlet Oxygen

Experiments carried out anoxically (or at least very low levels of oxygen, as a glove box may not be 100% oxygen free) showed no change in the observed rate coefficients of OMP photodegradation in SRFA, but did show retardation of rate in PLFA by 56% (Table 2). Uncertainty for these experiments was considerably larger than non-
sparged experiments, because of oxygen diffusion back into the solution during the transfer from the sparging set up to the glove box, or trace levels of oxygen within the glove box. Thus, it appears that OMP reaction with triplet DOM is not an important pathway.

Addition of Rose Bengal, a known producer of singlet oxygen in the presence of light (Gollnick et al., 1970), to photolysis solutions containing reference fulvic acids resulted in a substantial increase (5x) in the observed degradation coefficient for OMP. Sodium azide and furfuryl alcohol, two compounds known to react with singlet oxygen (Wilkinson et al., 1995; Haag et al., 1984), were unsuccessfully used as quenchers in OMP photodegradation. Furfuryl alcohol co-eluted with OMP, while azide quenched the entire indirect photolytic pathway (Table 4.2). This latter quencher is suspected to participate in side reaction pathways that can further participate in the degradation of the target compound. Azide has been shown to react with hydroxyl radical, and the second order reaction rate of $N_3^-$ with hydroxyl radical was been determined through pulse radiolysis methods (Alfassi and Schuler, 1985).

Furfuryl alcohol was used as a probe to determine singlet oxygen steady state concentrations in the bulk phase of fulvic acid solutions in the presence of UV light and corrected for light screening (Table 4.3, Figure 4.4). Based on error calculations, these values were determined not to be statistically different from each other for all of the fulvic acids studied even though their compositional differences are substantial.

A competition kinetics experiment similar to that conducted to attempt to determine the second order reaction rate of OMP with hydroxyl radical was performed to find the reaction rate with singlet oxygen. I used ascorbate as a singlet oxygen
competitor because it does not suffer from the same analytical issues as furfuryl alcohol. Ascorbate reactions with singlet oxygen according to the following equation to form hydrogen peroxide and dehydroascorbic acid (Kramarenko et al., 2006):

\[ ^1\text{O}_2 + \text{AscH}^- + \text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{DHA} \]

Unfortunately, the ormetoprim photoproducts co-eluted with the ascorbate and so concentrations of these species could not be determined in order to calculate the reaction rates using this method.

4.3.3. Characterization of DCFA and CPFA:

Preliminary characterization data for DCFA and CPFA are presented in Table 4. Excitation-emission matrices (Figure 4.6) reveal an FI of 1.40 and 1.64 in DCFA and CPFA respectively. SUVA values at 254 nm show that DCFA has a SUVA value similar to SRFA, and CPFA has a SUVA value similar to PLFA. Also, fulvic acid isolate of DCFA represents 50% of the total carbon pool in Deer Creek water, while CPFA represents only 25% of the total carbon pool in catfish pond water.

4.3.4. Effect of SDM on the Photodegradation of OMP:

SDM and OMP photodegradation were run together in a 5:1 ratio (5 µM and 1 µM) in Milli-Q water and fulvic acid solutions. Rates of degradation in Milli-Q water were \(-0.0635 \pm 0.002 \text{ hr}^{-1}\) and \(-0.0139 \pm 0.002 \text{ hr}^{-1}\), respectively, and are virtually statistically indifferent from either compound’s direct photolysis observed rate coefficients. However other experiments run as a combination of the two antibiotics together in DOM solutions were inconclusive and are thus not reported.

4.4. Discussion

Direct photolysis of ormetoprim is rather slow, with a half-life of just under 70
hrs in the solar simulator, whose intensity is about 4.5x that of natural sunlight at 40º N in June (Guerard et al., 2009). Thus, in natural sunlight (for example at 40º N in June) and taking into account about 12 hrs a day for darkness, the environmental half-life of OMP from direct photolysis alone (assuming no other reactions and light attenuation is minimal) would be expected to be on the order of 26 days. Given the low light exposure available in the study conducted by Lunestad et al. (1995), these results would seem corroborate their findings of the photo-stability of OMP in seawater where DOM levels are typically very low (< 80 µM carbon) even though these investigators did not actually report carbon levels and seawater contains a number of other constituents such as salts that can interfere with photolytic pathways.

It is interesting to note that there does not seem to be much difference between DOM samples, implying that source may not be as important in the degradation of OMP as other compounds, such as SDM. However, the fulvic acid samples from the Mississippi waters were considerably slower than the IHSS reference fulvic acids. Moreover, the catfish pond whole water and CPFA resulted in the same observed rate coefficients from OMP degradation. This is significant because it suggests that the entire fraction of the organic matter that is reactive to OMP is captured within the fulvic acid isolate.

Deer Creek whole water exhibited the lowest reactivity to OMP of all the DOM containing water samples, with 38% of degradation being due to direct photolysis. This could be due to light screening or other inhibitory components in the water. Unfortunately no analysis was run for anions/cations to assess the existence of other photosensitizers e.g., nitrate. Nonetheless, ALL samples containing organic matter (either
fulvic acid or from whole waters) did facilitate the photochemical degradation of OMP.

Over half of the OMP photodegradation was quenched in the presence of the hydroxyl radical scavenger methanol and provides evidence that reaction between OMP and OH• is an important pathway. Surprisingly, there appear to be minimal differences between the IHSS reference samples. Although alcohol scavengers are highly reactive toward hydroxyl radicals, they can undergo a number of other side reactions, e.g., S_N 1 substitution pathways (Solomons and Fryhle, 2002), which may result in the additional degradation of OMP. Nonetheless, the addition of methanol did not quench all of the reaction in any fulvic acid solution, meaning that there is at least one other pathway of degradation besides the hydroxyl radical.

A dark Fenton reaction using competitive kinetics of OMP with acetophenone for hydroxyl radical was carried out to determine the second order rate constant of OMP with OH•. OMP degradation did not follow first order degradation and it is possible that iron may form a complex with OMP in the reaction solution. However, I was unable to study the complexation between OMP and iron. As such, a second order rate constant could not be determined, and it will have to be sufficient enough to say that OMP does indeed react with OH•.

Mechanistic experiments were only run in IHSS reference fulvic acids, as they are the most characterized DOM samples of the ones used in this study. Anoxic experiments did not show much difference in observed rate coefficient of OMP photodegradation in the presence of SRFA, but slowed considerably in the presence of PLFA. This indicates that some other ROS may also be important in the degradation of OMP. If triplet excited DOM (³DOM) played a significant role as in SDM degradation (Guerard et al. 2009),
then the rate would have increased as removal of oxygen would have meant removal of a 
$^3$DOM scavenger allowing more reaction of $^3$DOM with OMP. But this is not the case, 
indicating that one or more ROS species is involved.

Singlet oxygen is a selective and highly reactive electrophilic non-radical ROS, 
and as such, can participate in a number of oxidation reactions. Singlet oxygen may be 
formed by DOM through the following series of reactions (Cory et al., 2009):

\[
\text{DOM} + h\nu \rightarrow \text{DOM}^* \\
\text{DOM}^* + ^3\text{O}_2 \rightarrow \text{DOM} + ^1\text{O}_2
\]

Thus, retardation of OMP degradation in an anoxic system may indicate the presence of 
singlet oxygen.

Singlet oxygen has been shown to react with model compounds containing 
phenol/phenolate functional groups (McNally et al., 2005). And while OMP has no 
phenol groups, OMP does have electron donating functional groups that make its rings 
electron rich and thus more susceptible to reaction with singlet oxygen. The methoxy 
and alkyl groups on the aromatic ring (Figure 4.1) are both electron donating. However, 
given the size and close proximity of these groups to each other, one would estimate that 
steric hindrances would inhibit reactions of singlet oxygen in this location. The amine 
groups on the pyrimidine ring are also electron donating, however, and would contribute 
to a higher electron density within the N-containing ring. Singlet oxygen has been shown 
to contribute to degradation of a number of compounds with pyrimidine bases, namely 
nucleic acids, but even caffeine to some extent (Rosenthal and Pitts Jr., 1971). Ravanat 
et al. (2006) have proposed that such oxidation by singlet oxygen may occur via an 
endoperoxide addition onto N-containing rings. Given this evidence, it is definitely
possible for singlet oxygen to be reactive towards OMP, even if I could not definitively measure its second order reaction rate constants.

Addition of Rose Bengal showed that OMP reacts with singlet oxygen, and this might be the ROS responsible for at least some of the indirect photodegradation observed that was not accounted for in the OH• scavenging experiments. While addition of a singlet oxygen sensitizer indicates susceptibility of OMP to singlet oxygen, it does not give any information as to the extent of the OMP photodegradation by singlet oxygen in the presence of photo-irradiated DOM. To assess this, singlet oxygen quenchers were added to the system. Furfuryl alcohol (FFA) was added to scavenge singlet oxygen during OMP photolysis. Unfortunately, no information could be obtained as FFA co-eluted with the OMP, and thus an accurate quantitative determination of OMP concentrations over the course of the experiment could not be obtained. Sodium azide (NaN$_3$) was also investigated, but side reactions involving azide radicals may have scavenged other ROS (Cawley et al., 2009). Thus, I could not use this data to determine extent of singlet oxygen participation in OMP indirect photodegradation pathways.

I measured the steady state singlet oxygen concentration produced in irradiated fulvic acid solutions (Table 4.3). Unfortunately, experimental error showed that the amount of $^1$O$_2$ produced could not be attributed to DOM composition. My results however are on par with other singlet oxygen steady state measurements in the bulk phase (Haag and Hoigne, 1986). Measurements of singlet oxygen steady state concentrations were determined to be appropriate here because OMP is relatively polar (calculated log $K_{ow}$ =1.41) and is not expected to reside completely in microheterogeneous hydrophobic pockets within the organic matter, where much higher
singlet oxygen concentrations have been measured (Latch and McNeill, 2006; Grandbois et al., 2008). Thus, OMP would not partition very strongly into the hydrophobic areas of DOM and would remain mostly in the bulk aqueous phase.

Degradation of SDM and OMP together in MilliQ water do not show any differences from direct photolysis rates when they were photolyzed separately. This is not unexpected, as in MilliQ water there are no indirect degradation processes for the two compounds to compete with each other. Since, in the presence of DOM, the compounds could not be quantitatively resolved, results are inconclusive as to whether one antibiotic affects the other’s degradation. However, given that SDM and OMP appear to have affinity towards different reaction pathways, one could expect the degradation of these compounds to be independent of each other, especially given their low concentrations compared to the amount of organic carbon within the ponds.

Mississippi fulvic acid solutions also showed considerable enhancement towards SDM photodegradation, with observed rate coefficients comparable to experiments conducted in reference fulvic acids (Chapter 3). This is different from observed OMP reaction in Mississippi fulvic acid solutions, where observed degradation was considerably slower than in reference fulvic acids. Such difference in reactivity of these antibiotics in these fulvic acids also suggests that these compounds degrade through different pathways.

4.5. Conclusions

Ormetoprim direct photolysis is much slower than SDM, but indirect photolysis is enhanced much more in the presence of DOM. Indirect reaction pathways appear to be through hydroxyl radical and singlet oxygen attack, though more information is needed to
know which of these pathways is dominant in the catfish pond waters. Future work identifying and quantifying OMP photoproducts may help answer this question. Mississippi fulvic acid samples appear to be end members of the DOM source spectrum, with CPFA being similar to PLFA and DCFA being similar to SRFA. OMP shows no difference in degradation enhancement between the Mississippi fulvic acid samples.

4.6. Acknowledgments

I would like to acknowledge Dr. Patricia Gaunt and the Mississippi State Delta Research and Extension Center for access to ponds and logistical assistance, and Collin Ward and Sheela Agrawal for helping with sampling. This research was funded by EPA STAR FP-91678401-0 awarded to J. J. G.
4.7. Tables

Table 4.1. Photodegradation data for OMP in various DOM solutions.

1Light screening factor for wavelengths 290-350 nm. 2$k_{obs}$ represents the observed pseudo first-order rate degradation coefficient measured from a least squares fit of degradation kinetics. 3$t_{1/2}$ represents half-life calculated from $t_{1/2} = \ln (2) / k_{obs}$. 4$k_{dp}$ represents the rate coefficient predicted for contribution of direct photolysis to the overall degradation in a given DOM solution. 5$k_{ip}$ represents the rate coefficient predicted for contribution of indirect photolysis to the overall degradation. 6The percentage contribution of direct (% DP) and indirect photolysis (% IP) to the overall observed degradation.

<table>
<thead>
<tr>
<th>Medium</th>
<th>$S_{290-350}$</th>
<th>$k_{obs}$ x 100 (hr$^{-1}$)$^2$</th>
<th>$t_{1/2}$ (hrs)$^3$</th>
<th>$k_{dp}$ x 100 (hr$^{-1}$)$^4$</th>
<th>$k_{ip}$ x 100 (hr$^{-1}$)$^5$</th>
<th>%DP$^6$</th>
<th>%IP$^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MilliQ</td>
<td>------</td>
<td>1.01 ± 0.5</td>
<td>68.6</td>
<td>1.01</td>
<td>------</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>SRFA</td>
<td>0.85</td>
<td>10.40 ± 0.8</td>
<td>6.7</td>
<td>0.94</td>
<td>10.89</td>
<td>8</td>
<td>92</td>
</tr>
<tr>
<td>PLFA</td>
<td>0.93</td>
<td>11.83 ± 0.2</td>
<td>5.9</td>
<td>0.86</td>
<td>9.54</td>
<td>8</td>
<td>92</td>
</tr>
<tr>
<td>CPFA</td>
<td>0.86</td>
<td>5.90 ± 1.1</td>
<td>11.7</td>
<td>0.87</td>
<td>5.03</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>DCFA</td>
<td>0.86</td>
<td>4.60 ± 1.1</td>
<td>15.1</td>
<td>0.87</td>
<td>3.73</td>
<td>19</td>
<td>81</td>
</tr>
<tr>
<td>CPWW</td>
<td>0.96</td>
<td>6.71 ± 0.3</td>
<td>10.3</td>
<td>0.97</td>
<td>5.74</td>
<td>14</td>
<td>86</td>
</tr>
<tr>
<td>DCWW</td>
<td>0.93</td>
<td>2.45 ± 0.3</td>
<td>28.3</td>
<td>0.94</td>
<td>1.51</td>
<td>38</td>
<td>62</td>
</tr>
</tbody>
</table>
Table 4.2. Observed rate coefficients of degradation for OMP in SRFA or PLFA in the presence of various solution amendments. $k_{\text{obs}}$ for furfuryl alcohol addition is not reported due to inability to resolve peaks through HPLC methods.

<table>
<thead>
<tr>
<th>DOM</th>
<th>DOM Only</th>
<th>Anoxic</th>
<th>40µM RB Addition</th>
<th>N$_3$ Addition</th>
<th>FFA Addition</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRFA</td>
<td>10.40 ± 0.8</td>
<td>9.83 ± 1.2</td>
<td>49.01 ± 11.2</td>
<td>1.43 ± 0.2</td>
<td>-----</td>
</tr>
<tr>
<td>PLFA</td>
<td>11.83 ± 0.2</td>
<td>5.21 ± 0.9</td>
<td>62.52 ± 9.7</td>
<td>1.25 ± 0.2</td>
<td>-----</td>
</tr>
</tbody>
</table>
Table 4.3. Singlet oxygen steady state concentrations (corrected for light screening) in the bulk phase as determined by furfuryl alcohol photodegradation rates (Haag and Hoigné, 1986).

<table>
<thead>
<tr>
<th>DOM</th>
<th>$[^1\text{O}<em>2]</em>{ss} \times 10^{-13} \text{(M)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLFA</td>
<td>3.58 ± 0.38</td>
</tr>
<tr>
<td>SRFA</td>
<td>3.63 ± 0.44</td>
</tr>
<tr>
<td>CPFA</td>
<td>3.90 ± 0.35</td>
</tr>
<tr>
<td>DCFA</td>
<td>5.66 ± 1.00</td>
</tr>
</tbody>
</table>
Table 4.4. Characterization data for fulvic acids. FI represents fluorescence index, ratio of fluorescence at excitation 370 nm, emission 470/520nm. SUVA is specific UV absorbance at 254 nm. %C is the percentage of the carbon pool that the fulvic acid represents. ¹Guerard et al., 2009 ²McKnight et al., 2001 ³Chin et al., 1994

<table>
<thead>
<tr>
<th>DOM</th>
<th>FI</th>
<th>SUVA₂₅₄</th>
<th>%C</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLFA</td>
<td>1.453 ¹</td>
<td>207 ¹</td>
<td>25% ¹</td>
</tr>
<tr>
<td>SRFA</td>
<td>1.30 ²</td>
<td>389 ³</td>
<td>50% ²</td>
</tr>
<tr>
<td>DCFA</td>
<td>1.4042 ³</td>
<td>362</td>
<td>50%</td>
</tr>
<tr>
<td>CPFA</td>
<td>1.6404</td>
<td>252</td>
<td>25%</td>
</tr>
</tbody>
</table>
4.8. Figures

Figure 4.1. Structure of ormetoprim.
Figure 4.2. Degradation of OMP in PLFA in the presence of either methanol or isopropanol. Observed degradation rate coefficients are statistically indifferent for either hydroxyl radical probe.
Figure 4.3. Photodegradation of OMP in DOM samples and with methanol addition.
**Figure 4.4.** Photodegradation of furfuryl alcohol in fulvic acids. Degradation rates were used to determine singlet oxygen steady state concentrations in photolyzed fulvic acid solutions.
Figure 4.5. Photodegradation of OMP in fulvic acid solutions made up in D$_2$O. A. OMP degradation fitted to a pseudo first order curve. B. OMP degradation fitted to a second order curve.
Figure 4.6. Spectroscopic characterization data of DCFA and CPFA. A. EEM of Deer Creek fulvic acid. B. EEM of Catfish Pond fulvic acid. C. UV/Vis absorbance curves of CPFA, DCFA.
4.9. References


Chapter 5: Conclusions and Future Work

To date, very few studies have considered how dissolved organic matter (DOM) source composition may have an impact on its ability to promote the photosensitized degradation of organic contaminants. Most studies focus on uncharacterized DOM in water samples collected from a specific water body or have used fulvic and humic acids from the International Humic Substances Society or other suppliers. Natural waters from locations worldwide have been used to investigate the photochemical fate of a number of contaminants. The advantage to this method is its simplicity and unbiased representation of the entire organic carbon pool in the water sample. Unfortunately, the DOM in these waters typically remains uncharacterized and, in some cases, other chemical species in the sample (nitrate, pH, presence of metals) could influence photodegradation, making it difficult to delineate DOM’s exact role in the contaminant’s fate (Wilson and Mabury, 2000; Miller and Chin, 2005).

The other common approach to study DOM’s role as a photosensitizer is to use a solution containing a subset of the organic matter pool such as the fulvic acids used in this study. Fulvic acids represent a fraction (20 – 50 %) of the DOM pool and, unlike a whole water sample, are stable in its lyophilized state. In addition, evidence in the literature show that the fulvic acid fraction of DOM typically accounts for the dominant share of light absorption (McKnight et al., 2001). While fulvic (and to a lesser extent
humic) acids are useful for inter-comparative studies, the results are applicable to that specific isolate and cannot be used to interpret overall DOM photoreactivity from other water bodies.

However, characterization studies on DOM from different sources (such as from Pony Lake) can provide insight on the differences in chemical composition between organic matter samples. Future work using a more systematic approach to assess contaminant fate in the presence of multiple organic matters from various origins may prove to be much more useful in predicting fate and transformation in natural environments, depending on a natural water body’s characteristics. It would also be interesting to combine end-member reference fulvic acids in solutions of different proportions and conduct photodegradation experiments of contaminants, in order to discern exactly how much DOM source contributes to the photofate of those compounds.

This work has shown that DOM mediated indirect photolysis of organic compounds can occur via a number of possible pathways and is in part driven by the contaminant's chemical reactivity. However, there exists a paucity of data that examines the role of DOM composition on photosensitized reactions. In this study, the indirect photolysis of sulfadimethoxine (SDM) and ormetoprim (OMP) was consistent with predominantly triplet DOM mediated pathway and ROS mediated pathways, respectively. DOM showed different reactivities towards promoting the enhancement of indirect degradation of these compounds. SDM photodegradation was very sensitive to source composition, with microbially-derived fulvic acids increasing photochemical transformation rates the most. However, with OMP, such a trend in indirect enhancement with source composition was not observed, suggesting that some reactive pathways promoted by the
presence of DOM may be more source dependent than others.

Future work needs to be done to obtain a clearer appreciation of the exact pathways and mechanisms of degradation of the antibiotic compounds studied. SDM reacts by triplet excited materials (Chapter 3). It would be helpful to isolate the photoproducts from SDM phototransformation separately and run NMR analysis (if possible) on them in order to combine with the mass spectra data and actually determine the structures of the photoproducts. This information would be very useful in working to elucidate specific mechanisms of reaction. OMP reacts by the hydroxyl radical and singlet oxygen according to the current evidence (Chapter 4). Future work needs to be done to better resolve HPLC methods in competition kinetics experiments in order to obtain accurate second order rate constants for reaction with hydroxyl radical and singlet oxygen. Also, isolation and identification of the photoproducts from OMP breakdown need to be characterized and quantified.

I planned, but was unable to carry out bacterial efficacy studies on the photoproducts of these antibiotics. If the breakdown products exhibit antibiotic properties, then this could have implications about the possibility of developing resistant strains of bacteria in the environment. The plan would have been to use *Escherichia coli* as a bacterial probe to look at the antibiotic effectiveness of these compounds before and after photolysis, as *E. coli* has been shown to be sensitive to both SDM and OMP (Lunestad et. al, 1995). The procedure for this would involve growing cultures of *E. coli* in a broth and then putting them onto agar plates. Sterile diffusion disks saturated with samples of these antibiotics before and after photolysis could be placed in the center of
the plate, and then the resultant zone of inhibition between the disk and the bacteria could be measured to assess relatively antibacterial efficacy.

Further work needs to be done to study the photolyses of these antibiotics combined together, particularly in the presence of DOM. Mimicking the situation in the catfish ponds as closely as possible will lead to better results and more accurate predictions as to the fate of these compounds. One thing that was planned, but was not executed due to logistical reasons, was to sample directly from the ponds during the medicated feed application period, that is, to monitor in real time the degradation of these compounds within the ponds.

Further characterization beyond cursory spectroscopy studies would be valuable in associating any trends observed in Mississippi DOM reactivity towards these compounds with their composition. It would also be interesting to do more work looking at reactive oxygen species (ROS) production within the Mississippi organic matter samples (rather than just singlet oxygen steady state concentrations) to see if any trends between ROS production rates and reactivity with these antibiotics occur. This information would prove to be insightful when assessing the fate of these compounds within the actual catfish ponds.

An ever-present gap in the state of the art of knowledge about organic matter reactivity is understanding how source composition affects DOM reactivity in the natural environment. The studies shown here only scratch the surface of this area of research, and I would really like to in the future work make a better assessment of this phenomenon. It would be interesting to investigate trends in ROS production across DOM samples from various sources, and compare the photoreactivity of compounds that
degrade by specific mechanisms within such DOMs. The problem with this approach is a lack of characterization data and ROS production in many DOMs, and lack of current systematic study of source composition within the context of photochemical reactivity. A larger across the board study of a number of well-characterized organic matter samples with different contaminant compounds with well established structure-reactivity relationships would be a very large task to undertake, but would help us understand DOM’s very complex, but important role in not only contaminant fate, but biogeochemical processes in general.

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