The Photofate of Pesticides and Dissolved Organic Matter in Diverse Aquatic Systems

DISSERTATION

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By

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Abstract

The application of pesticides to agricultural fields has impaired receiving water quality and possibly affected both ecosystem function and biodiversity in lotic systems. Photochemical reactions may be important pesticide attenuation mechanisms in shallow sunlit waters. However, the ubiquitous presence of ill-defined dissolved organic matter (DOM) coupled with the presence of iron (Fe) have added to the difficulty in understanding and predicting the environmental fate of pesticides in diverse water bodies over diurnal cycles.

This study investigated the impact of sunlight intensity, DOM, and Fe on the transformation of two pesticides: isoproturon and acetochlor. Change in light intensity, especially the natural diurnal cycle, was found to be important for isoproturon photofate due to its “apparent” photodegradation coupled to regeneration in the dark. For acetochlor, the addition of Fe in natural wetland waters was found to be especially beneficial at moderately and highly acidic pH values. In a highly acidic aqueous environment i.e., acid mine drainage (AMD) impacted waters, which are common in coal-producing regions of the country, the presence of high Fe levels impacted both phototransformation and photomineralization of acetochlor and DOM for which rates were orders of magnitude faster than most natural water bodies. This high photoreactivity
may make AMD impacted waters a potential resource for pesticide attenuation, but the effects on receiving water ecosystems by transformed/mineralized DOM also need to be considered.
Dedication

Dedicated to the Sun
Acknowledgement

This dissertation could not start and finish without all the help.

I would like to thank my advisors, Linda Weavers and Yo Chin, for your wisdom to guide me along the road, for your trust to let me fall, stand up, walk freely and wonder with curiosity and hope, and for the home you give me financially and spiritually.

I would like to thank all my committee members, collaborators and professor friends, for your brilliant ideas, for your way of thinking, and for your efforts to strength my research.

I would like to thank all the funding agencies and taxpayers, for your financial support and your trust in pure and applied science.

I would like to thank my parents, Xianji Yuan and Jianyun Cheng, for your open mind to support me studying abroad, for your suggestions for life, and for your hard work to take care of yourselves.

I would like to thank all my dear friends and lab mates, my academic sister, my aquatic soul mate, and my occasional enlightener for your existence, company and support, both close and far.

I would like to thank the Sun for rising and setting, thank the waters for flowing and mirroring, and thank the forests for sprouting and defoliating.

Finally, I would like to thank myself for continuing to dream.
Vita

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1.1 Important components in the aquatic system

1.1.1 Dissolved organic matter

Dissolved organic matter (DOM) in aquatic systems is operationally defined as the organic material that can pass through a 0.45 μM filter. Originating from both terrestrial (e.g., higher land plants) and microbial precursors (e.g., algae and microorganisms found in natural waters), DOM is considered a polydisperse mixture of biomolecules, hydrophilic acids and humic substances. Among this DOM mixture, aquatic humic substances (HS, mostly fulvic acid (FA) and some humic acid (HA)) accounts for about 50% of the DOM pool and a significant fraction of its sunlight absorption chromophoric composition (CDOM). HS are currently described as yellow-colored heterogeneous amphiphilic macromolecules that self-assemble into a supramolecular association. As such its actual composition is difficult to elucidate. Figure 1.1 depicts a hypothetical FA macromolecule of 1060-Da molecular weight and 39% aromatic carbon.

DOM is ubiquitous to all aquatic waters, with concentrations ranging from < 1mg-C L\(^{-1}\) in oligotrophic lakes to >> 30 mg-C L\(^{-1}\) in wetlands and estuaries. The ecological and environmental functions of DOM are multidimensional and interconnected. Firstly,
DOM is among the most reactive carbon reservoirs on earth.\(^6\) The assimilation, mineralization, and transport of DOM are important parts of the carbon cycle. Secondly, DOM serves as a significant energy, carbon, and/or nutrient source for both aquatic autotrophs and heterotrophs.\(^8\)–\(^10\) Thirdly, CDOM (mostly from HS) controls the photic zone depth, absorbs cell-damaging ultra-violet (UV) light and converts larger and complex organic matter components to simpler, more bioavailable compounds.\(^11\)–\(^15\) Fourthly, photochemically-produced reactive intermediates (PPRI) from DOM are capable of transforming anthropogenic compounds released to the natural water bodies.\(^16\)–\(^21\) Finally, the diverse functional groups on DOM affect its pH buffering capacity, redox potential and trace metal complexation.\(^2,22\)–\(^24\)

1.1.2 Fe and its interaction with DOM

Iron (Fe), as the fourth most abundant element (4.7 % by weight) in the earth’s crust, is ubiquitous in aquatic systems.\(^25,26\) The speciation of Fe in natural waters depends on a variety of reactions, including precipitation-dissolution, photolytic or thermal oxidation-reduction, complexation with inorganic and organic ligands, sorption-desorption, and biological utilization as summarized in Table 1.1.\(^26,27\)

Water constituents such as pH and DOM have a great impact on Fe speciation, leading to differences in its redox potential, solubility, and other interactions.\(^26,28\) At neutral pH, Fe is sparingly soluble with concentrations in the nM range; the presence of DOM increases Fe solubility to \(\mu\)M levels. Fe complexes with oxygen or nitrogen centered functional groups in DOM in a monodentate or multi-dentate fashion.\(^29\) In more acidic or reducing
environments, Fe becomes more soluble because reactions governing dissolution become more favored. In the extreme case, acid mine drainage (AMD) impacted waters may have sub mM Fe at pH values lower than 3, due to the weathering of pyrite. These Fe-containing systems at different pH values are discussed in depth in Chapters 3 and 4 of this dissertation.

1.1.3 Agrichemicals in the aquatic system
In the U.S., about 700 million pounds of pesticides per year are applied to agricultural lands since the 1980s. The application of pesticides in agricultural fields can impair the water quality and biodiversity in downstream water bodies. In a 10-year study (1992-2001) by the USGS National Water Quality Assessment (NAWQA) Program, more than 90% of the streams in agricultural areas had detectable levels of pesticides and 10% of the streams had pesticide levels higher than human health standards and guidelines. The environmental fate of these non-point source pollutants has been an area of active study in recent years. Two representative pesticides, isoproturon and acetochlor (Figure 1.2), are the topic of study in Chapters 2 and 3, respectively. Both herbicides are applied in grain fields as pre-emergent weed control.

1.2 Aquatic photochemical initiation processes
1.2.1 Reactive species formed from photolysis of DOM
As one of the most important photosensitizers and light attenuators in waters, DOM absorbs over a large portion of the UV range of the solar spectrum and is promoted to an
excited state ($^1\text{DOM}^*$). Once excited, DOM may generate various reactive intermediates in the presence of $\text{H}_2\text{O}$, $\text{O}_2$, $\text{CO}_3^{2-}$, $\text{Br}^-$, and other water constituents.\textsuperscript{16,34,35} These photochemically produced reactive intermediates (PPRI) include the hydroxyl radical ($\cdot\text{OH}$)$^{36-42}$, triplet excited states of DOM ($^3\text{DOM}^*$)$^{43-45}$, singlet oxygen ($^1\text{O}_2$)$^{5,44,46-49}$, the superoxide radical anion ($\text{O}_2^\cdot$), hydrogen peroxide ($\text{H}_2\text{O}_2$)$^{50-52}$, and the carbonate radical ($\text{CO}_3^{\cdot-}$)$^{53,54}$.

A number of tools exist that helps us understand the production of PPRI from photoexcited DOM. The quantum yield ($\Phi$) has been used to quantify the extent of PPRI production normalized to the number of photons absorbed.\textsuperscript{55} The apparent quantum yields of these PPRI is specific to the wavelength of light absorbed,\textsuperscript{56,57} the composition of DOM,\textsuperscript{21,58} and other water conditions e.g., presence of oxygen, nitrate, etc. As will be demonstrated elsewhere in this dissertation, DOM property plays an important role in its photochemistry. For example its composition is highly linked to its precursors and most natural water DOM falls on a continuum between microbially (autochthonous) and terrestrially (allochthonous) derived organic matter.\textsuperscript{3,59,60} DOM from terrestrial sources are usually more aromatic in composition, while DOM from microbial sources possess more aliphatic and carbohydrate moieties.\textsuperscript{35,61,62}

1.2.2 Photochemistry of Fe in the absence and presence of DOM

$\text{Fe}^{(\text{III})}$, free or when complexed with inorganic/organic ligands, has been shown to be an important photosensitizer in aquatic systems, leading to the generation of $\text{Fe}^{(\text{II})}$ and PPRI such as free $\cdot\text{OH}$ or low-energy hydroxylating species and carbon centered radicals.\textsuperscript{63-66}
\[
\begin{align*}
FeOH^{2+} + hv & \rightarrow Fe^{2+} + \cdot OH \quad (1) \\
Fe^{3+} + hv & \rightarrow Fe^{2+} + \cdot OH \quad (2) \\
Fe^{(II)}DOM + hv & \rightarrow Fe^{(III)} + DOM^{**} \quad (3)
\end{align*}
\]

Besides direct photolysis, Fe is actively involved in photo-Fenton like reactions, where photoreduced Fe(II) reacts with photogenerated H₂O₂ to form \(\cdot OH\) or other hydroxylating species depending on the pH or complexed ligands:⁶⁵,⁶⁷,⁶⁸

\[
Fe^{(II)} + H₂O₂ \rightarrow Fe^{(III)} + \cdot OH \quad (4)
\]

where Fe(II) may be free or complexed with CO₃²⁻, DOM, and other ligands. The presence of DOM not only shifts the Fe solubility and speciation in the aquatic system, but is also capable of changing the quantum yields of PPRI.⁶⁹–⁷² Thermodynamic and kinetic models have been developed to fit experiment data under these complicated conditions.⁷³,⁷⁴

1.3 Environmental impact: Photolytic contaminant degradation and DOM transformation

1.3.1 Attenuation of agrichemicals

Photochemical processes have been considered as important transformation pathways for anthropogenic compounds in addition to hydrolysis, gas exchange, sorption, sedimentation, and biodegradation.¹⁷,⁷⁵–⁷⁷ Agrichemicals released into aquatic systems may undergo direct photolysis if they absorb light in the solar spectrum. In addition, pesticides may undergo indirect photolysis in the presence of the PPRI generated by photochemical reactions sensitized by the presence of DOM, Fe, nitrate, and other water
constituents. Attenuation rates of pesticides depend on pesticide structure and the reactivity, selectivity, and steady state concentration of these PPRI. A variety of studies have been conducted on the photolysis of agrichemicals, including pesticides, insecticides, fungicides, herbicides, lampricides, and aquaculture antibiotics. A better understanding of the photolysis of agrichemicals may help to predict their environmental fate.

1.3.2 DOM phototransformation

As an important component in the carbon cycle, DOM continuously undergoes transformation. The photofate of DOM includes direct light absorption followed by electron or energy transfer and indirect pathways whereby DOM reacts with PPRI. These PPRI may be formed from DOM itself, or from Fe, nitrate, anthropogenic organic compounds, and other light absorbing constituents in the water. As a result, DOM can be mineralized to inorganic carbon, degraded to low molecular weight molecules, condensed to more aromatic molecules, or aggregated to form particulate organic matter. The transformation of DOM may largely change its photochemical, biogeochemical, and ecological functions.

1.3.3 Variable sunlight intensity and diurnal circle

All of the aforementioned photochemical process, either the degradation of agrichemicals or the transformation of DOM, depends on the sunlight intensity, which is spatiotemporally variable. The variations in sunlight irradiance is dependent upon cloud
cover, diurnal cycles, and seasonal turnover. When evaluating the photofate of a chemical, the additivity of solar fluence (i.e., summation of photons impinging on the target compound(s)) is often assumed for photochemical experiments and models. However, under certain circumstances, dark phase thermal or biological reactions of photo-formed intermediates may alter this additive nature of irradiance, resulting in over-prediction or under-prediction based upon the continuous irradiance assumption. For example, trebolone acetate metabolites have been shown to undergo direct photolysis during the day, but undergo product-to-parent transformation during the night. Moreover, Fe(III) in acid mine drainage impacted waters is photochemically reduced during the day but biologically reoxidized during the night. In Chapter 2, the herbicide, isoproturon, was investigated to study this unusual diurnal process.

1.4 Scope of research

As depicted in Figure 1.3, DOM and Fe are important chromophores responsible for the production of PPRI in aquatic systems. Both direct light absorption and indirect (sensitized) photochemical reactions initiate important pathways for the transformation of organic contaminants and DOM in various aquatic systems under variable solar irradiation. The overall objective of this dissertation is to foster an understanding of how diurnal solar irradiation transforms both natural (DOM) and anthropogenic (pesticide) organic substrates in aquatic systems, with an emphasis on reactive species photochemically generated from DOM and Fe. To accomplish this goal, a variety of water bodies with diverse light absorbing characteristics was investigated in this study.
In Chapter 2, I explored the indirect photolysis of the herbicide, isoproturon, induced by \(^3\text{DOM}\)*, which most likely occurs in sunlit receiving waters. The impact of diurnal processes was investigated as photodegradation and dark-regeneration of isoproturon. This study added to the limited knowledge about the regeneration of the parent compound by thermally labile phototransformation intermediates.

In Chapter 3, I studied the photolysis of acetochlor with •OH formed from the reaction of photo-irradiated Fe and DOM in natural and engineered wetlands. To understand and capitalize on photolysis as a herbicide attenuation pathway, different concentrations of Fe were dosed into different waters to determine an optimal concentration. This study shed light on important water constituents such as pH and DOM in •OH mediated pesticide degradation.

In Chapter 4, I investigated the photolysis of DOM induced by •OH in Fe rich acid mine drainage (AMD) impacted waters. I report for the first time, fast (hours) phototransformation and mineralization of DOM in AMD water bodies, which could alter the DOM initiated photochemistry and its role in downstream ecosystems.

Throughout this dissertation, the objective of testing the role of Fe and DOM was achieved by studying waterbodies on a Fe-DOM continuum whereby one boundary is defined by DOM dominated photochemistry and the other is Fe dominated. The results of this dissertation are an important step in understanding the photofate of organic anthropogenic compounds and DOM in aquatic systems.
1.5 Figures and tables

Figure 1.1. Hypothetical 3D structure of a representative fulvic acid (C\textsubscript{47}H\textsubscript{50}O\textsubscript{25}NS).

C: grey, H: white, O: red, N: blue, S: yellow. Redraw from Figure 18.10 in Brezonik et. al.\textsuperscript{2} with ChemBio3D software.
<table>
<thead>
<tr>
<th>major interaction</th>
<th>representative Fe species$^{28,69,101,102}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>precipitation-dissolution</td>
<td><strong>dissolved:</strong> Fe$^{3+}$; <strong>solids:</strong> amorphous Fe(OH)$<em>3$(s), α-FeO(OH)$</em>{(s)}$</td>
</tr>
<tr>
<td>oxidation-reduction</td>
<td>metal: Fe$^{(0)}$; ferric: Fe$^{(III)}$; ferrous :Fe$^{(II)}$; ferryl: Fe$^{(IV)}$=O$^{2+}$; ferrate: Fe$^{(VI)}$O$_4^{2-}$</td>
</tr>
<tr>
<td>complexation</td>
<td><strong>free:</strong> Fe$^{3+}$, Fe$^{2+}$; <strong>complexed:</strong> FeCO$_3$(aq), FeOH$^{2+}$, Fe$^{(III)}$-DOM</td>
</tr>
<tr>
<td>sorption-desorption</td>
<td>desorbed: Fe$^{4+}$; sorbed: Fe-POM (particular organic matter)</td>
</tr>
<tr>
<td>biological</td>
<td><strong>abiotic:</strong> Fe$^{2+}$, FeSO$_4$$^{+}$; <strong>biotic:</strong> ferritin</td>
</tr>
</tbody>
</table>
Figure 1.2. Structures of the herbicides, isoproturon and acetochlor.
Figure 1.3. Summary of important photochemical reactions discussed in this dissertation.
Chapter 2: Isoproturon Reappearance after Photosensitized Degradation in the Presence of Triplet Ketones or Fulvic Acids
2.1 Introduction

Direct or indirect (sensitized) photochemical reactions have been identified as important initiation steps in the natural attenuation of a variety of synthetic organic chemicals (SOCs) in shallow sunlit waters.\(^{55,103}\) However, sunlight intensity is spatiotemporally variable due to sun angle changes and cloud cover. As a result, the dominant transformation pathways may vary with sunlight intensity. Under sunny conditions, photolysis of SOCs generates unstable intermediates which, in turn, react by both photochemical and thermal routes. When light intensity becomes low or absent, the photogenerated intermediates can only react thermally, possibly forming different products compared to those formed at high light intensity. These thermally formed products may even include the parent compound. In this case, the intermediate-to-parent reversion leads to the persistence of the parent compound, which may not be accurately predicted based upon continuous photodegradation studies. A fast reversion from a short-lived intermediate will inhibit the overall degradation rate even in the presence of light,\(^{21}\) whereas a slow reversion from a long-lived intermediate may result in a diurnal cycling of the parent chemical in the aquatic system, such as trenbolone acetate metabolites,\(^{99}\) 2,4,6-trimethylphenol,\(^{104}\) and substituted naphthalene.\(^{105}\) Isoproturon (IPU,\(\text{C}_{12}\text{H}_{18}\text{N}_{2}\text{O}\), 3-(4-isopropylphenyl)-1,1-dimethylurea) is a compound that reappears thermally after indirect photolysis.\(^{106}\) As a substituted phenylurea herbicide, IPU is often applied to grain fields to kill broad-leaf weeds by inhibiting photosynthesis. However, IPU potentially promotes liver tumors in rats and impedes periphytic bacterial communities and aquatic invertebrates.\(^{107,108}\) Decades of extensive
use of IPU in several European and Asian countries have led to occasionally elevated concentrations in surface water and groundwater. In sunlit waters, natural attenuation of IPU induced by photoexcited triplet states of dissolved organic matter (\(^3\)DOM\(^*\)) may be an important degradation pathway in addition to microbial degradation. In a study using aromatic ketones (benzophenone and 3’-methoxy-acetophenone) as model \(^3\)DOM\(^*\) photosensitizers, the reappearance of IPU in the dark after initial photolysis was first reported and proposed to result from a hydrophilic peroxide intermediate (IPU\(_{\text{int}}\)). However, the identity of IPU\(_{\text{int}}\), the connection between IPU\(_{\text{int}}\) and IPU, and the detailed diurnal kinetics of photodegradation and dark-reappearance of IPU were not further reported.

The objectives of this chapter were as follows: (1) to confirm the cycling of IPU in the presence of model DOM, i.e. aromatic ketones and aquatic fulvic acids; (2) to quantify the significance and efficiency of this dark back reaction; and (3) to elucidate the diurnal transformation mechanism of IPU in aquatic systems by identifying the molecular structure of the reversible intermediate and by testing the effect of water constituents on the back reaction.

2.2 Experimental section

2.2.1 Chemicals and analytical techniques

All chemicals were obtained from commercial sources and used without further purification. For a complete list of chemicals, see the Appendix A (Text A.1). Suwannee River fulvic acid (SRFA) and Pony Lake fulvic acid (PLFA) were obtained from the
International Humic Substances Society. Analytical techniques including High Performance Liquid Chromatography (HPLC) analysis and computational details are detailed in the Appendix A (Text A.1).

2.2.2 Photochemical and sequential dark reaction kinetics

Similar experiments were independently designed and conducted in Columbus and Dübendorf.

Methods Used in Columbus. (a) *Photochemical Experiments.* Solutions of 10 μM IPU and 25 μM acetophenone (ACP, used as a triplet photosensitizer) dissolved in pH 8 buffers (1 mM NaHCO₃) were sealed in quartz tubes (9 mm internal diameter) and irradiated in an Atlas Suntest CPS+ solar simulator equipped with a 500 W/m² xenon lamp and solar standard filter for 7 h at 25 ± 2°C. A subset of the light experiments replaced NaHCO₃ and ACP solutions with 5.5 or 11 mgC/L PLFA or SRFA solutions and was adjusted to pH 8 with HCl or NaOH. Another subset used argon-saturated solution (see Text A.1). Control experiments in the absence of any photosensitizer were also conducted to measure IPU’s direct photolysis. (b) *Experiments in the Dark.* Photolyzed solutions at selected time points were transferred to amber vials and kept in a dark room (22 ± 2°C) for 4 days. In selected ACP experiments to elucidate the dark reaction mechanism, adjustments including temperature adjustment with water bath/refrigeration and pH adjustment (acidic pH) with HCl immediately after photolysis were performed.
Methods Used in Dübendorf. (a) Photochemical Experiments. A DEMA 125 merry-go-round photoreactor (Hans Mangels, Bornheim-Roisdorf, Germany) equipped with a Heraeus Noblelight TQ 718 medium-pressure mercury lamp (operated at 500 W), a borosilicate glass cooling jacket, and a filter solution cooled at 10 °C was used. The experimental setup is described in detail elsewhere.\textsuperscript{112,113} The photoreactor was operated using two different filter solutions. For irradiations of solutions containing 4-carboxybenzophenone (CBBP) as a photosensitizer, a 0.15 M sodium nitrate filter solution was used, whereby irradiation wavelengths <320 nm were cut off. For experiments with disodium 9,10-anthraquinone-1,5-disulfonate (1,5-AQDS) as a photosensitizer, a filter solution containing 0.25 M sodium nitrate and 0.05 M sodium nitrite was employed, whereby irradiation wavelengths <370 nm were cut off. These filters were applied to reduce possible direct photolysis of IPU and its main product (IPU\textsubscript{int}) while sustaining an efficient absorption of light by the photosensitizer. Aerated aqueous solutions (16-20 mL) containing 10 µM IPU, phosphate buffer (typically 5 mM) and a single photosensitizer (either 80 µM CBBP or 50-200 µM 1,5-AQDS) were filled into glass-stoppered quartz tubes (15 mm internal diameter) and equilibrated prior to irradiation for 15 min in a water bath at 10 °C. (b) Experiments in the Dark. After irradiations were completed, the quartz tubes were transferred to a thermostatic bath set at a constant temperature (stability ± 0.1 °C) and 400 µL samples were withdrawn for analysis at various time intervals. Except for the time needed for sample withdrawal, the solutions were protected from ambient light using aluminum foil. Alternatively, 300 µL of the concentrate containing the isolated IPU\textsubscript{int} (see Text A.1 for isolation method) were
added to 15 mL of buffer solution (basic pH, contained in the quartz tubes) that had been equilibrated at the selected temperature. The dark kinetic experiment was performed as described above.

2.2.3 Reaction of IPU with thermally generated excited singlet state of oxygen ($^1$O$_2$, performed in Dübendorf)

Peroxidation of IPU was performed using the hydrogen peroxide-molybdate system similarly as described by Aubry and co-workers.$^{114,115}$ The reaction was conducted under magnetic stirring in a 350 mL cylindrical Pyrex® glass reaction vessel equipped with a cooling jacket, through which water at 25 °C was recirculated. First, 225 mL of an aqueous 100 μM IPU solution and 25 mL of a 500 mM bicarbonate/carbonate buffer (pH 10.0) were filled into the vessel. Then, 7.5 g of sodium molybdate dihydrate were added. After complete dissolution of this salt, the pH of the solution was adjusted to 9.0 by adding sodium bicarbonate. To produce $^1$O$_2$, 7.5 mL aliquots of concentrated hydrogen peroxide (30 %) were added sequentially at the following times (0 corresponds to the first addition): 0, 15, 30, 45, 60, and 70 min. A short time (≈10 s) after each addition of the hydrogen peroxide a 400μL sample of the reaction solution was withdrawn for HPLC analysis. After the last addition of hydrogen peroxide, four quartz tubes were filled with 20 mL aliquots of the reacted solution, and each aliquot was adjusted at a different pH (initially 7.0, 8.0, 9.0 and 10.0). The four tubes were kept in a water bath at 25.0 °C and protected from light using an aluminum foil. Samples of 400 μL were then withdrawn.
after 0, 120, 270, 360, 1320, 1560, and 1790 min from each tube and submitted to HPLC analysis.

2.2.4 IPU<sub>int</sub> purification and identification (performed in Columbus)

To maximize IPU<sub>int</sub> production for structure identification, solutions of 300 µM IPU and 12 mM ACP were irradiated in phototubes in the Atlas Suntest CPS+ solar simulator (500 W/m<sup>2</sup> xenon lamp) for 15 min. 900 mL of photolyzed solution (containing ACP, IPU, and produced IPU<sub>int</sub>) was consolidated by combining batches of phototubes and was then extracted with ethyl acetate (EA, 5×80 mL). The EA extract was desiccated with Na<sub>2</sub>SO<sub>4</sub> and then concentrated to 2 mL using rotary evaporation. This 2 mL concentrated EA extract was purified by silica column chromatography using EA/hexanes mixture (ramping from 1:4 to 4:1, v/v) as the eluent. The eluate fraction containing IPU<sub>int</sub> (confirmed with HPLC) was rotary evaporated to dryness. The resulting IPU<sub>int</sub> isolate was redissolved in acetonitrile and analyzed with a Bruker maXis electrospray ionization quadrupole time-of-flight tandem mass spectrometer (ESI-QTOF-MS/MS). Details of MS analysis are reported in the Text A.1. Another batch of IPU<sub>int</sub> isolate was redissolved in dimethyl sulfoxide-<sup>d6</sup> (DMSO-<sup>d6</sup>) or acetonitrile-<sup>d3</sup> (CD<sub>3</sub>CN) for NMR spectroscopic analysis (400 MHz, Bruker NMR Spectrometer). NMR spectra are reported in Appendix A (Figure A1, Figure A2).

2.3 Results and discussion

2.3.1 IPU photodegradation and dark-reappearance
Consistent with previous work,\textsuperscript{106} we observed negligible direct photolysis of IPU over the course of 36 h with no detectable products formed (the half-life is $164 \pm 19$ h in Suntest CPS+, Figure A3.a, all errors given are 95\% confidence intervals). The addition of 25 \textmu{}M acetophenone (ACP) as a model excited triplet state photosensitizer shortened the half-life of IPU phototransformation to $6.2 \pm 0.8$ h, although a small deviation from first-order kinetics was observed (Figure A3a), which is likely due to the thermal back reaction discussed below.

Notably, a hydrophilic intermediate, IPU$_{\text{int}}$, appeared in the HPLC (UV detector) chromatogram when IPU was irradiated in the presence of ACP (Figure 2.1b insert). As depicted in Figure 2.1a, we monitored IPU and IPU$_{\text{int}}$ in a diurnal experiment consisting of two phases: the photolysis phase from time $t_{p,0}$ to $t_{p,\text{max}}$ ($t_{p,\text{max}} = 1, 2,$ or $5$ h), and the dark-reaction phase from time $t_{d,0}$ to $t_{d,\text{max}}$ ($t_{d,\text{max}} - t_{d,0} \approx 100$ h), where $t_{p,\text{max}} = t_{d,0}$. The dark transformation of IPU$_{\text{int}}$ followed first-order kinetics ($R^2>0.98$) with a half-life of $42 \pm 2$ h at $22 \degree$C at pH 8 in 1 mM NaHCO$_3$ and 25 \textmu{}M ACP (Figure 2.1a). Our rate corroborates the previous study where a half-life of 37 h at $25 \degree$C at pH 8 in 5mM phosphate buffer was reported.\textsuperscript{106} Figure 2.1a shows that the gradual decrease in IPU$_{\text{int}}$ peak area (PA) in the dark was accompanied by an increase in the parent compound IPU over the 4-day monitoring period. For purposes of this paper we assume that the PA is proportional to the concentration of IPU$_{\text{int}}$ based upon Beer’s law, and did not directly compare PA between IPU and IPU$_{\text{int}}$ due to difference in their molar extinction coefficients. To gain insight into the connection between IPU$_{\text{int}}$ loss and IPU increase in the dark, we explored the relative changes in the peak areas of IPU and IPU$_{\text{int}}$ by plotting

\textsuperscript{106}
(IPU \( PA_{t_{d,i}} \) – IPU \( PA_{t_{d,0}} \)) versus (IPU\(_{\text{int}}\) \( PA_{t_{d,i}} \) – IPU\(_{\text{int}}\) \( PA_{t_{d,0}} \)) at each combination of \( t_{d,i} \) and \( t_{d,0} \) for \( t_{p,\text{max}} = 1, 2, \) or 5 h in Figure 2.1b. The plot shows a significant negative correlation between the change of IPU and IPU\(_{\text{int}}\) PA in the dark reaction phase (\( P<0.05 \)), suggesting that IPU\(_{\text{int}}\) transformed to IPU in the dark. Additionally, the dark reversion to IPU occurred with purified IPU\(_{\text{int}}\) (Figure A4), confirming the causal link between IPU\(_{\text{int}}\) decay and IPU rebound and indicating that photosensitizers like ACP are not necessary for the rebound reaction.

The photodegradation (Figure A3.b) and dark-reappearance (Figure 2.2) cycling of IPU also occurred in the presence of selected fulvic acids at pH 8. We first irradiated IPU in the presence of two end-member fulvic acids (SRFA, representing an allochthonous DOM, and PLFA, representing an autochthonous DOM\(^3\)) at environmentally relevant concentrations (5.5 and 11 mgC/L). After three selected photolysis time points, we monitored the reappearance of IPU and the decay of IPU\(_{\text{int}}\) in the dark (\( t_{p,\text{max}} = 2, 4 \) or 6 h, \( t_{d,\text{max}} - t_{d,0} \approx 170 \) h). Figure 2.2 depicts that in all FA experiments, the loss of IPU\(_{\text{int}}\) was connected to the reversion of IPU. As to the kinetics, the dark transformation from IPU\(_{\text{int}}\) to IPU in the presence of fulvic acids not only was slower (Figure A5, overall half-life = 63 ± 9 h) but also deviated from first-order kinetics compared to experiments with ACP/NaHCO\(_3\). This retardation and change in kinetic order were not explored further, but may be attributable to the interaction between IPU\(_{\text{int}}\) decay intermediates and the fulvic acids in a manner that did not occur with ACP/NaHCO\(_3\), thereby circumventing conversion back to IPU. The retardation can also be explained by the lack of an inorganic buffer or changes in ionic strength in the fulvic acid experiments. We observed an
increase in the dark transformation rate of $\text{IPU}_{\text{int}}$ with the increase of phosphate concentration in a similar experiment at the pH of 8.0 and also observed a difference in the rates between phosphate and carbonate buffers. Mechanisms behind these observations were not explored in this paper.

Although IPU reappeared consistently in the dark for whichever photosensitizer was used, it did not rebound to its initial concentration and the extent of reversion varied. For example, Figure 2.1a shows that about 5.5 μM IPU ($[\text{IPU}]_{\text{p,max}} - [\text{IPU}]_{\text{p,0}}$) was lost after photolysis but only 2 μM IPU ($[\text{IPU}]_{\text{td,max}} - [\text{IPU}]_{\text{td,0}}$) was recovered after the whole diurnal experiment. The deviation from full mass balance indicates that byproducts (which are incapable of reverting back to IPU) were formed during photolysis and/or dark reaction. The observation of several very small unknown peaks in the HPLC-UV chromatogram further supports this hypothesis. The net amount of IPU loss after the diurnal experiment depended on the irradiation, the photosensitizer used, and other experimental conditions. In the dark reaction, the ratio of $(\text{IPU} \text{ PA}_{\text{td,i}} - \text{IPU} \text{ PA}_{\text{td,0}}) / (\text{IPU}_{\text{int}} \text{ PA}_{\text{td,i}} - \text{IPU}_{\text{int}} \text{ PA}_{\text{td,0}})$, or the slopes in Figures 2.1b and 2.2, can be used to determine the impact of different photosensitizers on the rebound extent. Comparing Figures 2.1b and 2.2, smaller absolute slopes were observed for the fulvic acids suggesting that a larger fraction of irreversible byproducts was formed in solutions with fulvic acids as compared to that in the presence of ACP/NaHCO$_3$. Surprisingly, although SRFA tends to induce a slightly larger rebound slope, no statistically significant difference in slopes was present between either fulvic acid types (Figure 2.2) or fulvic acid concentrations. There might exist sufficient amounts of responsible functional
groups at all concentrations of the fulvic acids needed to enhance side reactions and/or inhibit the rebound reaction. Another explanation might be that fulvic acids are not involved in the rebound reaction; instead, the presence of NaHCO$_3$ in the ACP experiment enhanced the rebound reaction over side reactions.

2.3.2 Factors influencing the rate and extent of dark reaction

To elucidate the reactivity of IPU$_{int}$ under a range of conditions and its possible transformation mechanisms, the first-order reaction rate constant (k) for IPU$_{int}$ degradation was investigated under different temperatures and pH values. As depicted in Figure 2.3, Arrhenius plots in both carbonate and phosphate buffers show a linear relationship over the observed temperature range. The derived apparent activation energy ($E_a$) was 121 ± 29 kJ/mol in the carbonate buffer at pH 8 and 101 ± 10, 118 ± 15 and 132 ± 16 kJ/mol in phosphate buffers at pH 7, 8, and 9, respectively (see Text A.1 for the calculation method). These large energy barriers from the reactant to the transition state suggest that a more favorable rebound to the parent compound occurs at higher temperatures. The corresponding entropy of activation ($\Delta S^\ddagger$) calculated from the Arrhenius plots ranges from 40 to 52 J/(mol·K) at 22°C and pH 8 for different buffers (see Text A.1 for the calculation method). These positive $\Delta S^\ddagger$ values might be indicative of a less ordered or dissociative transition state, as discussed below. However, this piece of evidence can be inconclusive due to large 95% confidence intervals (30-100J/(mol·K)) for $\Delta S^\ddagger$. 
Figure 2.4 shows that both acidic and basic conditions accelerated the disappearance of IPU_{int}, and this overall trend was consistent across different investigated buffer types and temperatures. However, the extent of dark reversion of IPU is much smaller under acidic as compared to basic conditions as quantified as rebound ratios in Table A.1. The increase in pH from neutral to basic values assured the rebound of IPU and caused a nonlinear increase in k with respect to OH\(^-\) concentration. This excludes a direct collision between IPU_{int} and OH\(^-\) to initiate the reaction; instead, it may imply that IPU_{int} can deprotonate at higher pH and the acid/conjugate base forms have different reversion rates. When acid was added, however, it appeared to react with IPU_{int} to form byproducts other than IPU. This phenomenon was most apparent below pH 4 since nearly no rebound was observed (Table A.1). Thus, the rate and extent of IPU reversion are sensitive to pH changes, but, in well-buffered surface waters where pH change is not dramatic, the change in reactivity is most likely to be small.

2.3.3 Identifying the structure of IPU_{int}

Understanding the structure of IPU_{int} is a crucial step in elucidating the mechanism of its reversion back to IPU and additional reactions leading to other potentially important products. Using ESI-QTOF-MS, the accurate mass to charge ratio (m/z) of the most abundant peak in the purified and enriched IPU_{int} sample was determined to be 261.1218 (Figure 2.5). The mass error of this peak is 1 ppm based on the calculated monoisotopic mass of the IPU-sodium adduct plus two oxygen atoms (IPUOONa, C\(_{12}\)H\(_{18}\)N\(_2\)O\(_3\)Na\(^+\), 261.1215\(_{116}\)). The corresponding proton (IPUOOH, C\(_{12}\)H\(_{18}\)N\(_2\)O\(_3\)H\(^+\), calculated m/z =
239.1396\textsuperscript{116} and potassium adducts (C\textsubscript{12}H\textsubscript{18}N\textsubscript{2}O\textsubscript{3}K\textsuperscript{+}, calculated m/z = 277.0955\textsuperscript{116}) are also present in the mass spectrum within a 2-ppm error. The major remaining ions in Figure 2.5 likely correspond to in-source fragments formed from either IPUOONa or IPUOOH, and match the in-source fragments formed from IPUOOH in MS/MS mode (discussion below). Therefore, the molecular formula of IPU\textsubscript{int} is established to be C\textsubscript{12}H\textsubscript{18}N\textsubscript{2}O\textsubscript{3}. This structure may either be an IPU peroxide or a dihydroxylated IPU. The MS/MS spectrum was then collected to further characterize IPU\textsubscript{int}. The protonated IPU\textsubscript{int} was susceptible to in-source fragmentation with no collision energy applied (Table A2). Fragments tabulated in Table A2 show the loss of 17, 33, or 42 mass units from the protonated IPU\textsubscript{int}, corresponding to the loss of -OH, -OOH and -C\textsubscript{3}H\textsubscript{6} groups, respectively. This fragmentation pattern does not match that of dihydroxylated IPU derivatives formed from reaction with hydroxyl radical, which usually contain unfragmented OH groups or have a loss of water.\textsuperscript{117} Therefore, this finding favors the incorporation of an oxygen molecule, i.e. peroxide formation, over the addition of two hydroxyl groups into IPU to form IPU\textsubscript{int}. In an attempt to gain more structural information, an increase in collision energy decreased the intensity of the parent ion but did not result in any useful fragmentation patterns.

\textsuperscript{1}H NMR (Figure A.1) and \textsuperscript{13}C NMR (Figure A.2) of IPU\textsubscript{int} were conducted to elucidate the type (a hydroperoxide vs an endoperoxide) and position of the peroxide functional group. The \textsuperscript{1}H NMR spectrum of IPU\textsubscript{int} in DMSO (Figure A.1a) shows a highly deshielded peak at 11.5 ppm consisting of one proton. We assigned this proton to a hydroperoxide functionality (existing in hydroperoxides) instead of an amide proton.
(existing in endoperoxides), the latter of which is likely to be less deshielded as in $^1$H NMR of IPU (6.2 ppm, Figure A.1b). Previous studies show the chemical shift of a hydroperoxide proton to be 7.6 to 9.2 ppm in CDCl$_3$.\textsuperscript{118,119} In the presence of a polar solvent such as DMSO, H-bonding would further deshield this proton, consistent with the observed value. The hypothesis of IPU\textsubscript{int} being a hydroperoxide is, therefore, more favored.

We analyzed the NMR spectra for the position of the hydroperoxide functional group. The splitting of proton resonances of the isopropyl -CH and -CH$_3$ groups in IPU\textsubscript{int} (Figure A.1a) remains intact compared to IPU, excluding the attachment of the -OOH group to the isopropyl moiety. However, their chemical shifts in IPU\textsubscript{int} (1.95, 0.82/0.83 ppm; Figure A.1a) occur upfield compared to IPU (2.86, 1.22 ppm; Figure A.1b) and therefore do not suggest the existence of an isopropyl group attached to an aromatic ring as found in IPU. The two chemically inequivalent isopropyl -CH$_3$ groups also appear as overlapping doublets (0.82 and 0.83 ppm, Figure A.1a) in contrast to a simple doublet (1.22 ppm, Figure A.1b) in IPU. These two observations of the isopropyl group suggest that IPU\textsubscript{int} lacks an aromatic structure, likely because of the attachment of the -OOH unit to the ring.

Based upon the MS/MS and NMR data, we assign the IPU\textsubscript{int} structure as illustrated in Figure 2.6. Both isopropyl and the -OOH group are attached to the 4-position of the ring with respect to the urea group. Attachment of the -OOH group at C4 combined with cis/trans geometrical isomerism due to imine functionality at C1 introduces chirality in IPU\textsubscript{int} as shown in Figure A.6. As a result of the -CH$_3$ groups of the isopropyl unit
becoming diastereotopic, they individually couple with the -CH proton to generate overlapping doublets in the $^1$H NMR spectrum as discussed above. Rotation around the N-C=O bond of the amide is also restricted and therefore the methyl groups in -N(CH$_3$)$_2$ appear as separate singlets in the $^1$H NMR spectra of IPU$_{int}$. The position of the hydroperoxide group is further confirmed by the largely upfield chemical shift (84.8 ppm, Figure A.2a) of the corresponding carbon in the $^{13}$C NMR as compared to an aromatic carbon (121–144 ppm for IPU, Figure A.2b).

Further support of the proposed IPU$_{int}$ assignment was conducted by comparing calculated (using density functional theory) and experimental UV-vis spectra as depicted in Table A.3 and Figure A.7. Compared to IPU ($\lambda_{max} = 240$ nm), the addition of the hydroperoxide functional group on IPU$_{int}$ leads to a red shift in excitation energy ($\lambda_{max} = 248$ nm). For the proposed IPU$_{int}$ structure, the calculated vertical energy of high oscillator strength (quantifying the probability of absorption) is 278 and 250 nm in the gas phase and 272 and 241 nm in water. The overall $\lambda_{max}$ is an overlap of these two excited states (Figure A.7). Considering the uncertainties in calculating the vertical energy and especially oscillator strength for large molecules like IPU$_{int}$, the calculated result is in reasonable agreement with the experimental data.

2.3.4 Proposed pathways and environmental significance

Figure 2.6 illustrates our proposed mechanism for the cycling of IPU and IPU$_{int}$ in a diurnal system (the degradation of IPU and the formation of IPU$_{int}$ in the photoreaction phase, and the reappearance of IPU and the depletion of IPU$_{int}$ in the dark-reaction
phase). As depicted in Figure 2.6, a variety of photochemically generated reactive species in sunlit waters may take part in the photoreaction phase, including $^3$DOM*, $^1$O$_2$, and superoxide radical anion (O$_2^-$).$^{16}$ IPU was previously determined to photochemically degrade primarily through a $^3$DOM* pathway rather than by reaction with $^1$O$_2$.$^{111}$ We observed enhanced IPU degradation under argon-saturated conditions (high $^3$DOM* and low $^1$O$_2$) compared to air-saturated systems, corroborating this pathway through $^3$DOM* (Figure A.8, Figure 2.6). Moreover, negligible photosensitized transformation of IPU was observed using 2-acetonaphthone, a good $^1$O$_2$ photosensitizer which supports the lack of $^1$O$_2$ reactivity.$^{106}$ However, the formation of IPU$_{int}$ from IPU by reaction with $^1$O$_2$ was observed in this study using the hydrogen peroxide-molybdate system as a nonphotochemical source of $^1$O$_2$ (Figure A.9). Identification of IPU$_{int}$ was done according to its identical HPLC retention time, electronic absorption spectrum, and comparable kinetic decay as the product from indirect photolysis. This reaction of IPU with $^1$O$_2$, through the possible mechanism of single-step concerted [4+2] cycloadditions,$^{120}$ is probably not important in sunlit surface waters but has high relevance from a mechanistic point of view.

Overall, in Figure 2.6, $^3$DOM* can induce electron transfer followed by deprotonation (or direct hydrogen-atom abstraction, to a lesser degree) to form IPU radicals (structures (I) and (II) are two possibilities).$^{106,121}$ It may, in turn, undergo a second electron-proton/hydrogen-atom abstraction to form compound (III). The formation of IPU$_{int}$ requires further steps for dioxygen species (such as $^1$O$_2$, O$_2$, O$_2^-$) to be incorporated to IPU. Both compounds (II) and (III) may directly react with O$_2^-$ and H$^+$ or O$_2$ followed
by hydrogen-atom transfer\textsuperscript{122} to form \textit{IPU}_{int}. It is worth mentioning that the reaction of intermediate (II) with \textsuperscript{3}DOM* appears to be less likely due to the low steady-state concentration of \textsuperscript{3}DOM* compared to \textit{O}_2\textsuperscript{−}. Because of the necessity of dioxygen species, the impact of oxygen on the \textit{IPU}_{int} formation rate and on the IPU degradation rate may be different. In the argon-saturated system, an increased level of \textsuperscript{3}DOM* (thus faster IPU radical (I) formation in Figure 2.6) and a decreased level of oxygen species (thus slower reaction from IPU radicals to \textit{IPU}_{int}) result in contrasting impacts on \textit{IPU}_{int} formation. This is illustrated by an initial modest enhancement followed by a decrease in the formation rate of \textit{IPU}_{int} as compared to an air-saturated system (Figure A.8). Several reactions in Figure 2.6 may account for the side reactions that do not participate in IPU reversion at environmentally relevant conditions i.e., compounds with an imine functional group may be hydrolyzed\textsuperscript{123,124} to side products (VII) and (VIII) and direct/indirect photolysis of \textit{IPU}_{int}.

The dark reaction mechanism is proposed as a base-catalyzed reaction in Figure 2.6. After deprotonation, \textit{IPU}_{int} can undergo nucleophilic conjugate addition to form an oxygen bridge (compound (V)).\textsuperscript{125} Its subsequent thermolysis may be closely related to a group of aromatic endoperoxides including furans such as 2,5-diphenylfuran,\textsuperscript{126} many polycyclic aromatics such as anthracenes,\textsuperscript{120} monocyclic aromatics such as 2,4,6-trimethylphenol,\textsuperscript{104,127} and nonaromatic compounds such as ascaridole.\textsuperscript{128} Most of the previous work focused on \textit{^1O}_2/superoxide as a reactant to form endoperoxides. However, in our system, although \textit{^1O}_2 is capable of generating \textit{IPU}_{int}, the role of excited triplets to initiate the reaction is important. Among the previously reported endoperoxides, 2,4,6-
trimethylphenol endoperoxide has been extensively studied in water and was proposed to be in equilibrium with its corresponding hydroperoxide, and both of which were able to regenerate the parent compound but at different rates.\textsuperscript{104,127} The reported activation energy (105 kJ/mol, at pH 13) and base-catalyzed reaction (fast reversion observed when pH>10) was similar to ours (132 kJ/mol, at pH 9).

At acidic pH, IPU\textsubscript{int} decays quickly to products other than IPU. Some possibilities include acid-catalyzed imine hydrolysis in water to form irreversible products (such as compound (VIII) in Figure 2.6) or O–O bond cleavage to form corresponding epoxides or phenols,\textsuperscript{125} leading to no rebound of IPU. Another possibility is the acidic hydrolysis at the urea group which has been well documented for phenylureas including IPU, but it tends to react at a much slower rate than what was observed for IPU\textsubscript{int}.\textsuperscript{129,130} It is noteworthy that DOM may not be directly involved in the simple dark reaction scheme, but it may work as an acid/base source. The interaction between DOM and intermediates in the dark reaction has not been mechanistically revealed in this study.

This work shows dark reversion of a phenylurea herbicide after indirect photolysis. The mechanism builds on the limited knowledge of environmentally related reversible reactions.\textsuperscript{99} In addition to the obvious diurnal cycling, this reversion may also apply to situations when the intermediate is physically transferred from the photic zone to areas in a water body that screen out the relative wavelengths responsible for the formation of reactive oxygen species and \(^3\)DOM\textsuperscript{*}. At these environmentally relevant conditions, the deoxygenation of IPU peroxide is likely to be responsible for the partial rebound of IPU, which would take several days in the absence of light. Variation of pH, temperature,
inorganic ion concentrations, and DOM properties will also alter the rate and significance of reversion. Therefore, the persistence of IPU may be underestimated if reversion is not considered. The detailed structure and mechanisms reported in this study help to predict similar processes occurring with other related compounds.
2.4 Figures and tables

Figure 2.1. Back reaction of IPU in the presence of ACP.

a. Light and dark reactions of IPU and IPU\textsubscript{int} in the presence of ACP. Labeled time points (t\textsubscript{p,0}, t\textsubscript{p,max} = t\textsubscript{d,0}, t\textsubscript{d,max}) in the figure are detailed in the text. Only data with t\textsubscript{p,max} = 5 h are shown in panel a for clarity. The depletion of IPU\textsubscript{int} in the dark is fitted to first-order kinetics: ln([IPU\textsubscript{int}] / [IPU\textsubscript{int}]\textsubscript{0}) = -(0.018 h\textsuperscript{-1}) \times \text{time} (R\textsuperscript{2} = 0.991). b. Peak area changes of IPU and IPU\textsubscript{int} in the dark in the presence of ACP (slope = −0.97 ± 0.06, R\textsuperscript{2} = 0.979). All data with t\textsubscript{p,max} = 1, 2, and 5 h are shown in panel b. ∆IPU = (IPU \ PA\textsubscript{td,i} \ − \ IPU \ PA\textsubscript{td,0}), ∆IPU\textsubscript{int} = (IPU\textsubscript{int} \ PA\textsubscript{td,i} \ − \ IPU\textsubscript{int} \ PA\textsubscript{td,0}). Insert: HPLC chromatogram at t\textsubscript{p,max} = 5 h.

Experimental conditions for a and b: pH = 8, [NaHCO\textsubscript{3}] = 1mM, [ACP]\textsubscript{0} = 25 μM, [IPU]\textsubscript{0} = 10 μM. PA, HPLC peak area; Abs., absorbance; AU, absorbance unit.
Figure 2.2. Peak area changes of IPU and IPU\textsubscript{int} in the dark in the presence of SRFA and PLFA.

Concentration changes of IPU were also shown as a second y-axis. Slope = \(-0.65 \pm 0.07\), \(R^2 = 0.866\) for SRFA and slope = \(-0.53 \pm 0.04\), \(R^2 = 0.903\) for PLFA. Experimental conditions: pH = 8, [SRFA]\textsubscript{0} = 5.4 mgC/L and 10.7 mgC/L, [PLFA]\textsubscript{0} = 5.5 mgC/L and 10.9 mgC/L, [IPU]\textsubscript{0} = 10 μM, dark rebound reaction started after photolysis time (t\textsubscript{p,max}) of 2, 4, or 6 h. Abbreviations are the same as in Figure 1.1.
Figure 2.3. Arrhenius plots for the decay of $\text{IPU}_{\text{int}}$ in the dark under different conditions.

Experiments in carbonate buffer (1 mM) were conducted in Columbus, where $\text{IPU}_{\text{int}}$ formation was mediated by ACP. Experiments in phosphate buffer (5 mM) were conducted in Dübendorf, where $\text{IPU}_{\text{int}}$ formation was induced by 1,5-AQDS. Alternatively, purified $\text{IPU}_{\text{int}}$ was used in the phosphate buffer experiments at pH 8 in Dübendorf, which shows similar results and is omitted for clarity. The error bars represent 95% confidence intervals for replicate experiments. The solid lines are linear regression lines.
Figure 2.4. IPU\textsubscript{int} dark reaction half-lives measured at different pH values.

The legend refers to the buffer (except for the carbonate subset at a low pH where most of the H\textsubscript{2}CO\textsubscript{3} volatilized), the temperature, and the remaining photosensitizer in the dark reaction. The inserted reaction mechanisms under acidic/basic conditions are explained in the text. The error bars represent the pH range before and after the reaction.
Figure 2.5. Electrospray ionization mass spectrum for purified IPU_{int}.

m/z 261 for IPUOONa; m/z 239 for IPUOOH; m/z 277 for IPUOOK; m/z 180 for loss of C_{3}H_{6} and OH from IPUOOH; m/z 222 for loss of OH from IPUOOH; m/z 228 for loss of OOH from IPUOONa. m/z stands for mass to charge ratio.
Figure 2.6. Proposed transformation pathways for IPU and IPU$_{int}$ in a diurnal cycle.

Note: the dark reaction phase focuses on basic conditions; B$^-$ and BH represent a base and its conjugated acid in the aquatic system. The Roman numerals are intermediate structures described in the main text (2.3.4).
3.1 Introduction

Agricultural runoff commonly contains synthetic agrichemicals, like herbicides, that impair water quality in receiving waters. Wetlands, both natural and engineered, have long been proposed as economic buffer zones to treat contaminants. Recent studies have shown photochemical degradation as an important pathway for herbicide attenuation in wetlands due to their large sunlit surface area and shallow depths. However, an incomplete understanding of attenuation mechanisms and efficacy have hindered its wide-spread application.

The irradiation of wetland waters by sunlight creates various reactive intermediates. Among them, hydroxyl radical (OH) is of particular importance due to its highly reactive nature towards organic contaminants (k \( \cdot \text{OH} \sim 10^8 \text{ to } 10^{10} \text{ M}^{-1}\text{s}^{-1} \)). The photochemical formation mechanism of OH is still an active research area but is generally believed to include (1) Fe-dependent pathways such as the photo Fenton reaction (requiring hydrogen peroxide (H\(_2\)O\(_2\))) and the irradiation of Fe(III)-ligand complexes and (2) Fe-independent pathways such as nitrate/nitrite photolysis and dissolved organic matter (DOM) photosensitization. Fe-dependent pathways
constitute a large portion of *OH production depending on the water constituents.\textsuperscript{138} For example, less than 50\% of *OH production is contributed from Fenton pathways in irradiated Suwannee River Fulvic Acid (SRFA)\textsuperscript{36}, and more than 70\% of *OH production is contributed to Fe in iron-rich (5-14 μM) Satilla River water\textsuperscript{139}. However, \textit{optimizing} Fe levels in wetland waters to specifically enhance *OH photoproduction is lacking. While in one highly relevant study, Vermilyea et al. found that at neutral pH, sufficient Fe\textsuperscript{(II)} was generated in two natural water bodies and SRFA solutions spiked with 10 μM Fe colloids to act as the main sink of photochemically produced H\textsubscript{2}O\textsubscript{2}\textsuperscript{68} the effect of Fe concentration on *OH production under diverse water conditions remained to be studied. The purpose of this chapter was to determine the optimal Fe concentration to promote the photoproduction of *OH in selected wetland waters. Because pH is a primary driver in Fe chemistry due to change in Fe speciation, we especially examined •OH dependent herbicide degradation at different pH values in the presence of dissolved organic matter (DOM). Acetochlor (AC) is a commonly applied row-crop herbicide, and was used in this study due to its known •OH specific degradation pathway and frequent detection in streams.\textsuperscript{83,131,140}

3.2 Methods

3.2.1 Reagents

Acetochlor (AC; analytical standard, 96.0 \%) and 2-hydroxyterephthalic acid (hTPA; 97 \%) were obtained from Sigma Aldrich (St. Louis, MO). Disodium terephthalate (TPA; >
99 %) was obtained from Alfa Aesar (Tewksbury, MA). Suwannee River fulvic acid (SRFA) and Pony Lake fulvic acid (PLFA) were obtained from International Humic Substances Society. All reagents were used as received. Ultrapure deionized water generated by a Milli-Q (MQ) water system (EMD Millipore, Billerica, MA) was used in all solutions. All glassware and plastic containers were acid washed to remove trace levels of metals. A complete list of reagents can be found in Appendix B (Text B.1).

3.2.2 Water samples

Tested water samples consisted of three whole water samples and two fulvic acid solutions. The whole water samples were collected from Old Woman Creek National Estuarine Research Reserve (OWC, Huron, OH), Waterman Farm (WF, Columbus, OH) and, Essington Lake (EL, Clayton Township, OH) and were filtered through 0.7 μm combusted glass fiber filters (See Figure B.1 in Appendix B for location details). 6 mgC/L FA (SRFA or PLFA) working solutions were diluted from stock solutions and spiked with NaHCO$_3$ to yield a total carbon concentration of 1 mM. UV-vis spectra were scanned with a Shimadzu spectrophotometer (Kyoto, Japan). Dissolved organic carbon (DOC) was determined using a Shimadzu TOC-VCPN analyzer (Kyoto, Japan) in non-purgeable organic carbon mode. pH values were measured with a Beckman 240 pH / Temp Meter (Brea, CA) equipped with a glass electrode. Alkalinity was determined by titration using 0.01 M sulfuric acid. Total Fe was determined using a modified FerroZine method (as described in Text B.2).
3.2.3 Photochemical experiments

The aforementioned water samples (both whole waters and FA working solutions) and MQ control were spiked with either AC (to yield a 5 μM final concentration) to determine herbicide degradation rates or TPA (to a yield 1 mM final concentration) to measure *OH production. After pH adjustment to the designed values (2.7, 5.0 or 7.6), aliquots of Fe sulfate (1 mM as Fe³⁺, dissolved in 0.01 M HCl) were added to the solutions to yield a 2-10 μM final added concentration (Fe_{added}) followed by a second pH adjustment. The solutions were then stirred over-night without noticeable precipitation at all pH values tested. After a final pH adjustment, capped quartz tubes were filled with solutions and irradiated in an Atlas Suntest CPS+ solar simulator equipped with a 500 W m⁻² xenon lamp and a solar standard filter at 25 ± 2°C. At designated times, replicate sample tubes were withdrawn for pH, UV-vis and High Performance Liquid Chromatography (HPLC) measurements. The photolysis of 100 nM of TPA’s *OH adduct, hTPA, was also conducted in the same manner at pH 5.0 and 7.6 to measure the direct photolysis of hTPA under our light source.

3.2.4 Analytical methods

AC, TPA and hTPA were measured using a Waters HPLC (equipped with a 1525 binary HPLC pump and av717plus autosampler) connected to a Waters 2487 dual λ ultraviolet-visible (UV-vis) spectrophotometric detector and a Waters 2475 multi λ fluorescence detector. AC was assayed by HPLC using a modified version reported by Langlois et al., using a 60 % acetonitrile: 40 % MQ (v : v) mobile phase and a Restek C₁₈ column.
(Allure, 150×3.2 mm, 5μm) with detection at an absorbance of 220 nm. TPA and hTPA were separated using a 60 % 0.05% formic acid: 40 % methanol (v : v) mobile phase and a Restek C18 column (Pinnacle DB, 250×4.6 mm, 5μm). TPA was detected at the absorbance of 220 nm and hTPA was determined using the fluorescence detector with excitation at 240 nm and emission at 425 nm.141

3.2.5 *OH photoproduction rate (R\textsubscript{prod})

R\textsubscript{prod} was determined using TPA as a probe compound. TPA reacts with *OH to produce fluorescent hTPA at a yield of 35 %, which was monitored by HPLC at nM level concentrations.141 However, in our solar simulator, the direct photolysis of hTPA under wavelengths shorter than 360 nm cannot be ignored. Therefore, to obtain R\textsubscript{prod}, the net change of [hTPA] over time was fitted using Scientist 3.0 based on hTPA’s formation and decay kinetics as shown in equations (1) to (3):

\[
\frac{d[hTPA]}{dt} = 0.35 \times R_{prod} - k_{1,hTPA}[hTPA] \tag{1}
\]

\[
R_{prod} = k_{OH,TPA}[TPA][*OH]_{ss} \tag{2}
\]

\[
k_{1,hTPA} = k_{OH,hTPA}[*OH]_{ss} + k_{dir,hTPA} \times SF \approx k_{dir,hTPA} \times SF \tag{3}
\]

where, k\textunderscore OH\textunderscore TPA (4.4×10\textsuperscript{9} M\textsuperscript{-1} s\textsuperscript{-1}) and k\textunderscore OH\textunderscore hTPA (6.3×10\textsuperscript{9} M\textsuperscript{-1} s\textsuperscript{-1}) are the respective second order rate constants between *OH and TPA and hTPA,141 0.35 is the yield of hTPA from the reaction of TPA and *OH,141 k\textsubscript{dir,hTPA} is the direct photolysis rate constant of hTPA (experimentally determined in separate experiments), and SF is the DOM light screening factor (calculated over the wavelengths where the solar spectrum and target compounds’ absorbance overlap).19 The first-order decay rate constant of hTPA (k\textsubscript{1,hTPA})
is equal to the direct photolysis rate of hTPA (equation 3) because \([\cdot \text{OH}]_{ss}\) in the presence of 1 mM TPA is insignificant. Detailed information about \(R_{\text{prod}}\) determination is provided in Appendix B with complete results (Table B.1) and a representative fitting curve (Figure B.2).

3.2.6 Experimental \((k_{\text{exp},AC})\) and calculated \((k_{\text{cal},AC})\) AC degradation rates

AC degradation rates were both experimentally determined \((k_{\text{exp},AC})\) (see representative kinetics in Figure B.3) and calculated \((k_{\text{cal},AC})\) based on experimentally determined \(R_{\text{prod}}\) as shown in equations (4) and (5). \(k_{\text{cal},AC}\) consists of direct photolysis and \(\cdot \text{OH}\) mediated photolysis (other photolytic pathways were assumed insignificant).

\[
[\cdot \text{OH}]_{ss} = \frac{R_{\text{prod}}}{k_{\cdot \text{OH,DOM}} \times [\text{DOM}] + k_{\cdot \text{OH,AC}} \times [\text{AC}] + k_{\cdot \text{OH,CO}_3^-} \times [\text{HCO}_3^-]} \tag{4}
\]

\[
k_{\text{cal},AC} = k_{\text{dir,AC}} \times SF + k_{\cdot \text{OH,AC}} \times [\cdot \text{OH}]_{ss} \tag{5}
\]

where \(k_{\cdot \text{OH,DOM}}\) \((1.4-3.3 \times 10^4 \text{ L mg-C}^{-1} \text{s}^{-1})^{83}\), \(k_{\cdot \text{OH,AC}}\) \((6.9 \times 10^9 \text{ M}^{-1} \text{s}^{-1})^{83}\), and \(k_{\cdot \text{OH,CO}_3^-}\) \((1.5 \times 10^7 \text{ M}^{-1} \text{s}^{-1})^{142}\) are the second order rate constants between \(\cdot \text{OH}\) and the respective constituents in the water samples, and \(k_{\text{dir,AC}}\) \((2.1 \times 10^{-6} \text{ s}^{-1})\) is the direct photolysis rate constant of AC in MQ\(^{89}\).

3.3 Results and discussion

3.3.1 Water sample selection and properties

Whole waters of OWC (natural) and WF (engineered) wetlands receive agricultural runoff and provide examples of pesticide photochemical attenuation processes. EL (acid
mine drainage (AMD) impacted) water was included because of its potential use in agrochemical treatment without the need for Fe addition and pH adjustment and may serve as a model for the repurposing of acid mine drainage waters. In an effort to characterize the role of Fe, SRFA and PLFA were used as “Fe free” waters. SRFA and PLFA were also used to understand the role of DOM on attenuation mechanism because they are well-studied end members of terrestrial and microbial sources, respectively, on the DOM source continuum.\(^3\)

The chemical and optical properties of all the waters tested in this study are tabulated in Table 3.1. SUVA\(_{280}\) (specific ultraviolet absorbance at 280 nm) is the absorbance at 280 nm normalized to DOC concentration and is an indicator of DOM aromaticity.\(^61\) E2/E3 is the ratio of absorbances at 254 nm and 365 nm and is inversely related to H\(_2\)O\(_2\) quantum yield.\(^{143}\) FA standards usually contain < 30 nM total Fe (Fe\(^{\text{II}}\)+Fe\(^{\text{III}}\)) and are thus considered “Fe free” for the purpose of this study.\(^{58,144,145}\) The optical parameters (SUVA\(_{280}\) and E2/E3) show that WF DOM is similar to SRFA, which have a higher aromatic content and a higher quantum yield for H\(_2\)O\(_2\) production, while OWC DOM is more similar to PLFA. Both wetland waters are well buffered with bicarbonate at their natural pH (~7.6) and contain 1 - 2 μM total Fe (Fe\(^{\text{II}}\)+Fe\(^{\text{III}}\), Fe\(_{\text{native}}\)), which is mostly in colloid or complexed forms. In contrast, the AMD impacted EL is very acidic (pH = 2.7) with 100s of μM Fe\(_{\text{native}}\), making it ideal for \(\text{^\bullet OH}\) photoproduction as discussed.

3.3.2 The impact of added Fe on AC degradation and \(\text{^\bullet OH}\) production
As described below, $k_{\text{exp,AC}}$ and $R_{\text{prod}}$ as a function of $\text{Fe}_{\text{added}}$ in the form of ferric salt ($\text{Fe}_T = \text{Fe}_{\text{native}} + \text{Fe}_{\text{added}}$) were determined in selected water samples from highly acidic pH to neutral pH to explore the potential for enhancing photodegradation. pH affects Fe solubility, complexation, and redox potential, and is often considered an important factor in the photoproduction of $\cdot$OH. The role of DOM, which participates in both $\cdot$OH production (via Fe complexation or $\text{H}_2\text{O}_2$ production) and $\cdot$OH scavenging, were also explored under different pH scenarios.

**Neutral pH (7.6)**

The degradation of AC in OWC and WF with different $\text{Fe}_{\text{added}}$ (0, 5 and 10 μM) was monitored at the native pH of the waters. As shown in Figure 3.1a, AC degraded with a similar rate (half-life = 40 h) in OWC and WF in the absence of added Fe and is about twice its direct photolysis rate (half-life ~ 90 h). The dominant mechanism for AC indirect photolysis is dependent on water chemistry. For example Langlois et al., reported that $\cdot$OH accounted for almost all indirect photolysis of AC in Everglades water (pH = 7.9, $\text{Fe}_{\text{native}} = 1.1 \ \mu\text{M}, [\text{DOC}] = 25 \ \text{mg-C L}^{-1}$) by using t-butanol as a $\cdot$OH quencher. Our waters were very similar to that study as far as pH and total Fe are concerned, and thus reaction with $\cdot$OH is assumed the most important pathway for AC degradation.

The lack of change in $k_{\text{exp,AC}}$ with increasing Fe in Figure 3.1a clearly shows the addition of $\text{Fe}^{(III)}$ in the unamended wetland waters at their natural pH does not change AC degradation rates. This result implies similar levels of [$\cdot$OH]$_{ss}$ exist across the tested Fe
concentrations when degradation by $\cdot$OH is an important pathway. $R_{\text{prod}}$ ($\cdot$OH photoproduction rate) was determined for OWC and WF to verify that $R_{\text{prod}}$ also did not change with Fe addition (Figure 3.1b). We also assumed that $\cdot$OH scavenging does not change dramatically with Fe addition as DOM and bicarbonate are its major scavengers and it is likely that $\text{Fe}^{(II)}$ is rapidly re-oxidized by bimolecular oxygen.

The impact of Fe addition on $R_{\text{prod}}$ in fulvic acid solutions is slightly different compared to whole waters (Figure 3.1b). There is a noticeable increase in $R_{\text{prod}}$ between 0 $\mu$M and 2 $\mu$M $\text{Fe}_{\text{added}}$ for both FA types and the increase in the presence of SRFA is greater than PLFA. The rate at 2 $\mu$M $\text{Fe}_{\text{added}}$ in the SRFA experiment appears to be an outlier and we currently have no plausible explanation for this difference. Thus, this data point was not considered for our analysis. Similar experiments at pH 7 (Figure B.4) also showed greater enhancement for SRFA relative to PLFA. It appears that most of the enhancement occurred between 0 and 2 $\mu$M $\text{Fe}_T$, while higher $\text{Fe}_T$ showed no further effect. This could explain why we observed no changes in $k_{\text{exp,AC}}$ and $R_{\text{prod}}$ for OWC and WF since $\text{Fe}_{\text{native}}$ already exceeds the optimal Fe concentration. Therefore at neutral pH values, an increase of $\text{Fe}_T$ to 2 $\mu$M may be beneficial (10% increase in $\cdot$OH production with PLFA and 60% with SRFA) for wetlands that lack sufficient natural iron.

The mechanism behind the formation of $\cdot$OH at neutral pH has been proposed to be photo-Fenton reactions (reaction I)$^{138,68}$ Upon solar irradiation, both reactants in the Fenton process, Fe$^{(II)}$ and H$_2$O$_2$, may be photochemically produced with Fe$^{(II)}$ formed by ligand-to-metal charge transfer (reaction II) and H$_2$O$_2$ produced from photoexcited charge-separated species within DOM followed by O$_2$$\cdot^-$. dismutation (reaction III and
Although the photo-Fenton process is usually optimized at acidic pH (2 - 3) partially because of higher Fe solubility, the presence of DOM greatly enhanced Fe$^{(III)}$ solubility by complexation at neutral pH. Nevertheless, colloid Fe$^{(III)}$ is also likely present depending on the kinetics. At neutral pH, the competition from O$_2$ (reaction IV) for photoproduced Fe$^{(II)}$ lowers the •OH generation rate as compared to more acidic pH.

$$Fe^{(II)} + H_2O_2 \xrightarrow{k_{H_2O_2}} Fe^{(III)} + •OH + OH^- \quad (I)$$

$$Fe^{(III)} DOM \xrightarrow{hv} Fe^{(II)} + DOM_{ox} \quad (II)$$

$$DOM + O_2 \xrightarrow{hv} DOM^+ + O_2^- \quad (III)$$

$$Fe^{(II)} + O_2 \xrightarrow{k_{O_2}} Fe^{(III)} + O_2^- \quad (IV)$$

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \quad (V)$$

The above mechanism shows that DOM is a key factor in generating both Fe$^{(II)}$ and H$_2$O$_2$. In Figure 3.1b, negligible $R_{prod}$ in bicarbonate buffered deionized water (MQ) with Fe$_T$ = 0 and 10 μM confirmed the importance of DOM to $R_{prod}$. A simple, but useful theory explains why there is a limiting concentration for Fe enhancement:

$$R_{prod} = R_{nonFe} + R_{Fe} \xrightarrow{pH=7.6} R_{nonFe} + R_{Fenton} = R_{nonFe} + R_{H2O2} Y_c Y \cdot OH$$

$$= R_{nonFe} + Abs_{DOM} \Phi_{H2O2} Y_c Y \cdot OH \quad (6)$$

Both the Fe-independent photolysis ($R_{nonFe}$) and the Fe-dependent reaction ($R_{Fe}$), i.e., the photo-Fenton reaction ($R_{Fenton}$) in this case, contribute to $R_{prod}$. Since Fe functions as a catalyst, $R_{Fenton}$ is limited by the production of H$_2$O$_2$ ($R_{H2O2}$, which is a product of the light absorption flux by DOM ($Abs_{DOM}$) and H$_2$O$_2$ quantum yield ($\Phi_{H2O2}$)) multiplied by
the fraction of H₂O₂ that reacts with Fe (Yc) and the yield of •OH (Y •OH). We assume that R_{nonFe}, R_{H₂O₂}, and Y •OH are independent of Fe and therefore the addition of Fe changes Yc. According to reaction I, Yc is closely related to [Fe^{III}], which depends on FeT and competition from O₂ (reaction II and IV). Once Yc reaches unity, R_{Fenton} approaches a maximum, and the increase in FeT no longer changes R_{prod}.

The comparison of the Fe-assisted maximum •OH production between different waters revealed this trend: WF > SRFA > PLFA > OWC (Figure 3.1b). This trend is ascribed to differences in R_{nonFe}, R_{H₂O₂}, and Y •OH. Because SRFA and PLFA had similar R_{nonFe} (Figure 3.1b when FeT = 0), the difference between them is attributed to R_{H₂O₂} and/or Y •OH. Among the two terms, R_{H₂O₂} was predicted to be higher for SRFA due to its higher UV-vis absorbance (Table 3.1) and Φ_{H₂O₂} (estimated from its E₂/E₃ ratio, which is negatively correlated to the quantum yield of H₂O₂)¹⁴³. Y •OH, on the other hand, is harder to predict. The only available Y •OH is 0.26 ± 0.13 for SRFA at pH of 7,⁶⁸ however, no comparison could be made for PLFA or other waters samples. Y •OH may be affected by pH, and Fe^{III} complexation and perhaps the microenvironment of reaction I.¹⁴⁶,¹⁴⁷

A few photo-Fenton reaction studies have been conducted with SRFA at neutral pH.⁷²,⁶⁸,¹⁴⁶ Vermilyea et. al. determined that the presence of 5 mg-C L⁻¹ SRFA and 10 μM FeCl₃-initiated amorphous Fe oxyhydroxide had a R_{prod} of about 1 nM min⁻¹ at pH 7 using benzene as a probe.⁶⁸ Their result is one order of magnitude lower than ours at pH 7.6. This discrepancy may be ascribed to a combination of differences in light source, pH,
probe selection, time, and DOM concentration. Differences in our light sources may be the biggest reason but lack of information in this reference prevented us from making direct comparisons.

*Moderately acidic pH (5)*

The benefit of Fe amendment starts to show at this moderately acidic pH value. An increase of 50 % and 100%, respectively, in the AC degradation rate was observed for WF and OWC from 0 to up to 10 μM of Fe\text{added} (Figure 3.1c). There is no statistical difference between 5 and 10 μM Fe\text{added} or 6 and 11 μM Fe\text{T}, indicating an optimal Fe concentration is reached when Fe\text{T} ≤ 6 μM. As for the R\text{prod} depicted in Figure 3.1d, all tested waters show a continuous increase in R\text{prod} with a plateau ≥ 11 μM Fe\text{T}. Therefore, for moderately acidic waters with very low Fe\text{T} (<< 1 μM), adding a few μM Fe has the potential to double or even triple •OH production allowing for increased herbicide degradation.

The mechanism behind •OH production at around pH 5 could be similar as to the neutral pH aforementioned but with altered rate constants. Equation 6 may be used to shed light on the role of pH on •OH production. Maximum R_{Fe} is approximately one order of magnitude higher at pH 5 compared to pH 7.6. The decrease in pH tends to slightly decrease Φ_{H_2O_2}^{143}, which is in contrast to the significant increases in R_{Fe}. Negligible to no changes in Abs\text{DOM} also do not explain the pH dependence on R_{Fe}. The remaining terms to influence R_{Fe} are Y_{c} and Y_{•OH}. Reaction between O_2 with Fe^{(II)}, which could otherwise react with H_2O_2 to form •OH, is much less favorable at lower pH.\textsuperscript{148} As a
result, \( Y_c \) may be much higher at pH 5. As for \( Y \cdot _{\text{OH}} \), although more research needs to be done, low pH and complexation with organic ligands tends to increase \( Y \cdot _{\text{OH}} \).\(^{147,146}\)

*Highly acidic pH (2.7)*

The AMD impacted EL sample shows high reactivity towards AC degradation as depicted in Figure 3.2. We determined the impact of Fe addition on AC degradation with both OWC water and a MQ “control”. In contrast to higher pH values, where the addition of Fe in MQ tends to have no enhancement (Figure 3.1b and Figure 3.1d), the first order rate constants increased with the addition of Fe almost linearly for both MQ and OWC (Figure 3.2). No plateau was reached for the highest concentration tested (500 μM Fe).

The OWC water showed much less reactivity compared to MQ. This decreased reactivity is in contrast to the greater H\(_2\)O\(_2\) production from DOM photolysis in OWC water and is mainly ascribed to the higher \( \cdot \text{OH} \) scavenging and light screening from this sample (see Table 3.1). More reactivity with MQ suggests that could reveal that the photo-Fenton reaction is not important at this pH as will be described below.

We did not measure \( \cdot \text{OH} \) production with TPA due to its very low solubility under acidic conditions (solubility = 0.28 mM at 25 °C).\(^{149}\) Instead, we used a more water soluble probe (caffeine)\(^{150}\) and determined the \( \cdot \text{OH} \) production rate of EL waters to be 6100 ± 180 nM/min and an \( \cdot \text{OH} \) scavenging rate of 3.5 ± 0.7 × 10\(^{4}\) s\(^{-1}\) using the initial rates approach.\(^{151}\) Therefore, with this higher \( \cdot \text{OH} \) production rate, this type of AMD impacted water may serve as an available and economic reservoir for agriculture runoff treatment, resulting in pesticide half-lives on the *order of minutes* under natural sunlight.
The mechanism of $\bullet OH$ production in iron rich highly acidic waters does not require DOM as it does at higher pH values. Instead, this reactivity is largely due to the photochemical reaction of Fe hydroxyl complexes whose portion depends on the available ligands such as sulfate:\textsuperscript{152}

$$FeOH^2+ \stackrel{hv}{\rightarrow} Fe^{2+} + \bullet OH \quad (7)$$

Photo-Fenton reactions are usually optimized at highly acidic pH. However, without an external source of H$_2$O$_2$, $\bullet OH$ production from photo-Fenton reactions is limited by H$_2$O$_2$ production (R$_{H_2O_2}$) from DOM photolysis. R$_{H_2O_2}$ ranges from $10^{-3}$ - $10^2$ nM min$^{-1}$, which is orders of magnitude lower than the measured production of $\bullet OH$ in this study (Figure 3.2).\textsuperscript{68,153,154} This difference is not surprising since both the quantum yield of H$_2$O$_2$ ($10^{-3}$ - $10^{-4}$) from DOM and the light absorption by DOM are much lower than the quantum yield of $\bullet OH$ ($10^{-2}$ - $10^{-1}$) from FeOH$^{2+}$ and the light absorption by FeOH$^{2+}$.\textsuperscript{50,143,155} Therefore, the $\bullet OH$ production from photo-Fenton reactions is of minor importance at highly acidic pH values.

It is worth mentioning that the high reactivity of Fe-assisted $\bullet OH$ production at highly acidic pH could reach such following limits. Soluble Fe$^{(III)}$ under these conditions could absorb all available sunlight in the UV region within a few centimeters of the water column, which would then limit photoreactivity below this depth. A second limit lies in that the slow reoxidation of Fe$^{(II)}$ to Fe$^{(III)}$ at this pH could deplete the photosensitive Fe$^{(III)}$ quickly. Abiotic Fe$^{(II)}$ oxidation by O$_2$ could be very slow under acidic conditions, while microbial Fe$^{(II)}$ oxidation are typically orders of magnitude faster and dependent upon the microbial consortia.\textsuperscript{100,156}
3.3.3 Comparison of experimental and calculated AC degradation rate at different pH values

Next, with knowledge of both $\cdot$OH production and AC degradation, we can estimate $k_{AC}$ based on $R_{prod}$, which may be used to predict the fate of other pesticides if their second order rate constants are known. AC degradation rate constants were calculated as a summation of $\cdot$OH induced photolysis ($k_{\cdot OH,AC}$) using the experimental $R_{prod}$ and the screening factor adjusted direct photolysis ($k_{dir,AC,SF}$). Figure 3.3 shows $k_{\cdot OH,AC} + k_{dir,AC,SF} > k_{exp,AC}$ for almost all conditions. The magnitude of these differences, $(k_{\cdot OH,AC} + k_{dir,AC,SF} - k_{exp,AC})/k_{exp,AC}$, was between 50 % to 100 % at pH of 7.6 and 50 % to 200 % at pH of 5. This discrepancy may be due to experimental or mechanistic reasons and is discussed below.

First, we may have underestimated the $\cdot$OH scavenging rates for the DOM used in this study. We assumed that they would fall within the range of our assumption ($1.4 - 3.3 \times 10^4$ L mg-C$^{-1}$ s$^{-1}$) based upon a previous study$^{83}$, but others have reported a somewhat greater range of DOM scavenging rate constants (typically $1 - 7 \times 10^4$ L mg-C$^{-1}$ s$^{-1}$) depending on the DOM used and $\cdot$OH production mechanisms.$^{83,141,157–159}$ Nevertheless, in order to manipulate the model so estimates are in agreement with $k_{exp,AC}$, it would require DOM scavenging rate constants on the order of $6 \times 15 \times 10^4$ L mg-C$^{-1}$ s$^{-1}$. While possible at the lower end of this enhanced range we believe it unlikely to explain the discrepancies between model predictions and our observations.
The second reason for the model discrepancy is that the yield of hTPA in the presence of photo-irradiated Fe and our DOM is much higher than 0.35. Indeed, irradiated SRFA spiked with Fe has been shown to reduce the phenol yield from reaction between benzene and •OH by half.\textsuperscript{68} The same mechanism may apply to the yield of hTPA, which would enlarge the model discrepancy. An increase of hTPA yield to 0.85 has been observed when strong oxidants like IrCl\textsuperscript{2+} was used in place of O\textsubscript{2},\textsuperscript{160} however no such oxidants existed in our system, and we consider this hypothesis highly unlikely.

A third reason may be due to the side reactions emanating from the •OH probe. The addition of 1 mM TPA may produce additional H\textsubscript{2}O\textsubscript{2} or reduce Fe\textsuperscript{(III)} which increases the •OH production rate to above that anticipated for irradiated Fe and DOM. The carboxyl groups on TPA could bind Fe\textsuperscript{(III)}, and promote ligand-to-metal charge transfer for the photo-reduction of Fe\textsuperscript{(III)}. Future experiments that can measure nM level of H\textsubscript{2}O\textsubscript{2} and Fe\textsuperscript{(II)} could test this hypothesis.

The final reason lies in the heterogeneity of reactive hydroxylating species and the reaction selectiveness towards these species by different compounds (such as TPA and AC). Free •OH, low-energy hydroxylation, and higher oxidation state Fe species such as Fe\textsuperscript{(IV)} are among the hydroxylating species generated by the photolysis of DOM and Fe.\textsuperscript{68,161} The formation of Fe\textsuperscript{(IV)} has been proposed in Fenton-type reactions at circumneutral pH values in the absence of ligands.\textsuperscript{67,161} If Fe\textsuperscript{(IV)} was produced in our system and if TPA has a higher reactivity towards this species relative to AC, this could result in the over estimates of k\textsubscript{exp,AC}. 

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3.4 Conclusions

The effectiveness of Fe-assisted AC degradation or \( \bullet \)OH production in studied wetland waters increased with decreasing pH as a result of different reaction mechanisms i.e., the photo-Fenton reaction at mildly acidic to circumneutral pH values while photolysis of Fe\(^{\text{III}}\) aquo complexes was the dominant photosensitizer at low pH. Adding Fe in the form of Fe\(^{\text{III}}\) salts was beneficial in certain situations. At neutral pH (7.6) when \( \text{Fe}_T \) is \(<\!\!\!< 1 \ \mu\text{M} \), the addition of Fe to \( \text{Fe}_T \) of 1-2 \( \mu\text{M} \) could increase the degradation of AC by tens of percent. Under moderately acidic conditions (pH 5) when \( \text{Fe}_T \) is \(<\!\!\!< 1 \ \mu\text{M} \), addition of Fe to \( \text{Fe}_T \) of 6-11 \( \mu\text{M} \) could increase AC photo-attenuation by 50 – 100 \( \% \). For AMD impacted waters where the pH is highly acidic (2.7) and Fe\(^{\text{III}}\) is on the order of 100 \( \mu\text{M} \), AC was completely degraded in a few minutes under natural sunlight. However, the exact efficacy depends on the DOM type and concentration. At neutral pH, terrestrial DOM would favor the Fe-assisted \( \bullet \)OH relative to microbially derived DOM. This was not observed for moderately acidic pH waters where both DOM were equally impacted by iron addition. At pH 2.7, DOM inhibited Fe-assisted \( \bullet \)OH production. This bench-scale study may shed light to select the right pH and Fe concentration for future field application.
3.5 Figures and tables

Table 3.1. Properties of whole waters and fulvic acid (FA) solutions

<table>
<thead>
<tr>
<th>Source</th>
<th>Type</th>
<th>pH\textsuperscript{a}</th>
<th>[Fe] \textsuperscript{b} \text{µM}</th>
<th>[DOC] \text{mg-C L}^{-1}</th>
<th>SUVA\textsubscript{280} \text{L mg-C}^{-1} m\textsuperscript{-1}</th>
<th>E2/E3</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR</td>
<td>Suwannee River FA</td>
<td>−</td>
<td>0.0025\textsuperscript{b}</td>
<td>6.0±0.1</td>
<td>3.77</td>
<td>4.1</td>
</tr>
<tr>
<td>PL</td>
<td>Pony Lake FA</td>
<td>−</td>
<td>ND\textsuperscript{c}</td>
<td>6.0±0.1</td>
<td>2.60</td>
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<tr>
<td>OWC</td>
<td>Old Woman Creek FA</td>
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<td>1.2±0.1</td>
<td>5.7±0.1</td>
<td>2.26</td>
<td>6.0</td>
</tr>
<tr>
<td>WF</td>
<td>Waterman Farm Natural wetland</td>
<td>7.58</td>
<td>1.9±0.1</td>
<td>7.8±0.5</td>
<td>3.72</td>
<td>3.9</td>
</tr>
<tr>
<td>EL</td>
<td>Essington Lake Acid mine drainage</td>
<td>2.74</td>
<td>326</td>
<td>1.2±0.5</td>
<td>_\textsuperscript{d}</td>
<td>_\textsuperscript{d}</td>
</tr>
</tbody>
</table>

Note: \textsuperscript{a} pH value measured when whole water was collected; pH values for FA isolates are not applicable. \textsuperscript{b} determined using graphite furnace atomic absorption spectrometry (GFAAS). \textsuperscript{c} Fe in diluted (1:8) PL whole water was determined to be not detected (ND) using GFAAS. \textsuperscript{d} absorption by Fe-inorganic complexes dominates the spectrum, thus SUVA\textsubscript{280} and E2/E3 are not applicable.
Figure 3.1. AC degradation rate constants and \(^{\bullet}\)OH production rates at different iron concentrations.

a. AC degradation at pH 7.6; b. \(^{\bullet}\)OH production at pH 7.6; c. AC degradation at pH 5; d. \(^{\bullet}\)OH production at pH 5. Experimental conditions: \([AC]_0 = 5 \text{ μM}, [TPA]_0 = 1 \text{ mM}\). Note that \(Fe_T = Fe_{\text{native}} + Fe_{\text{added}}\). Error bars represent 95 % confidence interval of the fitted parameters (\(k_{\exp, ac}\) or \(R_{prod}\)), and are smaller than symbol when not shown.
Figure 3.2. First order degradation rates of AC at pH 2.7 in various waters with increasing Fe concentrations.

Experimental conditions: [AC]₀ = 5 μM. Error bars represent 95% confidence interval of the fitted parameter (k_{exp,ac}), and are smaller than symbol when not shown.
Figure 3.3. Comparison of experimental and calculated AC first order degradation rate constants at different pH values and Fe\textsubscript{added}.

Note that Fe\textsubscript{added} instead of Fe\textsubscript{T} is shown in the figure. In the legend, “Direct” represents $k_{\text{dir,AC}} \times \text{SF}$, and “OH” represents $k_{\text{HOH,AC}} \times [\text{OH}]_{ss}$, and “exp” represents $k_{\text{exp,AC}}$. Error bars represent the range of $k_{\text{cal,AC}}$ when using the range of DOM scavenging constants (1.4-3.3×10$^4$ L mg-C$^{-1}$ s$^{-1}$).
Chapter 4: The Photomineralization of Dissolved Organic Matter in Acid Mine Drainage Impacted Waters

4.1 Introduction

Acid mine drainage (AMD) impacted waters are present in nearly half of the states in the US.\textsuperscript{162} They usually contain high Fe and H\textsubscript{2}SO\textsubscript{4} concentrations due to the chemical and biological weathering of pyrite in abandoned mining areas (FeS\textsubscript{2}).\textsuperscript{30} Dissolved organic matter (DOM), ubiquitous in aquatic systems, is a heterogeneous mix of macromolecules derived from biological sources. It serves important ecological roles as part of the carbon cycle,\textsuperscript{6,7} as a carbon and nutrient source for microorganisms,\textsuperscript{8–10} and as a photo-reactive component that can filter cell-damaging ultra-violet (UV) light and generate reactive intermediates that participate in contaminant degradation and DOM transformation.\textsuperscript{11–21}

Typically, low concentrations of DOM (~ 1 mg-C L \textsuperscript{-1}) has been observed in AMD impacted waters and has mainly been attributed to adsorption by Fe oxides that are particularly abundant in these systems.\textsuperscript{163,164}

Photochemical reactions in AMD impacted waters have focused on diel Fe redox cycling, i.e. the production of Fe\textsuperscript{2+} by photoreduction in the day and its oxidation by microorganisms at night.\textsuperscript{66,100,156} In these studies, the photo dissociation of FeOH\textsuperscript{2+} was determined as the most important photochemical reaction resulting in the production of
reduced Fe and the hydroxyl radical (\(\cdot\)OH).\textsuperscript{152} Although the \(\cdot\)OH is highly reactive toward organic compounds, the phototransformation of DOM has never been evaluated in AMD impacted waters and we suspect that the extent of \(\cdot\)OH generation in these aquatic environments plays an oversized role with respect to its fate.

In this paper we studied the DOM phototransformation/mineralization rate, mechanism, and transformation products in AMD impacted waters from southeastern Ohio (1) by comparing the shaded and sunlit AMD impacted waters collected in the same watershed and (2) by monitoring the change in DOM, \(\cdot\)OH, and Fe in the DOM-dosed AMD impacted waters in simulated sunlight.

4.2 Materials and methods

4.2.1 Reagents

Suwannee River fulvic acid (SRFA) and Pony Lake fulvic acid (PLFA) were obtained from the International Humic Substances Society. Caffeine (CAF), Benzoic acid (BZA), Terephthalic acid (TPA), and t-butyl alcohol (TBA) was purchased from commercial sources. See Text C.1 in Appendix C for details.

4.2.2 Sample collection and preparation

Surface water samples from Essington Lake (EL) and a shaded upstream pond (SP) were collected and filtered through 0.45 \(\mu\)m Pall AquaPrep groundwater filters (Port Washington, NY). See Figure C.1 for the watershed map. For Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS) and Size Exclusion
Chromatography (SEC) measurement, DOM from these two waters was extracted by absorption onto Agilent PPL cartridges (Santa Clara, CA) as described by Dittmar et al. and freeze dried for storage.

4.2.3 Irradiation experiment

Quartz tubes (path length of 0.9 cm, sealed with Teflon lined o-rings and glass caps) filled with water samples were irradiated in Atlas Suntest CPS+ solar simulation system equipped with a 500 W/m² xenon lamp and a solar standard filter at 25 ± 2 °C (Mount Prospect, IL). At the designated times, sample tubes were withdrawn for TOC, iron, DO, and/or UV-vis determination. Water samples include EL, SP, and EL spiked with 6 mg-C L⁻¹ SRFA or PLFA. Irradiance was recorded with a Solar Light PMA2100 data-logging radiometer with a PMA2107 UVA+UVB detector at an interval of one second or one hour depending on irradiation time (Glenside, PA). For the anoxic experiment, 80 ml SP was purged with argon gas for 80 min and transferred into quartz tubes (lined with layers of Parafilm and Teflon to avoid possible gas exchange) in a glove box. The following photolysis and sample analysis were conducted in the same manner as the oxic experiments.

4.2.4 •OH determination and model predication

EL waters spiked with 0.5 - 300 μM CAF (8 concentrations) or 0.5 - 50 μM TPA (8 concentrations) were filled in quartz tubes and irradiated as described above. At designated time, phototubes were withdrawn for High Performance Liquid
Chromatography (HPLC) measurements. \( \cdot \)OH production (R \( \cdot \)OH) and background scavenging (S \( \cdot \)OH) was calculated by plotting \( \frac{1}{[\cdot \text{OH}]_{ss}} \) vs \([\text{probe}]_0\) based on the following equation:

\[
\frac{1}{[\cdot \text{OH}]_{ss}} = \frac{k_{\text{probe}, \cdot \text{OH}}}{R \cdot \text{OH}} [\text{probe}]_0 + \frac{1}{R \cdot \text{OH}} S \cdot \text{OH} \tag{1}
\]

where \( k_{\text{probe}, \cdot \text{OH}} \) is the second order rate constant of reaction between \( \cdot \)OH and the probe.\(^{141,166}\)

In order to easily estimate the R \( \cdot \)OH in AMD waters under different conditions, a preliminary model was developed based on the integral of the product of light absorption flux and \( \cdot \)OH quantum yield (\( \Phi \)) over the wavelengths where the solar spectrum and the absorbance of FeOH\(^{2+}\) overlap:\(^{167}\)

\[
R_{\text{OH}} = \int_{\lambda_{\text{min}}}^{\lambda_{\text{max}}} \frac{I_{\lambda}F_{\lambda\Phi}F_{c\lambda}}{l} \, d\lambda = \int_{\lambda_{\text{min}}}^{\lambda_{\text{max}}} \frac{I_{\lambda}(1-e^{-2.3a_{s\lambda}l})\epsilon_{\lambda}c\Phi_{\lambda}}{a_{s\lambda}l} \, d\lambda \tag{2}
\]

where \( I_{\lambda} \) (Einstein m\(^{-2}\)s\(^{-1}\)) is the photo flux in at a particular wavelength \( \lambda \) (nm) and was measured or calculated using the National Renewable Energy Laboratory’s (NREL) Simple Model of the Atmospheric Radiative Transfer of Sunshine (SMARTS).\(^{168}\) \( F_{\lambda \Phi} \) is the fraction of light absorbed by the system with an attenuation coefficient of \( \alpha_{s\lambda} \) (cm\(^{-1}\)), and \( F_{c\lambda} \) is the fraction of light absorbed by FeOH\(^{2+}\). \( c \) (M) is the concentration of FeOH\(^{2+}\) calculated with Visual MINTEQ 3.1, \( \epsilon_{\lambda} \) is its molar absorptivity (M\(^{-1}\)cm\(^{-1}\)) and \( \Phi_{\lambda} \) is its \( \cdot \)OH production quantum yield.\(^{63}\) \( l \) (cm) is the average photo path length of the studied water. See Text C.3 (Figure C.2 and Figure C.3) for more calculation details.
4.2.5 The roles of $\text{SO}_4^{2-}$

*SO*$_4^{\bullet^-}$ formation. The production of $\text{SO}_4^{\bullet^-}$ in EL was determined using two probes of different selectivity towards $^\bullet\text{OH}$ and $\text{SO}_4^{\bullet^-}$: Benzoic acid (BZA) has high reactivity towards both $^\bullet\text{OH}$ (bimolecular rate constant of $4.2 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$) and $\text{SO}_4^{\bullet^-}$ ($1.2 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$), while t-butyl alcohol (TBA) is a strong $^\bullet\text{OH}$ scavenger ($3.8 - 7.6 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$) but a weak $\text{SO}_4^{\bullet^-}$ quencher ($4 - 9.1 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$). The degradation of 30 $\mu$M BZA in EL in the presence of 0, 10, or 100 mM TBA was monitored in the solar simulator described above.

*The impact of $\text{SO}_4^{2-}$ on $^\bullet\text{OH}$ production.* The degradation of $^\bullet\text{OH}$ probe CAF was monitored in the presence of varying [SO$_4^{2-}$] (1 mM, 11 mM and 21 mM) in artificial AMD waters with controlled pH (2.7), [Fe(III)] (660 $\mu$M), and ionic strength (0.06 M from ClO$_4^-$, SO$_4^{2-}$, and Na$^+$).

4.2.6 Analytical techniques

TOC was measured using a Shimadzu TOC-VCPN analyzer (Kyoto, Japan). Water sample absorbance was determined with a Shimadzu spectrophotometer (Kyoto, Japan). TPA, CAF, BZA were measured using Waters HPLC. Dissolved oxygen (DO) was measured with a Lazer Research Laboratories micro-DO probe (Los Angeles, CA). Elements were determined using a Teledyne-Leeman Labs Prodigy Dual View Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Hudson, NM) by Service Testing and Research Laboratory at Ohio State University. Major anions were measured using Ion Chromatography. Extracted DOM samples were analyzed on a Bruker Daltonics 12 Tesla
Apex Qe FTICR-MS instrument with an Apollo II ESI ion source in the negative ionization mode, as described by Chen et al.93 See methods about iron measurement (FerroZine colorimetry) and size distribution determination (size exclusion chromatography) in Text C.1.

4.3 Results and discussion

4.3.1 Photomineralization rates of DOM in AMD contaminated waters

The 5.5 km² Perry State Forest watershed is representative of the more than 2000 km of AMD impacted streams in Ohio (see watershed map in Figure C.1).169,170 The majority of the drainage areas in Perry State Forest have been strip-mined for coal, releasing both Fe and H₂SO₄ to receiving waters. Two sites in this watershed were selected in this study: Essington lake water (EL), which is largely un-shaded and represents a natural photolytic reactor, and a highly shaded upstream pond (SP) in the same watershed to represent a less irradiated surface water body. While both waters had similarly low pH (2.7-3) and high total Fe concentrations (300-600 μM), SP had 5 times more TOC compared to EL (Table C.1).

Under simulated sunlight, which is similar to midday June irradiance at the latitude of the Perry State Forest watershed98 (see irradiation comparison in Figure C.1) the photomineralization i.e., complete conversion to CO₂ of both EL and SP waters were fast with pseudo-first order half-lives of 1.0 and 0.8 h, respectively (Figure 4.1). In an effort to assess DOM compositional differences we added fulvic acids isolated from the Suwannee River, GA (SRFA, which represents terrestrially derived DOM) and Pony
Lake, Antarctica (PLFA, which represents a microbiially derived DOM) to EL water to assess their photo-reactivity in AMD impacted waters. Compared to EL and SP DOM, SRFA and PLFA were mineralized more slowly with half-lives of 1.6 and 2.4 h, respectively. When interpreted in the form of rates in the first 1.5 hours, photochemical mineralization ranged from 0.3 to 3.3 mg-C L⁻¹ h⁻¹. This large variation is likely due to the difference in the generation of reactive species, DOM composition, and other radical scavengers, as discussed below. The DOM mineralization rates are generally one to two orders of magnitude higher than most natural surface waters at neutral pH values. ¹⁷¹,¹⁷²,¹⁷³

It is worth mentioning that the natural mineralization rate depends on local solar intensity and sun angle, so the comparison between studies should be handled with caution.

4.3.2 Light absorption and reactive species production in AMD impacted waters

Solar energy is absorbed by various species in sunlit surface waters including Fe complexes and DOM. We evaluated the importance of different light absorption constituents to the transformation of DOM as follows.

*Light absorption by FeOH²⁺ and *OH production*

*(a) *OH production mechanism*

\[
FeOH^{2+} \rightarrow Fe^{2+} + \cdot OH
\]  (I)

The photolysis of FeOH²⁺ (reaction I) is the main *OH production pathway in a variety of acidic Fe-rich waters such as AMD impacted water, clouds, fog, rain, and in certain wastewater treatment systems. ⁶⁵,¹⁰⁰,¹⁵²,¹⁷⁴ Because the electronic excitation wavelength
bands for FeOH$^{2+}$ overlaps with the solar spectrum in the region of 290 - 400 nm (Figure C.3a and Figure C.3d), the generation of •OH may be important in natural sunlight. The hydroxyl radical is a potent oxidant and reacts with DOM at rates of 1-7× 10$^4$ L mg-C$^{-1}$ s$^{-1}$.

We determined the •OH production rates ($R_{\cdot OH}$) and background steady state •OH concentrations ([•OH]$_{ss}$) in EL water using the •OH probes terephthalic acid (TPA) and caffeine (CAF), which is more soluble than TPA in AMD waters (Table 4.1). Reaction of the two probes in our irradiated AMD samples resulted in a consistent $R_{\cdot OH}$ of 1.4 × 10$^{-7}$ M s$^{-1}$ and [•OH]$_{ss}$ of 2 × 10$^{-12}$ M. Our model estimates of •OH production based on light absorption and the quantum yield of •OH from FeOH$^{2+}$ corroborates our experimental data (Table 4.1), and demonstrates that FeOH$^{2+}$ is the principal •OH photosensitizer in AMD waters. Experiments conducted under natural sunlight with waters collected hourly from 10 am to 2 pm on a sunny mid-Oct day revealed similar [•OH]$_{ss}$ (see details in Text C.2 and Table C.3). Our [•OH]$_{ss}$ is similar to the only other reported values measured in AMD waters from west-central Indiana$^{152}$, and is orders of magnitude higher than the values reported for sunlit natural waters that are not affected by AMD (10$^{-15}$ - 10$^{-18}$ M)$^{175-177}$.

(b) The impact of water constituents on •OH production and scavenging

Like most of AMD impacted waters, EL and SP had high metal and anion concentrations (Table C.1). The fluctuation of pH and the presence of ligands such as SO$_4^{2-}$, F$^-$ in AMD impacted waters may potentially impact the production and scavenging of •OH.
The FeOH$_{2}^{2+}$ concentration increases with [OH$^{-}$] when pH increases from 2 to 4. Using the SP sample as an example, our modeled R • OH values decreased by approximately 40% when pH decreased from its native pH (3.0) to the pH of EL (2.74). This modest decrease may in part explain the somewhat slower DOM mineralization kinetics in EL water relative to SP.

Equilibrium Fe speciation calculations using MINTEQ showed that FeSO$_4^{2+}$ is the most important complex (75 - 80 %), followed by FeOH$_{2}^{2+}$ (8 - 13 %), Fe(SO$_4$)$_2^{-}$ (4 - 5 %) and free aquo-Fe$_3^{3+}$ (3 - 4 %) (Table C.2), which is in agreement with past AMD speciation studies. Because Fe sulfate complexes have similar molar extinction coefficients as FeOH$_{2}^{2+}$, this may result in strong light absorption without production of •OH. We corroborated this phenomenon whereby photolysis in AMD-like artificial water solutions (similar pH, Fe, SO$_4^{2-}$, and ionic strength), revealed a roughly 5-fold decrease in •OH production with increasing [SO$_4^{2-}$] from 1mM to 21 mM (Figure 4.2a).

The complexation of F$^{-}$ with Fe$_3^{3+}$ is two orders of magnitude stronger than SO$_4^{2-}$. As a result, the 0.2 mM F$^{-}$ in EL would have complexed approximately 20 % of the total Fe$^{(III)}$ in the AMD and competes with both SO$_4^{2-}$ and OH$^{-}$ in the absence of other metals. Since FeF$_2^{+}$ does not absorb light in relevant wavelengths, its presence would decrease light attenuation in AMD waters compared to when Fe$^{(III)}$ exists in light-absorbing forms of FeSO$_4^{+}$ and FeOH$_{2}^{2+}$. However, the large amount of total Al$^{(III)}$ in EL out-competed Fe$_3^{3+}$ for nearly all the fluoride present making this ligand unimportant in Fe speciation. Unlike F$^{-}$, the sub mM Cl$^{-}$ mainly existed as a free anion in EL and SP and was an unimportant ligand in our system. At higher levels, Cl$^{-}$ ($k_{Cl}$ • OH$^{-}$~10$^9$ M$^{-1}$ s$^{-1}$, pH≈2) may be an
important sink for \( \cdot \text{OH} \), through the production of \( \cdot \text{Cl} \), which may in turn react with DOM.\(^{179}\) Thus, when predicting \( \cdot \text{OH} \) production for other AMD waters interference from strong ligands and competing metals should be considered.

*Light absorption by \( \text{FeSO}_4^{+} \) and sulfate radical (\( \text{SO}_4^{\cdot -} \)) production*

As the dominant Fe species, \( \text{FeSO}_4^{+} \) may also undergo photolysis to produce the more selective \( \text{SO}_4^{\cdot -} \) as shown in reaction (II).

\[
\text{FeSO}_4^{2-} \xrightarrow{\text{hv}} \text{Fe}^{2+} + \text{SO}_4^{\cdot -} \quad \text{(II)}
\]

The production of \( \text{SO}_4^{\cdot -} \) in EL waters was determined using benzoic acid (BZA) and t-butyl alcohol (TBA) which selectively scavenges \( \cdot \text{OH} \) without reacting with \( \text{SO}_4^{\cdot -} \). Figure 4.2b shows that the \( \text{SO}_4^{\cdot -} \) production rate was less than 5 % of \( \cdot \text{R} \cdot \text{OH} \). These results are consistent with the reported quantum yield for \( \text{SO}_4^{\cdot -} \) (~ 0.005) which is about 2 orders of magnitude lower than the first \( \text{Fe}^{(III)} \) hydrolysis complex for \( \cdot \text{OH} \) production.\(^{63}\) Finally, \( \text{SO}_4^{\cdot -} \) reaction kinetics with DOM is approximately 2 times slower than with \( \cdot \text{OH} \) and as such \( \text{SO}_4^{\cdot -} \) is probably at best a minor contributor to DOM transformation.\(^{180}\)

*Light absorption by \( \text{Fe-DOM complex} \) and \( \text{DOM decarboxylation} \)*

Fe-DOM complexes tend to absorb light at higher wavelengths found in sunlight relative to its inorganic complexes, due to ligand-to-metal charge transfer (LMCT).\(^{2,65}\)

\[
\text{Fe}^{(III)} - \text{DOM}(C_n) \xrightarrow{\text{hv}} \text{Fe}^{(II)} + \text{DOM}^\bullet(C_{n-1}) + \text{CO}_2 \quad \text{(III)}
\]
In AMD impacted waters, the presence Fe-DOM may be limited due to the protonation of DOM acidic groups plus competition from Al(III). Nonetheless, strong Fe complexing ligand sites exist in all DOM and may participate in decarboxylation pathways through LMCT. This has been observed in various simple carboxylic acids e.g., oxalate, nitrilotriacetic acid, etc. and both well-defined biogenic ligands e.g., aquachelin and DOM.\textsuperscript{65,181,182}

\textit{Light absorption by DOM and its direct photolysis}

\[ DOM + (O_2)^{hv} \rightarrow DOM_{ox} \]  \hspace{1cm} \text{(IV)}

Although DOM is one of the most important light attenuators in most natural waters, the fraction of light absorbed by DOM in AMD impacted waters is relatively low due to its low concentration and strong competition from Fe species. For example when 6 mg-C L\textsuperscript{-1} SRFA was added to EL water, the fraction of light absorbed by the fulvic acid increases from 20 % to 70 % in the UV-A and -B range of sunlight and is higher in the visible range where the total light absorption is extremely low in AMD waters. In the native EL water where DOM is less than 1 mg-C L\textsuperscript{-1}, this fraction light absorbed by DOM is less than 10 %. Thus, the direct photolysis of DOM results in a number of important pathways including decarboxylation, production of OH and other reactive species, which in turn ultimately participates in the transformation of DOM itself.\textsuperscript{173}

4.3.3 Thermal reactions initiated by photochemical processes
All the aforementioned major light absorption processes require Fe$^{(III)}$ as an electron acceptor. If we assume the oxidation states of carbon in DOM is approximately zero\(^9\) and Fe$^{(III)}$ is the only electron acceptor, approximately 0.25 mol of CO\(_2\) may be produced for every mol of Fe$^{(III)}$ reduced. Thus, we anticipate that for EL and SP waters, a maximum of 1.0 and 1.9 mg-C L\(^{-1}\) of DOM may be mineralized respectively based upon Fe$^{(III)}$ levels present in each sample. In contrast, we observed photomineralization of at least 4 mg-C L\(^{-1}\) SRFA and 3 mg-C L\(^{-1}\) PLFA in EL, and 4 mg-C L\(^{-1}\) native-DOM in SP (Figure 4.1). Therefore, other electron acceptors than Fe$^{(III)}$ must participate in thermal mineralization of DOM following the photomineralization of DOM mediated by \(\bullet OH\). Dissolved oxygen may be the most important electron acceptor after Fe$^{(III)}$. In surface waters at equilibrium with the atmosphere (\(~300\) μM saturated O\(_2\)) we estimate that it may mineralize 300 μM or 3.6 mg-C L\(^{-1}\) of DOM based upon redox stoichiometry. In a previous study that examined the photodegradation of 2,4-dichlorophenoxy acetic acid (pH 2.8, Fe = 1.0 mM), the presence of O\(_2\) enhanced mineralization by a factor of four.\(^181\) We observed an enhancement of a factor of less than two in the presence of initial air saturated oxygen levels as compared to in the absence of oxygen where water samples were purged with argon before the photolysis (Figure C.4). It is worth mentioning that even in the oxic experiments, the oxygen concentration dropped dramatically (as high as 80%) after photolysis. While in the field where oxygen is continuously replenished by wind, a higher enhancement in DOM mineralization is possible compared to what was determined in sealed phototubes. We propose that the reaction between O\(_2\) and photogenerated organoradicals to form organoperoxy radicals may be the first step in the thermal
transformation of DOM. These organoperoxyl radicals may be involved in the reoxidation of Fe\(^{\text{II}}\) back to Fe\(^{\text{III}}\) or in the generation of H\(_2\)O\(_2\) to restart the ferrous wheel.\(^{181}\)

4.3.4 DOM phototransformation products

DOM from SP and EL waters were extracted by solid phase extraction (PPL) cartridges in order to remove the high salt content and enrich DOM for characterization by spectroscopy and mass spectrometry. We experienced much lower extraction efficiencies for EL and SP (< 20 % and 50%, respectively) relative to other surface waters,\(^ {165}\) which may bias our results.

We employed size exclusion chromatography with UV detection (SEC-UV) to determine the molecular weight distribution (Figure 4.3) and Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS) to determine the molecular formula distributions (Figure 4.4). Figure 4.3 clearly shows the shift in size of major UV absorption molecules from about 1000 Da in SP to about 100 Da in EL (at the lower MW limits of SEC), suggesting the degradation of DOM from larger units to smaller moieties upon photolysis. The unshaded EL, which had been photolyzed extensively during lake retention time, revealed unusually high aliphatic content in van Krevalan diagrams based on the FTICR-MS spectrum (Figure 4.4) compared to both SRFA and PLFA.\(^ {183}\) However, we observed no black carbon-like compounds or “photo-condensation” products, in contrast to past work on hydroxyl radical initiated DOM photolysis. This
previous study in the presence of iron was conducted at much higher pH and DOM concentrations.¹³

4.4 Significance

In addition to adsorption to Fe minerals, we believe that very low levels of DOC in AMD impacted water is largely the result of exceedingly rapid photomineralization processes sensitized by FeOH²⁺. Upon solar irradiation, FeOH²⁺ generates steady state hydroxyl radical concentration ([OH]ₚ) of approximately 10⁻¹² M, which is 4 to 5 orders of magnitude higher than typical sunlit natural surface waters.¹⁷⁶ At these levels OH becomes the principal oxidant capable of the complete mineralization of DOM on a time scale of hours in the photic zone of AMD affected water bodies. The loss of DOM in these watersheds to CO₂ coupled with the existence of highly aliphatic residual carbon may impact the carbon cycle and downstream ecosystems.
4.5 Figures and tables

Figure 4.1. The photomineralization of DOM in EL and SP waters with or without the spike of SRFA or PLFA.

Fe levels: \([\text{Fe}^{(III)}]_0\) for all EL solutions with or without SRFA/PLFA = 280-295 μM, \([\text{Fe}^{(III)}]_0\) for SP-1 = 601 μM, \([\text{Fe}^{(III)}]_0\) for SP-2 = 420 μM. pH = 2.7-3.0.
Table 4.1. Summary of water chemistry and measured/modeled \(^{1} \text{OH}\) results in AMD impacted waters in quartz tubes.

<table>
<thead>
<tr>
<th>system</th>
<th>pH</th>
<th>Fe(^{\text{III}})</th>
<th>SO(_{4}^{2-})</th>
<th>probe</th>
<th>[^{[\text{probe}]}_{0}]</th>
<th>R( \cdot \text{OH} )</th>
<th>S( \cdot \text{OH} )</th>
<th>[^{[\text{OH}]}_{\text{ss}}]</th>
</tr>
</thead>
<tbody>
<tr>
<td>EL</td>
<td>2.72</td>
<td>330</td>
<td>17.2</td>
<td>CAF</td>
<td>0.5-300</td>
<td>1.39 ± 0.13</td>
<td>5.5 ± 4.3</td>
<td>2.5 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>2.74</td>
<td>330</td>
<td>17.2</td>
<td>TPA</td>
<td>0.5-30</td>
<td>1.53 ± 0.10</td>
<td>9.7 ± 4.6</td>
<td>1.6 ± 0.8</td>
</tr>
<tr>
<td>artificial</td>
<td>2.76</td>
<td>660</td>
<td>21.0</td>
<td>CAF</td>
<td>5-300</td>
<td>1.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>water</td>
<td>2.76</td>
<td>660</td>
<td>21.0</td>
<td>model(^{a})</td>
<td></td>
<td>0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP</td>
<td>2.99(^{c})</td>
<td>612</td>
<td>15.2</td>
<td>model(^{a})</td>
<td></td>
<td>2.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.74(^{d})</td>
<td>612</td>
<td>15.2</td>
<td>model(^{a})</td>
<td></td>
<td>1.26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: \(^{a}\)the modelled value has been multiplied by a correction factor of 2.2 to include irradiation from all directions in quartz tubes. \(^{b}\)S\(_{\text{OH}}\) were calculated using initial rate methods, because the first order rate method is too sensitive to small variations. \(^{c}\)native pH of SP water. \(^{d}\)an alternative pH calculated for SP to compare with EL data. \(^{e}\)the \[^{[\text{OH}]}_{\text{ss}}\] are on the higher end of range.
Figure 4.2. The effect of $SO_4^{2-}$ on $^*OH$ production.

a. Degradation of CAF with different sulfate concentrations in artificial AMD solutions. $[CAF]_0 = 30 \mu M, [Fe^{(III)}]_0 = 660 \mu M, I = 0.06 \text{ mM}, pH = 2.8$. Similar experiments were conducted for $[CAF]_0 = 5$ and $300 \mu M$ with similar results (not shown). b. Degradation of BZA with different [TBA] in the EL water. $[Fe^{(III)}]_0 = 330 \mu M, I = 0.04 \text{ mM}, pH = 2.9, [SO_4^{2-}] = 17.2 \text{ mM}$. Similar experiments were conducted for probe CAF with similar results (not shown). Modelled results were shown as the prediction lines.
Figure 4.3. Size exclusion chromatograph of PPL extracted EL and SP DOM.

The peaks were detected as light absorbance at 224 nm.
Figure 4.4. Van Krevelen Diagram for PPL extracted DOM from EL.

Number averaged Non-aromatic formulas \((0 < \text{Al}_{\text{mod}} < 0.5)\) were 95.3\%, aromatic formulas \((0.5 < \text{Al}_{\text{mod}} < 0.67)\) were 2.3\%, and condensed aromatic formulas \((\text{Al}_{\text{mod}} > 0.67)\) were 2.4\%. \text{Al}_{\text{mod}} stands for modified aromaticity index.\textsuperscript{185}
Chapter 5: Conclusions and Future Work

5.1 Diurnal photochemistry

Previous research and guidelines on the photofate of synthetic organic chemicals have been mostly focused on controlled studies of the target compounds under simulated or natural light sources.\textsuperscript{186} To date less attention has been given to the \textit{thermal and biological} stability of the photoproducts. Their fate could also contribute to the residual reactivity and toxicity during the transformation of the parent contaminants. The research in Chapter 2 illustrated such an example under day-night cycles or other intermittent irradiance conditions. The photolytic intermediate of the herbicide isoproturon (IPU) formed during the day was found to be able to thermally regenerate its parent compound in the dark. This example is similar to the product-to-parent transformation of the steroidal growth promoter trebolone, but undergoes a completely different transformation mechanism.\textsuperscript{99} Similarly, in Chapter 4, the photoproduction of the hydroxyl radical (\textbullet OH) from FeOH\textsuperscript{2+} during the day and the biological regeneration of Fe\textsuperscript{III} during the night revealed another diurnal photochemistry scenario in acid mine drainage (AMD) impacted waters.\textsuperscript{100} As a result, the \textbullet OH mediated phototransformation and photomineralization of organic water constituents, both pesticides and DOM, could be more important when considering
biological processes in the dark. Unlike the previous examples where the thermal reversion of the photoproduct was the main consequence of diurnal cycling, this example elucidated the role of biological reaction on the phototransformation of DOM, which in turn affected the bioavailability of the DOM pool in downstream communities. The proposed mechanism of IPU regeneration in Chapter 2 may be used to identify important functional groups needed to initiate the thermal reversion transformation. In addition, a whole body of future research on diurnal transformation/cycling may be extended to other groups of synthetic organic chemicals, where important thermal and biological transformation of photoproducts has been previously neglected. Another future research topic would be to determine the change in ecotoxicity and human health risk during the diurnal transformation of these substances.

5.2 Co-occurrence of DOM and Fe

The diverse aquatic systems investigated in this study range from predominantly fulvic acid containing waters in Chapter 2 where DOM is the major photosensitizer to AMD impacted waters in Chapter 4 where Fe becomes the major photosensitizer. Natural and engineered wetland waters with different levels of added Fe (Chapter 3) falls in the middle of the aforementioned DOM/Fe continuum. With the increase in Fe concentration and decrease in pH, the major photochemically produced reactive intermediates (PPRI) were found to shift from the coexistence of $^3$DOM*, $^1$O$_2$, $^.$OH and other species to $^.$OH-dominant systems. The change of PPRI concentrations, along with the specific reactivity of the target chemicals are needed to predict the photofate of these contaminants.$^{58}$
The addition of μM level of Fe$^{3+}$ to DOM containing waters with none-to-little native Fe was found to accelerate the degradation of acetochlor (AC) in a pH range of 3 to 8, especially at acidic pH values (Chapter 3). Except for in highly acidic pH environments, DOM is a necessary component for Fe-amended AC attenuation. The optimum concentrations and speciation of total Fe needed for the fastest degradation rates may be used as a guideline when considering seeding Fe in treatment wetlands.

The extremely high *OH steady state concentration in AMD impacted waters was found to degrade acetochlor (AC) in minutes and mineralize DOM in hours to days under natural solar irradiation. The effect of DOM in the Fe rich AMD impacted waters could be viewed as an inhibitor for the degradation of pesticides because of its role as a *OH scavenger. Moreover, DOM was transformed and eventually mineralized in this Fe sensitized aquatic system. However, the existence of residual DOM in natural AMD impacted waters shows that a fraction of the DOM pool appears to be resistant to degradation. Nonetheless, AMD impacted waters close to agricultural fields may be used to treat agricultural runoff contaminated by organic pesticides. To apply this theoretical treatment idea into practice, more research is required with respect to dosage, heavy metal effects, and other factors.

Future studies on the interconnected role of DOM and Fe in the aquatic photochemistry is dependent upon a good DOM and Fe speciation and reactivity model under different environmental conditions because of the difficulty in measuring this process experimentally. The best available models have been developed through fitting experimental reaction data to a serious of redox kinetics and complexation
thermodynamic equations.\textsuperscript{69,74} My data regarding \textsuperscript{•}OH production rates in different Fe/DOM systems may potentially be used to understand this process.
Appendix A: Supporting information for chapter 2

Text A.1. Experimental methods

Chemicals

(a) Materials used in Columbus. Isoproturon (3-(4-isopropylphenyl)-1,1-dimethylurea, IPU; 99.5%) was obtained from Chem Service (West Chester, PA). Acetophenone (ACP; ≥99.5%) was obtained from Sigma Aldrich (St. Louis, MO). Acetonitrile (HPLC grade), sodium bicarbonate (certified ACS, 100.2%), hydrochloric acid (certified ACS, 37.1%), ethyl acetate (certified ACS, 99.9%), hexanes (certified ACS grade, ≥98.5%), and anhydrous sodium sulfate (ACS grade, 98%) were obtained from Fisher Scientific (Pittsburgh, PA). Sodium hydroxide (ACS grade, >97%) was obtained from GFS chemicals (Powell, OH). Monobasic sodium phosphate (ACS grade, 100.5%) and dibasic sodium phosphate (Baker analyzed grade) were obtained from Mallinckrodt Baker (Phillipsburg, NJ). Dimethyl sulfoxide-$d_6$ (DMSO-$d_6$) and acetonitrile-$d_3$ (CD$_3$CN) were obtained from Cambridge Isotope Laboratory (Tewksbury, MA). Ultrapure deionized water was generated by a Milli-Q water system (EMD Millipore, Billerica, MA).
(b) Materials used in Dübendorf. Isoproturon (99.5 %) was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), sodium molybdate dihydrate (puriss. p.a.) was from Riedel-de Haën (Seelze, Germany), and sodium hydroxide (≥32%, puriss. p.a.) and hydrogen peroxide (35%, purum p.a.) were from Fluka (Buchs, Switzerland). Sodium dihydrogen phosphate monohydrate, disodium hydrogen phosphate dihydrate, hydrochloric acid (32%), anhydrous sodium carbonate, and sodium hydrogen carbonate (all p.a. grade) were obtained from Merck (Darmstadt, Germany). As photosensitizers, benzophenone-4-carboxylic acid (CBBP, 99%, from Aldrich) and disodium 9,10-anthraquinone-1,5-disulphonate (1,5-AQDS, 98%, from ABCR GmbH, Karlsruhe, Germany) were used.

Analytical and computational methods

(a) Analytical and computational procedures performed in Columbus.

(i) Analytical procedures. Analysis of IPU and its transformation products (including the reversible intermediate IPUint) was performed using a Waters High Performance Liquid Chromatography (HPLC) (1525 binary HPLC pump and 717plus autosampler) connected to a Waters 2487 dual λ ultraviolet-visible (UV-vis) spectrophotometric detector with the separation on a Restek C18 column (Allure, 150×3.2 mm, 5μm). The mobile phase was 35% acetonitrile and 65% water, the flow rate was 0.5 ml/min, the injection volume was 100 μL and the detection wavelength (λ) was 240 nm. For samples containing fulvic acids, however, a gradient mobile phase was used for the separation of IPUint and the fulvic acids starting with 10 % acetonitrile/90 % water, ramping to 65 %
acetonitrile/35 % water in 33 minutes, ramping back to 10 % acetonitrile/90 % water in 3 minutes, and holding at that composition for 15 minutes. The full UV-vis spectrum of IPU\(_{\text{int}}\) was obtained using Hewlett-Packard 1100 HPLC (Agilent Technologies) equipped with a diode array detector (from 200 to 900 nm with an interval of 2 nm) with a 35% acetonitrile/65% water mobile. Dissolved organic carbon (DOC) of the initial samples containing fulvic acids was measured with a Shimadzu TOC-VCPN analyzer (Kyoto, Japan). pH measurement was performed with a Beckman 240 pH / Temp Meter (Brea, CA) equipped with a glass electrode and daily calibration was conducted with standard buffers of pH 4.0, 7.0 and 10.0.

(ii) Calculation of activation energy (\(E_a\)) and entropy of activation (\(\Delta S^\ddagger\)) for IPU\(_{\text{int}}\) dark reaction. In the Arrhenius equation, \(k = A \times e^{(-\frac{E_a}{RT})}\), where \(k\) is the reaction rate constant, \(T\) is the absolute temperature, \(R\) is the ideal gas constant, and \(A\) is the pre-exponential factor. A least-squares linear regression of \(\ln(k)\) versus \(1/T\) was conducted with OriginPro 2016 software which fitted the intercept \(a\), slope \(b\), and the corresponding 95% confidence intervals. \(E_a\) was calculated as \(E_a = -RT\times b\). Based on the Arrhenius equation and transition state theory, the pre-exponential factor 
\[A = \frac{k_B \times T}{h} e^{(\frac{-\Delta S^\ddagger}{R})},\]
and thus
\[\Delta S^\ddagger = a \times R + R \times \ln\left(\frac{h}{k_B \times e \times T}\right),\]
where, \(k_B\) is Boltzman constant, \(h\) is the Planck constant, and \(e \approx 2.71828\). The 95% confidence intervals of \(E_a\) and \(\Delta S^\ddagger\) were derived from the 95% confidence intervals of \(a\) and \(b\) using error propagation method.

(iii) IPU\(_{\text{int}}\) identification with MS, NMR and computational calculation. The setting for MS/MS fragmentation is as follows: ion polarity, positive; capillary voltage, 4500 V;
nebulizer gas, 0.3 bar; dry gas, 3.0 L/min, 180°C; scan range, 50-1000 m/z; end plate offset, -500 V; collision cell RF, 500.0 Vpp. Collision-induced dissociation approach was attempted for MS/MS with collision energies ranging from 5 to 25 eV (data not shown), resulting in no further fragmentation information. UV-vis absorption maxima were calculated using density functional theory (see Table S2). The conformational space of the proposed IPU\textsubscript{int} structures was searched under semi-empirical AM1 level of theory using Spartan’14. The 10 most stable conformations of the IPU\textsubscript{int} candidate were optimized and confirmed (as local minima by means of frequency calculation) using Becke three-parameter exchange functional with a Lee-Yang-Parr correlation functional (B3LYP)\textsuperscript{1,2} and the 6-31+G(d) basis set in both gas and water (integral-equation formalism polarizable continuum model (IEF-PCM))\textsuperscript{3} phases in Gaussian 09.\textsuperscript{4} Time-dependent (TD) B3LYP calculation with 6-31+G(d) basis set was performed to compute the lowest 60 excited states using the optimized ground state geometries in the gas and water phases. The UV-vis spectrum was calculated in GaussView 5.0 by setting the UV-vis peak half-width at half-height be 0.333 eV (by default).

(b) Analytical procedures and IPU isolation performed in Dübendorf.

(i) Analytical procedures. The concentrations of IPU and IPU\textsubscript{int} were quantified by HPLC using an Agilent 1100 system equipped with a diode array detector and a fluorescence detector. Alternatively, an equivalent HPLC system Dionex model Ultimate 3000 was employed. Separation was performed on a reverse-phase column (Nucleosil C\textsubscript{18}, 5 µm, 125×4 mm, Macherey - Nagel, Oensingen, Switzerland) at room temperature. IPU and
IPU_{int} were quantified by UV absorbance at a wavelength of 242 nm and 248 nm, respectively. A mobile phase with a variable composition of acetonitrile and water was used. Measurement of the pH was performed at room temperature by means of a Metrohm pH meter (Model 632 or 691) equipped with a combined glass electrode (either Metrohm 6.0204.100 or Orion 8115SC) that was calibrated before each series of measurements using standard buffers of pH 7.0 and 9.0.

(ii) Concentration and isolation of IPU_{int}. Four quartz tubes (see the experimental section of the paper), each containing 16 mL of solution consisting of IPU (70 µM) 1,5-AQDS (200 µM) and phosphate buffer (5 mM, pH 7.0), were irradiated for 150 min. Subsequently, each of the four irradiated 16 mL-samples were passed through a pre-washed Oasis® HLB 6 cc cartridge with 200 mg sorbent, 30 µm particle size (Waters Corp., Milford, Massachusetts). After rinsing three times with 6 mL of water (pH 7), the sorbed material was eluted with 2 mL of methanol (HPLC grade). The methanol solution, collected in a reagent tube, was gently flushed with nitrogen until the volume was reduced to 150 µL and then mixed with 1 mL of water in an HPLC vial. The resulting four vials of concentrate were then injected into the HPLC system (total of 32 injections of 100 µL sample volume). For each of the 32 runs, the fraction containing the IPU_{int} (run time after injection: 1.70–2.05 min) was collected. The resulting solution (a total of 11 mL) containing IPU_{int} in a water/acetonitrile mixture (50/50 vol/vol) was stored in a glass vial in the dark at 5 °C.

*Photochemical kinetics of IPU and IPU_{int} in argon saturated conditions in Columbus*
Solutions of 10 μM IPU and 25 μM acetophenone (ACP) dissolved in a pH 8 buffer (1mM NaHCO₃) were made in equilibrium with air. Half of the solution was directly sealed in the quartz tubes. Another half of the solution was purged with argon gas at a rate of 1 min gas / mL-solution before being sealed in quartz tubes in the glove box. The small volatilization of ACP (10%) during argon purging was proved to have little impact on IPU transformation based on a replicate experiment using a higher ACP concentration. The phototubes were then irradiated in an Atlas Suntest CPS+ solar simulator equipped with a 500 W/m² xenon lamp and solar standard filter for 7 h at 25 ± 2°C. Phototubes were withdrawn at selected time points for HPLC measurement.
a. IPU\textsubscript{int}

Figure A.1. \textsuperscript{1}H NMR spectra of IPU\textsubscript{int} (a) and IPU (b).

Notes: (a) \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}_6): \(\delta\) 0.82 (3H, d, \(J = 6.8\) Hz), 0.83 (3H, d, \(J = 6.8\) Hz), 1.95 (1H, m), 2.72 (3H, s), 2.92 (3H, s), 6.35 (1H, m, \(J = \sim 10.4\) Hz), 6.43 (1H, m, \(J = \sim 10.4\) Hz), 6.57 (2H, m), 11.52 (1H, s). Note: The spikes at 1.3, 1.98 and 4.0 ppm are due to traces of ethyl acetate impurity in the sample. The insert represents an enlarged area from 0.5 to 3 ppm. The corresponding H is colored red in the structures near the peak. Continued.
Notes: (b) $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.22 (6H, d, J = 6.8 Hz), 2.86 (1H, m, J $\approx$ 6.8 Hz), 3.02 (6H, s), 6.23 (1H, s), 7.28 (2H, dd, J = 6.4, 2 Hz), 7.14 (2H, dd, J = 6.8, 2 Hz). The corresponding H is colored red in the structures near the peak.
Figure A.2. $^{13}$C NMR spectra of IPU_{int} (a) and IPU (b).

Notes: (a) $^{13}$C NMR (100 MHz, CD$_3$CN): $\delta$ 17.3, 35.1, 35.6, 37.1, 84.8, 125.0, 132.0, 143.7, 144.8, 158.5, 164.7. Note: During $^{13}$C NMR of the IPU$_{int}$ in CD$_3$CN we observed that some percentage of IPU$_{int}$ converted back to starting IPU at room temperature. Presence of water in CD$_3$CN is believed to be responsible for this conversion. Resulting $^{13}$C NMR spectrum has peaks due to both IPU and IPU$_{int}$. However, we were able to differentiate the $^{13}$C NMR peaks due to IPU and IPU$_{int}$ by comparing it to the $^{13}$C NMR of authentic IPU in CD$_3$CN and the circled chemical shifts were produced only by IPU$_{int}$. The red color highlights the chemical shift of the carbon connected to the hydroperoxide. Continued.
Figure A.2 continued.

Notes: (b) $^{13}$C NMR (100 MHz, CD$_3$CN): $\delta$ 24.4, 34.3, 36.8, 121.5, 127.3, 139.1, 144.1, 157.2.
Figure A.3. IPU photolysis in the presence of different photosensitizers.

a. IPU photolysis (direct and in the presence of ACP) and dark control. Light source: xenon lamp in Suntest CPS+ at 500 W/m². For direct photolysis and dark control, $[\text{IPU}]_0 = 5.5 \mu\text{M}, [\text{NaHCO}_3] = 1 \text{mM}, \text{pH} = 8$; for indirect photolysis, $[\text{IPU}]_0 = 10 \mu\text{M}, [\text{ACP}]_0 = 25 \mu\text{M}, [\text{NaHCO}_3] = 1 \text{mM}, \text{pH} = 8$. The lines in the figures are fitted from linear regression.

b. IPU photolysis in the presence of SRFA and PLFA. Light source: xenon lamp in Suntest CPS+ at 500W/m². Experimental conditions: $\text{pH} = 8, [\text{SRFA}]_0 = 12.8 \text{mgC/L}, [\text{PLFA}]_0 = 11.0 \text{mgC/L}, [\text{IPU}]_0 = 8.7 \mu\text{M}$. The lines in the figures are fitted from linear regression.
Figure A.4. HPLC chromatogram of the reversion of IPU$_{\text{int}}$ to IPU for purified IPU$_{\text{int}}$ in acetonitrile.

Experiments were done in Columbus. Note that IPU$_{\text{int}}$ in acetonitrile reverted to IPU at a slower speed than in water. Abs., absorbance at 240 nm. AU, absorbance unit.
Figure A.5. The dark transformation kinetics of IPU_{int} in the presence of fulvic acids. SRFA stands for Suwannee River fulvic acid and PLFA stands for Pony Lake fulvic acid. Averaged half-life for all experiments regardless of fulvic acid type, concentration and photolysis time (t_{p,max}) was 63 ± 9 hours for the first 100-h dark reaction period.
Table A.1. The extent of dark reversion from IPU\textsubscript{int} to IPU under different pH values.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>pH</th>
<th>IPU rebound ratio</th>
<th>Half-life (h)</th>
<th>Lab Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbonate (1mM), 22°C, ACP</td>
<td>2.4</td>
<td>0.01</td>
<td>0.11</td>
<td>Columbus</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>0.05</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>0.12</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>0.97</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Phosphate (1mM), 22°C, ACP</td>
<td>4.1</td>
<td>0.05</td>
<td>6.7</td>
<td>Columbus</td>
</tr>
<tr>
<td></td>
<td>4.1</td>
<td>0.06</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.6</td>
<td>0.90</td>
<td>103</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.6</td>
<td>0.90</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>Phosphate (5mM), 25°C, 1,5-ADQS</td>
<td>7.0</td>
<td>0.73</td>
<td>59</td>
<td>Dübendorf</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>1.40</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>1.00</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Phosphate (5mM), 25°C, CBBP</td>
<td>7.0</td>
<td>0.84</td>
<td>60</td>
<td>Dübendorf</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>0.82</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>0.88</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.9</td>
<td>0.91</td>
<td>1.4</td>
<td></td>
</tr>
</tbody>
</table>

Note: The experiment column refers to the buffer (except for the carbonate subset at low pH where most of the H\textsubscript{2}CO\textsubscript{3} volatilized), the temperature, and the remaining photosensitizer in the dark reaction. The IPU rebound ratio is the absolute slope of \((\text{IPU}_\text{PA}_\text{td,i} - \text{IPU}_\text{PA}_\text{td,0})\) versus \((\text{IPU}_\text{int PA}_\text{td,i} - \text{IPU}_\text{int PA}_\text{td,0})\) as described in the text. It is worth mentioning that the ratios determined in different labs were not necessarily comparable due to different detection wavelengths used in different labs. In Columbus, IPU and IPU\textsubscript{int} were both detected at 240nm, and in Dübendorf, IPU was detected at 242nm and IPU\textsubscript{int} at 248nm.
Table A.2. MS/MS fragmentation for IPU$_{int}$ at 0 eV collision energy.

<table>
<thead>
<tr>
<th>nominal mass</th>
<th>measured mass</th>
<th>theoretic mass</th>
<th>Δ mass</th>
<th>relative Δ mass (ppm)</th>
<th>Intensity (%)</th>
<th>loss from M+1</th>
<th>molecular formula</th>
<th>Structure (deprotonated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>239 (M+1)</td>
<td>239.1386</td>
<td>239.1396</td>
<td>-0.0010</td>
<td>4.1</td>
<td>100</td>
<td>-17</td>
<td>C$<em>{12}$H$</em>{19}$N$_2$O$_3$ $^+$</td>
<td>![Structure 1]</td>
</tr>
<tr>
<td>222</td>
<td>222.1359</td>
<td>222.1368</td>
<td>-0.0009</td>
<td>4.2</td>
<td>21</td>
<td>-33</td>
<td>C$<em>{12}$H$</em>{18}$N$_2$O$_2$ $^+$</td>
<td>![Structure 2]</td>
</tr>
<tr>
<td>206</td>
<td>206.1411</td>
<td>206.1419</td>
<td>-0.0008</td>
<td>3.9</td>
<td>3</td>
<td>-42</td>
<td>C$<em>{12}$H$</em>{18}$N$_2$O $^+$</td>
<td>![Structure 3]</td>
</tr>
<tr>
<td>197</td>
<td>197.0918</td>
<td>197.0926</td>
<td>-0.0008</td>
<td>4.2</td>
<td>5</td>
<td>-C$_3$H$_6$</td>
<td>C$<em>9$H$</em>{13}$N$_2$O$_3$ $^+$</td>
<td>![Structure 4]</td>
</tr>
</tbody>
</table>

Note: This data was acquired at a different time than the MS mode used in Figure 5; thus, the measured accurate mass was slightly different.
Figure A.6. The chirality in $\text{IPU}_{\text{int}}$. 
Table A.3. Density functional theory calculated UV-vis absorption maxima for IPU_{int}.

<table>
<thead>
<tr>
<th>Gas phase (nm, osc. str.)</th>
<th>Water phase (nm, osc. str.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(S1) 389.97 (0.0009)</td>
<td>(S1) 347.79 (0.0029)</td>
</tr>
<tr>
<td>(S2) 347.75 (0.0440)</td>
<td>(S2) 327.95 (0.0299)</td>
</tr>
<tr>
<td>(S2) 285.86 (0.0138)</td>
<td>(S3) 281.58 (0.0081)</td>
</tr>
<tr>
<td><strong>(S4) 277.63 (0.2701)</strong></td>
<td><strong>(S4) 271.87 (0.4210)</strong></td>
</tr>
<tr>
<td>(S5) 254.29 (0.0105)</td>
<td>(S5) 252.43 (0.0307)</td>
</tr>
<tr>
<td><strong>(S6) 249.55 (0.2543)</strong></td>
<td><strong>(S6) 240.66 (0.1771)</strong></td>
</tr>
</tbody>
</table>

Each entry is shown as (excited singlet state (S) number) $\lambda_{\text{max}}$ (oscillator strength, or osc. str.). Unit: nm. The numbers in bold are the major electric transitions (osc. str. $> 0.1$).
Figure A.7. Experimental (exp) UV-vis absorption spectrum and DFT calculated (cal, gas and water phase) absorption spectra for IPU\textsubscript{int}.

The experimental data was collected using a diode array detector with a 35% acetonitrile/65% water mobile phase. For the calculated spectrum, the UV-vis peak half-width at half-height is set to be 0.333 eV in GaussView 5.0 by default.
Figure A.8. Photolysis of IPU and formation of $\text{IPU}_{\text{int}}$ in the presence of ACP at different oxygen levels (argon/air saturated).

Experimental conditions: $\text{pH} = 8$, $[\text{NaHCO}_3] = 1 \text{ mM}$, $[\text{ACP}]_0 = 25 \mu\text{M}$, $[\text{IPU}]_0 = 10 \mu\text{M}$.

PA, HPLC peak area; AU, absorbance unit.
Depletion of IPU and formation of IPU\textsuperscript{int} in the hydrogen peroxide-molybdate system at pH 9, with subsequent transformation of IPU\textsuperscript{int} and reformation of IPU at pH 7 after the last hydrogen peroxide addition. Experiments were conducted in Dübendorf. PA, HPLC peak area; AU, absorbance unit.
Appendix B: Supporting information for chapter 3

Text B.1. Reagents

Formic acid (97%), Fe$^{III}$ sulfate (Fe$_2$(SO$_4$)$_3$•xH$_2$O, 74.4% anhydrous), ammonium Iron(II) sulfate hydrate ((NH$_4$)$_2$Fe(SO$_4$)$_2$•xH$_2$O), Puratronic, 99.9999% metals basis) were obtained from Alfa Aesar (Haverhill, MA). Methanol (HPLC grade, 99.9%), hydrochloric acid (trace metal grade, 34-37%), sulfuric acid (certified ACS plus, 95.9 w/w %), glacial acetic acid (trace metal, 99%) were obtained from Fisher Chemical (Pittsburgh, PA). Acetonitrile (HPLC grade, 99.8%) was obtained from EMD Millipore (Billerica, MA). FerroZine$^{TM}$ iron reagent (hydrate, 98%) was obtained from Acros Organics (Waltham, MA). Hydroxylamine hydrochloride (ACS reagent, 98.0%) was obtained from Sigma-Aldrich (St. Louis, MO). Sodium hydroxide (ACS reagent, 97.0%) was obtained from GFS Chemicals (Columbus, OH).
Figure B.1. Location of sampling sites.

Notes: OWC water was collected on 120815, WF water was collected on 122215, and EL water was collected on 100615.
Text B.2. Fe measurement using FerroZine method.

The determination of Fe by its colored complex with FerroZine was originally developed by Stookey\textsuperscript{187} and we modified the method based on Gibbs’ parameterization\textsuperscript{188}. An aliquot of 200 μl 1.44 M NH\textsubscript{2}OH was added to 20 ml whole water samples to reduce Fe\textsuperscript{III} to Fe\textsuperscript{II}. After 2 min of reaction time, aliquots of 1.6 ml 5 M acetate buffer (pH 4.8) and 200 μl 10 mM FerroZine were added to the above solution. The absorbance at 562 nm (\textit{A}\textsubscript{562}) was measured using Shimadzu spectrophotometer (Kyoto, Japan) after 15 min. \textit{A}\textsubscript{562} was then converted to [Fe] using standard addition calibration curves of known Fe\textsubscript{added} in case of matrix interference.
Table B.1. Water conditions and $R_{\text{prod}}$ results from hTPA formation experiments.

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Figure B.2. Representative experimental and best fit of hTPA formation.

Conditions: pH = 7.6, [SRFA] = 6 mg-C L⁻¹ SRFA, and Fe_{added} = 10 μM. Note that non-zero value of [hTPA] at time = 0 min represents the dark formation of \( \cdot \text{OH} \).\(^{24,189} \)
Figure B.3. Representative AC photolysis kinetics.

Conditions: pH = 7.6, and in the presence of WF and 10 μM Fe_{added}.
Figure B.4. •OH production rate at pH 7 with increasing Fe addition.

The experiments in SRFA were conducted twice. Error bars are smaller than symbol when not shown.
Appendix C: Supporting information for chapter 4

Text C.1. Reagents and analytical methods

*Reagents*

Disodium terephthalate (TPA; > 99 %) was obtained from Alfa Aesar (Tewksbury, MA). Fe\(^{\text{III}}\) sulfate (Fe\(_2\)(SO\(_4\))\(_3\)•xH\(_2\)O, 74.4% anhydrous), ammonium Iron(II) sulfate hydrate ((NH\(_4\))\(_2\)Fe(SO\(_4\))\(_2\)•xH\(_2\)O), Puratronic, 99.9999% metals basis) were obtained from Alfa Aesar (Haverhill, MA). Methanol (HPLC grade, 99.9%), glacial acetic acid (tracemetal grade, 99%), perchloric acid (HClO\(_4\), reagent ACS, 70 %), sodium sulfate (Na\(_2\)SO\(_4\); certified ACS, 100.2%), monobasic potassium phosphate (K\(_2\)HPO\(_4\), primary standard, 99.9%), and acetone (C\(_3\)H\(_6\)O, certified ACS, 99.7%) were obtained from Fisher Chemical (Pittsburgh, PA). Acetonitrile (HPLC grade, 99.8%) was obtained from EMD Millipore (Billerica, MA). FerroZine\(^{\text{TM}}\) iron reagent (hydrate, 98%) was obtained from Acros Organics (Waltham, MA). Hydroxylamine hydrochloride (ACS reagent, 98.0%), anhydrous sodium perchlorate (NaClO\(_4\); reagent ACS) was obtained from Sigma-Aldrich (St. Louis, MO). Sodium hydroxide (ACS reagent, 97.0%) was obtained from GFS Chemicals (Columbus, OH). 12-hydrate, dibasic sodium phosphate (Na\(_2\)HPO\(_4\)•12H\(_2\)O, ‘Baker Analyzed’® Reagent) was obtained from J. T. Baker (now part of Avantor
Performance Materials, Center Valley, PA). Sodium polystyrene sulfate (MW = 1, 5, 6, 18 k) were obtained from Polysciences Inc (Warrington, PA).

Iron determination

The determination of Fe by its colored complex with FerroZine was originally developed by Stookey\textsuperscript{187} and we modified the method based on Gibbs’ parameterization\textsuperscript{188}. Because AMD impacted waters contained ions such as Al\textsuperscript{3+} to complex with FerroZine without forming color, we added excess amount of FerroZine to ensure full color development for Fe. The known color forming ions, Co\textsuperscript{2+} and Cu\textsuperscript{+}, are of trace amount or below detection limit in our waters, therefore did not interfere with Fe determination.\textsuperscript{187} For total Fe (Fe\textsuperscript{III} + Fe\textsuperscript{II}), an aliquot of 50 μl 1.44 M NH\textsubscript{2}OH was added to 0.2 ml AMD impacted water samples to reduce Fe\textsuperscript{III} to Fe\textsuperscript{II}. After 2 min of reaction time, aliquots of 400 μl 5 M acetate buffer (pH 4.8), 50 μl 10 mM FerroZine, and 4.3 ml MQ were added to the above solution. The absorbance at 562 nm (A\textsubscript{562}) was measured using Shimadzu spectrophotometer (Kyoto, Japan) after 15 min. For ferrous iron (Fe\textsuperscript{II}) measurement, 0.2 - 2 ml of AMD water samples were diluted with MQ depending on the Fe\textsuperscript{II} concentration to ensure the appropriate absorbance reading. Aliquots of 300 μl 10 mM FerroZine and 400 μl 5 M acetate buffer (pH 4.8) were added to the above solutions. After 15 min, A\textsubscript{562} was determined with the spectrophotometer. Blanks and calibration curves were periodically checked for measurement accuracy.

Size exclusion chromatography
The size distribution of extracted DOM from AMD waters were measured the method developed by Chin. et al.\textsuperscript{61,190} In brief, high pressure size exclusion chromatography was performed with a Waters High Performance Liquid Chromatography (HPLC) (1525 binary HPLC pump and 717plus autosampler) connected to a Waters 2487 dual λ ultraviolet-visible (UV-vis) spectrophotometer. A Waters Protein-Pak\textsuperscript{TM} column (125 Å, 7.8 × 300 mm) was used to separate molecules based on size. The mobile phase was 2 mM Na$_2$HPO$_4$, 2 mM KH$_2$PO$_4$, 0.1 M NaCl at pH of 6.8, the flow was 1 ml min$^{-1}$, and the injection volume was 20 μl. Random soil sodium polystyrenesulfonate (MW = 1, 5, 6, and, 18 k) and acetone (MW = 58) were used as calibration standards.
Figure C.1. Perry State Forest watershed map.

The coordinates for sampling places: SP (39°45′47″ N, 82°12′38″ W) and EL (39°45′31″ N, 82°12′26″ W).
Table C.1. Water chemistry of EL and SP waters

<table>
<thead>
<tr>
<th>property</th>
<th>unit</th>
<th>EL</th>
<th>SP</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td>2.7</td>
<td>3</td>
</tr>
<tr>
<td>TOC</td>
<td>mg-C L(^{-1})</td>
<td>0.9</td>
<td>5.4</td>
</tr>
<tr>
<td>conductivity</td>
<td>mS cm(^{-2})</td>
<td>2.24</td>
<td>2.39</td>
</tr>
<tr>
<td>ionic strength</td>
<td>M</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>anions</td>
<td>mM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SO(_4^{2-})</td>
<td></td>
<td>17.2</td>
<td>15.2</td>
</tr>
<tr>
<td>Cl(^-)</td>
<td></td>
<td>0.23</td>
<td>0.12</td>
</tr>
<tr>
<td>F(^-)</td>
<td></td>
<td>0.19</td>
<td>0.12</td>
</tr>
<tr>
<td>Br(^-)</td>
<td>below detection limit (BDL)</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td>NO(_3^-)</td>
<td></td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td>PO(_4^{3-})</td>
<td></td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td>Fe</td>
<td>μM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe(^{II})</td>
<td></td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Fe(^{III})</td>
<td></td>
<td>332</td>
<td>621</td>
</tr>
<tr>
<td>elements</td>
<td>mM</td>
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</tr>
<tr>
<td>P</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
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<tr>
<td>K</td>
<td>0.17</td>
<td>0.31</td>
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</tr>
<tr>
<td>Ca</td>
<td>4.93</td>
<td>4.84</td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>6.47</td>
<td>6.63</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>15.1</td>
<td>15.1</td>
<td></td>
</tr>
<tr>
<td>Al</td>
<td>1.20</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.004</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>0.0002</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td>Fe</td>
<td>0.33</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>0.42</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Mo</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td>Na</td>
<td>0.47</td>
<td>0.37</td>
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<tr>
<td>Zn</td>
<td>0.015</td>
<td>0.011</td>
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<tr>
<td>As</td>
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<td>BDL</td>
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</tr>
<tr>
<td>Ba</td>
<td>0.0002</td>
<td>0.0003</td>
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<tr>
<td>Be</td>
<td>BDL</td>
<td>BDL</td>
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</tr>
<tr>
<td>Cd</td>
<td>0.00002</td>
<td>0.00002</td>
<td></td>
</tr>
<tr>
<td>Co</td>
<td>0.008</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>0.0003</td>
<td>0.0004</td>
<td></td>
</tr>
<tr>
<td>Li</td>
<td>0.002</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td>0.009</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>BDL</td>
<td>BDL</td>
<td>0.00004</td>
</tr>
<tr>
<td>Sb</td>
<td>0.0001</td>
<td>0.0001</td>
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<tr>
<td>Se</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
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<tr>
<td>Si</td>
<td>0.54</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>Sr</td>
<td>0.007</td>
<td>0.007</td>
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</tr>
<tr>
<td>Tl</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td>V</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td>Ag</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
</tr>
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Table C.2. Fe speciation calculation (%) for EL and SP waters with MINTEQ.

<table>
<thead>
<tr>
<th></th>
<th>EL pH=2.74</th>
<th>EL pH = 3.0</th>
<th>SP pH = 2.74</th>
<th>SP pH = 3.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe$^{3+}$</td>
<td>3.87</td>
<td>3.47</td>
<td>4.08</td>
<td>3.66</td>
</tr>
<tr>
<td>FeOH$^{2+}$</td>
<td>8.10</td>
<td>13.25</td>
<td>8.72</td>
<td>13.91</td>
</tr>
<tr>
<td>Fe(OH)$_2^+$</td>
<td>0.48</td>
<td>1.42</td>
<td>0.52</td>
<td>1.48</td>
</tr>
<tr>
<td>Fe$_2$(OH)$_2$$^{4+}$</td>
<td>0.03</td>
<td>0.07</td>
<td>0.05</td>
<td>0.14</td>
</tr>
<tr>
<td>FeF$^2+$</td>
<td>0.62</td>
<td>0.58</td>
<td>1.19</td>
<td>1.11</td>
</tr>
<tr>
<td>FeF$_2^+$</td>
<td>0.00</td>
<td>0.00</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>FeSO$_4^+$</td>
<td>81.27</td>
<td>75.76</td>
<td>80.36</td>
<td>74.81</td>
</tr>
<tr>
<td>Fe(SO$_4$)$_2^-$</td>
<td>5.62</td>
<td>5.43</td>
<td>5.05</td>
<td>4.88</td>
</tr>
</tbody>
</table>

Field experiment time: Oct 15, 2015

Vessel: phototube

Water: A (water collected on Oct 6, 15 and filtered in the lab); B (field collected water at the desired time point)

Irradiation time for field experiment: Ever hour from 10 am to 2 pm (solar time), each water sample is irradiated for 4 min (replicates)

Table C.3. Comparison of production of \( ^\cdot \mathrm{OH} \) in EL water in field and in lab.

<table>
<thead>
<tr>
<th>property</th>
<th>unit</th>
<th>A-filtered</th>
<th>A-filtered</th>
<th>A-filtered</th>
<th>B-onsite</th>
</tr>
</thead>
<tbody>
<tr>
<td>light</td>
<td></td>
<td>Suntest- 3 min</td>
<td>Suntest- 8 min</td>
<td>natural- 4 min</td>
<td>natural- 4 min</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>2.72</td>
<td>~2.74</td>
<td>~2.74</td>
<td>~2.7</td>
</tr>
<tr>
<td>Fe(^{\text{III}})</td>
<td>μM</td>
<td>330</td>
<td>~330</td>
<td>~330</td>
<td>NA</td>
</tr>
<tr>
<td>SO(_4^{2-})</td>
<td>mM</td>
<td>17.2</td>
<td>~17</td>
<td>~17</td>
<td>NA</td>
</tr>
<tr>
<td>[CAF](_0)</td>
<td>μM</td>
<td>0.5-300</td>
<td>20-40</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>R(\cdot)OH</td>
<td>(10^{-7}) M s(^{-1})</td>
<td>1.39 ± 0.13</td>
<td>1.2 ± 0.4</td>
<td>0.7 - 1.3</td>
<td>0.7 - 1.1</td>
</tr>
<tr>
<td>S(\cdot)OH</td>
<td>(10^{4}) s(^{-1})</td>
<td>5.5 ± 4.3</td>
<td>3.5 ± 7.4</td>
<td>3.5 (assumed)</td>
<td>3.5 (assumed)</td>
</tr>
<tr>
<td>([\cdot)OH]ss</td>
<td>(10^{-12}) M</td>
<td>2.5 ± 2.0</td>
<td>2.9 ± 5.9</td>
<td>2 - 4</td>
<td>2 - 3</td>
</tr>
</tbody>
</table>
Text C.3. \textsuperscript{\textcircled{1}}OH production calculation.

\textit{Light source calculation}

Figure C.2. UV spectra during summer (June 21, 2016, solar noon, calculated), winter (Dec 21, 2016, solar moon, calculated), and inside the Suntest solar simulator (measured).
Figure C.3. Calculation of *OH production rates in top cm at the summer scenario.

Continued
Figure C.3 continued

Notes: a. summer noon solar spectrum. b. the fraction of light absorbed the whole water normalized by the incident light. c. percentage of absorbed light absorbed by FeOH$^{2+}$ normalized by the total absorbed light. d. molar absorptivity of FeOH$^{2+}$. e. quantum yield of *OH production from FeOH$^{2+}$. f. calculated *OH production.
Figure C.4. DOM photomineralization in the presence and absence of initial oxygen.

SP-1 and SP-2 (in the presence of initial oxygen) were taken from Figure 4.1. SP-Argon (in the absence of initial oxygen) and SP-Oxygen (in the presence of initial oxygen) were conducted in the same batch.
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