**Correlating the Chemical and Spectroscopic Characteristics of Natural Organic
Matter with the Photodegradation of Sulfamerazine**

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Abstract

The role of aquatic natural organic matter (NOM) in the removal of contaminants of emerging concern has been widely studied. Sulfamerazine (SMR), a sulfonamide antibiotic detected in aquatic environments, is implicated in environmental toxicity and may contribute to the resistance of bacteria to antibiotics. In aquatic systems sulfonamides may undergo direct photodegradation, and, indirect photodegradation by generating reactive species. Because some forms of NOM inhibit the photodegradation there is an increasing interest in correlating the spectroscopic parameters of NOM as potential indicators of its degradation in natural waters.

Under the conditions used in this study, SMR hydrolysis showed to be negligible; however, direct photolysis is significant in most of the solutions studied. Photodegradation was investigated using standard solutions of NOM: Suwannee River natural organic matter (SRNOM), Suwannee River humic acid (SRHA), Suwannee River fulvic acid (SRFA), and Aldrich humic acid (AHA). The steady-state concentrations and formation rates of the reactive species and the SMR degradation rate constants (k_1) were correlated with NOM spectroscopic parameters determined using UV-vis absorption, excitation-emission matrix fluorescence spectroscopy (EEMs), and proton nuclear magnetic resonance spectroscopy (^1H NMR).

SMR degradation rate constants (k_1) were correlated with steady-state concentrations of NOM triplet-excited state ($[^3\text{NOM}^*]_{\text{ss}}$) and the corresponding formation rates ($^3\text{NOM}^*$) for SRNOM, SRHA, and AHA. The efficiency of SMR degradation was highest in AHA solution and was inhibited in solutions of SRFA. The steady-state concentrations of singlet oxygen ($[^1\text{O}_2]_{\text{ss}}$) and the SMR degradation rate constants with singlet oxygen ($k_{1\text{O}_2}$) were linearly correlated with the total

46 fluorescence and inversely correlated with the carbohydrate/protein content (^1H
47 NMR) for all forms of NOM. The total fluorescence and EEMs Peak A were
48 confirmed as indicators of $^1\text{O}_2$ formation. Specific ultraviolet absorbance at 254 nm
49 (SUVA_{254}) and aromaticity showed potential correlations with the steady-state
50 concentrations of hydroxyl radical ($[\text{HO}\cdot]_{\text{ss}}$) and the corresponding formation rates
51 ($\text{HO}\cdot$).

52

53 **Keywords:** EEMs; natural organic matter; photodegradation; sulfamerazine; ^1H
54 NMR.

55

56 **Introduction**

57 Pharmaceuticals and their photodegradation products are contaminants of
58 emerging concern because of their prevalence in many natural waters (Yan and Song
59 2014, Brooks et al. 2009, Celiz et al. 2009) and the lack of any environmental
60 regulations. Natural organic matter (NOM) can be either a sensitizer or an inhibitor of
61 photodegradation processes and the correlation of chemical and spectroscopic
62 parameters may assist in understanding these processes.

63 Sulfamerazine (SMR) is a sulfonamide antibiotic designed to treat human and
64 animal infections (Kümmerer 2009a, b) and is also used in formulated feed in
65 aquaculture (Sapkota et al. 2008). Adverse environmental effects of sulfonamides
66 include their toxicity to organisms such as the crustacean *Hyalella azteca* (Bartlett et
67 al. 2013) and their contribution to bacterial resistance (Pei et al. 2006). Understanding
68 the processes involved in the environmental fate of these antibiotics is important
69 because sulfonamides and their metabolites are not completely degraded in either
70 wastewater treatment plants (García-Galán et al. 2010) or in natural waters (Boreen et
71 al. 2003, 2004a, 2005a).

72 The environmental fate of pharmaceuticals in general and sulfonamides in
73 particular has been studied in natural waters and in NOM solutions (Boreen et al.
74 2004a, 2005a, Bahn Müller et al. 2014, Guerard et al. 2009a). The absorption of light
75 by NOM generates the singlet-excited state of NOM ($^1\text{NOM}^*$) that may lose energy
76 through intersystem crossing to triplet-excited state ($^3\text{NOM}^*$) (Zepp et al. 1985). The
77 $^3\text{NOM}^*$ reacts with di-oxygen ($^3\text{O}_2$) in aerated solutions to produce singlet oxygen
78 ($^1\text{O}_2$) (al Housari et al. 2010, Cooper et al. 1988). Sulfonamides undergo both direct
79 and indirect photodegradation in NOM solutions primarily through the triplet-excited

state of NOM (Bahnmüller et al. 2014, Boreen et al. 2005b, 2004b). Degradation can be accelerated in the presence of autochthonous (phytoplankton-derived) NOM (Guerard et al. 2009b) or inhibited by phenolic-like components of the light-absorbing fraction of NOM (Canonica and Laubscher 2008, Wenk and Canonica 2012). The electron donating capacities (EDC) and electron accepting capacities (EAC) of NOM have been linked to NOM inhibition (Aeschbacher et al. 2010, Aeschbacher et al. 2012). Natural waters and reference NOM standards including Suwannee River natural organic matter (SRNOM), Suwannee River humic acid (SRHA), and Suwannee River fulvic acid (SRFA) are commonly used for evaluating the environmental fate of antibiotics (Bahnmüller et al. 2014, Wenk and Canonica 2012, Wenk et al. 2011, Guerard et al. 2009b). Aldrich Humic Acid (AHA) is also frequently used to characterize NOM photoreactivity although it is derived from brown coal and is not an aquatic NOM (Minella et al. 2013, Appiani and McNeill 2015, Latch and McNeill 2006, Aguer and Richard 1996). AHA is one of the few forms of NOM able to generate triplet-excited states ($^3\text{NOM}^*$) instead of solvated electrons by laser flash photolysis (Cottrell et al. 2013).

Chromophoric NOM (also termed CDOM) is the light-absorbing component of NOM responsible for aquatic photochemistry (Zafiriou et al. 1984) arising from charge transfer (CT) interactions that generated the excited state species (Sharpless and Blough 2014). Three-dimensional excitation emission matrix fluorescence spectroscopy (EEMs), (Coble 1996, Her et al. 2003, Valencia et al. 2013) and UV-vis spectroscopy (Weishaar et al. 2003, Helms et al. 2008) are important tools in determining NOM structure and composition. Recent studies show that correlations between parameters such as quantum yield (Cawley et al. 2014) and spectroscopic characteristics can be potential predictors of NOM reactivity. Fluorescence is one

predictor of $^1\text{O}_2$ formation in natural waters (Shao et al. 1994) and from soil humic substances (Coelho et al. 2011). Fluorescence was also shown to correlate with the quantum yield of the hydroxyl radical in wastewater (Lee et al. 2013). The specific ultraviolet absorbance at 254 nm (SUVA_{254}) is correlated with aromatic carbon content (Weishaar et al. 2003).

Proton nuclear magnetic resonance (^1H NMR) provides high-resolution molecular information for the characterization of NOM (Simpson et al. 2012, Minor et al. 2014, Mopper et al. 2007). The chemical shifts or proton resonance frequencies are characteristic of the structural components of NOM that include aromatic, carbohydrate-protein, carboxylic acid rich (CRAM) in alicyclic compounds (Hertkorn et al. 2006), and material derived from linear terpenoids (MDLT) (Lam and Simpson 2009). The peak areas are proportional to proton resonance, making ^1H NMR a quantitative tool for analysis on a few milligrams of material (Cottrell et al. 2013b). While SUVA_{254} and fluorescence are source-dependent for natural waters (Timko et al. 2014), to our knowledge there are few direct comparison of these spectroscopic parameters for assessing the formation of reactive species from SRNOM, SRFA, SRHA and AHA solutions. The photodegradation of sulfamerazine has been well characterized in natural waters (Boreen et al. 2005b) and in advanced oxidation processes (Batista et al. 2014) making it a useful probe to study its photochemical fate with NOM from different sources. In this study SRNOM, SRHA, SRFA, and AHA were used to examine the production of hydroxyl radical ($\text{HO}\bullet$), singlet oxygen ($^1\text{O}_2$), and the triplet-excited state of NOM ($^3\text{NOM}^*$) and their effect on the sulfamerazine degradation using simulated sunlight. Spectroscopic parameters (SUVA_{254} , EEM, and ^1H NMR) of the NOM samples were used to determine potential correlations with

SMR degradation rate constant (k_1) and the steady-state concentrations and formation rates of reactive species.

2. Experimental Methods

2.1 Reagents

All the solutions were prepared with MilliQ-Q[®] water (Millipore, MA). Sulfamerazine (SMR, 4-amino-N- (4-methylpyrimidin-2-yl)benzene-1-sulfonamide, ACS reagent grade, MM= 264 g mol⁻¹), sorbic acid ($\geq 99.0\%$), terephthalic acid (98%), and furfuraldehyde (FAD, 99%) were purchased from Sigma-Aldrich. Acetonitrile and methanol (HPLC grade), ammonium acetate (97.8%), *o*-phosphoric acid (85%), and sodium hydroxide solution (10 mol L⁻¹) were purchased from Fisher Scientific. 2-hydroxyl terephthalic acid (TPA-OH), used for calibration, was synthesized as described elsewhere (Mason et al. 1994). Furfuryl alcohol (98%) was purchased from Acros Organics. Ultra high-purity nitrogen was obtained from Airgas. SRNOM (1R101N), SRFA (1S101F), and SRHA (2S101H) were obtained from the International Humic Substances Society (IHSS). Aldrich humic acid sodium salt (AHA) was obtained from Sigma-Aldrich. NOM was dissolved in 10 mmol L⁻¹ phosphate buffer (15 mg L⁻¹) and sonicated to dissolve.

2.2 Hydrolysis of sulfamerazine

Hydrolysis studies were performed in 10 mmol L⁻¹ phosphate buffer, at pH 7 and room temperature. Sulfamerazine solutions were prepared in 40 mL amber vials and aliquots (500 μ L) were taken over 12 h and analyzed by HPLC (injection vol. = 50 μ L).

2.3 Irradiation experiments

Direct and indirect photodegradation experiments with sulfamerazine were performed in 10 mmol L⁻¹ phosphate buffer (pH = 7) in the absence and presence of NOM using a Luzchem SolSim solar simulator (Ottawa, Canada) equipped with a rotating table. The output of the 300-W ceramic Xe lamp was adjusted daily (Reliability Direct AR823 power meter, USA). An 1/800 Esco optical glass filter was used to match the spectrum and approximately the intensity of the AM1.5 solar spectrum in the range 290-800 nm with power setting ~ 77%. Samples were irradiated in 4-mL quartz cuvettes (Starna, USA) sealed with silicon septa. The reaction mixture was subsampled (500 µL) over 360 min and analyzed by HPLC. The SMR degradation rate constant (k_1) was determined using pseudo first-order kinetics. In this study, standard deviations were calculated from three replicates of the experiments.

2.4 Determining the formation rate and steady state concentrations of reactive species

The formation rate and steady state concentrations of hydroxyl radical ($\bullet$ OH), singlet oxygen (1 O₂), and the triplet excited-state of NOM (3 NOM^{*}) were determined in 10 mmol L⁻¹ phosphate buffer at pH 7 with 15 mg L⁻¹ NOM (Timko et al. 2014). Reactions for determining 3 NOM^{*} were performed in oxygenated solutions for determining $\bullet$ OH and 1 O₂, and in de-aerated (15 min de-aeration with nitrogen). All the reactions were performed in triplicate.

2.4.1 Singlet oxygen

The formation rate and steady-state concentrations of 1 O₂ were measured using furfuryl alcohol (FFA) (Xu et al. 2011, Wang et al. 2012, Haag et al. 1984). The

initial rate of FFA loss (R_{FFA}) was determined from the change in FFA concentration (initial conc. = 1.5 mmol L⁻¹) with time. The bimolecular reaction rate constant of SMR and ¹O₂ was determined by a competitive kinetics study using furfuraldehyde (FAD) with Rose Bengal as a photosensitizer for ¹O₂ production in solution (Razavi et al. 2011, Xu et al. 2011). The pseudo first-order SMR degradation rate constant with singlet oxygen, $k_{1\text{O}_2}$, was then calculated using the bimolecular reaction rate constant ($7.93 \times 10^4 \text{ L mol}^{-1} \text{ s}^{-1}$, see Supplemental Information, Fig. SI 2) (Xu et al. 2011). Aliquots of samples (500 µL) were taken over 2 h and analyzed by HPLC (injection vol. = 50 µL).

2.4.2 Hydroxyl radical

The formation rate and steady-state concentrations of •OH were measured using 0.60 mmol L⁻¹ terephthalic acid (TPA) (Page et al. 2010). The oxidation of TPA by •OH generates 2-hydroxyl terephthalic acid (TPA-OH), with a reaction yield of 35% (Mark et al. 1998). Hydroxyl radical concentrations were measured using terephthalic acid (TPA) as a kinetic probe. TPA and TPA-OH concentrations were monitored by HPLC (Luo et al. 2012, Razavi et al. 2011). There was no loss of 2-hydroxyl terephthalic acid (TPA-OH) in irradiations < 100 min (Timko et al. 2014).

To measure the SMR degradation rate constant with hydroxyl radical, $k_{\bullet\text{OH}}$, in NOM solutions the pseudo first-order rate constant was calculated from the steady-state concentrations of •OH and the bimolecular reaction rate constant of SMR and •OH [$(7.8 \pm 0.3) \times 10^9 \text{ L mol}^{-1} \text{ s}^{-1}$] obtained by Mezyk et al. (2007). Aliquots of samples (500 µL) were taken over 1 h and analyzed by HPLC (injection vol. = 50 µL).

2.4.3 Triplet-excited state of NOM

The formation rate and steady-state concentrations of $^3\text{NOM}^*$ were determined using sorbic acid (trans,trans-hexadienoic acid, t,t-HDA) to quench the triplet excited state (Grebel et al. 2011, Timko et al. 2014). The concentrations of cis–trans isomer of sorbic acid (cis,trans-hexadienoic acid, c,t-HDA) were measured during irradiation of six initial concentrations of sorbic acid (t,t-HAD) in de-oxygenated solution containing 15 mg L^{-1} NOM (10 mmol L^{-1} phosphate buffer at pH 7). The reactions were performed over 1 h without any observed quenching of the $^3\text{NOM}^*$. The data were analyzed by linearization of the kinetic expression to calculate the formation rate of cis–trans isomer of sorbic acid (c,t-HDA). The values of c, t-HDA formation rate were divided by the yield, 0.18, to obtain the removal rate of $^3\text{NOM}^*$ by sorbic acid, R_p . The formation rates ($^3\text{NOM}^*$) and steady-state concentrations, $[^3\text{NOM}^*]_{ss}$, of triplet excited-state of NOM were calculated from regressing $[t,t\text{-HAD}]/R_p$ against $[t,t\text{-HAD}]$ yields according to previous studies (Grebel et al. 2011, Timko et al. 2014). To avoid air introduction during sampling, the aliquots were taken from 4-mL quartz cuvettes (Starna, USA) sealed with silicon septa using a glass syringe fitted with a 9-in stainless-steel needle (Sigma-Aldrich, USA). The role of $^3\text{NOM}^*$ was studied in the presence or absence of the t,t-HAD (0.18 mmol L^{-1}). To measure the SMR degradation rate constant with respect to $^3\text{NOM}^*$, $k_{3\text{NOM}^*}$, the pseudo first-order rate constant was calculated in de-aerated NOM solutions. Aliquots of samples ($500 \text{ }\mu\text{L}$) were taken over 2 h and analyzed by HPLC (injection vol. = $50 \text{ }\mu\text{L}$).

2.5 HPLC analysis

HPLC analysis was performed using an Agilent 1200 HPLC, equipped with diode array (DAD G1315C) and fluorescence (FLD G1321A) detectors. Sulfamerazine (SMR) was analyzed using a sample injection volume of 50 μL . The eluents were (A) H_2O + 0.2% acetic acid and (B) acetonitrile at 85:15 ratio and 0.8 mL min^{-1} flow rate. The DAD detection wavelength was 268 nm. The retention time was 2.77 min using a Gemini C18 column (50 mm $\times$ 4.60 mm, 3 μm). An instrumental calibration curve was determined for SMR. Under these conditions, SMR detection limit was 4.4 $\mu\text{mol L}^{-1}$ and the corresponding quantification limit was 13.5 $\mu\text{mol L}^{-1}$.

For the quantification of furfuryl alcohol (FFA) the sample injection volume was 10.0 μL . The eluents were (A) methanol and (B) 30 mmol L^{-1} ammonium acetate buffer (pH 4.72) at 10:90 ratio and 1.0 mL min^{-1} flow rate. An instrumental calibration curve was determined for FFA. The DAD detection wavelength was 219 nm. The retention time was 2.5 min using a Gemini C18 column (50 mm $\times$ 4.60 mm, 3 μm).

For the quantification of terephthalic acid (TPA) and 2-hydroxyterephthalic acid (TPA-OH) the sample injection volumes were 3.0 μL and 40 μL , respectively. The eluents were (A) methanol and (B) 0.08% H_3PO_4 at 50:50 ratio and 1.00 mL min^{-1} flow rate. The DAD detection wavelength was 254 nm for TPA; for the FLD detection of TPA-OH, the excitation and emission wavelengths were 240 nm and 425 nm, respectively. An instrumental calibration curve was determined for TPA and TPA-OH. The retention times were 5.6 min for TPA and 7.2 min for TPA-OH using a Gemini C18 column (250 mm $\times$ 4.60 mm, 5 μm).

For sorbic acid (t,t-HDA) and its isomer (c,t-HDA) the sample injection volumes were 10 and 100 μL , respectively. An instrumental calibration curve was determined for t,t-HDA. As isomer standard of cis–trans isomer of sorbic acid (c,t-HDA) was not commercially available, molar absorption coefficient correction factors at 254 nm relative to t,t-HAD were used to determine the corrected calibration curve for the isomer product according to a previous study (Grebel et al. 2011, Timko et al. 2014). The eluents were (A) acetonitrile and (B) 30 mmol L^{-1} ammonium acetate buffer (pH 4.72) at 15:85 ratio and 1.00 mL min^{-1} flow rate. The DAD detection wavelength was 254 nm. The retention times were 15.7 min for t,t-HDA and 14.5 min for c,t-HDA, using a Gemini C18 column (250 mm $\times$ 4.60 mm, 5 μm).

2.6 Specific ultraviolet absorbance at 254 nm (SUVA_{254})

The UV-Vis absorption spectra of NOM solutions were measured with a Varian Cary 100 Bio UV-Vis Spectrophotometer using a 1-cm path length quartz cuvette. SUVA_{254} ($\text{L.NOM mg}^{-1}.\text{m}^{-1}$) was determined for SRNOM, SRFA, SRHA, and AHA (15 mg L^{-1} in 10 mmol L^{-1} phosphate buffer) at pH 5, 7 and 9. SUVA_{254} was calculated by dividing the absorbance at 254 nm (m^{-1}) by NOM concentration (mg NOM L^{-1}) (Weishaar et al. 2003).

2.7 Excitation-emission matrix fluorescence spectroscopy

Excitation-emission analyses fluorescence (EEMs) spectra were obtained using a FluorMax-4 spectrometer (Horiba Jobin Yvon, Inc.) with a 1-cm path length quartz cuvette. The EEMs were measured in the range 240-600 nm at 5 nm intervals. Spectra were corrected for Raman scattering using FL Toolbox 1.91 for Matlab[®] (Zepp et al. 2004). Corrections for inner filtering effects were performed using the absorbance-

based approach (Kothawala et al. 2013). The $\lambda_{\text{ex}}/\lambda_{\text{em}}$ peak maxima for Peaks A: 250/450 nm (SRNOM, SRFA), 250/482 nm (AHA), 250/478 nm (SRHA) Peak C: 340/425 nm, Peak T: 275/345, and Peak M 310/400 nm.

2.8 ^1H NMR of NOM

Proton NMR (^1H NMR) was performed on Suwannee River natural organic matter, Suwannee River fulvic acid, Suwannee River humic acid, and Aldrich humic acid. NOM was dissolved in D_2O (7 mg mL^{-1}) at pH = 8 (NaOD). Solutions were filtered through combusted (450 $^{\circ}\text{C}$) glass wool. Analysis was performed on a Bruker AdvanceTM 500 MHz NMR using a 5-mm probe. Spectra were collected using water suppression with presaturation (Topspin 3.x). The proton resonance of the four major chemical shift regions aromatic, carbohydrate protein, carboxylic acid rich alicyclic compounds CRAM (Hertkorn et al. 2006), and MDLT (material derived from linear terpenoids) (Lam and Simpson 2009) were integrated and expressed as the percent of total resonance intensity.

3. Results and discussion

3.1 Hydrolysis of sulfamerazine

The hydrolysis of SMR (0.01 mmol L^{-1}) was determined at pH 7 (Fig. 1), 5, and 9 (Table 1). There was negligible loss (0.5 – 1.0%) of SMR due to hydrolysis in agreement with a previous study (Boreen et al. 2005).

3.2. Direct and indirect photolysis of sulfamerazine under simulated sunlight.

i) Direct Photolysis.

Direct photolysis of SMR (0.01 mmol L⁻¹ in 10 mmol L⁻¹ phosphate buffer solutions at pH 7) resulted in a 61.0 ± 0.2% sulfamerazine loss (Fig. 1) and SMR degradation rate constant (k_1) of (2.96 ± 0.20)×10⁻³ min⁻¹ with a half-life of 231 min (Table 1).

ii) Indirect Photolysis.

Indirect photolysis was determined using 15 mg L⁻¹ SRNOM, SRHA, and SRFA in 10 mmol L⁻¹ phosphate buffer at pH 7 (Fig. 1) and the results are summarized in Table 1. There was 100% loss of SMR in the presence of AHA after only 3 hours of irradiation [$k_{1\text{AHA}} = (11.2 \pm 0.30) \times 10^{-3} \text{ min}^{-1}$] where SMR was below the detection limit (4.4 µmol L⁻¹). The percent loss of SMR (79.4 ± 0.7%) with SRNOM was higher than by direct photolysis (≈ 18%) after 6 hours of irradiation ($k_{1\text{SRNOM}} = (4.13 \pm 0.30) \times 10^{-3} \text{ min}^{-1}$). There was a slight increase in SMR loss (2.5%) in the presence of SRHA (63.6 ± 0.2%), ($k_{1\text{SRHA}} = (3.00 \pm 0.10) \times 10^{-3} \text{ min}^{-1}$). SRFA inhibited SMR degradation (approximately 41%) over the same time period ($k_{1\text{SRFA}} = (1.21 \pm 0.20) \times 10^{-3} \text{ min}^{-1}$). This inhibitory effect of SRFA was reported for other sulfonamides (Bahnmüller et al. 2014) and was attributed to the antioxidant properties of SRFA (Aeschbacher et al. 2012). The high electron donating capacity of SRFA may reduce the degradation product back to its parent compound, inhibiting contaminant oxidation in water systems (Wenk and Canonica 2012).

3.3 The role of reactive species in sulfamerazine degradation in NOM-containing solutions

The SMR degradation rates (Fig. 2, Fig. SI 1- Supplemental Information) due to the individual reactive species are summarized in Table 1. The degradation rate of SMR with SRFA was not determined because of its inhibitory effect on SMR photolysis. The SMR degradation rate constants in deoxygenated solutions ($k_{3\text{NOM}^*}$) increased for SRNOM (1.8 fold), SRHA (1.5 fold), and AHA (1.8 fold). Addition of sorbic acid (t,t-HAD), a known $^3\text{NOM}^*$ quencher (Velosa et al. 2007, Grebel et al. 2011), decreased SMR degradation rate constants ($k_{t,t\text{-HAD}}$) by SRNOM (22%), SRHA (34%), and AHA (88%), confirming $^3\text{NOM}^*$ as the main reactive species.

The reaction rate with SRNOM, SRHA and AHA for SMR and singlet oxygen ($k_{^1\text{O}_2}$) were of the order of 10^{-6} min^{-1} , while those with hydroxyl radical ($k_{\bullet\text{OH}}$) were an order of magnitude higher (10^{-5} min^{-1}). These values are much lower than the SMR degradation rate with the triplet-excited state of NOM (of the order of 10^{-3} min^{-1}), in agreement with earlier studies showing $^3\text{NOM}^*$ as the main reactive species during the NOM-sensitized photodegradation of SMR (Boreen et al. 2005b).

3.4 Determining steady-state concentrations and formation rates of reactive species generated from SRFA, SRNOM, SRHA and AHA in aqueous solution

The formation rates and steady-state concentrations of $^3\text{NOM}^*$, $\bullet\text{OH}$ and $^1\text{O}_2$ are summarized in Table 2. The steady-state concentrations of $\bullet\text{OH}$ for SRNOM, SRHA, and AHA were ($\approx 10^{-16} \text{ mol L}^{-1}$) in agreement with previous studies (al Housari et al. 2010, Xu et al. 2011, Wang et al. 2012) and the $\bullet\text{OH}$ formation rates ($10^{-12} \text{ mol L}^{-1} \text{ s}^{-1}$) were similar to those reported for natural waters (Nakatani et al. 2007, Takeda et al. 2004). However, the $\bullet\text{OH}$ formation rate and the corresponding steady-state concentration for SRFA were an order of magnitude lower than with the other forms

of NOM (see Supplemental Information, Fig. SI 3), suggesting an important difference in their chemical composition.

The formation rate of $^1\text{O}_2$ for all NOM samples was of the same order of magnitude ($10^{-8} \text{ mol L}^{-1} \text{ s}^{-1}$) as values reported for fresh and estuarine waters (al Housari et al. 2010, Timko et al. 2014). The measured steady state concentrations of $^1\text{O}_2$ ($10^{-13} \text{ mol L}^{-1}$) were also in agreement with previously reported values (al Housari et al. 2010, Timko et al. 2014, Kohn and Nelson 2006). The formation rate and steady-state concentration of $^1\text{O}_2$ were 1.5 – 2 times higher for AHA and SRFA in comparison with SRNOM and SRHA (see Supplemental Information, Fig. SI 4). For SRNOM, SRFA and AHA, the formation rate of $^1\text{O}_2$ was higher than that for $^3\text{NOM}^*$ possibly due to trace levels of O_2 in the reaction mixture. The steady-state concentrations of $^3\text{NOM}^*$, as well as of singlet oxygen, for both AHA and SRFA were approximately twice the value for SRHA and SRNOM (see Supplemental Information, Fig. SI 5), indicating that the inhibitory effect of SRFA on the photodegradation of some contaminants is unrelated to the production of photochemically reactive species.

3.5 Correlation of spectroscopic parameters and NOM reactivity for SMR degradation

The importance of optical parameters for evaluating NOM solution was recently reviewed (Sharpless and Blough 2014). The spectroscopic properties including ^1H NMR were evaluated in this study as potential indicators of reactive species generation from SRNOM, SRFA, SRHA and AHA (Table 3). Correlations with confidence values of $R^2 > 0.8$ were considered strongly positive correlations. Correlations determined by excluding SRFA data (due to its inhibitory effect on the

SMR degradation) significantly increased the R^2 values in only a limited number of cases (values in parentheses in Table 3).

The SMR degradation rate constant (k_1) was correlated with $[^3\text{NOM}^*]_{\text{ss}}$ ($R^2 = 0.83$) and the triplet excited state formation rate ($R^2 = 0.98$) for SRNOM, SRHA, and AHA. These findings are in agreement with prior studies that identified $^3\text{NOM}^*$ as the major reactive species in sulfonamide degradation (Ryan et al. 2011). There is also a strong correlation between the SMR degradation rate constant (k_1), the total fluorescence, and the $[^1\text{O}_2]_{\text{ss}}$ ($R^2 \geq 0.97$) and its formation rate ($R^2 = 0.99$) for these three forms of NOM. Although singlet oxygen plays a minor role in SMR degradation (Boreen et al. 2005a) these correlations with singlet oxygen may be related to the fact that $^3\text{NOM}^*$ is a precursor of singlet oxygen. However, additional studies using additional NOM reference samples are needed. The lack of correlation between k_1 and $[\text{OH}\cdot]_{\text{ss}}$ ($R^2 = 0.39$) reflects the minor contribution of hydroxyl radicals to SMR degradation (Boreen et al. 2005b).

The specific UV absorbance (SUVA_{254}), (see Supplemental Information, Fig. SI 6), and EEMs fluorescence (Table 4 and Fig. SI 7- Supplemental Information) were measured for SRNOM, SRFA, SRHA, and AHA at pH 7. The relative functional group distribution (aromatic, carbohydrate-protein, CRAM, and MDLT) for the same NOM set was determined by ^1H NMR (See Fig. 3 and Table 5).

As expected, SUVA_{254} was linearly correlated ($R^2 = 0.95$) with NOM aromaticity (Weishaar et al. 2003). There was also a positive correlation between aromaticity and SUVA_{254} and the $[\text{HO}\cdot]_{\text{ss}}$ ($R^2 \geq 0.97$) and its formation rate ($R^2 \geq 0.98$). There was a possible correlation between the formation rate and steady state concentrations of $^3\text{NOM}^*$. However, unlike the Everglades study of natural waters

(Timko et al. 2014) there was no correlation between $SUVA_{254}$ and the formation rate and steady-state concentration of singlet oxygen.

The EEM spectra of SRNOM, SRFA, SRHA, and AHA (see Supplemental Information, Fig. SI 7) illustrate the typical NOM fluorescence profiles (peaks A, C, M, and T) (Coble 1996, Zepp et al. 2004). The EEMs spectra were dominated by peak A (65 – 71% fluorescence), and the Peak A emission maximum was shifted to longer wavelengths for SRHA and AHA (Table 4). The total AHA fluorescence is 20% higher than SRFA and approximately twice that of SRNOM, and SRHA (Table 4). The total fluorescence and Peak A fluorescence for all forms of NOM were positively correlated with $[^1O_2]_{ss}$ ($R^2 \geq 0.95$), its formation rate ($R^2 \geq 0.95$), and SMR degradation rate constant ($R^2 \geq 0.95$). The relationship between fluorescence and singlet oxygen production was reported for both humic substances (Coelho et al. 2011) and lake water (Shao et al. 1994). Excluding SRFA, there was also a positive correlation between total fluorescence and $[^3NOM^*]_{ss}$ ($R^2 = 0.98$) and with their corresponding formation rates ($R^2 = 0.86$). This relationship could be attributed to the fact that $^3NOM^*$ deactivation in oxygenated waters forms singlet oxygen.

The aromatic functional group content determined by 1H NMR (Table 5) showed the expected correlation ($R^2 = 0.95$) with $SUVA_{254}$. AHA, derived from brown coal, had the highest aromatic content (37%) in comparison with SRHA (29%), SRFA (22%) and SRNOM (21%). The 1H NMR spectrum of AHA was very similar to a previously published spectrum (Grasso et al. 1990) and the values for aromaticity were in good agreement with those obtained by ^{13}C NMR (Aeschbacher et al. 2012). In contrast, the carbohydrate/protein content (chemical shift = 3.2 - 4.5 ppm) showed a very strong inverse correlation with $[^1O_2]_{ss}$ ($R^2 = 0.97$), 1O_2 formation rate ($R^2 = 0.97$) and with both the total fluorescence ($R^2 = 0.99$) and Peak A

fluorescence ($R^2 = 0.99$). This inverse correlation is perhaps not unexpected because protein-like material in NOM is a sink for singlet oxygen (Janssen et al. 2014, Rosado-Lausell et al. 2013) and quenches fluorescence (Wang et al. 2015).

4. Conclusions

It is recognized that the absolute concentration of the SMR used in this study was at a concentration higher than normally found in typical environmental samples and this was necessary to allow the detection of the compound and over time in the reactions. The removal of SMR results from a series of second order reactions while both hydrolysis and direct photolysis are described as first order reactions (i.e. the rate of loss of SMR is independent of concentration.) The reactions resulting from indirect photolysis are all second order reactions and dependent on both the steady-state concentration of the reactive species and the initial concentration of the SMR. The steady state concentration of the reactive species is dependent on the concentration of NOM and the concentrations used in this study are reasonable for many natural waters. However, the relative loss of SMR would not change at the lower concentrations likely to be encountered in 'natural' waters. For example, it is clear that for AHA, where a triplet state has been confirmed (Cottrell et al. 2013), this reaction plays an important role in the loss of SMR and this would be true regardless of the concentration of SMR. Where this may not be true would be at concentrations higher than used in these studies, but those are not likely to be found in natural waters.

Sulfamerazine degradation under sunlight radiation depends on the chemical composition of natural organic matter (NOM) in aqueous solution. Aldrich humic acid (AHA) with high aromatic and low protein/carbohydrate content was found to be

the most reactive NOM for SMR degradation, resulting in SMR concentrations below the detection limit after 3 hours of irradiation. AHA, while not representative of aquatic DOM, exhibited relatively high steady-state concentrations of $^3\text{NOM}^*$, high total fluorescence intensity, high SUVA_{254} and aromatic content in comparison with Suwannee River natural organic matter (SRNOM), Suwannee River humic acid (SRHA), and Suwannee River fulvic acid (SRFA). Fluorescence was correlated with the generation of singlet oxygen even though it does not have a significant role in SMR photodegradation. Aromaticity and SUVA_{254} are potential indicators of NOM hydroxyl radical reactivity but additional research is needed. SRFA was an outlier in some of these correlations because of its inhibitory effect on SMR photodegradation. Future studies to elucidate correlation among these parameters should include a larger sampling of NOM and compounds whose photodegradation is inhibited and enhanced by NOM.

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Figure 1. The loss of sulfamerazine (SMR) due to hydrolysis ($\blacksquare$); direct photolysis ($\blacktriangle$); and, indirect photolysis in the presence of: ($\bullet$) SRFA; ($\blacktriangledown$) SRHA; ($\blacktriangleleft$) SRNOM; ($\blacktriangleright$) AHA. Conditions: $[SMR]_0 = 0.01 \text{ mmol L}^{-1}$; NOM = 15 mg L^{-1} ; 10 mmol L^{-1} phosphate buffer (pH 7).

Figure 2. Loss of sulfamerazine due to the triplet excited state of NOM ($^3\text{NOM}^*$). (a) SRNOM; (b) SRHA; (c) AHA. Conditions: $[\text{SMR}]_0 = 0.01 \text{ mmol L}^{-1}$; $\text{NOM} = 15 \text{ mg L}^{-1}$; 10 mmol L^{-1} phosphate buffer (pH 7).

Figure 3. ^1H NMR spectra of Suwannee River NOM (SRNOM), Suwannee River fulvic acid (SRFA), Suwannee River humic acid (SRHA), and Aldrich humic acid (AHA) in D_2O . The four principle chemical shift regions are: aromatic (6.0-9.0 ppm), carbohydrate/protein (3.2-4.5 ppm), CRAM (carboxyl-rich alicyclic molecules) (1.7-3.2 ppm), and MDLT (material derived from linear terpenoids, aliphatic) (0.7-1.7 ppm).

Table 1. Losses of sulfamerazine (initial concentration 0.01 mmol L⁻¹) by hydrolysis, direct and indirect photolysis (10 mmol L⁻¹ phosphate buffer, NOM = 15 mg L⁻¹) under simulated sunlight after 6 hours of irradiation for SRNOM, SRFA, and SRHA (3 h for Aldrich humic acid).

	Hydrolysis			Direct photolysis (pH 7)	Combined processes: direct and indirect photolysis (pH 7)			
	pH 5	pH 7	pH 9		SRNOM	SRHA	SRFA	AHA
SMR loss, %	1.0	0.7	0.5	61.0 ± 0.2	79.4 ± 0.7	63.6 ± 0.2	35.5 ± 0.5	100 ^(a)
SMR half-life, min	–	–	–	231	165	192	577	63
SMR degradation rate constant (k_d), 10 ³ min ⁻¹	–	–	–	2.96 ± 0.20	4.13 ± 0.30	3.00 ± 0.10	1.21 ± 0.20	11.20 ± 0.30 ^(a)
^(b) SMR degradation rate constant (k_{HO_2}), 10 ⁶ min ⁻¹	–	–	–	–	8.33 ± 0.30	9.03 ± 0.30	1.17 ± 0.30	9.41 ± 0.30
^(c) SMR degradation rate constant (k_{HO_2}), 10 ⁶ min ⁻¹	–	–	–	–	1.80 ± 0.50	2.68 ± 0.20	1.27 ± 1.00	3.86 ± 0.20
SMR degradation rate constant (N ₂) (k_{3NOM}), 10 ³ min ⁻¹	–	–	–	–	7.49 ± 0.70	4.59 ± 0.10	nd	19.60 ± 0.60
SMR degradation rate constant (t,t-HAD) ($k_{t,t-HAD}$), 10 ³ min ⁻¹	–	–	–	–	3.20 ± 0.30	1.97 ± 0.10	nd	1.30 ± 0.10

(a) SMR concentration below the detection limit (DL = 4.4 μmol L⁻¹) after 3 hours of irradiation.

(b) Calculated from the steady state concentration of •OH and the bimolecular reaction rate constant of SMR and •OH (Mezyk et al. 2007).

(c) Calculated from the steady-state concentration of ¹O₂ and the bimolecular reaction rate constant of SMR and ¹O₂ (Figure SI 2) according to Xu et al. (2011).

nd – not determined

Table 2. Rates of formation and steady-state (SS) concentrations of reactive species in the absence of sulfamerazine for Suwannee River natural organic matter (SRNOM), Suwannee River fulvic acid (SRFA), Suwannee River humic acid (SRHA), and Aldrich humic acid (AHA) (15 mg L⁻¹ NOM and 10 mmol L⁻¹ phosphate buffer solution, pH 7).

NOM	HO•		¹ O ₂		³ NOM*	
	(10 ¹² mol L ⁻¹ s ⁻¹)	[HO•] _{ss} (10 ¹⁶ mol L ⁻¹)	(10 ⁸ mol L ⁻¹ s ⁻¹)	[¹ O ₂] _{ss} (10 ¹³ mol L ⁻¹)	(10 ⁸ mol L ⁻¹ s ⁻¹)	[³ NOM*] _{ss} (10 ¹⁴ mol L ⁻¹)
SRNOM	2.50 ± 0.10	1.78 ± 0.10	9.51 ± 0.20	3.80 ± 0.50	6.83 ± 0.30	3.20 ± 0.10
SRHA	2.70 ± 0.20	1.93 ± 0.10	6.65 ± 0.20	2.66 ± 0.20	7.46 ± 0.60	4.24 ± 0.50
SRFA	0.35 ± 0.70	0.25 ± 0.50	14.1 ± 0.30	5.65 ± 1.00	11.80 ± 0.10	6.50 ± 0.50
AHA	2.82 ± 0.50	2.01 ± 0.30	20.3 ± 0.1	8.12 ± 0.20	14.70 ± 0.40	6.89 ± 0.20

Table 3. The summary of apparent correlations (R^2) between the spectroscopic parameters, reaction rate constants, formation rates, and steady-state concentrations of reactive species for Suwannee River Fulvic Acid (SRFA), Suwannee River Humic Acid (SRHA), Suwannee River Natural Organic Matter (SRNOM), and Aldrich Humic Acid (AHA). Numbers in bold show correlations with $R^2 > 0.8$. Numbers in parentheses show the correlations calculated without SRFA.

	Aromatic Content (NMR)	Carb/protein (NMR) inverse ⁽⁶⁾ corr	SUVA ₂₅₄ (L·NOM mg ⁻¹ ·m ⁻¹)	k_1 (10 ³ min ⁻¹)	Total FU ⁽⁵⁾	[HO•] _{ss} (10 ⁻¹⁶ mol L ⁻¹)	[¹ O ₂] _{ss} (10 ⁻¹³ mol L ⁻¹)	[¹ NOM*] _{ss} (10 ⁻¹⁴ mol L ⁻¹)	Formation rate of HO• (10 ⁻¹⁶ mol L ⁻¹ s ⁻¹)	Formation rate of ¹ O ₂ (10 ⁻¹³ mol L ⁻¹ s ⁻¹)	Formation rate of ¹ NOM* (10 ⁻¹⁴ mol L ⁻¹ s ⁻¹)
Aromatic Content (NMR)	-	-	0.95	0.71	-	0.32 (0.97)	0.34	0.27 (0.94)	0.32 (0.98)	0.33 (0.56)	0.39 (0.81)
Carb/protein (NMR) – inverse corr ⁽⁶⁾	-	-	-	-	0.99	-	0.97	-	-	0.97	-
SUVA ₂₅₄ (L·NOM mg ⁻¹ ·m ⁻¹)	0.95	-	-	0.62	-	0.50 (1.00)	-	-	0.40 (0.99)	-	-
k_1 10 ³ min ⁻¹ ⁽¹⁾	0.71	-	0.62	-	0.36 (0.96)	0.39	0.51 (0.99)	0.15 (0.83)	0.39	0.52 (0.99)	0.37 (0.96)
$k_{HO•}$ 10 ⁵ min ⁻¹ ⁽²⁾	0.32 (0.97)	-	0.50 (0.99)	0.45*	-	1.00	-	-	0.99	-	-
k_{1O2} 10 ⁶ min ⁻¹ ⁽³⁾	0.33	0.97	-	0.51 (0.99)**	0.96	0.01	0.99	0.72	0.43	0.99	(0.99)
k_{NOM} 10 ³ min ⁻¹ ⁽⁴⁾	0.27**	-	0.41**	0.99**	(0.97)**	0.41**	0.99**	0.80**	0.00	1.00**	0.94**
[HO ₂] _{ss} 10 ⁻¹⁶ mol L ⁻¹	0.32 (0.82)	-	0.50 (1.00)	0.39	-	0.00	0.01	0.15 (0.83)	0.98	0.01	0.39
[¹ O ₂] _{ss} (10 ⁻¹³ mol L ⁻¹)	0.34	0.97	-	0.51 (0.99)	0.96	0.01	-	0.72 (0.79)	0.06	1.00	0.92
[¹ NOM*] _{ss} (10 ⁻¹⁴ mol L ⁻¹)	0.27 (0.94)	-	-	0.15 (0.83)	0.69 (0.98)	0.15 (0.83)	0.72 (0.79)	-	0.26	0.72 (0.79)	0.92
Total FU ⁽⁵⁾	0.17	0.99	-	0.36 (0.96)	-	0.06	0.97	0.69 (0.98)	0.03	0.99	0.86
EEMs A (FU)	0.15	0.99	-	0.36 (0.95)	0.99	0.06	0.95	0.65	0.14	0.95	0.82

(**) Calculated with SRNOM, SRHA, and AHA; reaction rate constant not determined for SRFA.

(1) SMR degradation rate constant.

(2) SMR reaction rate constant with respect to •OH radicals.

(3) SMR reaction rate constant with respect to ¹O₂.

(4) SMR reaction rate constant in de-aerated solution (N₂).

(5) Total fluorescence (corrected for inner filtering) = A+C+M+T.

(6) The correlation has a negative slope indicating an inverse relationship.

Table 4. Total EEMs fluorescence (Peaks A, C, M, T) and the peak totals corrected for inner filtering: Suwannee River humic acid (SRHA), Suwannee River natural organic matter (SRNOM), Aldrich humic acid (AHA), and Suwannee River fulvic acid (SRFA) in 10 mmol L⁻¹ phosphate buffer solutions at pH 7.

NOM	A Ex ₂₅₀	C Ex ₃₄₀ /Em ₄₂₅	M Ex ₃₁₀ /Em ₄₀₀	T Ex ₂₇₅ /Em ₃₄₅	Total Fluorescence
SRNOM	Em ₄₅₀ 3.95 x 10 ⁵	0.92 x 10 ⁵	0.81 x 10 ⁵	0.11 x 10 ⁵	5.79 x 10 ⁵
SRHA	Em ₄₇₈ 2.74 x 10 ⁵	0.59 x 10 ⁵	0.52 x 10 ⁵	0.11 x 10 ⁵	3.96 x 10 ⁵
SRFA	Em ₄₅₀ 5.44 x 10 ⁵	1.10 x 10 ⁵	1.15 x 10 ⁵	0.12 x 10 ⁵	7.81 x 10 ⁵
AHA	Em ₄₈₂ 6.90 x 10 ⁵	1.15 x 10 ⁵	1.25 x 10 ⁵	0.13 x 10 ⁵	9.43 x 10 ⁵

Table 5. The functional group distribution of the NOM samples determined by ^1H NMR. Distribution is expressed as percent of the total integrated proton resonances.

Functional group	Aromatic	Carbohydrate/protein	CRAM	MDLT (aliphatic)
Chemical shift (ppm)	6.0-9.0	3.2-4.5	1.6-3.2	0.7-1.6
SRNOM	21 %	21 %	33 %	25 %
SRHA	29 %	23 %	26 %	22 %
SRFA	22 %	18 %	36 %	24 %
AHA	37 %	16 %	20 %	27 %

CRAM: carboxylic-rich alicyclic molecules (Hertkorn et al. 2006).

MDLT: material derived from linear terpenoids (Lam and Simpson 2008).