Citation for published version:
Wenk, J, Aeschbacher, M, Sander, M, Von Gunten, U & Canonica, S 2015, 'Photosensitizing and inhibitory effects of ozonated dissolved organic matter on triplet-induced contaminant transformation', Environmental Science & Technology, vol. 49, no. 14, pp. 8541–8549. https://doi.org/10.1021/acs.est.5b02221

DOI:
10.1021/acs.est.5b02221

Publication date:
2015

Document Version
Early version, also known as pre-print

Link to publication

University of Bath

Article

Photosensitizing and inhibitory effects of ozonated dissolved organic matter on triplet-induced contaminant transformation

Jannis Wenk, Michael Aeschbacher, Michael Sander, Urs von Gunten, and Silvio Canonica

Just Accepted

1　**Photosensitizing and inhibitory effects of ozonated dissolved**

2　**organic matter on triplet-induced contaminant transformation**

3

4　Jannis Wenk[†, ‡,∥], Michael Aeschbacher[‡], Michael Sander[‡] , Urs von Gunten[†,‡,§] and Silvio

5　Canonica[*†]

6

7　[†]Eawag, Swiss Federal Institute of Aquatic Science and Technology

8　CH-8600, Dübendorf, Switzerland

9　[‡]Institute of Biogeochemistry and Pollutant Dynamics, ETH Zürich, CH-8092, Zürich,

10　Switzerland

11　[§]School of Architecture, Civil and Environmental Engineering (ENAC), Ecole Polytechnique

12　Fédérale de Lausanne (EPFL), CH-1015, Lausanne, Switzerland

13　[∥] Present address: Department of Chemical Engineering and Water Innovation & Research

14　Centre (WIRC), University of Bath, Claverton Down, Bath BA2 7AY, United Kingdom
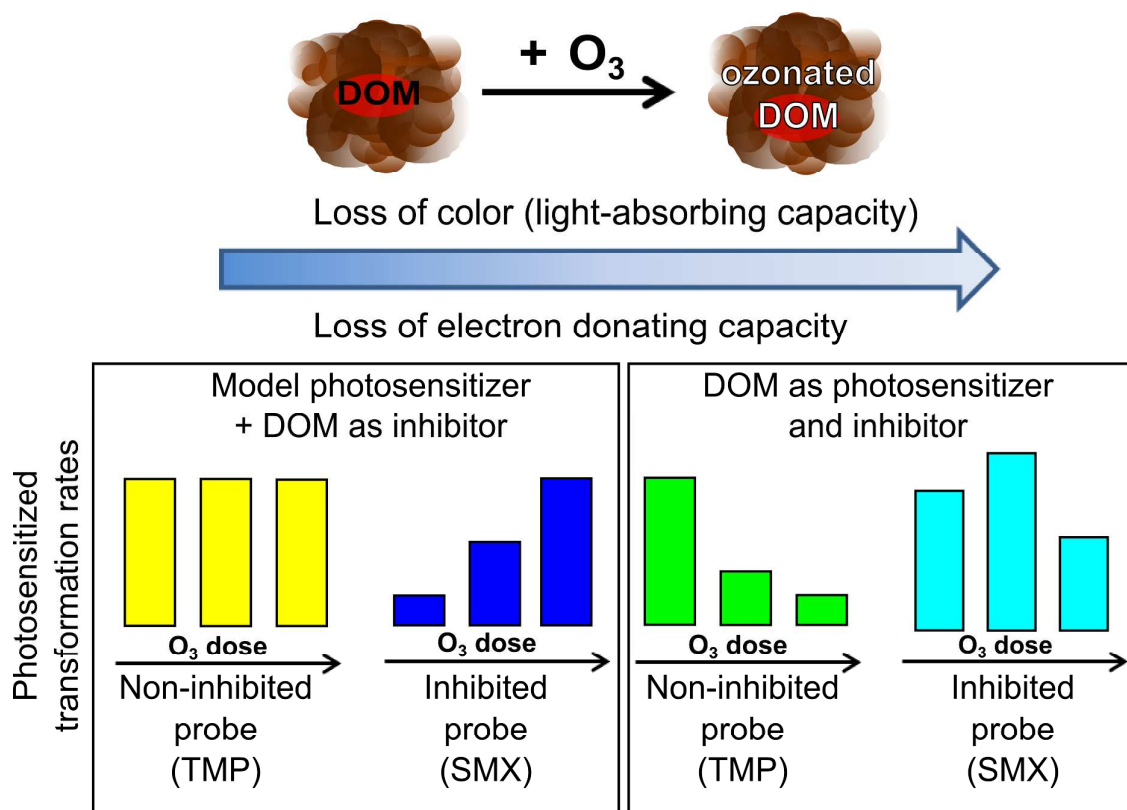
15

17

18

19　Number of pages: 28

20　Number of figures: 4

21　Number of tables: 0

22

23

24

25　[*]Corresponding Author:

26　Phone +41-58-765-5453; Fax +41-58-765-5028; email: silvio.canonica@eawag.ch.

27

1

28

29 **Graphical Abstract**

Abstract

Dissolved organic matter (DOM) is both a promoter and an inhibitor of triplet-induced organic contaminant oxidation. This dual role was systematically investigated through photochemical experiments with three types of DOM of terrestrial and aquatic origins that were pre-oxidized to varying extents by ozonation. The inhibitory effect of DOM was assessed by determining the 4-carboxybenzophenone photosensitized transformation rate constants of two sulfonamide antibiotics (sulfamethoxazole and sulfadiazine) in the presence of untreated or pre-oxidized DOM. The inhibitory effect decreased with increasing extent of DOM pre-oxidation, and was correlated to the loss of phenolic antioxidant moieties, as quantified electrochemically, and to the loss of DOM ultraviolet absorbance. The triplet photosensitizing ability of pre-oxidized DOM was determined using conversion of the probe compound 2,4,6-trimethylphenol (TMP), which is unaffected by DOM inhibition effects. DOM-photosensitized transformation rate constants of TMP decreased with increasing DOM pre-oxidation and were correlated to the concomitant loss of chromophores (i.e., photosensitizing moieties). The combined effects of DOM pre-oxidation on the inhibiting and photosensitizing properties were assessed by phototransformation experiments of the sulfonamides in DOM-containing solutions. At low extents of DOM pre-oxidation, sulfonamide phototransformation rate constants remained either unchanged or slightly increased, indicating that the removal of antioxidant moieties had larger effects than the loss of photosensitizing moieties. At higher extents of DOM pre-oxidation, transformation rates declined, mainly reflecting the destruction of photosensitizing moieties.

51   **Introduction**

52   Dissolved organic matter (DOM) is a heterogeneous, complex mixture of organic molecules

53   and is ubiquitous in natural waters.[1] DOM plays a central role in aquatic photochemical

54   processes[2-4] that are important for biogeochemical element cycles as well as pollutant

55   dynamics. While DOM has long been known to enhance organic pollutant

56   phototransformation by acting as a sensitizer, it was recently demonstrated that DOM may

57   also play the role of an inhibitor of triplet-induced contaminant transformation [5-7] as well as

58   direct photooxidation reactions.[8]

59   The formation of excited triplet states of DOM ($^3DOM^*$) is initiated by the absorption of light

60   by chromophoric moieties of DOM followed by the formation of excited singlet states of

61   DOM ($^1DOM^*$). Subsequent rapid intersystem crossing converts $^1DOM^*$ to $^3DOM^*$. The

62   latter are key reactive species initiating the oxidative transformation of various aquatic

63   contaminants.[3, 9, 10] The importance of $^3DOM^*$ as photooxidants was verified by using low

64   molecular weight aromatic ketones as models mimicking the photosensitizing characteristics

65   of DOM.[11-14]

66   More recently, the inhibitory properties of DOM on $^3DOM^*$-induced contaminant

67   transformations were recognized.[5-7] Inhibition is hypothesized to result from contaminant

68   intermediates, formed via the oxidation of the parent compound through a reactive encounter

69   with $^3DOM^*$, being transformed back to the parent compound by accepting an electron from

70   antioxidant moieties present in the DOM. More specifically, phenolic moieties are considered

71   to be the major antioxidant groups in DOM.[15] The importance of phenols as antioxidants in

72   DOM was supported by demonstrating that low molecular weight model phenolic compounds

73   at micromolar concentrations were capable of inhibiting triplet-induced reactions.[7]

74   Despite the successful use of model aromatic ketones and phenolic compounds, the detailed

75   chemical structure and nature of the moieties responsible for the photosensitizing and

76   inhibiting effects of the DOM on triplet-induced contaminant transformation remain to be

77   clarified in more detail. A promising approach to identify a specific group of moieties in the

78   DOM involves exposing DOM to specific chemical reactants that inactivate the target

79   moieties. Such an approach was recently used by Blough, del Vecchio and coworkers who

80   assessed the role of aromatic ketones and quinone moieties in the electronic absorption and

81   fluorescence spectra of DOM.[16] Treatment of the DOM with the reducing agent sodium

82   borohydride ($NaBH_4$) transformed the target carbonyl groups into hydroxy (alcohol) groups,

83   resulting in a preferential loss of DOM absorption in the visible range as well as an enhanced

84   fluorescence with blue-shifted spectra. These results support the hypothesis that DOM

85   electronic absorption in the visible range is largely due to donor-acceptor complexes between

86   electron-rich aromatic donors and carbonyl-containing acceptors.[4, 17, 18] Two follow-up

87   studies showed that borohydride treatment reduced the rate of the triplet-induced

88   transformation of the probe compound 2,4,6-trimethylphenol[19] and of photosensitized singlet

89   oxygen production,[20] reinforcing the role of aromatic ketones as key DOM photosensitizer

90   moieties in these processes.

91   The main motivation for the present study was to selectively deplete the antioxidant moieties

92   in the DOM to test their involvement in the inhibition of triplet-induced transformations of

93   contaminants. With this objective in mind, in a recent study, we oxidized different DOMs

94   with three chemical oxidants, namely ozone, chlorine and chlorine dioxide, that are currently

95   used in water treatment.[21] Both the electronic absorption spectrum and the electron donating

96   capacity (EDC) of treated and untreated DOM were measured to characterize the chemical

97   changes of the DOM. The EDC was measured using mediated electrochemical oxidation

98   (MEO) as described elsewhere.[22] It expresses the number of electrons that can be withdrawn

99   from a unit mass of material under well-defined applied reduction potentials and solution pH.

100   EDC values are well suited as quantitative descriptors of the antioxidant capacities of a

101   material,[23-25] and, for DOM, they were shown to be well correlated to DOM phenolic

102   contents.[15] Treatment of DOM with any of the three aforementioned oxidative methods led to

103   decreases in the EDC values and, at the same time, in the UV and visible absorption. The

104   differential decreases in EDC values and absorption coefficients indicated that not only

105   antioxidant moieties, but also further chromophoric DOM components were lost by

106   oxidation.

107   In this study, we investigated the effect of DOM ozonation on the triplet-induced

108   transformation of sulfamethoxazole (SMX) and sulfadiazine (SD), two sulfonamide

109   antibiotics chosen as representatives for contaminants exhibiting concomitantly promotion

110   and inhibition of the transformation rates in the presence of DOM.[5-7] In a first series of

111   kinetics experiments, the model photosensitizer 4-carboxybenzophenone (CBBP) was

112   employed to induce the phototransformation in the presence of untreated and ozone-treated

113   DOM. This set of experiments assessed the inhibitory effects of the DOM. In a second,

114   analogous experimental series, untreated and O_3-treated DOM served as both photosensitizer

115   and inhibitor of the phototransformation. Besides SMX and SD, the well-established

116   photochemical probe compound 2,4,6-trimethylphenol (TMP),[11] which is not affected by

117   inhibition, was used to benchmark the modified photosensitizing strength of the treated

118   DOMs.

119

Materials and Methods

121   **Chemicals and humic substances.** All chemicals were from commercial sources and used as

122   received: 2,4,6-trimethylphenol (TMP) [CAS 527-60-6] (EGA Chemie, 99%),

123   sulfamethoxazole   (SMX)   [723-46-6],   sulfadiazine   (SD)   [68-35-9]   (≥99%),   4-

124   carboxybenzophenone (CBBP) [611-95-0] (>99%, all Sigma-Aldrich), *tert*-butanol (t-BuOH)

125   [75-65-0] (≥99.7%), all inorganic chemicals were either from Fluka or Merck. Humic

126   substances: Suwannee River humic acid (SRHA, catalogue number: 2S101H), Suwannee

127   River fulvic acid (SRFA, 2S101F) and Pony Lake fulvic acid (PLFA, 1R109F) were obtained

128   from the International Humic Substances Society (IHSS, St. Paul, MN). Chemicals used for

129   electrochemical analyses are specified elsewhere.[22]

130   **Preparation of solutions.** Aqueous solutions (including ozone stock solutions and HPLC

131   eluents) were prepared using deionized water from Milli-Q (Millipore) or Barnsteadt water

132   purification systems. Organic chemical stock solutions (all 1 mM) and standard DOM stock

133   solutions (100 mg_C L^{-1}) were prepared with buffered water (5 mM phosphate, pH 8). Ozone

134   stock solutions were produced and standardized as described previously.[21]

135   **Ozonation.** DOM solutions (nominal concentration after reagent mixing of 0.83 $mmol_C$ L^{-1} =

136   10 mg_C L^{-1}) and blank solutions (containing no DOM) at pH 7 (all 50 mM phosphate buffer)

137   were ozonated in a series of identical glass reaction vessels (50 or 100 mL, Schott, Germany)

138   in the absence and presence (5 mM) of t-BuOH as a hydroxyl radical scavenger. Aliquots of

139   the ozone stock solution were added to reaction vessels under vigorous mixing at volumes

140   yielding specific ozone doses of 0–1.12 $mmol_{ozone}$ $(mmol_C)^{-1}$. After addition of ozone, the

141   vessels were closed, removed from the stirrer and stored at room temperature (22 °C) for 2h.

142   Subsequently, residual ozone was removed by purging with helium for 20 min. The effect of

143   t-BuOH as a hydroxyl radical scavenger on changes in EDC and optical properties during

144   ozonation was discussed in detail in our previous study.[21]

145   **Irradiation experiments.** A merry-go-round photoreactor system was employed equipped

146   with a medium pressure mercury lamp (Heraeus Noblelight model TQ 718, operated at

147   500W) and a 0.15 M sodium nitrate filter solution that minimizes direct phototransformation

148   reactions. The experimental setup was described in detail previously.[6] Aliquots of ozonated

149   DOM solutions and blanks were supplemented either with only the target compounds (i.e.,

150   SMX, SD and TMP) or additionally with the excited triplet state sensitizer CBBP and diluted

151   to yield final concentrations of 0.19 $mmol_C$ L^{-1} = 2.3 mg_C L^{-1} for DOMs, 5 μM for target

152   compounds and 50 μM for CBBP. The solution pH was adjusted to 7.0 by addition of

153   phosphoric acid (11.5 mM final buffer concentration) prior to irradiation. This pH was

154   chosen to match the pH used in EDC measurements.[21] A 20 mL sample of each solution was

155   filled into capped quartz-glass tubes and irradiated for 5 min (all CBBP – target compound

156   combinations), 100 min (DOMs – TMP), 225 min (DOMs – SMX/SD). These irradiation

157   times were determined based on preliminary kinetic irradiation experiments. During

158   irradiation six aliquots of each 400 μL were withdrawn at equidistant time intervals and

159   analyzed, immediately or stored at 4°C, by high-performance liquid chromatography

160   (HPLC). Details on HPLC equipment and methods employed to quantify the concentration of

161   the target compounds (SMX, SD and TMP) are available elsewhere.[6, 7] To confirm that t-

162   BuOH had no effect on the phototransformation kinetics of the target compounds, control

163   irradiation experiments were conducted with DOM solutions that were ozonated in the

164   absence of t-BuOH but were subsequently amended with 5 mM t-BuOH prior to the

165   irradiations.

166   **Kinetic data analysis.** Pseudo-first-order rate constants for the transformation of the target

167   compounds were determined by linear regression of natural logarithmic concentration data

168   versus irradiation time. These rate constants were submitted to correction depending on the

169   type of experiment, as described in the following. (1) Irradiation experiments with CBBP as

170   the photosensitizer: The correction procedure is described in detail elsewhere.[6] Briefly, in a

171   first step, the rate constant for CBBP-photoinduced transformation was corrected for

172   contributions from other phototransformation pathways (i.e., sensitization by DOM and direct

173   photolysis), as detailed in the Supporting Information (SI), Tables S4–S9. In a second

174   correction step, the light screening by DOM was accounted for (see correction factors in the

175   SI, Tables S1–S3). The obtained corrected rate constants are denoted as $k^{(2)}_{CBBP,DOM,(O_3-dose)}$ (s⁻

176   ¹), the subscript '(O$_3$-dose)' indicating the ozone dose used to treat the DOM prior to the

177   irradiation experiments. (2) Irradiation experiments with untreated and ozonated DOM as the

178   photosensitizer: The rate constants were corrected for light screening following a previously

179   described method[6] (see correction factors in the SI, Tables S1–S3). Subsequently, the

180   contributions from direct phototransformation, which became important in experiments

181   conducted with highly ozonated DOM, were subtracted from the rate constants. The corrected

182   rate constants after these two correction steps are denoted as $k^{(2)}_{DOM,(O_3-dose)}$, in analogy to the

183   terminology used above.

184

185   **Results and Discussion**

186   **Inhibitory effect of untreated or ozonated DOM on CBBP-induced**

187   **phototransformations.** The phototransformation kinetics of TMP, SMX and SD were

188   measured using CBBP as a model photosensitizer and untreated or ozonated DOM as

189   potential inhibitors. CBBP was chosen because its photoexcited triplet state was previously

190   employed to investigate the inhibitory effect of DOM on the triplet-induced oxidation of

191   aquatic contaminants.[5, 6] From the pseudo-first-order rate constants, obtained and corrected as

192   described in the previous section (see SI, Tables S4–S9 for the rate constant values), the

193   inhibition factor, *IF*, was calculated according to equation 1.

194    $$IF = k^{(2)}_{CBBP,DOM,(O_3-dose)} / k^{(2)}_{CBBP}$$ (1)

195 where $k_{CBBP}^{(2)}$ is the corrected rate constant determined for samples without DOM. The results

196 of the present series of experiments are displayed in Figure 1 in terms of *IF* versus the ozone

197 dose applied in the pre-oxidation treatment of each DOM. Note that an *IF* value of unity

198 describes systems in which DOM has no inhibitory effect. *IF* values <1 and >1 signify

199 inhibited and enhanced transformation in the presence of DOM, respectively.

200 We first consider the *IF* of DOM for the transformation of TMP, a commonly used probe

201 compound for excited triplet states in the aquatic environment[19, 26, 27] that is not subject to

202 inhibition by DOM.[5] Inhibition factors for TMP were close to unity and independent of the

203 ozone dose used for DOM pre-treatment (Figure 1a−c), demonstrating that neither the excited

204 triplet state of CBBP (^{3}CBBP*) nor the transformation of TMP were affected by untreated

205 and ozonated DOM. Independent direct evidence that the lifetime of ^{3}CBBP* is not affected

206 by the presence of DOM at concentrations <30 mg_C L^{-1} has been provided in a recent ^{3}CBBP*

207 quenching study.[28]

208 In contrast to the TMP data, *IF* values for SMX and SD were significantly lower than unity in

209 experiments with untreated DOMs (Figure 1d−f), revealing the inhibitory effect of the DOM.

210 The *IF* values increased monotonically − and hence inhibition decreased − with increasing

211 specific O_3 doses for DOM pre-treatment. The differential increase in *IF* values became

212 smaller at higher specific ozone doses. All DOMs showed similar trends with one exception,

213 for which we currently do not have an explanation: the *IF* values of SRHA ozonated in the

214 presence of the hydroxyl radical scavenger t-BuOH exhibited a maximum of *IF*≈0.8 at a

215 specific O_3 dose of ≈0.25 $mmol_{ozone}$ $mmol_C^{-1}$ and subsequently decreased for more extensive

216 ozonation (Figure 1d). The initial *IF* values for untreated SRHA and SRFA were ~0.3,

217 whereas for PLFA they varied between 0.53 (SMX) and 0.7 (SD). These values confirm

218 previous findings[6] showing that allochthonous (terrestrially-derived) aquatic DOMs, such as

219   SRHA or SRFA, are better inhibitors than mostly autochthonous aquatic DOMs such as

220   PLFA. Moreover, for SRFA and PLFA *IF* values appeared to reach a limit of ≈ 1 at high

221   ozone doses (Figure 1e, f), indicating that the inhibitory effect was entirely eliminated. In

222   general, for SMX the increases in *IF* values with an increasing degree of DOM ozonation

223   were more pronounced for ozonation in the presence of t-BuOH than in its absence (Figure

224   1d–f). Furthermore, for SMX the *IF* values slightly exceeded unity for pre-treatment of

225   PLFA with high ozone doses in the presence of t-BuOH. Values larger than unity may have

226   resulted from the formation of DOM moieties with photosensitizing character, which could

227   enhance the phototransformation of SMX. In general, however, for the phototransformation

228   of SMX and SD the increases in *IF* values with increasing specific ozone doses applied in

229   DOM pre-oxidation are consistent with the expectation: Antioxidant moieties of the DOM

230   were increasingly removed at increasing oxidant doses, resulting in decrease of the inhibitory

231   effects of DOM on triplet-induced transformation of SMX and SD.
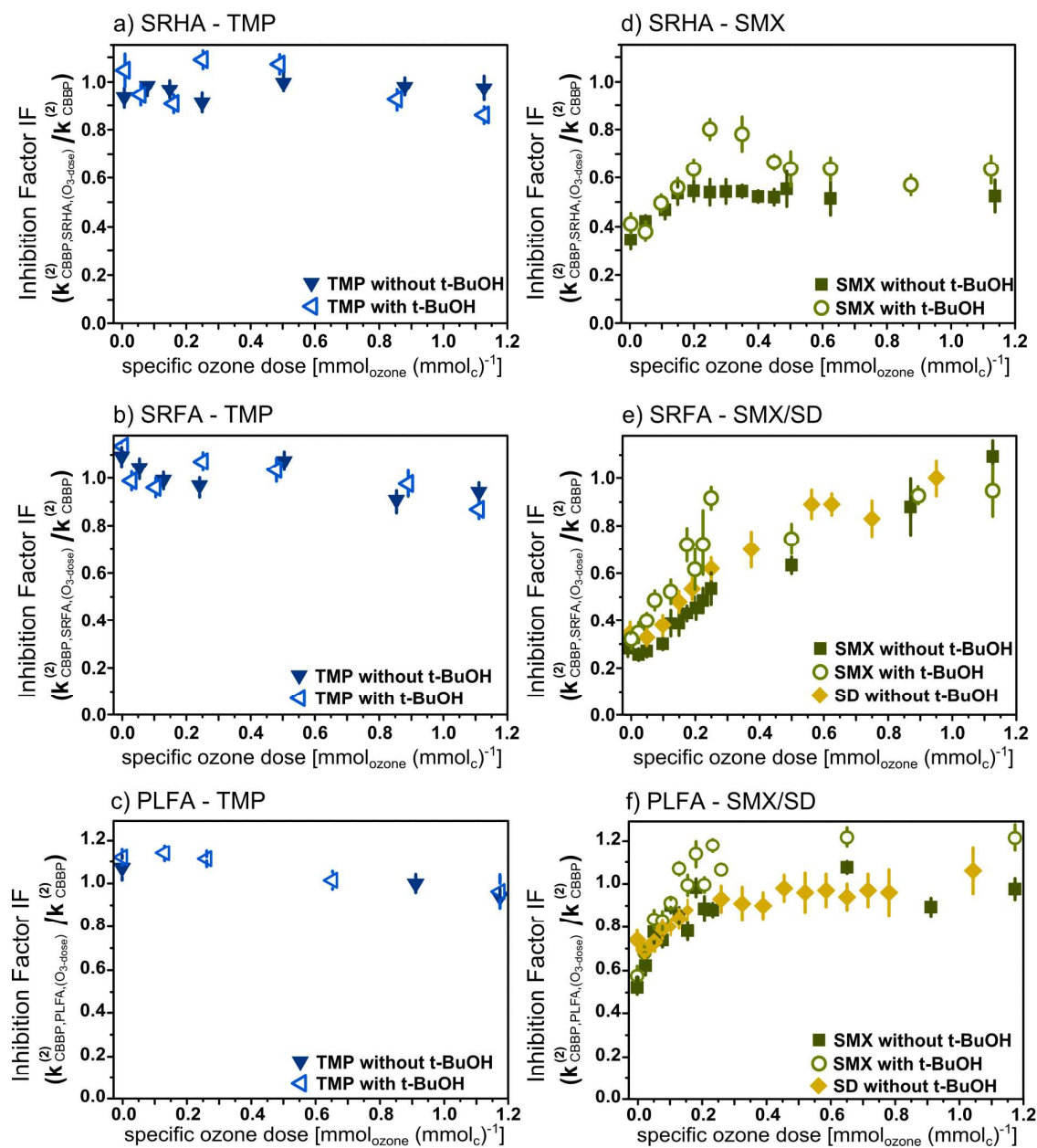
Figure 1. Inhibition factor (*IF*) of dissolved organic matter (DOM, 2.3 mg$_C$ L^{-1}) on the ^{3}CBBP*-induced phototransformation of the target compounds 2,4,6-trimethylphenol (TMP), sulfamethoxazole (SMX) and sulfadiazine (SD) as a function of the specific ozone dose (mmol$_{ozone}$ (mmol$_C$)$^{-1}$). The three standard DOMs Suwannee River humic acid (SRHA), Suwannee River fulvic acid (SRFA) and Pony Lake fulvic acid (PLFA) were investigated. Ozonation was carried out both in the absence and presence of t-BuOH. a–c (left-hand side

12

239 panels): Data for TMP in the presence of the selected DOMs. d–f (right-hand side panels):

240 Data for SMX/SD in the presence of the selected DOMs. Experiments for the combination

241 SD/SRHA were not conducted. Error bars give 95% confidence intervals.

242

243 **Relationship between inhibition factor (*IF*) for CBBP-induced phototransformation and**

244 **electron donating capacity (EDC) of ozonated DOM.** In our previous studies,[6, 7] the *IF*

245 values for the triplet-induced transformation of anilines and sulfonamide antibiotics were

246 shown to be related to the concentration of a specific DOM or model antioxidant (AO). For

247 both SMX and SD, a one-channel reaction model[6] and the corresponding equation (of the

248 type of the following equation 2) were found to satisfactorily fit the data.

249
$$IF([AO]) = \frac{1}{1 + [AO]/[AO]_{1/2}}$$
(2)

250 where $[AO]_{1/2}$ is the concentration of antioxidant needed to slow down the reaction by 50%.

251 While the concentration and type of antioxidant moieties in the untreated and ozonated DOM

252 are not characterized in detail, the electron donating capacity (EDC) is a useful indicator of

253 such moieties[15] and is available from a recent study.[21] In a simple model, we assume the EDC

254 for a given type of DOM, untreated or subjected to ozonation, to be directly proportional to

255 the concentration of antioxidant moieties in the same DOM, as expressed by the

256 proportionality constant κ (equation 3).

257
$$EDC = \kappa \times [AO]$$
(3)

258 Inverting equation 2 and substituting [AO] using equation 3 leads to the following linear

259 relationship between (1/*IF*) and *EDC* (equation 4):

260
$$\frac{1}{IF} = 1 + EDC/EDC_{1/2}$$
(4)

261   with $EDC_{1/2} = \kappa \times [AO]_{1/2}$.

262   Figure 2a displays plots of ($1/IF$) versus EDC values for the tested DOMs and the

263   corresponding linear regression lines (see SI, Table S10 for a collection of fitting

264   parameters). Linear regression fits were performed grouping data for SRFA and PLFA as

265   well as data with and without t-BuOH used in the ozonation pre-treatment of DOM. The

266   SRHA data were fitted separately from the two fulvic acids because of markedly different

267   trends between SRHA the fulvic acids. SMX and SD data were also treated separately, given

268   that $[AO]_{1/2}$ (see equations 2-4) are expected to be compound-specific.[5-7] Data fits were

269   satisfactory in all cases. This is particularly true for the fulvic acid data that yielded high

270   coefficients of determination ($R^2 \approx 0.92$). These high R^2 values suggest that EDC is an

271   adequate descriptor variable of the inhibition efficiency of DOM on the triplet-induced

272   phototransformation of these sulfonamides. For SMX, the slope parameter value (i.e.,

273    $1/EDC_{1/2}$) determined for SRHA was significantly smaller (i.e., by a factor of ≈ 2.8) than that

274   determined for the fulvic acids. This finding implies that a much larger decrease in the EDC

275   of the humic acid than of fulvic acids was required to obtain the same effect on IF, suggesting

276   a more effective inhibition by the antioxidant moieties in the fulvic acids than in SRHA. This

277   conclusion is consistent with the stronger inhibition (i.e., smaller IF) obtained with untreated

278   SRFA than with untreated SRHA (see Figure 1d, e), even though the EDC of SRHA is much

279   larger (by about 80%) than that of SRFA.[15] For SD almost the same slope parameter value

280   was obtained as for SMX, confirming the similarity in the photochemical behavior of these

281   two sulfonamides that has been recently described elsewhere.[29] The ordinate intercepts of the

282   regression lines in Figure 2a are generally below the value of 1 predicted by equation 4 and

283   indicate a deviation of the experimental data from the simple model described above. It is

14

284   possible that the deviation resulted from the presence of antioxidant moieties in the DOMs

285   that contributed to the EDC (measured by MEO) but that were not reactive with triplet-

286   induced transformation intermediates of SD and SMX. Regressions were also performed for

287   the individual data sets (see SI, Figure S1 and Tables S11-S13). The results of these

288   regressions were consistent with those obtained from the regressions of the pooled data but

289   had larger errors in the fitting parameters, reflecting the smaller data sets.

290   As EDC changes were found to be positively correlated with the specific absorption

291   coefficient (SUVA) changes during ozonation of the three DOMs used in this study,[21]

292    $SUVA_{254}$ (the coefficient for the wavelength of 254 nm) was employed as a secondary proxy

293   to predict inhibition of triplet-induced transformations. Corresponding overall correlations

294   and linear regression lines as for the EDC are displayed in Figure 2b. While the trends in

295   Figure 2b are in good qualitative agreement with those shown in Figure 2a (see the preceding

296   discussion), the scattering of the data is larger and the quality of the fits lower (see SI, Table

297   S14). For the individual data sets (see SI, Figure S2 and Tables S15-S17) the larger scattering

298   in the data as compared to the regressions using the EDC is confirmed. Despite the weaker

299   performance of $SUVA_{254}$ than EDC as a predictor of inhibition of triplet-induced

300   transformation, $SUVA_{254}$ might be a useful proxy when EDC data are missing and/or cannot

301   be readily determined.

302
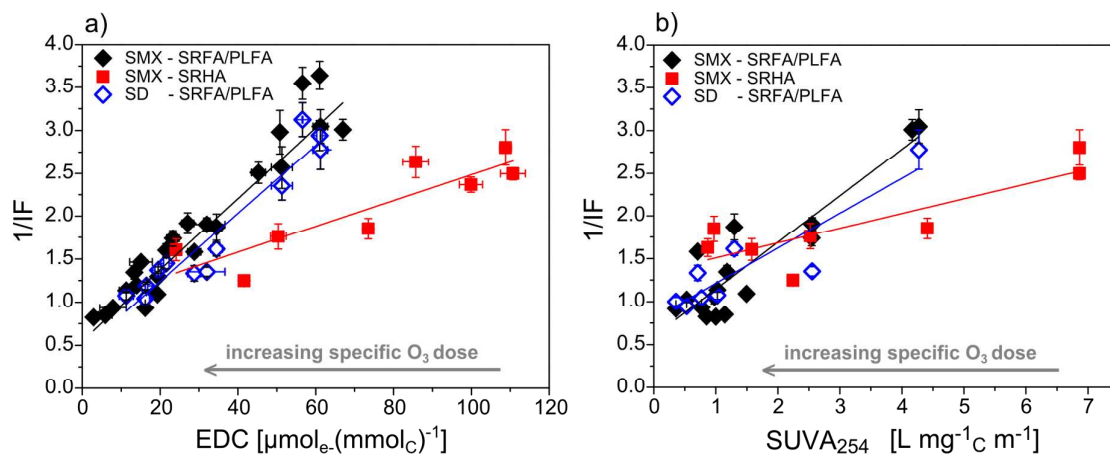
Figure 2. Correlations between the inverse inhibition factor (1/*IF*) and a) the electron donating capacity (*EDC*) and b) the specific absorption coefficient (*SUVA*$_{254}$) of DOMs ozonated in the presence and absence of t-BuOH for the CBBP-induced phototransformation of sulfamethoxazole (SMX) and sulfadiazine (SD). For SMX, separate correlations were performed for the pooled SRFA/PLFA data and for the SRHA data. Horizontal error bars correspond to the standard deviations of two duplicate EDC measurements. Vertical error bars give 95% confidence intervals of single kinetic measurements. 1/IF > 1: Inhibition; 1/IF < 1: Enhanced transformation.

Phototransformation sensitized by ozonated DOM. Figure 3 displays the pseudo-first-order rate constants for the transformation of TMP and SMX photosensitized by increasingly ozonated SRHA, SRFA and PLFA, normalized to the respective rate constants obtained in experiments with the corresponding non-ozonated DOMs, i.e. $k^{(2)}_{DOM,(O_3-dose)} / k^{(2)}_{DOM,(O_3-dose)=0}$ (see the SI, Figures S3−S5 for the raw kinetic data, and Tables S18−S23 for the numerical values of the rate constants and the applied corrections, according to the details given in the *Material and Methods* section). The relative rate constants of TMP transformation decreased with increasing specific ozone doses in a similar manner for all three DOMs (Figure 3a−c).

321　　DOMs treated with high specific ozone doses retained a residual photochemical activity for

322　　TMP transformation (i.e., 7–20% in terms of rate constants with respect to untreated DOM).

323　　This activity was possibly associated with photosensitizing moieties in the DOM that either

324　　reacted slowly with ozone and/or were newly formed during ozonation. Aromatic ketones are

325　　plausible candidates for such photosensitizing moieties resistant to ozone and formed during

326　　ozonation of DOM.[30] In general, the indirect phototransformation rates of TMP were

327　　expected to correlate to DOM light absorption – and therefore to the absorption coefficient of

328　　the DOM – and to the capability of the chromophores to form oxidizing excited triplet states.

329　　We previously reported the specific spectral absorption coefficients a_λ (L mg$_C^{-1}$ m^{-1}) for all

330　　three studied DOMs, both for the untreated and the ozonated materials.[21] We here used the

331　　reported coefficients to calculate wavelength-weighted specific absorption coefficient ratios

332　　$\dfrac{\overline{a}_{DOM,(O_3-dose)}}{\overline{a}_{DOM,(O_3-dose)=0}}$ (Figure 3a–c; calculation details and numerical values are provided in the SI,

333　　Text S1 and Tables S24 and S25). Except for SRHA ozonated in the absence of t-BuOH, the

334　　specific absorption coefficient ratios decreased more strongly with increasing ozone dose

335　　than the relative TMP indirect phototransformation rate constants. This finding is also

336　　evidenced by the non-linear dependence of TMP rate constants versus absorption coefficient

337　　ratios shown in the SI, Figure S6 (panels b and c). It may indicate that chromophores

338　　involved in the indirect phototransformation of TMP were more resistant to ozonation than

339　　the whole ensemble of DOM chromophores absorbing at the considered wavelengths. A

340　　similar finding was recently reported for ozonated wastewaters irradiated with simulated

341　　solar light: the quantum yield of singlet oxygen formation increased with increasing applied

342　　ozone doses.[31]

343　　Compared to TMP, the relative rate constants of SMX (Figure 3d–f) showed very different

344　　dependencies on the specific ozone doses (see also the SI, Figure S6 for an alternative

345   representation of the data). For SRHA and SRFA (both ozonation treatments), and PLFA

346   ozonated in the absence of t-BuOH, at low specific doses (i.e., <0.2 $mmol_{ozone}$ $(mmol_C)^{-1}$) the

347   relative rate constants of SMX were nearly unchanged, at values of approximately 1−1.25. At

348   higher specific ozone doses these relative rate constants tended to decrease, albeit to smaller

349   extents than determined for TMP. The data set for PLFA ozonated in the presence of t-BuOH

350   deserves special consideration as relative SMX phototransformation rate constants reached

351   much higher values than in the absence of t-BuOH. The relative rate constants for PLFA

352   ozonated in the presence of t-BuOH doubled at low specific ozone doses with respect to

353   untreated PLFA, decreased markedly at specific doses larger than 0.2 $mmol_{ozone}$ $(mmol_C)^{-1}$),

354   and finally converged with the values measured for PLFA ozonated in the absence of t-

355   BuOH. The marked increase in the rate constants at low specific ozone doses for the O_3/t-

356   BuOH-treated PLFA correlates well with the observed strong reduction in EDC of treated

357   PLFA[21] and virtual disappearance of any inhibition of CBBP-induced phototransformation of

358   SMX (Figure 1f). In conclusion, the higher relative rate constants for SMX than for TMP

359   phototransformation observed with ozonated DOM may be explained by the partial removal

360   of antioxidant moieties, which weakens the inhibitory effect of DOM on SMX

361   phototransformation.
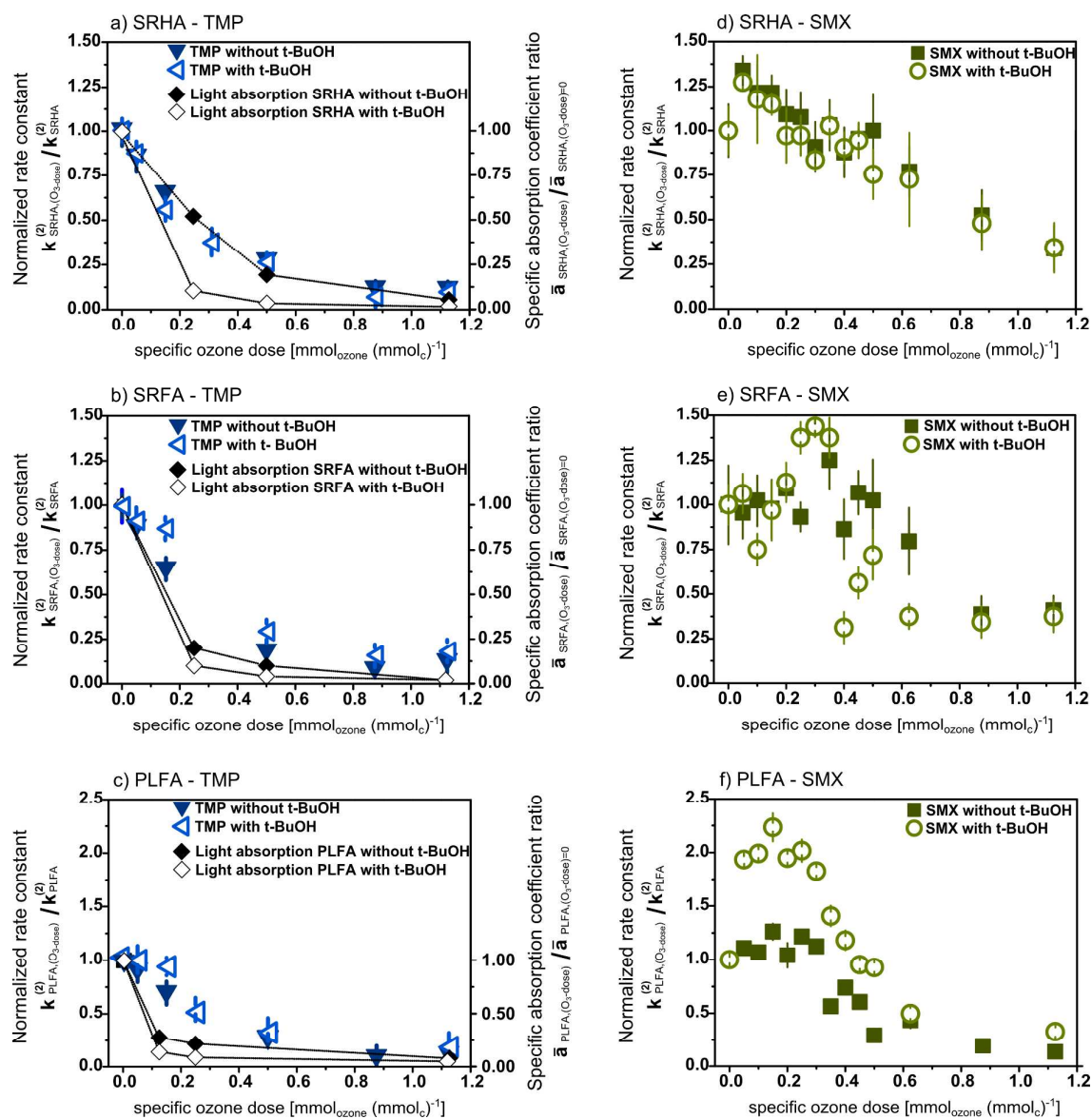
Figure 3. Normalized phototransformation rate constants, $k^{(2)}_{DOM,(O_3-dose)} / k^{(2)}_{DOM,(O_3-dose)=0}$, of

(a–c) 2,4,6-trimethylphenol (TMP) and (d–f) sulfamethoxazole (SMX) sensitized by the three

standard DOMs, Suwannee River humic acid (SRHA), Suwannee River fulvic acid (SRFA)

and Pony Lake fulvic acid (PLFA), as a function of the specific ozone dose applied during

DOM pre-oxidation (in the presence or absence of t-BuOH as a hydroxyl radical scavenger).

The specific absorption coefficient ratios (see text for definition) of the treated DOMs are

369   also shown in (a–c) for comparison (second y-axis). Note the different y-axes scales for

370   PLFA. Error bars indicate 95% confidence intervals.

371

372   In the following we present an attempt to assess exclusively the inhibitory effect of pre-

373   oxidized DOM on the phototransformation of SMX, using the data from Figure 3 and

374   compensating for the changes in photosensitizing activity of the DOM. TMP is assumed to be

375   unaffected by the inhibitory effect of untreated or pre-oxidized DOM, as supported by the

376   results of previous studies and of the experiments with CBBP presented above (Figure 1). Let

377   us further assume that the rate constants for the transformation of SMX photosensitized by

378   untreated and ozonated DOM are directly proportional to the corresponding rate constants for

379   TMP. Then, the ratio of the normalized rate constants for SMX and TMP, as given in Figure

380   4, can be considered as a relative inhibition factor (normalized to the inhibition factor of the

381   untreated DOM). Since the data set for SMX was larger than the one for TMP, the relative

382   rate constants for the phototransformation of TMP were linearly interpolated from its data in

383   Figure 3 to match the specific ozone dose values of the DOM used for SMX

384   phototransformation. Figure 4 shows that treatment of all three DOMs with low specific

385   ozone doses (smaller than ≈ 0.3 mmol$_{ozone}$ (mmol$_C$)$^{-1}$) resulted in a marked monotonic increase

386   in the ratio of the relative rate constants. This trend is qualitatively similar to the one

387   observed for the CBBP photosensitization experiments (Figure 1). The ratio of relative rate

388   constants tended to level off or progress through a maximum for the DOMs treated with

389   higher specific ozone doses. The maximum of the ratios was particularly pronounced for

390   PLFA ozonated in the presence of t-BuOH (Figure 4c). For this data series, the enhancement

391   of SMX phototransformation upon PLFA ozonation reached much higher values (≈ 3.8) than

392   the maximum enhancement factor observed in CBBP experiments (≈ 1.9, Figure 1f). Such an

393   additional enhancement can either be attributed to a stronger inhibition of the DOM

394   antioxidant moieties on $^3DOM^*$-induced than on $^3CBBP^*$-induced transformation of SMX, or

395   to the formation of photosensitizing moieties during DOM pre-treatment with ozone.

396   Carbonyl compounds are one example of such possible photosensitizing moieties, since they

397   are known to be produced by reaction of ozone with unsaturated organic compounds such as

398   olefins and aromatics.[30, 32]

399

400

22
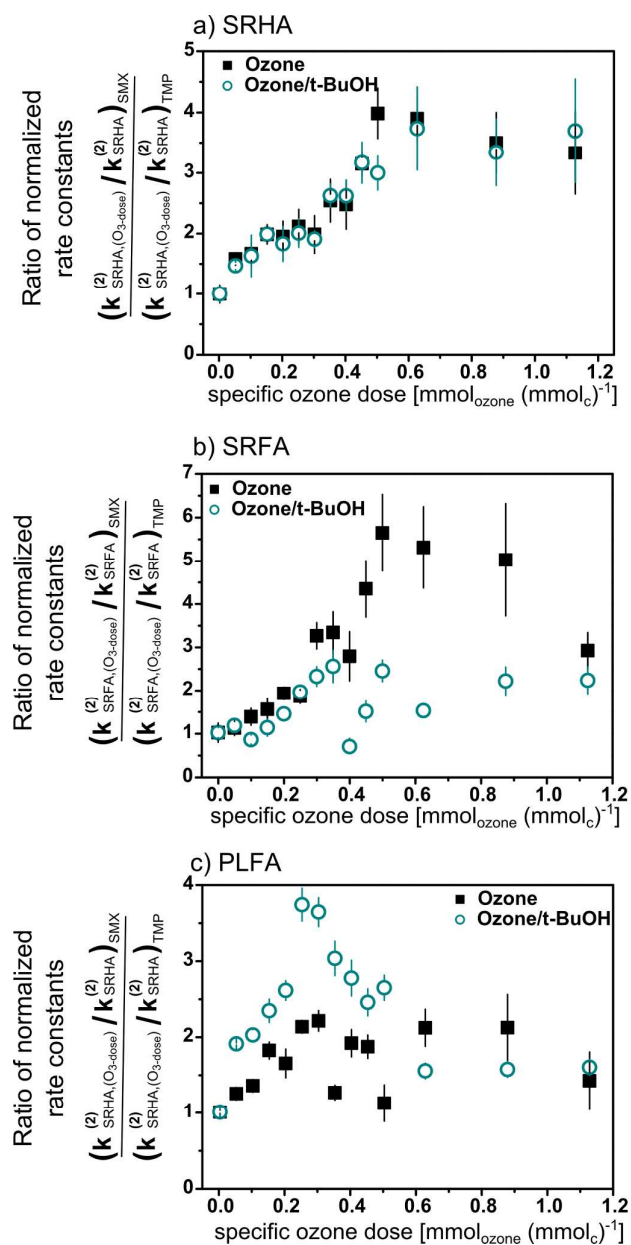
401   **Figure 4**. Ratio of normalized phototransformation rate constants of sulfamethoxazole

402   (SMX) and 2,4,6-trimethylphenol (TMP) sensitized by the three standard DOMs, (a)

403   Suwannee River humic acid (SRHA), (b) Suwannee River fulvic acid (SRFA) and (c) Pony

404   Lake fulvic acid (PLFA), versus the specific ozone dose applied during DOM pre-oxidation.

405   Error bars were calculated using the Gaussian error propagation law from the original rate

406   constants and are shown as 95% confidence intervals.

407

408　**Environmental implications**

409　The results of this study have provided solid evidence that antioxidant moieties of DOM are

410　involved in the inhibition of the triplet-induced transformation of two sulfonamide

411　antibiotics, namely SMX and SD. Considering our previous investigations,[5-7] many other

412　contaminants occurring in surface waters, particularly those exhibiting easily oxidizable

413　aromatic nitrogen moieties, are expected to undergo the same kind of inhibition effect. For

414　the $^3CBBP^*$-induced transformation of SMX and SD, we demonstrated a strong positive

415　correlation between the inverse inhibition factors (expressing the inhibition capacity) and the

416　EDC values of partially oxidized DOM. This correlation suggests that the concentration of

417　electron-donating moieties (measured as EDC×DOC, DOC=dissolved organic carbon) in a

418　natural water is a suitable proxy for its inhibitory effect on triplet-induced oxidations. Such a

419　hypothesis will have to be verified experimentally for a consistent and representative

420　collection of natural water samples.

421　Ozonation is being increasingly applied in advanced wastewater treatment for removal of

422　micropollutants[33-36] and it is one of the main options, besides adsorption to powdered

423　activated carbon, that will be implemented for the advanced treatment of municipal

424　wastewater in Switzerland.[37] The results of the present study allow a qualitative prediction of

425　the impact of ozonation of wastewater on the optical and photochemical properties of the

426　receiving water bodies, provided that a final filtration step would not alter the characteristics

427　of effluent organic matter (EfOM) significantly. Typical specific ozone doses that are applied

428　in wastewater treatment range from 0.5 to 1.0 g_{ozone} $(g_C)^{-1}$ (C measured as DOC; on a molar

429　base these doses correspond to 0.13−0.25 $mmol_{ozone}$ $(mmol_C)^{-1}$). While quantitative data on

430　optical changes in EfOM during wastewater ozonation are available,[38, 39] integrative data sets

431　addressing the optical and photochemical properties as well as EDC properties of EfOM

432   during ozonation are, to the best of our knowledge, missing. In a recent study it was shown

433   that EfOM undergoes similar changes in these properties upon ozonation as the studied fulvic

434   acids.[40] By inspecting the fulvic acid data from our previous paper[21] and from Figure 3 (b, c,

435   e, and f) the following changes after application of a specific ozone dose of 0.25 $mmol_{ozone}$

436   $(mmol_C)^{-1}$ are expected: (1) Absorption coefficients of DOM for the UV-A are reduced by

437   >75%; (2) The rate constants for the phototransformation of TMP (indicative of the

438   photosensitizing efficiency of DOM) are reduced by ≈50%; (3) EDC also decreases by

439   ≈50%; (4) The rate constants for the phototransformation of SMX remain unchanged. Thus,

440   the main effect of wastewater ozonation that may be relevant to phototransformation rate

441   constants of contaminants in receiving surface waters is expected to be de-colorization of

442   EfOM. Correspondingly, there will be an increase in transparency of receiving water bodies

443   that goes along with an increase in direct phototransformation rate constants averaged over

444   the photic zone, as compared to water bodies receiving non-ozonated wastewater. Predicting

445   changes in triplet-induced phototransformation rate constants is more complex: While

446   contaminants behaving like TMP, may show a decrease in rate constants, contaminants

447   behaving like SMX may have higher rate constants and hence experience enhanced

448   phototransformation. More detailed predictions can only be made after knowing the ratio of

449   the released EfOM to the background DOM that is already present in the receiving water

450   body. However, an ultimate quantitative assessment of phototransformation rate constants

451   will require that the specific wastewaters and natural waters are investigated with the

452   methods delineated in this work.

453

454 **Associated content**

455 Additional information as noted in the text. This material is available free of charge via the

456 Internet at http://pubs.acs.org.

457 **Author information**

458 *Corresponding Author:

459 Phone +41-58-765-5453; email: silvio.canonica@eawag.ch.

460 Notes: The authors declare no competing financial interests.

461 **Acknowledgements**

464

465 **References**

466 1. Leenheer, J. A.; Croué, J. P. Characterizing aquatic dissolved organic matter. *Environ.*
467 *Sci. Technol.* **2003,** *37* (1), 18A-26A.

468 2. Zafiriou, O. C.; Joussot-Dubien, J.; Zepp, R. G.; Zika, R. G. Photochemistry of
469 natural waters. *Environ. Sci. Technol.* **1984,** *18* (12), A358-A371.

470 3. Richard, C.; Canonica, S. Aquatic phototransformation of organic contaminants
471 induced by coloured dissolved natural organic matter. In *The Handbook of Environmental*
472 *Chemistry*, Hutzinger, O., Ed. Springer: Berlin, Germany, 2005; Vol. 2, Part M, pp 299-323.

473 4. Sharpless, C. M.; Blough, N. V. The importance of charge-transfer interactions in
474 determining chromophoric dissolved organic matter (CDOM) optical and photochemical
475 properties. *Environ. Sci.-Process Impacts* **2014,** *16* (4), 654-671.

476 5. Canonica, S.; Laubscher, H.-U. Inhibitory effect of dissolved organic matter on
477 triplet-induced oxidation of aquatic contaminants. *Photochem. Photobiol. Sci.* **2008,** *7* (5),
478 547-551.

479 6. Wenk, J.; von Gunten, U.; Canonica, S. Effect of dissolved organic matter on the
480 transformation of contaminants induced by excited triplet states and the hydroxyl radical.
481 *Environ. Sci. Technol.* **2011,** *45* (4), 1334-1340.

482 7. Wenk, J.; Canonica, S. Phenolic antioxidants inhibit the triplet-induced
483 transformation of anilines and sulfonamide antibiotics in aqueous solution. *Environ. Sci.*
484 *Technol.* **2012,** *46* (10), 5455-5462.

485 8. Janssen, E. M. L.; Erickson, P. R.; McNeill, K. Dual roles of dissolved organic matter
486 as sensitizer and quencher in the photooxidation of tryptophan. *Environ. Sci. Technol.* **2014,**
487 *48* (9), 4916-4924.

488 9.   Canonica, S. Oxidation of aquatic organic contaminants induced by excited triplet
489 states. *Chimia* **2007,** *61* (10), 641-644.

490 10.   Zeng, T.; Arnold, W. A. Pesticide photolysis in prairie potholes: Probing
491 photosensitized processes. *Environ. Sci. Technol.* **2013,** *47* (13), 6735-6745.

492 11.   Canonica, S.; Jans, U.; Stemmler, K.; Hoigné, J. Transformation kinetics of phenols in
493 water - Photosensitation by dissolved natural organic matter and aromatic ketones. *Environ.*
494 *Sci. Technol.* **1995,** *29* (7), 1822-1831.

495 12.   Canonica, S.; Hellrung, B.; Wirz, J. Oxidation of phenols by triplet aromatic ketones
496 in aqueous solution. *J. Phys. Chem. A* **2000,** *104* (6), 1226-1232.

497 13.   Gerecke, A. C.; Canonica, S.; Müller, S. R.; Schärer, M.; Schwarzenbach, R. P.
498 Quantification of dissolved natural organic matter (DOM) mediated phototransformation of
499 phenylurea herbicides in lakes. *Environ. Sci. Technol.* **2001,** *35* (19), 3915-3923.

500 14.   Canonica, S.; Hellrung, B.; Müller, P.; Wirz, J. Aqueous oxidation of phenylurea
501 herbicides by triplet aromatic ketones. *Environ. Sci. Technol.* **2006,** *40* (21), 6636-6641.

502 15.   Aeschbacher, M.; Graf, C.; Schwarzenbach, R. P.; Sander, M. Antioxidant Properties
503 of Humic Substances. *Environ. Sci. Technol.* **2012,** *46* (9), 4916-4925.

504 16.   Ma, J.; Del Vecchio, R.; Golanoski, K. S.; Boyle, E. S.; Blough, N. V. Optical
505 properties of humic substances and CDOM: Effects of borohydride reduction. *Environ. Sci.*
506 *Technol.* **2010,** *44* (14), 5395-5402.

507 17.   Del Vecchio, R.; Blough, N. V. On the origin of the optical properties of humic
508 substances. *Environ. Sci. Technol.* **2004,** *38* (14), 3885-3891.

509 18.   Boyle, E. S.; Guerriero, N.; Thiallet, A.; Del Vecchio, R.; Blough, N. V. Optical
510 properties of humic substances and CDOM: Relation to structure. *Environ. Sci. Technol.*
511 **2009,** *43* (7), 2262-2268.

512 19.   Golanoski, K. S.; Fang, S.; Del Vecchio, R.; Blough, N. V. Investigating the
513 mechanism of phenol photooxidation by humic substances. *Environ. Sci. Technol.* **2012,** *46*
514 (7), 3912-3920.

515 20.   Sharpless, C. M. Lifetimes of triplet dissolved natural organic matter (DOM) and the
516 effect of NaBH$_4$ reduction on singlet oxygen quantum yields: Implications for DOM
517 photophysics. *Environ. Sci. Technol.* **2012,** *46* (8), 4466-4473.

518 21.   Wenk, J.; Aeschbacher, M.; Salhi, E.; Canonica, S.; von Gunten, U.; Sander, M.
519 Chemical oxidation of dissolved organic matter by chlorine dioxide, chlorine, and ozone:
520 Effects on its optical and antioxidant properties. *Environ. Sci. Technol.* **2013,** *47* (19), 11147-
521 11156.

522 22.   Aeschbacher, M.; Sander, M.; Schwarzenbach, R. P. Novel electrochemical approach
523 to assess the redox properties of humic substances. *Environ. Sci. Technol.* **2010,** *44* (1), 87-
524 93.

525 23.   Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C.
526 Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free*
527 *Radic. Biol. Med.* **1999,** *26* (9-10), 1231-1237.

528 24.   Pellegrini, N.; Del Rio, D.; Colombi, B.; Bianchi, M.; Brighenti, F. Application of the
529 2,2 '-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation assay to a flow injection
530 system for the evaluation of antioxidant activity of some pure compounds and beverages. *J.*
531 *Agric. Food Chem.* **2003,** *51* (1), 260-264.

532   25.   Ozgen, M.; Reese, R. N.; Tulio, A. Z.; Scheerens, J. C.; Miller, A. R. Modified 2,2-
533   azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant
534   capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP)
535   and 2,2 '-diphenyl-1-picrylhydrazyl (DPPH) methods. *J. Agric. Food Chem.* **2006,** *54* (4),
536   1151-1157.

537   26.   Halladja, S.; Ter Halle, A.; Aguer, J.-P.; Boulkamh, A.; Richard, C. Inhihition of
538   humic substances mediated photooxygenation of furfuryl alcohol by 2,4,6-trimethylphenol.
539   Evidence for reactivity of the phenol with humic triplet excited states. *Environ. Sci. Technol.*
540   **2007,** *41* (17), 6066-6073.

541   27.   Canonica, S.; Freiburghaus, M. Electron-rich phenols for probing the photochemical
542   reactivity of freshwaters. *Environ. Sci. Technol.* **2001,** *35* (4), 690-695.

543   28.   Wenk, J.; Eustis, S. N.; McNeill, K.; Canonica, S. Quenching of excited triplet states
544   by dissolved natural organic matter. *Environ. Sci. Technol.* **2013,** *47* (22), 12802-12810.

545   29.   Bahnmüller, S.; von Gunten, U.; Canonica, S. Sunlight-induced transformation of
546   sulfadiazine and sulfamethoxazole in surface waters and wastewater effluents. *Water Res.*
547   **2014**, under review.

548   30.   von Gunten, U. Ozonation of drinking water: Part I. Oxidation kinetics and product
549   formation. *Water Res.* **2003,** *37* (7), 1443-1467.

550   31.   Mostafa, S.; Rosario-Ortiz, F. L. Singlet oxygen formation from wastewater organic
551   matter. *Environ. Sci. Technol.* **2013,** *47* (15), 8179-8186.

552   32.   von Sonntag, C.; von Gunten, U. *Chemistry of Ozone in Water and Wastewater
553   Treatment - From Basic Principles to Applications*. IWA Publishing: London - New York,
554   2012; p 302.

555   33.   Lee, Y.; von Gunten, U. Oxidative transformation of micropollutants during
556   municipal wastewater treatment: Comparison of kinetic aspects of selective (chlorine,
557   chlorine dioxide, ferrate(VI), and ozone) and non-selective oxidants (hydroxyl radical).
558   *Water Res.* **2010,** *44* (2), 555-566.

559   34.   Hollender, J.; Zimmermann, S. G.; Koepke, S.; Krauss, M.; McArdell, C. S.; Ort, C.;
560   Singer, H.; von Gunten, U.; Siegrist, H. Elimination of Organic Micropollutants in a
561   Municipal Wastewater Treatment Plant Upgraded with a Full-Scale Post-Ozonation Followed
562   by Sand Filtration. *Environ. Sci. Technol.* **2009,** *43* (20), 7862-7869.

563   35.   Zimmermann, S. G.; Wittenwiler, M.; Hollender, J.; Krauss, M.; Ort, C.; Siegrist, H.;
564   von Gunten, U. Kinetic assessment and modeling of an ozonation step for full-scale
565   municipal wastewater treatment: Micropollutant oxidation, by-product formation and
566   disinfection. *Water Res.* **2011,** *45* (2), 605-617.

567   36.   Gerrity, D.; Snyder, S. Review of Ozone for Water Reuse Applications: Toxicity,
568   Regulations, and Trace Organic Contaminant Oxidation. *Ozone Sci. Eng.* **2011,** *33* (4), 253-
569   266.

570   37.   Eggen, R. I. L.; Hollender, J.; Joss, A.; Schärer, M.; Stamm, C. Reducing the
571   discharge of micropollutants in the aquatic environment: The benefits of upgrading
572   wastewater treatment plants. *Environ. Sci. Technol.* **2014,** *48* (14), 7683-7689.

573   38.   Nanaboina, V.; Korshin, G. V. Evolution of Absorbance Spectra of Ozonated
574   Wastewater and Its Relationship with the Degradation of Trace-Level Organic Species.
575   *Environ. Sci. Technol.* **2010,** *44* (16), 6130-6137.

28

576   39.   Gerrity, D.; Gamage, S.; Jones, D.; Korshin, G. V.; Lee, Y.; Pisarenko, A.; Trenholm,
577   R. A.; von Gunten, U.; Wert, E. C.; Snyder, S. A. Development of surrogate correlation
578   models to predict trace organic contaminant oxidation and microbial inactivation during
579   ozonation. *Water Res.* **2012,** *46* (19), 6257-6272.

580   40.   Chon, K.; Salhi, E.; von Gunten, U. Combination of UV absorbance and electron
581   donating capacity to assess degradation of micropollutants and formation of bromate during
582   ozonation   of   wastewater   effluents.   *Water   Res.*   **2015,**   *in   press*.   (DOI:
583   10.1016/j.watres.2015.05.039)

584