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Phenolic antioxidants inhibit the triplet-induced transformation of anilines and sulfonamide antibiotics in aqueous solution

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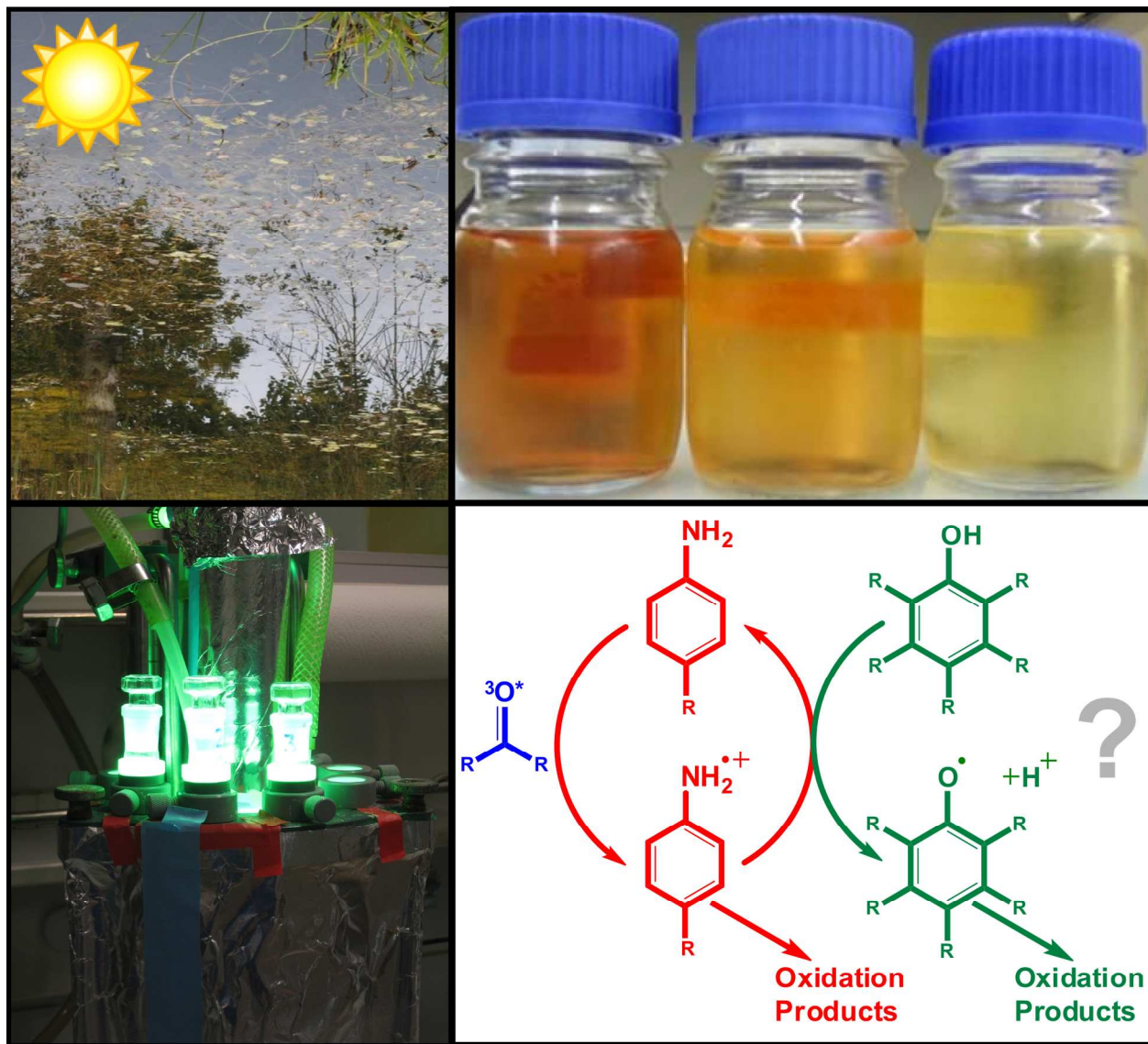
27 **Abstract**

28 Recent studies have shown that dissolved organic matter (DOM) may inhibit the excited triplet-

29 induced oxidation of several aromatic water contaminants, in particular those containing an

30 aniline functionality. Such an inhibition was ascribed to antioxidant moieties of DOM. The

31 present study was conducted with the aim of verifying whether well-defined antioxidants could

32 act as inhibitors in analogy to DOM. Various substituted phenols exhibiting antioxidant character

33 were able, at micromolar concentration, to slow down the photo-induced depletion of several

34 anilines and sulfonamides in aerated aqueous solution containing 2-acetonaphthone as the

35 photosensitizer. A concomitant accelerated degradation of the phenols in the presence of such

36 contaminants was observed. This reinforces the hypothesis of reduction of oxidation

37 intermediates of the contaminants by the phenols. Phenol (unsubstituted) was found to be a useful

38 inhibitor even in the case of DOM-photosensitized transformations. Phenolic antioxidants are

39 proposed as diagnostic tools to investigate the aquatic photochemistry of aromatic amines.

40

Introduction

41

42   Oxidation induced by reaction with photo-generated excited triplet states is important for the

43   fate of contaminants in sunlit surface waters.[1] The source of these excited triplet states is

44   constituted by the chromophoric components of dissolved organic matter (DOM), which are

45   generally the main absorber of sunlight in surface waters.[2] Moreover, DOM is involved as a

46   photosensitizer in other reactions leading to an enhanced transformation of contaminants.[3]

47   However, DOM may also play an opposite role and slow down the sunlight-induced

48   transformation of contaminants by various mechanisms. Besides light screening and scavenging

49   of photooxidants (e.g. hydroxyl radical[4-6] and carbonate radical[7, 8]), DOM has been shown to

50   inhibit the excited triplet-induced transformation of certain aromatic compounds. In two

51   preceding studies[9, 10] we characterized these inhibition reactions by employing two aromatic

52   ketones as excited triplet state precursors and using various types of DOM as inhibitors.

53   Compounds containing amine moieties, in particular the broad class of the anilines, including 4-

54   aminophenyl-sulfonamides that are used as antibiotics (simply referred to as sulfonamides in the

55   following), were significantly affected by inhibition. The observed inhibition generally increased

56   with DOM concentration and could be explained in terms of a one- or two-channel reaction

57   model.[10] DOM of terrestrial origin with high aromaticity was shown to be a better inhibitor than

58   DOM of aquatic origin with low aromaticity. To interpret the findings we proposed that an

59   oxidation intermediate (possibly but not necessarily the radical resulting from one-electron

60   oxidation of the target compound) was reduced by some antioxidant moieties present in the DOM.

61   The occurrence of such moieties is likely because DOM, in particular its humic fraction,

62   possesses a relevant electron donating capacity (EDC)[11, 12] and is even capable to reduce mild

63   oxidants such as ferric citrate and hexacyanoferrate.[13] The chemical complexity of DOM impairs

64   the direct identification of the antioxidant moieties thought to be responsible for the observed

65   inhibition effect. However, probable candidates may be found in the class of phenolic moieties,

66   which are abundant in DOM.[14, 15] Phenols, especially those bearing electron-rich substituents, are

67   known to exhibit antioxidant activity.[16] Phenolic antioxidants (AOs) are either present in nature

68   to prevent oxidative damage to living cells, as in the case of α-tocopherol (vitamin E) and various

69   phenolic compounds produced in plants, or added on purpose to inhibit the degradation of

70   numerous goods such as foods, pharmaceuticals and personal care products, and plastic

71   materials.[17]

72   The utilization of model AOs offers new options to explore the inhibition of triplet-induced

73   oxidation, as illustrated in Scheme 1. The left-hand part of the scheme shows the two alternatives

74   we have used to date to induce oxidations by excited triplet states. Chromophores of either a

75   model aromatic ketone or DOM, both acting as photosensitizers, are promoted by absorption of

76   light to their excited triplet states, which oxidize a contaminant (P) to an intermediate

77   contaminant radical cation ($P^{\bullet+}$, representing here for simplicity a suite of possible oxidation

78   intermediates). The model photosensitizer or DOM are recycled via formation of a radical anion

79   and subsequent reaction with molecular oxygen, which takes up the exceeding electron thus

80   forming the superoxide radical anion ($O_2^{\bullet-}$). The right-hand part of the scheme considers two

81   alternatives for inhibition of oxidation, consisting in the reduction of the oxidation intermediate

82    $P^{\bullet+}$, either by reaction with a model AO or with DOM, yielding back the parent contaminant P.

83   The reduction reaction competes with the formation of oxidation products (P_{Ox}) which cannot

84   react back to give P.

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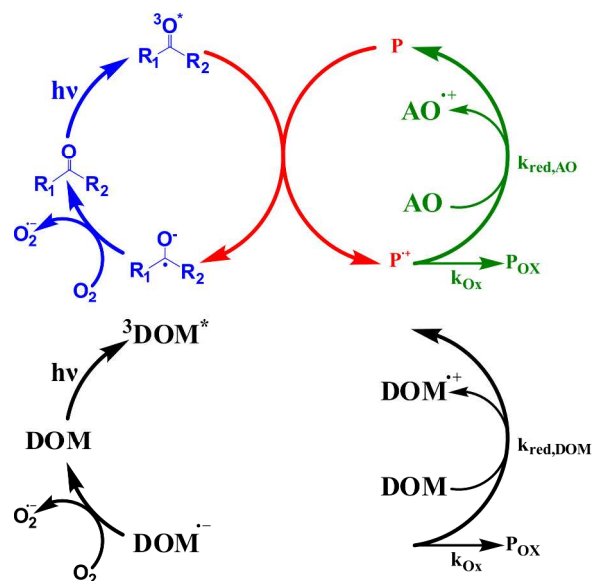
Scheme 1. Conceptual model for the triplet-induced oxidation of a contaminant (P) and subsequent inhibition: Alternative systems with model and natural photosensitizers, and with model and natural antioxidants/inhibitors.

There are four different ways to combine the various photosensitizer and inhibitor reaction elements: (1) A model aromatic ketone is used as the photosensitizer in combination with a model AO (upper part of Scheme 1); (2) DOM is used as the photosensitizer together with a model AO as the inhibitor (black arrows in the lower left part combined with green arrows); (3) A model aromatic ketone is used in combination with DOM as the inhibitor (blue arrows combined with black arrows in the lower right part); (4). DOM is both the photosensitizer and the inhibitor in the oxidation of P (lower part of the scheme, black arrows). This last combination mode represents the situation occurring in a DOM solution or a natural water, but is not suitable for mechanistic investigations, so it is not further considered in this paper. In our previous studies[9, 10] the combination mode (3) was employed. The application of the combination modes (1) and (2) are presented for the first time in this paper.
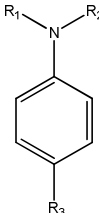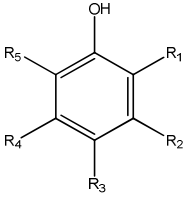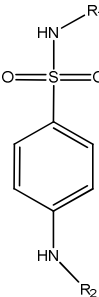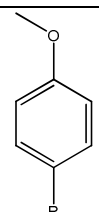
111   The main objective of the present study was to demonstrate that phenolic compounds (as

112   model AOs) are able to inhibit the triplet-induced oxidative transformation of certain

113   contaminants as observed for DOM, thus providing a proof of concept for the proposed

114   mechanistic interpretation. This was achieved by conducting steady-state irradiation experiments

115   of solutions containing a given target contaminant and appropriate combinations of

116   photosensitizers and model AOs. The depletion kinetics of both target contaminant and AO was

117   determined for each single experiment.

118   For a successful implementation, particular attention had to be paid to the selection of the

119   target compounds, the model AOs and the model photosensitizers. As target compounds, several

120   anilines and sulfonamides were chosen, because these classes of compounds have proven to be

121   significantly affected by inhibition of oxidation.[9, 10] Moreover, anilines represent basic functional

122   units of many water contaminants,[18] and sulfonamide antibiotics are frequently detected aquatic

123   contaminants,[19] the photochemical transformation of which is of great interest.[20-23] Little is

124   known about the inhibiting effect of phenols on photoinduced reactions.[24-26] In selecting the

125   model phenolic AOs, the general ability of phenols to undergo direct[27, 28] and indirect[29-34]

126   phototransformation in water was considered. As main phenolic antioxidants to be used in this

127   study phenol and 4-methylphenol were preferred, due to their relative stability against triplet-

128   induced oxidation[32] and the consequent reduction of the risk of interference by side-reactions, but

129   we also extended our selection to include several hydroxybenzoic acids, because they probably

130   better match the chemical structure and reactivity of the phenolic units present in DOM. 2-

131   Acetonaphthone (2AN) was chosen as the model photosensitizer due to the low reactivity of its

132   excited triplet state toward phenols.[35]

133

134 **Table 1. Substances tested in this study**

(a) anilines		
	aniline	ANI
	4-chloroaniline	4CA
	N-methylaniline	NMA
	N,N-dimethylaniline	DMA

(b) sulfonamides		
	sulfadiazine	SD
	sulfathiazole	STZ
	sulfamethoxazole	SMX
	sulfachloropyridazine	SCPD
	acetylsulfadiazine	ASD
	acetylsulfathiazole	ASTZ

(c) anisoles		
	anisole	
	4-methylanisole	4MA

(d) phenols (including hydroxybenzoic acids)		
	phenol	
	4-methylphenol	4MP
	catechol	
	resorcinol	
	L-tyrosine	TYR
	p-coumaric acid	CMA
	3-hydroxybenzoic acid	3HBA
	4-hydroxybenzoic acid	4HBA
	3,4-dihydroxybenzoic acid	3,4DHBA
	2,4-dihydroxybenzoic acid	2,4DHBA
	3,5-dihydroxybenzoic acid	3,5DHBA
	2,3-dihydroxybenzoic acid	2,3DHBA
	5-hydroxy-2-methylbenzoic acid	5H2MBA
phenolic controls	2,4,6-trimethylphenol	TMP
	3,4-dimethoxyphenol	DMOP
	trolox	

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137   **Experimental Section**

138   **Materials and chemicals.** The organic substances used in this study and their abbreviations

139   are shown in Table 1 and more comprehensively in the Supporting Information (SI, Figure S1).

140   Anilines (a) and sulfonamides (b) served as target compounds (TCs) for the photosensitized

141   oxidation experiments. Phenols (d), including hydroxybenzoic acids, were mostly employed as

142   antioxidants (AOs). However, TMP, DMOP and trolox did not serve as AOs but as control TCs.

143   These electron-rich compounds were expected not to undergo inhibition of oxidation[9] and were

144   therefore used as indicators of possible side-reactions. Two anisoles (c) were chosen as non-AO

145   controls. For a complete list of all chemicals used and preparation of solutions see the SI, Text S1.

146   Standard DOMs: Nordic Aquatic Fulvic Acid (NAFA), Pony Lake Fulvic Acid (PLFA), and

147   Suwannee River Fulvic Acid (SRFA) were obtained from the International Humic Substances

148   Society. Freshwater samples (all from Switzerland) were taken from: 1) River Murg, Switzerland

149   upstream (Latitude: 47°34'4.66"N; Longitude: 8°53'41.34"E; [DOM]=1.9 mg C L^{-1}; pH 8.1) and

150   downstream (47°34'43.42"N;   8°53'9.04"E; [DOM]=2.3 mg C L^{-1}, pH 8.1) of Frauenfeld's

151   municipal waste water treatment plant (WWTP); 2) River Thur (47°35'27.26"N; 8°46'19.81"E;

152   [DOM]=2.3 mg C L^{-1}; pH 8.2); 3) Lake Greifensee effluent (47°22'44.20"N; 8°38'47.82"E;

153   [DOM]=3.8 mg C L^{-1}; pH 8.0). Samples were immediately filtered (0.45 μm, cellulose nitrate,

154   Sartorius AG, Goettingen, Germany) and stored in glass bottles at 4°C. Experiments were

155   conducted within four weeks after sample collection.

156   **Irradiation experiments.** Irradiation conditions are described in detail elsewhere.[10] A central

157   requirement for the present study was to avoid quenching of the excited triplet state of 2AN

158   (^{3}2AN*) by the added model AO, to ensure that triplet quenching was not the cause of any

159   decreased transformation rates of TCs. For any AO a maximum second-order quenching rate

160 constant of 3.1×10^9 M^{-1} s^{-1} can be assumed[35], which translates into a first-order quenching

161 constant of 3.1×10^4 s^{-1} for the generally employed AO concentration of 10 μM. Comparing this

162 constant with the deactivation constant of $^3 2AN^*$ in aerated aqueous solution (6.44×10^5 s^{-1})[35],

163 one obtains a maximum quenching contribution of 4.6 % due to the presence of AO. With phenol

164 and 4MP the quenching contribution for 10 μM is even much below this value (0.05 % and

165 0.13 %, respectively), and is still small even at an AO concentration of 500 μM (2.5 % and 6.1 %,

166 respectively). We therefore conclude that quenching by AO in any of the experiments performed

167 in this study was lower than the experimental uncertainty (typically $\approx$15 %) and thus negligible.

168 Irradiation experiments were performed in quartz glass tubes at a temperature of 25.0 ± 0.5 °C.

169 The irradiated solutions (20 mL) were buffered at pH 8.0 (5 mM phosphate) except for the case

170 of the natural waters (which had a pH near 8). The solutions contained various combinations of a

171 TC (5 μM), a sensitizer (either 2AN at 10 − 50 μM, or standard DOMs, [DOM] = 2.5 − 5 mg C

172 L^{-1}, or the DOM of the natural waters), an AO (10 μM for the majority of irradiations and 1 − 750

173 μM for the study of the concentration dependence) and a control compound. For a full overview

174 of all combinations see Text S3 and Table S3 in the SI. Samples of 400 μL were withdrawn from

175 each tube at six equidistant time intervals during irradiation.

176 **Analytical methods.** High-performance liquid chromatography (HPLC) using UV-vis

177 absorbance and fluorescence detection was employed to quantify the concentration of organic

178 compounds over the course of the irradiations. Details on the HPLC equipment and methods, and

179 on pH and spectrophotometric measurements are given in the SI, Text S2, Table S1 and S2.

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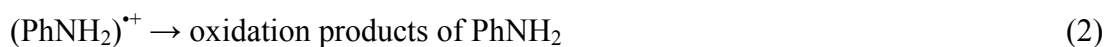41
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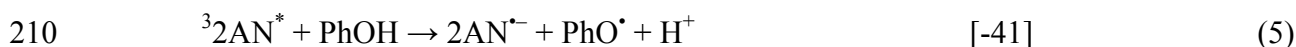51
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183   **Results and Discussion**

184   **Phototransformation of target compounds (TCs) induced by excited triplet 2-**

185   **acetonaphthone (2AN): Inhibition by phenolic antioxidants (AOs).** The depletion kinetics of

186   each of the studied TCs (anilines and sulfonamides) and the AOs was followed in sets of steady-

187   state irradiation experiments as illustrated in Figure 1. The behavior shown for aniline as a TC

188   and phenol as an AO is typical for all studied TC/AO pairs (see SI). Aniline (Figure 1a) is

189   efficiently depleted in the presence of 2AN, and the presence of phenol (10 μM) strongly inhibits

190   the reaction. Moreover there is negligible aniline depletion in the absence of 2AN (with or

191   without phenol present) and no inhibitory action of anisole (used as a negative control for the

192   antioxidant activity). Phenol (Figure 1b) is appreciably depleted only in the presence of both

193   2AN and aniline, while it is not in the absence of one of these components. Summarizing, phenol

194   inhibits the transformation of aniline, which, at the same time, catalyzes the transformation of

195   phenol. Since the quenching of ^{3}2AN* by phenol is negligible (see Experimental Section), only

196   mechanisms involving interaction of phenol with transients formed from aniline transformation

197   may explain the observed behavior. We postulate the following reaction sequence (aniline =

198   PhNH$_2$; phenol = PhOH; $\Delta G°$/(kJ mol^{-1}) in square brackets).

199   $\qquad ^3\text{2AN}^* + \text{PhNH}_2 \rightarrow \text{2AN}^{\bullet-} + (\text{PhNH}_2)^{\bullet+}$ $\qquad\qquad$ [-31] $\qquad\qquad$ (1)

200   $\qquad (\text{PhNH}_2)^{\bullet+} \rightarrow$ oxidation products of PhNH$_2$ $\qquad\qquad\qquad\qquad$ (2)

201   $\qquad (\text{PhNH}_2)^{\bullet+} + \text{PhOH} \rightarrow \text{PhNH}_2 + \text{PhO}^\bullet + \text{H}^+$ $\qquad$ [-11] $\qquad\qquad$ (3)

202   $\qquad \text{PhO}^\bullet \rightarrow$ oxidation products of PhOH $\qquad\qquad\qquad\qquad\qquad$ (4)
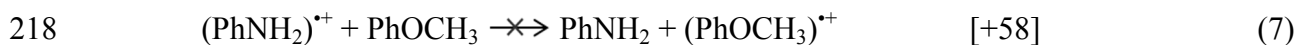
203   Note that reactions 1 and 3 are both exergonic at pH 8.0, since the standard one-electron

204   reduction potential (all the following values in water, vs. NHE) of ^{3}2AN* (1.34 V[36]) is higher

205   than that of (PhNH$_2$)$^{\bullet+}$ (1.02 V[37]), which in turn is higher than that of (PhO$^\bullet$/H$^+$) at pH = 8.0 (0.91

206   V, calculated according to Li and Hoffman[38]). In accordance with this, the reaction of ^{3}2AN* with

207   phenol (eq. 5) is even more exergonic (at pH 8.0) than the reaction of ^{3}2AN* with aniline, but its

208   kinetics is slow, since the rate-determining step is an endergonic electron transfer reaction (eq.

209   6).[35]

210 $$^3\text{2AN}^* + \text{PhOH} \rightarrow \text{2AN}^{\bullet-} + \text{PhO}^\bullet + \text{H}^+ \qquad\qquad [-41] \qquad\qquad (5)$$

211   $$^3\text{2AN}^* + \text{PhOH} \rightarrow \text{2AN}^{\bullet-} + \text{PhOH}^{\bullet+} \qquad\qquad [+15] \qquad\qquad (6)$$

212   We would like to point out that eq. 3 is written in a simplified form, since the aniline radical

213   cation $(\text{PhNH}_2)^{\bullet+}$ can deprotonate yielding the corresponding conjugate base, $(\text{PhNH})^\bullet$. However,

214   taking this acid base equilibrium into account $(pK_a = 7.05)$[39], the reaction is still exergonic and

215   the same conclusions hold. The missing inhibitory effect in the presence of anisole can also be

216   explained based on thermodynamic arguments. For anisole (PhOCH_3), eq. 3 is substituted by eq.

217   7:

218   $$(\text{PhNH}_2)^{\bullet+} + \text{PhOCH}_3 \xrightarrow{\;\;\times\;\;} \text{PhNH}_2 + (\text{PhOCH}_3)^{\bullet+} \qquad [+58] \qquad\qquad (7)$$

219   This reaction is highly endergonic and hence extremely slow, owing to the high reduction

220   potential of $(\text{PhOCH}_3)^{\bullet+}$ (1.62 V[40]). Consequently, $(\text{PhNH}_2)^{\bullet+}$ is not reduced by anisole.

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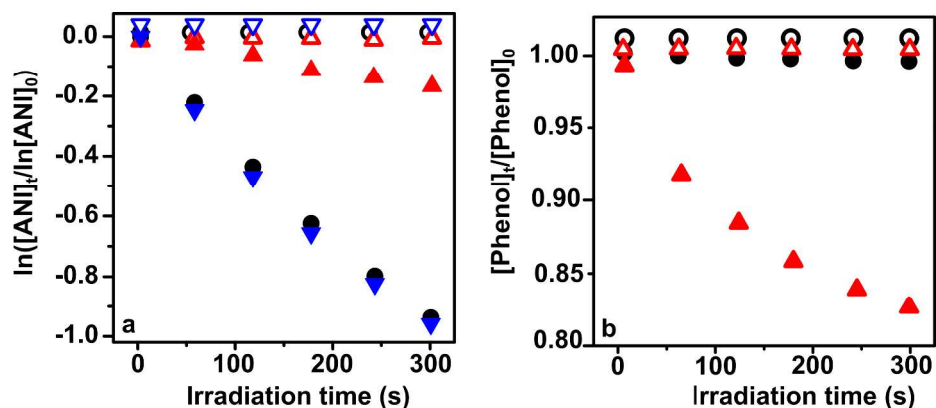
Figure 1. Depletion kinetics of (a) aniline (ANI) (5 μM) and (b) phenol (10 μM) in irradiated

aqueous solutions (pH 8.0), in the presence (closed symbols) and absence (open symbols) of the

photosensitizer 2-acetonaphthone (25 μM). Solution composition: (●, ○) either aniline or phenol,

(▼, ▽) aniline and anisole (10 μM), (▲, △) aniline and phenol.

233

The representative example of the inhibited aniline transformation in the presence of phenol

serves as a proof of concept and indicates that such a system can mimic the inhibition of triplet-

induced oxidation observed in the presence of DOM.[9, 10] Since the mentioned inhibition by DOM

was observed with various target compounds, and since DOM is expected to include a great

variety of phenolic AOs, investigations were extended to consider a series of TCs and a series of

phenolic AOs, comprising some natural compounds. Firstly, for each TC, kinetic experiments as

shown in Figure 1 were performed, using either phenol or 4-methylphenol as the AO (see SI,

Figures S2 – S10). In a second series of experiments (see SI, Figures S11 – S21) the TC (aniline)

was fixed, while different AOs were tested. The results of these experiments are shown in Figure

2. The pseudo-first-order rate constants for the depletion of each TC in the presence, $k_{Sens,AO}$, and

244 absence, k_{Sens}, of a given AO (the subscript *Sens* is used to denote the used photosensitizer) were

245 determined and the inhibition efficiency (IE) was calculated according to eq. 8.

$$IE = 1 - k_{Sens,AO} / k_{Sens} \tag{8}$$

246 Rate constants for the depletion of each AO were also determined.
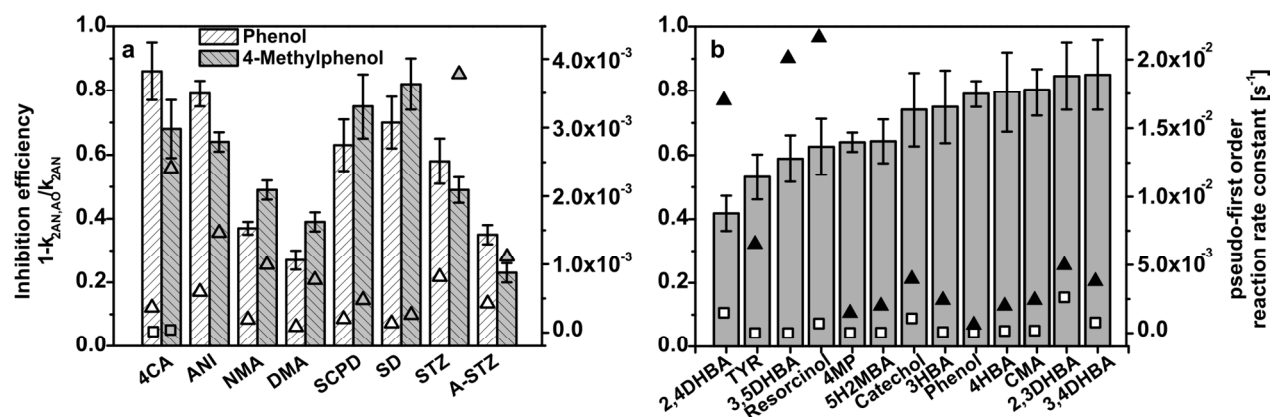
253 **Figure 2.** Inhibition efficiency (bars, left scale) of (a) phenol and 4-methylphenol (4MP) toward

254 triplet-induced oxidation of various target compounds (anilines and sulfonamides), and (b)

255 various AOs toward triplet-induced oxidation of aniline (ANI). Error bars indicate 95%

256 confidence intervals. Symbols represent pseudo-first-order AO depletion rate constants in the

257 presence of the photosensitizer 2AN only (squares, in (a) for clarity of presentation only shown in

258 the first column) and in presence of both 2AN and the respective target compound (triangles).

259

260   The depletion of all selected TCs was inhibited in the presence of 10 μM phenol or 4-

261 methylphenol (Figure 2a), but not in the presence of the negative control compounds, anisole and

262 4-methylanisole. A significant trend for IE is observed for anilines, being highest for 4CA and

263 decreasing from ANI over NMA to DMA. As discussed above, a probable oxidation intermediate

264 of an aniline is the corresponding aniline radical cation, which can react with a phenolic AO to

265 give the corresponding phenoxyl radical under reformation of the aniline. For a given AO, IE is

266   expected to increase with the lifetime of the aniline radical cation and with its reduction potential.

267   While such lifetimes are not known, the trend found for IE corresponds adequately well to that of

268   measured standard one-electron reduction potentials (in aqueous solution) for the radical cations

269   of the tested anilines: 4CA: 1.02 V;[37] ANI: 1.02 V;[37] NMA: 0.95 V;[41] DMA 0.87 V.[42] For

270   sulfonamides a comparison between IE and the reduction potentials is not possible, because the

271   latter are not available. Moreover, the nature of the radical formed upon sulfonamide oxidation is

272   not known. It could be an aniline type radical as well as a radical resulting from the oxidation of

273   the heterocyclic substituent on the sulfonamide group. This is evident when considering the

274   results for the sulfonamide metabolites (see Table 1 (b), R_2=acetyl). While ^{3}2AN* apparently

275   cannot oxidize the acetylated aniline group of ASD, for which no depletion is observed, it leads

276   to a depletion of ASTZ with a rate constant that is only 25% lower than that of STZ (SI Figures

277   S09, S10 and Table S3). We conclude that the thiazole group in ASTZ is the moiety undergoing

278   oxidation by ^{3}2AN*.

279   There is no clear answer to the question whether 4MP is a better oxidation inhibitor than

280   phenol. For half of the tested TCs, 4MP shows a lower IE than phenol (Figure 2a). Generally

281   4MP should be a more effective AO than phenol because the additional *p*-methyl group makes it

282   a better electron donor, which is expressed in a lower standard one-electron reduction potential

283   and oxygen–hydrogen bond dissociation energy.[43, 44] However, in aqueous solution the acid–base

284   speciation of the phenolic AO and of the radical formed during oxidation of the TC are expected

285   to play an important role in the determination of IE, and, in the absence of detailed information

286   on the reaction kinetics of the various species involved, it is impossible to make reasonable

287   predictions about IE of the different AOs.

288   Figure 2b shows that all 13 different phenolic compounds are good inhibitors of the triplet

289   induced oxidation of aniline, IE (bars) ranging from 0.4 (2,4DHBA) to 0.85 (3,4DHBA). As in

290 the case of phenol as an AO, the ^{3}2AN*-induced depletion of all tested AOs is catalyzed by

291 aniline. The corresponding depletion rates are weakly correlated to IE (r^2 = 0.30). One should be

292 aware that, in contrast to phenol and 4MP, many of the used phenolic AOs reacted at significant

293 rates with ^{3}2AN*. Substantial formation of reactive AO intermediates, particularly phenoxyl

294 radicals, and AO transformation products may hinder a detailed understanding of the kinetic

295 results. A further unknown in the studied oxidation reactions concerns the role of the superoxide

296 anion radical, which should be generated by reaction of molecular oxygen with the ketyl radical

297 of 2AN.[1, 32, 35] Superoxide can either add to the radicals resulting from oxidation, leading to

298 oxygenated products, or donate an electron to such radicals leading to their reduction. These

299 reactions may be in mutual competition, as observed for various phenoxyl radicals.[45] Moreover,

300 the depletion of some of the investigated AOs, such as resorcinol, 2,4DHBA, and 3,5DHBA (all

301 of which have two hydroxyl groups in *m*-position to each other) appears to be autocatalyzed,

302 showing that transformation products of these phenolic AOs can lead to complex kinetics.

303 An additional series of experiments was performed using TCs that were expected or

304 previously shown[9] not to undergo inhibition of oxidation in the presence of DOM. The choice

305 was limited to TCs that could be readily oxidized by ^{3}2AN*, and so the electron-rich phenols

306 TMP, Trolox and 3,4DMOP[32, 35] were selected. For all these electron-rich TCs, no inhibition of

307 ^{3}2AN*-induced oxidation was observed using phenol, 4MP and 3HBA as AOs (see SI, Figure

308 S22), which confirms the expectations. We ascribe the absence of inhibition effect for the

309 oxidative transformation of these electron-rich phenols to the low reduction potential of their

310 phenoxyl radicals (<0.50 V vs. NHE)[35], preventing their reduction by the used AOs.

311 **Dependence of the inhibition effect on antioxidant concentration.** As in the case of

312 inhibition by DOM[9, 10], and also following the reaction mechanism proposed above (see eq. 3), an

313 increase of inhibition with AO concentration was expected. To verify this hypothesis, the pseudo-

314 first-order rate constants ($k_{Sens,AO}([AO])$) for the ^{3}2AN*-induced oxidation of four selected TCs,

315 namely aniline, NMA, DMA and SD, was measured at different concentrations of added phenol

316 and 4MP (Figure 3). For both phenolic AOs the rate constants follow qualitatively the expected

317 decrease with increasing [AO] up to [AO] $\cong$ 50 μM. At higher [AO], the decrease continues in

318 the case of phenol, whereas a slight increase is observed for 4MP. A possible explanation of this

319 unanticipated behavior is that 4MP reacts with ^{3}2AN* at higher rates than phenol, contributing to

320 an increased formation of 4MP-derived phenoxyl radicals and transformation products which

321 might increase the depletion of the TC. For a quantitative analysis, the previously developed

322 relationships (eq. 7 combined with eq. 8 from our previous study[10]) were adapted by substitution

323 of [DOM] with [AO] to give eq. 9, which was used for data fitting (the nonlinear curve fit

324 procedure for rational functions as provided by the Origin software version 8.0 (OriginLab) was

325 employed).

$$\frac{k_{Sens,AO}([AO])}{k_{Sens}} = \frac{f}{1+[AO]/[AO]_{1/2}} + (1-f) \tag{9}$$

326 The underlying kinetic model considers two parallel reaction channels for the oxidation of a

327 given TC, one of which (channel 1) undergoes inhibition by AO, while the other channel is not

328 affected by AO. In eq. 9, k_{Sens} is the measured rate constant in the absence of AO, while the

329 fitting parameters f and $[AO]_{1/2}$ represent the yield of the channel 1 reaction in the absence of AO

330 and the concentration of AO needed to halve this yield, respectively.

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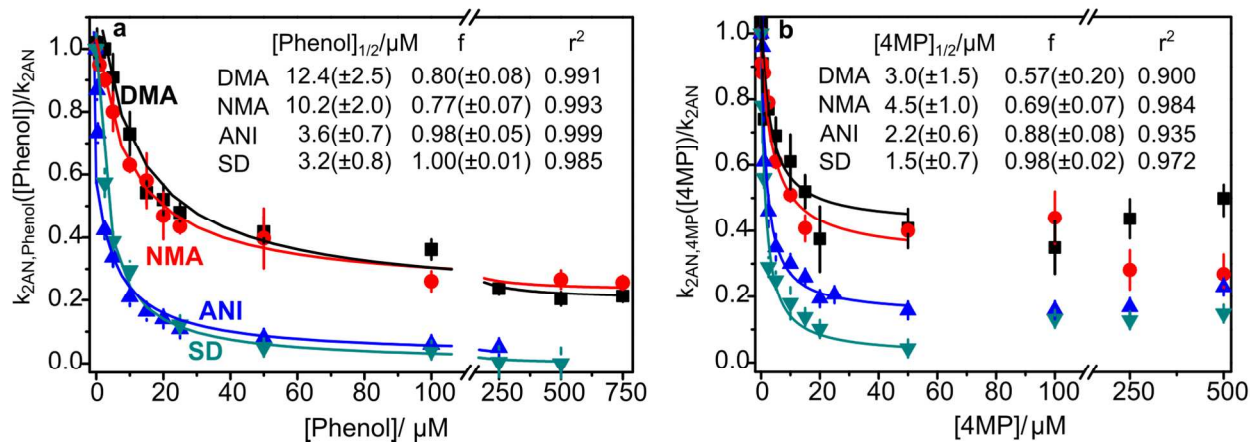
Figure 3. Relative reaction rate constants for the oxidation of the four target compounds *N,N*-dimethylaniline (DMA; ■), N-methylaniline (NMA; ●), aniline (ANI; ▲), and sulfadiazine (SD; ▼) as a function of (a) phenol and (b) 4MP concentration. Curves represent nonlinear fits to equation 9. Inserted tables give numeric results of data fitting. Error bars display 95% confidence intervals.

344

For the reasons given above, fitting of the 4MP data was restricted to the concentration range of $0 - 50$ μM. Overall, eq. 9 fits well to the data, with correlation coefficients that are higher for phenol than for 4MP. The fitted values of $[AO]_{1/2}$ are $2 - 4$ times higher for phenol than for 4MP, which is reflected in the lower steepness of curves for phenol at low $[AO]$. This means that, according to the kinetic model, 4MP is the better antioxidant than phenol. The fitted *f*-values for 4MP are lower than for phenol, meaning that the yield of the non-inhibited reaction pathway $(1 - f)$ is higher. As discussed in the qualitative analysis of the data, this effect might be related to additional reactions following oxidation of 4MP by $^{3}2AN^{*}$. The consistent picture obtained for all 4 TCs, indicating that 4MP is a better inhibitor of $^{3}2AN^{*}$-induced oxidation than phenol, are apparently in contrast to the mixed results of the IE values from Figure 2a. However, one should

355   consider that the IE is affected by *f,* and so the information given by IE is less detailed than the

356   one obtained from the [AO] concentration dependence.

357   The fitted $[AO]_{1/2}$ values increase in the order SD < aniline < NMA < DMA for phenol, while

358   the order of NMA and DMA is reversed for 4MP. For the anilines and phenol as the AO, this

359   order is in line with the decrease in reduction potential of the corresponding aniline radical

360   cations (see above), providing further evidence that these could be the relevant oxidation

361   intermediates involved in the inhibition of oxidation. Considering SD as a substituted aniline, one

362   can roughly predict the reduction potential of its radical cation to be on the order of 1.2 V vs.

363   NHE,[1] perfectly matching the order predicted from $[AO]_{1/2}$.

364   **Phototransformation of target compounds induced by DOM: Inhibition by phenolic**

365   **antioxidants.** This sub-section deals with the application of combination mode 2 (see Scheme 1

366   and the *Introduction*) to obtain information about a possible inhibition effect caused by

367   antioxidants on $^3DOM^*$-induced oxidation of TCs. The implementation of the irradiation

368   experiments is more critical than for the case of a model photosensitizer, since the concentration

369   of DOM has to be kept quite low to avoid excessive inhibition of the reaction by DOM itself,

370   which acts of course both as a photosensitizer and an inhibitor. Owing to the less efficient

371   photosensitizing activity of DOM with respect to model aromatic ketones,[32] required irradiation

372   times to study the depletion kinetics of the TC were found to be at least one order of magnitude

373   higher than for the corresponding experiments using 2AN. Under these conditions, the

374   phototransformation of some TCs, such as aniline and SCPD, in blank solutions (i.e. without

375   DOM) was very important. Consequently, such TCs were considered to be inappropriate for the

376   sake of the present study. Also, under DOM photosensitization and such long reaction times,

377   4MP was depleted rapidly in comparison to the TCs, and was therefore considered to be

378   inappropriate as a model AO in this case. SMX and SD were found to undergo negligible

379   transformation in irradiated blank solutions and were therefore selected. The detailed kinetic data

380   are given in SI, Figures S23-S25, while Figure 4 displays the inhibition, expressed as IE, for the

381   depletion of these two TCs obtained using phenol (10 µM) as the AO and various DOM solutions

382   and natural waters as the photosensitizers.
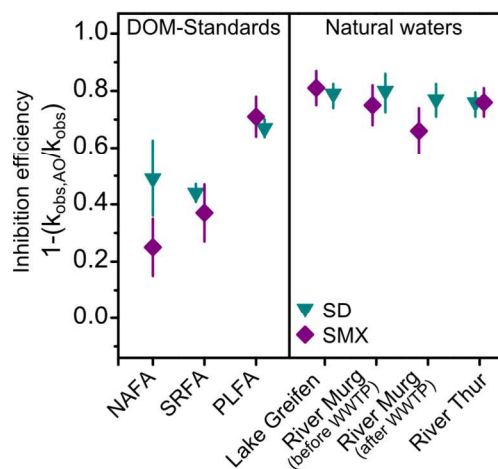
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391   **Figure 4.** Inhibition efficiency of 10 µM phenol on the oxidation of sulfadiazine (SD) and

392   sulfamethoxazole (SMX) irradiated in standard DOM solutions (5 mg C L^{-1}) and natural waters

393   (1.9 – 3.8 mg C L^{-1}). Error bars display 95% confidence intervals.

394

395   The inhibition follows for both TCs the same trend, with IE being significantly lower for the

396   mainly allochthonous fulvic acids than for the mainly autochthonous PLFA and the natural

397   waters. The interpretation appears straightforward, because the allochthonous DOMs are

398   expected to inhibit more efficiently the triplet-induced oxidation than less aromatic DOMs[10] such

399   as PLFA and the organic matter in the used freshwaters, thus partially neutralizing the potential

400   for further inhibition. For PLFA and the natural waters, IE for SD is almost the same as

401   determined with 2AN as a photosensitizer (Figure 2a), suggesting a low concentration of

402   inhibitors (and AOs) in such systems. In contrast to the results obtained using 2AN as the

403   photosensitizer, the depletion of the AO (phenol in this case, see Figure S25 of SI) was already

404   important in the presence of DOM only, and was not significantly accelerated by the presence of

405   TC.

406   **Environmental relevance.** Our results clearly show that phenolic compounds with

407   antioxidant character are capable of inhibiting the excited triplet-induced transformation of

408   several anilines and sulfonamide antibiotics in aerated aqueous solution. Thereby excited triplet

409   state quenching was excluded as a possible cause of inhibition. The effect was observed using a

410   model photosensitizer, the aromatic ketone 2AN, as well as various types of DOM (in dissolved

411   extracts or as present in freshwater samples) as natural photosensitizers. The used phenolic AOs

412   are apparently capable of mimicking DOM as an inhibitor of excited triplet-induced oxidations.[9,]

413   [10] This property of phenolic AOs can be exploited as a diagnostic tool to characterize the

414   degradation pathway of anilines, sulfonamides and possibly other important contaminants in the

415   aquatic environment. For instance, if the indirect phototransformation of a given contaminant is

416   efficiently inhibited by addition of phenol (or another appropriate AO) at a relatively low

417   concentration (<10 μM), this finding would support excited-triplet induced oxidation as a

418   relevant transformation mechanism. Within our research group, this method is currently under

419   development and is being applied for an improved understanding of the aquatic photochemistry

420   of sulfonamides, including those which have been the subject of recent studies.[22, 23]

421   We now address the question whether phenolic AOs are sufficient to explain the inhibition of

422   oxidation that occurs in the presence of DOM. Owing to the complexity of DOM, not only

423   phenolic AO moieties but also a variety of further mechanisms might be responsible for its

424   inhibitive action. The phenolic content of various DOM extracts has been estimated by acid/base

425   titration.[15] For NAFA and SRFA it was found to be ≈ 3 meq (g C)$^{-1}$, implying that 1 mg C

426   corresponds to 3 μmol of phenolic moieties in the DOM. Only a certain part of these moieties is

427   expected to have AO character, thus the value has to be considered as an upper limit for the

428   phenolic AO content. Using the $[DOM]_{1/2}$ values previously determined[10] and converting them to

429   phenolic moiety concentrations, one obtains values of $0.4 - 0.6$ μM and ≈ 6.5 μM for DMA and

430   SMX as TC, respectively (data for 2AN as a photosensitizer). In the case of DMA, the level of

431   DOM phenolic moieties to achieve a 50% inhibition is much lower (by a factor of $5 - 30$) than

432    $[AO]_{1/2}$ determined here (Figure 3) with phenol and 4MP. A possible explanation of such an

433   underestimation of the antioxidant activity of DOM is that the used model AO are less reactive

434   with oxidation intermediates of DMA than the corresponding moieties in DOM. This may well

435   be the case, since NAFA and SRFA possess ≈ 1 meq (g C)$^{-1}$ of electron donating groups that can

436   be oxidized at potentials as low as 0.61 V at pH 8,[12] which is much lower than the potential

437   required to oxidize phenol and 4MP, and also much lower than the redox potential of the DMA

438   radical cation. An analogous comparison between $[DOM]_{1/2}$ for SMX and $[AO]_{1/2}$ for SD shows

439   that they have similar values. Thus, the assumption that AO moieties of DOM are responsible for

440   its inhibition of oxidation appears to be reasonable.

441

Acknowledgements

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Supporting Information Available

449   3 texts, 3 tables and 25 figures are available for further information addressing materials,

450   experimental procedures, and additional data. This information is available free of charge via the

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

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