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Photodegradation of Ormetoprim in Aquaculture and Stream-Derived Dissolved Organic Matter

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Supporting Information

ABSTRACT: Ormetoprim (OMP) is an antibiotic approved for use in the United States to prevent the spread of disease in freshwater aquaculture. It has been shown in the previous literature to be photochemically stable to direct photolysis, but the role of photosensitization processes in the presence of dissolved organic matter (DOM) on the rate of degradation is not well understood. The present results show that water and DOM (specifically the fulvic acid fraction) isolated from a eutrophic aquaculture catfish pond and a nearby stream (Deer Creek) at the Mississippi State University Delta Research and Extension Center facility in Stoneville, MS, significantly increased the phototransformation of OMP relative to direct photolysis. Similar results were reported for reference fulvic acids obtained from the International Humic Substances Society. Results from a combination of scavenging experiments and experiments conducted under anoxic conditions indicate the indirect photodegradation pathway occurs by hydroxyl radical, singlet oxygen attack, and reaction with triplet excited-state DOM.

KEYWORDS: *ormetoprim, photodegradation, dissolved organic matter, singlet oxygen, reactive oxygen species, hydroxyl radical, triplet excited states*

INTRODUCTION

Ormetoprim (OMP) is an antibiotic used in conjunction with sulfadimethoxine (SDM) in a 5:1 mixture of medicated feed for aquaculture use (Figure 1). These antibiotics are used in combination to fight enteric septicemia of catfish, a common cause of disease outbreak in catfish farming.

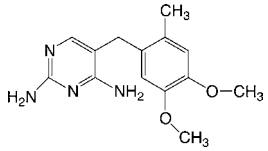

Figure 1. Structure of ormetoprim.

Currently there is very little knowledge of the fate of ormetoprim in waters or its photochemical behavior. Catfish ponds, which are shallow and lack significant shade, may allow photodegradation to be an important process governing its transformation. Ormetoprim, which absorbs light in the UV region (Supporting Information, Figure S1), has been found at concentrations as high as 12 μg L^{-1} in fish hatchery waters during treatment, although these maximum detected levels drop to <0.69 μg L^{-1} during nontreatment periods.[1] In simulated environments Bakal et al. observed that light had little to no effect on ormetoprim concentrations over the course of a year even when salinity, pH, or the amount of artificial sediment was varied.[2] In natural sunlight experiments containing seawater samples from Bergen, Norway, OMP was also found to be stable.[3] Neither study, however, examined the specific role of photosensitization processes on OMP's environmental fate. We suspect that the presence of dissolved organic matter (DOM), a known and ubiquitous photosensitizer, may play an important synergistic (or possibly antagonistic) role in the photofate of OMP. To date, there is no information about the degradation mechanisms of ormetoprim or any interaction with DOM in natural waters in the presence of sunlight.

Dissolved organic matter source composition has been shown to affect the chemical reactivity of DOM and thus may also affect contaminant fate through the production of reactive oxygen species (ROS) and other reactive transients, such as, triplet forms of DOM and carbon-centered radicals, in the presence of sunlight.[4−13] For example, the source of DOM can profoundly affect the photochemical degradation of sulfadimethoxine (SDM), an antibiotic used in conjunction with OMP in aquaculture.[4,5] In a recent study we demonstrated this phenomenon, whereby the composition of DOM derived from water bodies hundreds of meters apart resulted in opposite effects on the photofate of SDM.[5] Unaltered DOM derived directly from ponds used in catfish farming had a significant photosensitization effect on SDM, whereas DOM from an adjacent stream retarded its photolytic transformation. Thus, 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.

In this study we investigated the degradation of OMP using a series of photochemical probe experiments to determine reaction pathways in the presence of light and fulvic acid isolated from the same catfish aquaculture pond and nearby stream (Deer Creek) in rural Mississippi. The fulvic acids were characterized by absorbance and fluorescence spectroscopy to

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delineate compositional differences, and we sought to relate any trends in DOM composition to the photodegradation of OMP in an effort to elucidate compound-specific pathways.

MATERIALS AND METHODS

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, MS. Samples were shipped to The Ohio State University, where they were filtered to 0.45 μM and the DOM was isolated by XAD-8 chromatography to capture the fulvic acid fraction. Details regarding DOM isolation are provided in Guerard et al.[5]

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, USA). 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, furfuryl alcohol, sodium ascorbate, and isopropanol were purchased from Fisher Scientific (Pittsburgh, PA, USA). 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, USA) as described previously.[5,14] Dissolved organic carbon (DOC) measurements were taken at multiple places during the processes to determine the extraction efficiency of this isolation method.

DOM Characterization. Absorbance scans of natural waters were run on a Varian Cary 13 UV−vis spectrophotometer from 200 to 600 nm. Specific UV absorbance (SUVA) was obtained by normalizing absorbance values at 254 nm to the dissolved organic carbon (DOC) concentration of the sample.[15] Dissolved organic carbon analysis was carried out on a TOC-5000 Shimadzu carbon analyzer using potassium hydrogen phthalate standards. All fulvic acid solutions used in this study were made at 10 mg C L^{-1}, Deer Creek whole water was measured at 6.23 mg C L^{-1}, and Pond 29 whole water was measured at 11.28 mg C L^{-1}.

Fluorescence excitation−emission matrices (EEMs) of sample waters and DOM solutions were run on a Varian Cary Eclipse at excitation wavelengths of 240−450 nm (at 5 nm intervals) and emission wavelengths of 300−600 nm (at 2 nm intervals). Solutions were diluted to avoid inner filter effects (absorbance at 254 nm was ≤0.05 unit).[16] Fluorescence results were blank subtracted, scaled for dilution, and corrected in Matlab[17] using correction curves provided by the manufacturer. The fluorescence index (FI) as defined by Cory and McKnight[18] was obtained by taking a five-point moving average of the emission scan for smoothing purposes and then taking the ratio of corrected fluorescence intensity from 370 nm excitation and emissions at 470 and 520 nm.

Photolysis Experiments. Solutions of either 0.1 or 1.0 μM OMP dissolved in Milli-Q water, 0.45 μm 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. Samples were irradiated in a Suntest CPS + (Atlas, IL, USA) solar simulator with a 500 W xenon lamp (set to 450 W) over a length of 2−3 half-lives to determine both the reaction order and rate constants. Actinometer experiments showed that our solar simulator operated at ∼4.5 times the intensity of average sunlight at 40° N at noon in June.[5] 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 OMP was performed via reverse phase HPLC using a Restek Ultra IBD column (3.2 μm, 3.5 mm × 150 mm) at 0.5 mL min^{-1}, 70/30% v/v of 25 mM KH_2PO_4 in water (pH 3)/methanol, and UV detection set at 230 nm. Photodegradation was semilog-linearized and fitted to a pseudo-first-order model when applicable. Rates were corrected for light screening of fulvic acids and natural waters.[19] Light screening factors were used to determine contributions of indirect and direct photolysis to overall observed rate coefficients and are explained in the Supporting Information.

Amendments to the above experiments were conducted to elucidate the potential reactive pathways of OMP photodegradation in the presence of DOM. The role of ROS in the photofate of OMP was investigated by sparging solutions with argon gas (1 min mL^{-1}) and preparing them in an anaerobic glovebox fitted with a dioxygen scrubber. The role of the hydroxyl radical ($OH^{\bullet}$) in OMP degradation was determined using 25 mM methanol or isopropanol as an OH• scavenger. We also investigated the photo-Fenton pathway by adding iron (20 μM $FeCl_3$) to filtered samples and fulvic acid solutions.

To determine the susceptibility of OMP to singlet oxygen ($^1O_2$), we added 40 μM Rose Bengal, a known $^1O_2$ photosensitizer, to sample solutions. Finally, we conducted experiments using DCFA or CPFA solutions made up in deuterium oxide (D_2O) to assess kinetic solvent effects on the efficacy of singlet oxygen reactivity on OMP.

Methods for dark Fenton reactions used acetophenone and Fenton's reagent to measure the specific second-order reaction rate constant between OMP with $^{\bullet}OH$. In addition, we conducted steady state measurements of $^1O_2$ concentration using furfuryl alcohol. Details for both experiments are provided in the Supporting Information.

RESULTS AND DISCUSSION

Characterization of DCFA and CPFA. The properties of unaltered Pond 29 and Deer Creek water DOM were presented previously,[5] whereas characterization data for DCFA and CPFA are presented in Table 1. Excitation−emission matrices (Supporting Information, Figure S2) reveal an FI of 1.40 and 1.64 in DCFA and CPFA, respectively.[18] Surprisingly, the FI for DCFA is similar to the terrestrial end member SRFA value, whereas the CPFA is significantly higher. CPFA is derived from a highly eutrophic water body and has a higher FI than DCFA, which has significantly more allochthonous organic matter precursors. Indeed, SUVA at 254 nm show that DCFA has a SUVA value similar to that of SRFA, whereas CPFA's SUVA value resembles that of PLFA (Table 1). Moreover, the fulvic acid isolate of Deer Creek represents 50% of the total carbon pool in Deer Creek water, whereas CPFA represents only 25% of the total carbon pool in catfish pond water and is similar to recoveries reported for SRFA and PLFA, respectively.[20−22]

Table 1. Characterization Data for Fulvic Acids[a]

fulvic acid	FI	$SUVA_{254}$	%C	$[^1O_2]_{ss} \times 10^{-13}$ (M)
PLFA	1.453[b]	207[b]	25[b]	3.58 ± 0.4
SRFA	1.300[c]	389[d]	50[c]	3.63 ± 0.4
DCFA	1.404	362	50	3.90 ± 0.4
CPFA	1.640	252	25	5.66 ± 1.0

[a]FI represents fluorescence index, ratio of fluorescence at excitation 370 nm, emission 470/520 nm. SUVA is specific UV absorbance at 254 nm in absorbance units L (mol C)$^{-1}$. %C is the percentage of the carbon pool that the fulvic acid represents. Singlet oxygen steady state concentrations (corrected for light screening) in the bulk phase as determined by furfuryl alcohol photodegradation rates. All fulvic acid solutions used in the single oxygen steady state measurements were at 10 mg C L^{-1}. [b]Reference 4. [c]Reference 31. [d]Reference 15.

Our data show that the DOM from the Mississippi sites is spectroscopically similar to the DOM reference standards SRFA and PLFA. Deer Creek is a highly colored fluvial system bordered by cypress trees, and its riparian environment is similar to that of the Suwannee River watershed in GA. We anticipate that a significant pool of organic matter is terrestrially

Table 2. Photodegradation of OMP in Various Dissolved Organic Matters and Natural Water[a]

sample	$S_{S290-350}$[b]	$k_{obs} \times 100$[c] (h^{-1})	half-life (h)	$k_{dp} \times 100$[d] (h^{-1})	$k_{ip} \times 100$[e] (h^{-1})	%DP[f]	%IP[f]
Milli-Q		1.01 ± 0.5	68.6	1.01		100	0
SRFA	0.85	10.40 ± 0.8	6.7	0.86	9.54	8	92
PLFA	0.93	11.83 ± 0.2	5.9	0.94	10.89	8	92
CPFA	0.86	5.90 ± 1.1	11.7	0.87	5.03	15	85
DCFA	0.86	4.60 ± 1.1	15.1	0.87	3.73	19	81
CPWW	0.96	6.71 ± 0.3	10.3	0.97	5.74	14	86
DCWW	0.93	2.45 ± 0.3	28.3	0.94	1.51	38	62

[a]All fulvic acid solutions were at 10 mg C L^{-1}. The carbon concentration of Deer Creek whole water was 6.23 mg C L^{-1} and catfish pond whole water was 11.28 mg C L^{-1}. [b]Light screening factor for wavelengths 290−350 nm. [c]Observed pseudo-first-order rate degradation coefficient measured from a least-squares fit of degradation kinetics. [d]Rate coefficient predicted for contribution of direct photolysis to the overall degradation in a given DOM solution. [e]Rate coefficient predicted for contribution of indirect photolysis to the overall degradation. [f]The percentage contribution of direct (% DP) and indirect photolysis (% IP) to the overall observed degradation.

derived from higher plants. In contrast, both the catfish ponds and Pony Lake, Antarctica, are hypereutrophic and hydrologically closed, that is, no inflow or outflow systems, and the major organic matter precursors are derived from algal primary production in the water column. Indeed, Pony Lake has no higher plant inputs whatsoever due to its location. Thus, our field site may be a very unique environment in that DOMs of such disparate composition exist within a few hundred meters of each other.

OMP Photolysis. Direct ormetoprim photodegradation obeyed pseudo-first-order kinetics (Table 2). Direct photolysis of ormetoprim is rather slow, with a half-life of just under 70 h in our solar simulator. Thus, in natural sunlight (at 40° N in June) at constant irradiance and taking into account about 12 h a day for darkness, the photolytic half-life of OMP from direct photolysis only (assuming no other reactions and minimal light attenuation) would be expected to be on the order of 26 days. These results seem to corroborate the findings of Lunestad et al.,[3] who observed photostability of OMP in seawater.[3] However, even though DOC levels were not actually reported in their study, DOM levels in seawater are typically very low (<1 mg/L DOC), and seawater also contains a number of other constituents such as salts that can interfere with photolytic pathways. For example, bromide can quench photochemically formed hydroxyl radical in seawater.[23−26]

We observed enhanced OMP degradation in both PLFA and SRFA solutions, and our data obeyed pseudo-first-order kinetics (Figure 2). Rate constants were an order of magnitude higher than those observed for direct photolysis. Indirect photolysis in these samples accounted for >90% of the observed OMP degradation even after light screening by DOM was taken into account. Surprisingly, OMP indirect photolytic rate coefficients were statistically similar in both Suwannee River and Pony Lake fulvic acid solutions.

OMP photodegradation in solutions of CPFA and DCFA were similarly statistically indistinguishable, but roughly *half* the enhancement observed in PLFA and SRFA solutions (Figure 3). Observed OMP rate coefficients in filtered (0.45 μM) catfish pond water were not statistically different from those of the fulvic acid solutions, but in Deer Creek whole water, OMP photodegradation was *slower* than in comparable DCFA solutions (only 62% of the overall photolytic rate constant can be due to indirect photolysis compared to >80% of the overall rate constant in the fulvic acid solutions; Table 2). Carbon-normalized observed rate coefficients were used to compare across samples of different DOM concentrations, which assumes that carbon concentration is proportional to the

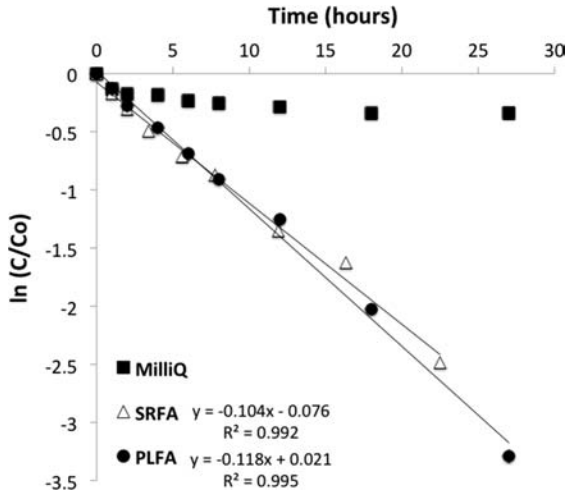

Figure 2. OMP degradation in Milli-Q water and Pony Lake and Suwannee River fulvic acid. All fulvic acid solutions were at 10 mg C L^{-1}.

reactive DOM content. The difference between Deer Creek whole water and DCFA is less pronounced, but OMP photodegradation is still significantly slower in the whole water. Observed OMP rate coefficients in catfish pond water DOM and CPFA remain statistically similar to each other. This suggests that an important fraction of the organic matter photoreactive to OMP is captured within the fulvic acid isolate. Conversely, other substances could also scavenge reactive transients in the Deer Creek whole water sample, such as naturally occurring constituents in the water column or certain moieties in Deer Creek DOM that were removed in the isolation process. Finally, unlike the large differences in SDM photoreactivity observed for these identical water samples, we see no statistical difference in OMP degradation rates.[4,5]

OMP Reaction with Hydroxyl Radical. Methanol and isopropanol were each tested to assess their effectiveness as hydroxyl radical scavengers, because both compounds may be prone to side reactions that may cause overestimates of the apparent importance of the hydroxyl radical. Both 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 statistically the same (Supporting Information, Figure S3). Therefore, throughout the course of this study, we used methanol as the hydroxyl radical quencher. In the presence of methanol, OMP photolysis in PLFA, SRFA, CPFA, and DCFA solutions slowed by 66, 53,

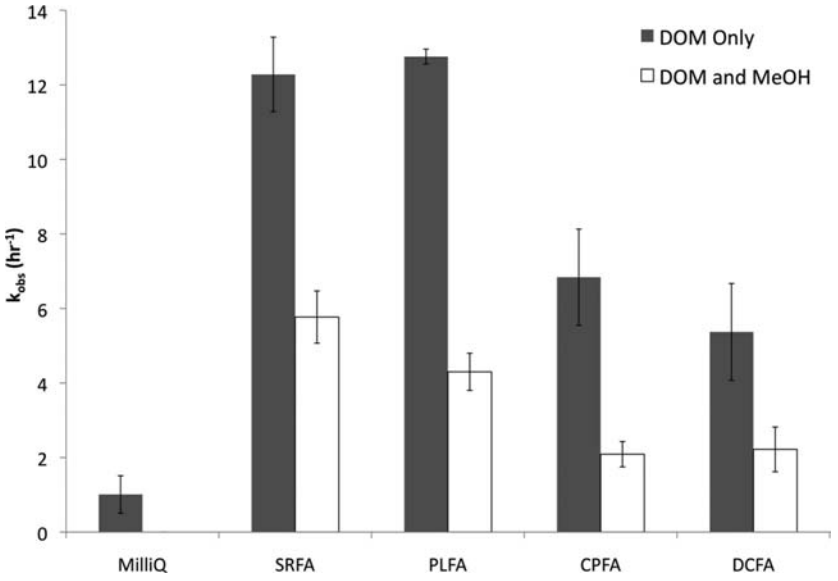

Figure 3. Photodegradation of OMP in Milli-Q water and DOM samples with and without methanol addition. All fulvic acid solutions were at 10 mg C L^{-1}.

70, and 59%, respectively (Figure 3). Thus, over half of the OMP photodegradation was quenched in the presence of methanol, providing evidence that reaction between OMP and $OH^{\bullet}$ is an important pathway. Nonetheless, the addition of methanol did not quench the entire reaction in any fulvic acid solution, implying the existence of at least one and possibly more degradation pathways.

We measured OMP's $OH^{\bullet}$ second-order rate constant with a competitive $OH^{\bullet}$ probe, acetophenone, which has a known second-order rate constant, and Fenton's reagent to generate $OH^{\bullet}$ in the absence of light. A plot of $\ln(S/S_o)$ versus $\ln(R/R_o)$ (Supporting Information, Figure S4, where S is OMP and R is acetophenone) yielded a highly linear correlation ($y = 1.13x + 0.84$, $R^2 = 0.98$) and a $k_{^{\bullet}OH}$ value of 6.7×10^9 M^{-1} s^{-1}. Using this value with steady state $OH^{\bullet}$ concentrations reported from White et al.[27] that ranged from 1 to 32×10^{-16} M for a variety of DOM (ranging from an Antarctic Lake to black water environments from the eastern United States), we estimate first-order hydroxyl radical rate constants (i.e., $k_{obs\,^{\bullet}OH} = k_{^{\bullet}OH}[OH^{\bullet}]_{ss}$) ranging from 0.002 to 0.077 h^{-1}. The upper range of our estimates is similar to our observed rate constants determined in the presence of methanol (Table 3).

OMP Reaction with Singlet Oxygen. Singlet oxygen, unlike hydroxyl radical, is a much more selective electrophilic nonradical 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:[28]

Table 3. Observed Rate Coefficients of Degradation for OMP in SRFA or PLFA in the Presence of Various Solution Amendments[a]

	$k_{obs} \times 100$ (h^{-1})			
sample matrix	DOM only	MeOH addition	anoxic	Rose Bengal addition
SRFA	10.40 ± 0.8	5.77 ± 0.5	5.21 ± 0.9	49.01 ± 11.2
PLFA	11.83 ± 0.2	4.30 ± 0.4	9.83 ± 1.2	62.52 ± 9.7

[a]All fulvic acid solutions were at 10 mg C L^{-1}.

$$DOM + h\nu \rightarrow {}^3DOM^*$$

$$^3DOM^* + {}^3O_2 \rightarrow DOM + {}^1O_2$$

Even though singlet oxygen has been shown to react predominately with dienes (via an endoperoxide intermediate), it has also been demonstrated to react with phenol/phenolate compounds with electron-donating groups.[29] Whereas OMP lacks diene or phenolic moieties, it *does* have strongly electron-donating functional groups and thus could be more susceptible to reaction with singlet oxygen, particularly the amine groups, as amines have been known to be oxidized by singlet oxygen.[30] Singlet oxygen has also been shown to contribute to the degradation of a number of compounds with pyrimidine bases.[31] For example, Ravanat et al. have proposed that such oxidation by singlet oxygen may occur via an endoperoxide addition onto N-containing rings.[32] Another possible pathway is the formation of an OMP cation radical, which donates an electron to singlet oxygen that subsequently forms superoxide.

We measured singlet oxygen steady state concentrations $[^1O_2]_{ss}$ in our fulvic acid solutions using furfuryl alcohol (refer to the Supporting Information for details). We observed very little variability in $[^1O_2]_{ss}$ as a function of fulvic acid composition (Table 1) with the possible exception of CPFA, which is marginally higher (statistically) than the other fulvic acids. The relatively uniform effects of $OH^{\bullet}$ on the indirect photolysis of OMP based upon the methanol scavenging experiments (see above) and the relatively consistent measured $[^1O_2]_{ss}$ levels could in part explain the lack of variability in OMP's reaction rate for fulvic acids of such disparate composition if these two ROS dominate OMP's photofate.

Photolysis of OMP was also conducted in fulvic acid solutions made up in D_2O instead of water, as the former is a much less efficient $^1O_2$ quencher,[33] and we would anticipate faster OMP kinetics if OMP is susceptible to $^1O_2$. We observed significantly enhanced OMP degradation, but our data no longer obeyed first-order kinetics (Figure 4). Nonetheless, we observed good first-order fits for the initial rates of OMP transformation in D_2O (calculated from the 15 and 10% of OMP degradation in CPFA and DCFA, respectively). In both

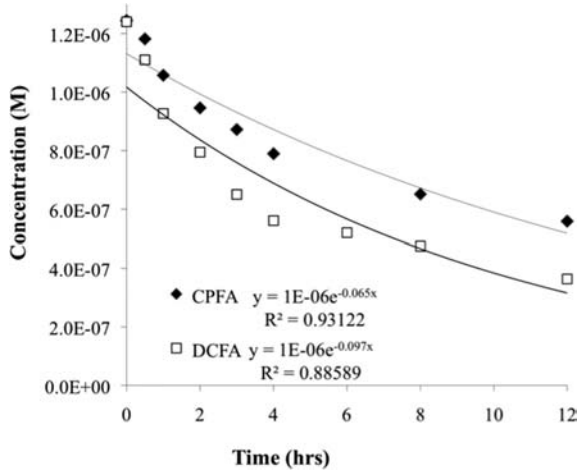

Figure 4. Photodegradation of OMP in fulvic acid solutions made up in D_2O, fitted to a pseudo-first-order degradation kinetics. All fulvic acid solutions were at 10 mg C L^{-1}.

cases the pseudo-first-order rate constants were significantly higher than experiments conducted in water. In CPFA, OMP photodegradation was 0.163 h^{-1}, nearly 3 times faster than rate constants measured in water, and in DCFA, OMP photodegradation was 0.221 h^{-1}, which is nearly 5 times faster (Table 1). Such increased reactivity is strongly indicative of singlet oxygen involvement in the indirect photolysis of OMP.

To further investigate the singlet oxygen pathway, we conducted additional experiments in the presence of 40 μM Rose Bengal, a known producer of singlet oxygen in the presence of light.[34] Photolyzed Rose Bengal in the presence of the reference fulvic acids resulted in a substantial increase (5 times) in the observed degradation coefficient for OMP. Whereas the addition suggests susceptibility of OMP to singlet oxygen, it does not conclusively demonstrate singlet oxygen involvement. Like DOM, Rose Bengal produces $^1O_2$ via excitation to the triplet state (^{3}RB), where it reacts with triplet molecular oxygen. Thus, it is possible that ^{3}RB could be an important oxidant and could be more reactive to OMP relative to ^{3}DOM*, particularly if the reduction potential of Rose Bengal leads to more favorable electron transfer than the triplet DOM. We suspect that in the presence of Rose Bengal, OMP reacts with singlet oxygen as well as with the triplet species.

OMP Reaction with Triplet DOM and Long-Lived Radicals. We also investigated OMP's reaction with "long-lived" radicals, for example, peroxyl and phenoxyl radicals using the "initial concentration" approach.[35,36] Photolysis at an initial OMP concentration of 0.1 μM was statistically similar to that of the 1 μM solutions (SRFA, k_{obs} was 0.1614 ± 0.0429 h^{-1}, and in PLFA, k_{obs} was 0.0948 ± 0.019 h^{-1}). Our large experimental error bars made it difficult to accurately assess whether true enhancement occurred as we were near the limits of detection for OMP. Nonetheless, it appeared unlikely that the presence of long-lived radicals is an important phototransformation pathway for OMP.

Photolyses of OMP were run in the absence of oxygen to test for the contribution of triplet excited states to the degradation of OMP, as triplet oxygen is a quencher of triplet species. In the anoxic systems, ROS production would be suppressed, whereas triplet reactivity would be enhanced. There was no statistical difference in the observed OMP rate coefficients between anoxic and air-equilibrated photolytic experiments in PLFA solutions, whereas OMP photolysis slowed slightly in the anoxic SRFA solutions (Table 3). Experimental uncertainty was considerably larger than in air-equilibrated experiments, because of possible oxygen back diffusion into the solution during the transfer from the sparging solutions (using a Schlenk line) to the glovebox. At first glance our results suggest that ^{3}DOM* is considerably less important than ROS in OMP's photofate. However, we would anticipate a *significant* decrease in overall reactivity if ROS were the only transients involved in the transformation process. OMP has several electron-donating functional groups capable of reacting with triplet species, and the lack of difference in degradation rates may instead be indicative of competing effects that include decreased ROS involvement and increased triplet reactivity in the absence of oxygen. For example, the observed rate coefficient for OMP degradation in the presence of PLFA in the sparged experiments is higher than in the $^\bullet$OH quenching experiments with methanol, indicating that some enhancement apparently involving triplet DOM may have occurred. In the case of SRFA, there is little difference compared to the $^\bullet$OH quenching experiment, which, although not quantitative, shows similar trends for triplet enhancement by these DOM as observed previously.[5,13] As stated previously, triplet oxygen is an important scavenger of ^{3}DOM to produce $^1O_2$. Whereas this would decrease OMP's reaction rate through the loss of $^1O_2$, this could be compensated by its reaction with ^{3}DOM. Thus, our results could indicate the presence of both pathways having an influence on the photodegradation rate of OMP as the net loss of one reactive species is offset by gains in the increased analyte reactivity with triplet species. Such competing effects in deoxygenated solutions have also been observed by Felcyn et al.[37]

Ormetoprim direct photolysis is slow, with half-lives on the order of days. In contrast, we demonstrated that DOM (specifically the fulvic acid fraction) significantly mediates the indirect photolysis of OMP. The indirect reaction pathways appear to be through hydroxyl radicals (~60%) and both singlet oxygen and triplet excited organic matter, although 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. Surprisingly, rates of OMP photodegradation, unlike many other organic contaminants, appear to be independent of fulvic acid composition.

■ ASSOCIATED CONTENT

Supporting Information

Methods and graphical results for competition kinetics experiments to determine rates of reaction with hydroxyl radical, measurements of single oxygen steady state concentrations, and an OMP absorbance spectrum, as well as the spectroscopic characterization of Deer Creek and Catfish Pond fulvic acids. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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