Sunlight-induced transformation of sulfadiazine and sulfamethoxazole in surface waters and wastewater effluents

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Abstract

Sulfadiazine (SD) and sulfamethoxazole (SMX) are widely used sulfonamide antibiotics, which are present as contaminants in surface waters and are known to undergo phototransformation. This kinetic study was conducted to identify the processes responsible for their phototransformation in sunlit surface waters. Water samples from the Thur River (Switzerland) and from a pilot wastewater treatment plant, as well as aqueous solutions of two well-characterized natural dissolved organic matter (DOM) extracts, namely Suwannee River and Pony Lake fulvic acids (SRFA, PLFA), were examined. Both sulfonamides were found to undergo direct and indirect phototransformation, with contributions of excited triplet states of DOM and of effluent organic matter (EfOM) and possibly of hydroxyl radical and other unidentified reactive species. Under simulated sunlight, SMX mainly reacted through direct phototransformation, with a certain contribution of indirect phototransformation occurring for a wastewater effluent. The behavior of SD was found to be more diverse. For river waters, wastewater effluents and PLFA solutions, indirect phototransformation was predominant, while for SRFA solutions direct phototransformation prevailed. The rates of phototransformation of SD were interpreted as the result of a complex interplay between the photosensitizing and the inhibitory effect of DOM/EfOM, with an additional component related to the nitrite ion as a source of photoproduced hydroxyl radical. For typical conditions found in surface waters comparable to the Thur River, phototransformation half-lives on the order of 3–13 d were estimated for the two studied sulfonamides.

Keywords: Phototransformation; DOM; Excited triplet states; Hydroxyl radical; Sulfonamide antibiotics; Antioxidants.
1. Introduction

Pharmaceuticals and personal care products have been identified as contaminants of concern owing to their ubiquitous occurrence and the risk that they might cause to the aquatic environment (Boxall et al., 2003, Daughton and Ternes, 1999, Kolpin et al., 2002). Among the pharmaceuticals, sulfonamide antibiotics are antibacterial compounds widely used in human and veterinary medicine (Hirsch et al., 1999). Depending on their application, they may enter the aquatic environment through wastewater effluents (Götz et al., 2010), leaching from manure (Sukul et al., 2008), or direct use in aquaculture ponds (Guerard et al., 2009a). Sulfonamide antibiotics, including the two title compounds, are frequently encountered at concentrations of 0.01–2 μg/L in surface waters (Hirsch et al., 1999, Hollender et al., 2009, Kolpin et al., 2002). Besides the possible risks connected to the presence of a low-concentration cocktail of pharmaceuticals (Pomati et al., 2006), the main concern is that bacteria in the environment might develop resistance against these antibiotics in spite of the low exposure levels (Dodd et al., 2009, Dodd, 2012, Gilliver et al., 1999, Witte, 1998).

The fate of sulfonamide antibiotics in the aquatic environment has been the subject of various studies in recent years. Although the capability of certain bacterial strains to degrade sulfonamide antibiotics has been newly demonstrated (Bouju et al., 2012, Zhang et al., 2012), investigations in an aquatic outdoor field microcosm lead to the conclusion that biodegradation was not an important loss process for sulfamethoxazole (SMX) and suggested photodegradation as the main depletion pathway (Lam et al., 2004). Boreen and co-workers characterized the phototransformation of ten sulfonamides containing five-membered (Boreen et al., 2004) and six-membered (Boreen et al., 2005) heterocyclic groups. While compounds of the first class were shown to undergo uniquely direct phototransformation under sunlight, the sulfonamides with the
six-membered substituents were subject to both direct and indirect phototransformation, the latter apparently initiated by excited triplet states of dissolved organic matter (DOM), denoted hereafter as $^3\text{DOM}^*$. The photochemical kinetic behavior of sulfonamide antibiotics is complicated by the acid–base speciation of these compounds, which exhibit two pK$_a$s, typically in the range of $\approx 1.5$–3 and $\approx 5$–8, respectively (Boreen et al., 2004, 2005). The direct phototransformation kinetics could be described satisfactorily by considering the distinct species involved (protonated, neutral, anionic) and assigning them a constant quantum yield (Boreen et al., 2004, 2005), a method which was also applied to describe UVC-induced phototransformation of SMX (Canonica et al., 2008). At neutral pH, direct phototransformation half-lives (for mid-latitude, mid-summer, surface of water body conditions) were on the order of $\approx 3$–30 h, which should translate into half-lives of a few days to weeks if one considers the attenuation of light in a water body, daily and seasonal cycles, and meteorological variability of solar irradiance (Zepp and Cline, 1977). For SMX, an average half-life of 19 d was found in the mentioned microcosm study (Lam et al., 2004), compatible with the just mentioned rough estimation. The indirect phototransformation was studied in more detail for SMX (Lam and Mabury, 2005), sulfadimethoxine (Guerard et al., 2009a, Guerard et al., 2009b) and sulfadiazine (SD) (Sukul et al., 2008). The transformation of sulfadimethoxine induced by simulated sunlight was found to be accelerated in the presence of autochthonous (phytoplankton-derived) DOM, while allochthonous (from soil runoff) DOM did not enhance the transformation (Guerard et al., 2009a, Guerard et al., 2009b). The study on SD also showed an accelerating effect on the phototransformation rate induced by fulvic and humic acid. By contrast, the phototransformation of SMX was slowed down by DOM, which was attributed to the light screening effect. A recent study (Ryan et al., 2011) showed that SMX may indeed undergo indirect phototransformation in wastewater effluent,
and excited triplet states of effluent organic matter (EfOM), denoted hereafter as $^{3}\text{EfOM}^*$, as well as the hydroxyl radical were proposed to be the key species involved.

Besides the just mentioned investigations, the inhibiting effects of DOM on triplet-induced phototransformation of aquatic contaminants have to be considered as well (Canonica and Laubscher, 2008, Wenk et al., 2011, Wenk and Canonica, 2012). Along with several other contaminants and model aromatic compounds, sulfonamides were shown to be importantly affected by such an inhibition. Allochthonous DOM was found to be a better inhibitor than autochthonous DOM, meaning that indirect phototransformation rates were lower in the presence of allochthonous DOM, in striking coincidence with the results by Guerard and coworkers on sulfadimethoxine (Guerard et al., 2009a, Guerard et al., 2009b). To date such an inhibiting effect of DOM on the indirect phototransformation of sulfonamides, induced by the chromophoric components of DOM itself, has only been studied under laboratory conditions using the UV-A and visible emission of a medium-pressure mercury lamp (Wenk and Canonica, 2012). An important result of the latter study was that phenolic antioxidants were successfully used to mimic the inhibiting effect caused by DOM, and phenol was able to inhibit the transformation of SMX and SD initiated by $^{3}\text{DOM}^*$. However, the validity of such a method still has to be demonstrated for the case of irradiation using (simulated) sunlight.

The present study was conceived to identify the various factors that affect the rates of sunlight-induced transformation of sulfonamide antibiotics in surface freshwaters, with a particular focus on DOM and EfOM. SMX and SD were selected as representative compounds of the mentioned two categories of sulfonamides identified by Boreen and coworkers (Boreen et al., 2004, 2005).

In the pH range investigated in this study (7.9–8.7), no influence of pH on transformation rates of both sulfonamide antibiotics is expected, since both compounds are present almost exclusively
 (>96%) in their anionic form (pK_a,2 = 5.7 for SMX and 6.4 for SD (Boreen et al., 2004, 2005)).

The photochemical transformation kinetics of SMX and SD was investigated under simulated sunlight for solutions of two well-characterized fulvic acids, in surface waters and wastewaters, and in a number of water samples taken along the course of the Thur River (North-East Switzerland). Additional mechanistic tests were performed by adding, to the irradiation experiments, isopropanol and phenol to scavenge hydroxyl radical and oxidation intermediates of the sulfonamides, respectively.

2. Experimental Section

2.1. Chemicals

The organic compounds sulfadiazine (SD, Sigma-Aldrich, >99%), sulfamethoxazole (SMX, Sigma-Aldrich >99%), 2,4,6-trimethylphenol (TMP, EGA-Chemie, 99%), isopropanol (Sigma-Aldrich, >99%) and phenol (Fluka, ≥99.5%) were used without further purification. All inorganic chemicals were analytical grade from common commercial sources. Ultrapure water was produced by the “barnstead nanopure” water purification system from Thermo Scientific. Stock solutions of SD, SMX and TMP were made in ultrapure water at pH 8 with 5 mM phosphate (Na_2HPO_4/NaH_2PO_4). Pony Lake fulvic acid (PLFA) and Suwannee River fulvic acid (SRFA) were used as standard DOMs and obtained from the International Humic Substances Society. Stock solutions of standard DOMs were made in ultrapure water with an approximate concentration of 100 mgC L^{-1}, the exact DOM concentration was measured after preparation of the stock solutions.
2.2. River water and wastewater effluent samples

River water samples were taken from the Thur River, a tributary of the Rhine River located in North-East Switzerland. The Thur River valley has no natural or artificial lake in its course. The annual average discharge (1904–2011) is about 47 m³ s⁻¹. The catchment is characterized by its rural environment and agricultural activity. If not further specified, Thur River water samples were taken at a single sampling point near Niederneunforn (47° 35′ 60″ N, 8° 46′ 60″ E). A detailed description of the field site is given elsewhere (Diem et al., 2013, Schneider et al., 2011). Sampling points of a one-day field campaign along the course of the Thur River are described in detail in the Supplementary Information (SI), Text S1. Wastewater effluent was taken from a wastewater pilot plant of Eawag, receiving municipal wastewater of the city of Dübendorf, Switzerland (mixed household and industrial origin). Selected water quality parameters of the sampled river waters and wastewaters, which are relevant for the phototransformation studies, are given in Table 1. All sampled river waters and wastewaters were directly filtered under vacuum by using pre-rinsed cellulose nitrate membrane filters of 0.45 μm pore size. The filtered samples were stored in glass bottles in the dark at 4 °C. A fraction of the filtered wastewater sample A1 was treated by electrodialysis (PCCell GmbH, Heusweiler, Germany) to remove salts, in particular nitrate and nitrite, and the filtrate is termed as wastewater A2 (Table 1). More water quality parameters of river and wastewater samples, and river water samples of the one-day field campaign are listed in the SI, Tables S1 and S2, respectively. Note that in all used water samples iron was below the limit of detection (< 10 μg L⁻¹) as measured by ICP-OES.
2.3. Irradiation experiments

Steady state irradiations were performed in glass-stoppered quartz tubes (external diameter 18 mm, internal diameter 15 mm) using primarily a solar simulator (Heraeus model Suntest CPS+). In brief, the sample tubes were exposed to the simulated solar light in a temperature-controlled ultrapure water bath (25.0±0.5 °C) located under the solar simulator. A detailed description of the experimental set up is given elsewhere (Huntscha et al., 2008). Alternatively, a DEMA 125 merry-go-round photoreactor (Hans Mangels GmbH, Bornheim-Roisdorf, Germany) equipped with a Heraeus Noblelight TQ 718 medium-pressure mercury lamp (MP Hg lamp) was employed. It was operated with a borosilicate glass cooling jacket and a 0.15 M sodium nitrate filter solution (permitting only transmission of wavelengths, $\lambda > 320$ nm) (Wegelin et al., 1994), a typical configuration used to study photosensitized reactions and reduce the contribution of direct phototransformation to the total photoinduced transformation (Canonica et al., 1995). For both irradiation setups, light screening effects caused by absorption of light in the irradiated samples, in particular by the DOM or EfOM present in the aqueous solutions and real water samples, were neglected because smaller than the typical relative experimental error of ~15% for the determination of phototransformation rate constants. For the solar simulator, they were estimated to be <7.6% for SRFA solutions and <5.0% for all other waters, based on the absorption coefficients of these samples at 310 nm, while for the merry-go-round photoreactor they were even lower (calculations performed using the method described in Wenk et al. (2011)).

During irradiation experiments, 400 µL samples were withdrawn from each tube at six equidistant time intervals during irradiation. With the solar simulator, water samples containing SD, SMX and TMP were irradiated for a total time of 6, 8 and 2 hours, respectively. With the merry-go-round photoreactor, overall irradiation times were 2 hours, 4 hours and 18 min,
respectively. Solutions containing standard DOM were buffered at pH 8.0 with 5 mM phosphate (Na$_2$HPO$_4$/NaH$_2$PO$_4$). The pH of filtered river water and wastewater samples was in the range of 7.5–8.5 (Table 1) and was not modified for the experiments. Each tube was filled with 16 mL either with ultrapure water, river water, wastewater or standard DOM and spiked with an appropriate amount of stock solution of a single target compound (SD, SMX or TMP) yielding a concentration of 1–5 μM. To assess the photosensitizing capacity of DOM, TMP was used as a reference compound since the phototransformation of TMP is not inhibited by DOM (Canonica and Laubscher, 2008). Isopropanol (10 mM) was used as a hydroxyl radical scavenger. Phenol (10 μM) was applied as a model compound to investigate the triplet-induced transformation of SD and SMX and to gain information about the antioxidant or inhibitive potential of DOM/EfOM in the water samples.

Actinometry was performed using an aqueous solution containing p-nitroanisol (10 μM) and pyridine (600 μM) as described elsewhere (Dulin and Mill, 1982, Kari et al., 1995). The photon fluence rate in the range of 300–400 nm was calculated according to the method described in Canonica et al. (2008), using a wavelength-independent quantum yield of 0.000544 mol einstein$^{-1}$ for the depletion of p-nitroanisol and spectral data that are given in the SI (Text S2, Table S3).

2.4. Analyses

High-performance liquid chromatography (HPLC) using UV absorbance and fluorescence detection was employed to quantify the concentration of SD, SMX and TMP over the course of the irradiations. Both an Agilent 1100 HPLC system equipped with a diode array detector and a fluorescence detector or an equivalent Dionex Ultimate 3000 HPLC system were employed. Compounds were analyzed with isocratic programs at a flow rate of 1 mL min$^{-1}$ on a reverse-
phase column (Nucleosil C18-5 μm, 125 x 3 mm Macherey-Nagel, Düren, Germany) at room
temperature. The mobile phase consisted of a mixture of acetonitrile (ACN)/10mM phosphoric
acid (pH 2.1) and was run at a proportion of 10/90 for SD, 20/80 for SMX and 50/50 for TMP,
respectively. SD and SMX were detected by UV absorption at a wavelength of 270 nm, and TMP
was detected by fluorescence at an excitation wavelength of 225 nm and an emission wavelength
of 316 nm. Pseudo-first-order depletion rate constants of the target compounds, termed in the
following as phototransformation rate constants ($k_p$), were obtained by linear regression of the
logarithmic concentration values at each sampling time.

DOM concentrations of all filtered (0.45 μm) water samples and fulvic acid solutions were
measured as dissolved organic carbon (DOC) using a Shimadzu model TOC-V CPH analyzer
(Shimadzu Corporation, Kyoto, Japan). Carbonate/bicarbonate concentrations were measured as
alkalinity by titration with 0.1 M hydrochloric acid (endpoint pH 4.5) using a Titrando 809
potentiometric titrator (Metrohm, Zofingen, Switzerland). Nitrite was analyzed colorimetrically
by addition of a reagent composed of aminobenzenesulphonamide and N-(1-Naphthyl)-
ethylendiaminhydrochloride. The absorbance of the resulting red azo dye was measured at 543
nm using a Cary 100 spectrophotometer (Varian AG, Zug, Switzerland). Ammonium
concentrations were measured based on the reaction of ammonium with hypochlorite under
alkaline conditions to give chloramine. This further reacts with salicylic acid to a blue dye
complex (dichlorophenolindophenol), whose absorbance is measured at 690 nm. Nitrate
concentrations were measured with a Metrohm model 761 Compact ion chromatograph
(Metrohm Schweiz AG, Zofingen, Switzerland).
3. Results and Discussion

3.1. Phototransformation rate constants of sulfadiazine and sulfamethoxazole in various waters and aqueous solutions

3.1.1. Irradiation with simulated sunlight (λ > 290 nm)

The UV photon fluence rate (determined for the wavelength range of 300–400 nm) in the experiments performed in the solar simulator was ≈135 μeinsteins m$^{-2}$ s$^{-1}$ (±15%), corresponding to a half-life of p-nitroanisol of ≈4.5 h. This value compares well with previous studies (Dulin and Mill, 1982) for irradiations under genuine sunlight and indicates an irradiation intensity equivalent to the strength of sunlight between summer and autumn at 40° N latitude on a cloud-free day. The results of simulated sunlight-induced phototransformation rate constants of SD and SMX for various water samples and aqueous solutions are displayed in Figure 1.

(Figure 1)

Figure 1. Solar simulator experiments (λ > 290 nm): Phototransformation rate constants of (a) SD and (b) SMX (initial concentration: 1 μM for SD and 5 μM for SMX) in selected water samples in the presence and absence of scavengers (isopropanol: hydroxyl radical scavenger; phenol: inhibitor for triplet-induced oxidation). The DOM concentration is given in brackets as mgC L$^{-1}$. Error bars indicate 95% confidence intervals obtained from linear regression.

For SD (Figure 1a) phototransformation rate constants for all water samples and DOM solutions, in the presence and absence of scavengers, are significantly higher than for ultrapure water solution, indicating that indirect phototransformation is likely to be a significant process. In the
absence of scavengers, the phototransformation rate constants for spring water (0.09 h\(^{-1}\)) and river water A (0.13 h\(^{-1}\)) are enhanced by a factor of 2.4 and 3.5, respectively, compared to the rate obtained for ultrapure water (0.037 h\(^{-1}\)), which is assumed to correspond to the rate constant for direct phototransformation. Interestingly, the phototransformation rate constants in DOM-containing waters do not seem to strongly depend on the DOM concentration. Wastewater B exhibits the highest rate (0.22 h\(^{-1}\)), although its DOM concentration is in the same range as for the other wastewaters. The increased rate is possibly related to the high nitrite concentration of this wastewater (Table 1, see also discussion below). Regarding the solutions of the two DOM extracts, PLFA shows a rate similar to those for the river waters, while the rate for SRFA is clearly lower, but still higher than the one for ultrapure water.

To identify the factors controlling the phototransformation rate constants of SD, experiments using isopropanol as a scavenger of the hydroxyl radical and phenol as an inhibitor of the triplet-induced transformation of sulfonamides were performed. The addition of 10 mM isopropanol (second-order rate constant for the reaction with the hydroxyl radical: \(k_{\text{OH, isopropanol}} = 1.9 \times 10^9 \text{ M}^{-1}\text{s}^{-1}\) (Buxton et al., 1988)) results in an increment of the (first-order) hydroxyl radical scavenging rate constant by 1.9\(\times\)10\(^7\) s\(^{-1}\). Using the total scavenging rate constants of hydroxyl radical given in Table S4 (0.8–3.1\(\times\)10\(^5\) s\(^{-1}\)), we calculated that isopropanol scavenged 98.4–99.6% of the hydroxyl radical for the waters considered in this study. Isopropanol addition significantly affected only the phototransformation rate constant for wastewater B, causing a 36% decrease. This is an indication that the hydroxyl radical, which is generated by photolysis of nitrate, nitrite and other water components, may be an important photooxidant initiating the transformation of SD in this wastewater (see below). With the addition of 10 \(\mu\)M phenol two effects are anticipated: First, triplet-induced oxidation of SD is expected to be reduced at an extent varying upon
DOM/EfOM composition (Wenk and Canonica, 2012); second, phenol is also a hydroxyl radical scavenger ($k_{\text{OH, phenol}} = 1.3 \times 10^{10}$ M$^{-1}$s$^{-1}$, average of three values from (Buxton et al., 1988)), resulting in an increase of the hydroxyl radical scavenging rate constant by $1.3 \times 10^5$ s$^{-1}$. Thus, for the studied waters a significant fraction (46%–62%) of the hydroxyl radical is scavenged in the presence of 10 μM phenol. Figure 1a shows that the presence of phenol significantly reduced the phototransformation rate constants of SD for all waters, though at a variable extent. The reduction for the spring water and river water was similar to the sample containing PLFA. With the assumption that indirect phototransformation rate constants can be calculated by subtraction of the rate obtained for ultrapure water from the overall rate, one obtains reductions in indirect phototransformation of about 60% (±6%) upon phenol addition, very much in line with the previous observations with PLFA and several surface water samples irradiated using the merry-go-round photoreactor (λ > 320 nm) (Wenk and Canonica, 2012). Such a strong reduction was attributed to the relatively low concentration of antioxidant moieties in PLFA and the studied surface waters. By contrast, the reduction of phototransformation rate constant upon phenol addition was minor for the SRFA solution and the wastewaters A1 and A2, probably indicating a higher concentration of antioxidant moieties in these waters. For wastewater B the rate constant reduction upon phenol addition was quite substantial and larger than the reduction obtained upon isopropanol addition (see above). Therefore, both inhibition of triplet-induced oxidation and hydroxyl radical scavenging appear to be responsible for the observed reduction of the rate constant. Finally, it has to be noted that even for ultrapure water phenol addition causes a slight reduction of the phototransformation rate constant, possibly indicating that direct phototransformation involves reaction intermediates that may be reduced by antioxidants such as phenol.
Figure 1b displays the phototransformation rate constants of SMX obtained for various water samples under simulated sunlight. Except for wastewater C, the rates are essentially constant and unaffected by the presence of isopropanol or phenol, indicating that the depletion of SMX is dominated by direct phototransformation. However, in wastewater C a significantly higher phototransformation rate constant was observed, which was reduced by the addition of isopropanol or phenol to the values typical for direct phototransformation. The enhanced depletion of SMX in wastewater C is possibly caused by the presence of photogenerated hydroxyl radical or by excited triplet states of the EfOM, as proposed elsewhere (Ryan et al., 2011). For a further discussion of the role of the hydroxyl radical, see below.

3.1.2. Irradiation with UV-A and visible light (λ>320 nm)

The results of irradiation experiments performed with the merry-go-round photoreactor (Figure 2) confirm on the one hand some trends observed for SD under simulated sunlight but yield on the other hand a completely new picture concerning the phototransformation of SMX. For SD (Figure 2a) the enhancement of phototransformation with PLFA, river water A and wastewater B with respect to the other waters is higher than for simulated sunlight (Figure 1a), suggesting, for these three water samples, a higher proportion of photo-active chromophores under these irradiation conditions than under simulated sunlight. The effects of scavenger addition are overall very similar for both types of irradiation, in agreement with the assumption that the phototransformation mechanisms should essentially be the same. For SMX (Figure 2b) direct phototransformation appears to be largely suppressed, in agreement with the observation that SMX barely absorbs any light above 320 nm (see Figure S4 for its electronic absorption spectrum). In contrast, indirect phototransformation is clearly observed for all water samples, with the highest rate for wastewater C. The relative phototransformation rate constants of SMX
follow the same order and approximate magnitude as for SD, and the effect of scavenger addition
is also very similar. In wastewater B for SD and wastewater C for SMX, the contribution of
hydroxyl radical to phototransformation is indicated by the significant rate reduction upon
isopropanol addition, and the even greater reduction observed upon addition of phenol points to
an important contribution of triplet-induced phototransformation.

(Figure 2)

Figure 2. Merry-go-round photoreactor experiments (λ > 320 nm): Phototransformation rate
constants of (a) SD and (b) SMX (initial concentration: 1 μM for SD and 5 μM for SMX) in
different water samples in the presence and absence of scavengers. The DOM concentration is
given in brackets as mgC L⁻¹. Error bars indicate the 95% confidence intervals obtained from
linear regression.

The results for SMX obtained under UV-A and visible irradiation may be relevant to its
phototransformation in surface waters. At the surface of sunlit natural waters, direct
phototransformation of SMX is very dominant in most cases, as can be concluded from solar
simulator experiments (Figure 1b). However, the relative contribution of indirect
phototransformation is expected to increase with depth in a water body, since the spectrum of
solar light at increasing depth becomes more and more red-shifted due to the wavelength-
dependent absorption caused by DOM/EfOM. This is expected to reduce the rate of light
absorption by SMX (see Figure S4) more strongly than the rate of light absorption by
DOM/EfOM (which extends largely in the UV-A spectral range), and consequently direct
phototransformation should undergo a stronger reduction than indirect phototransformation. Thus, indirect phototransformation possibly plays a more important role in the fate of SMX in surface waters than can be deducted from the present solar simulator experiments.

3.1.3. Estimating the contribution of the hydroxyl radical

To understand the role of the hydroxyl radical in the phototransformation of SD in wastewater B and of SMX in wastewater C, calculations of hydroxyl radical photoproduction rates under solar simulator conditions and of hydroxyl radical steady-state concentrations in the various waters were performed considering the various sources, namely nitrate, nitrite and EfOM (see SI, Text S3 and Table S4). The contribution of nitrate as a hydroxyl radical precursor was found to be negligible, which is backed by the absence of reduction in SD phototransformation rate constant upon isopropanol addition for wastewater A1 (which has the highest nitrate concentration of all studied samples, see Figure 1a and Table 1). Nitrite and EfOM, as sources of photoproduced hydroxyl radical, were estimated to be responsible for only part of the phototransformation rate constant reductions observed upon addition of isopropanol (which scavenges >99% of the hydroxyl radical in the studied water samples). Besides a possible inaccuracy in the determination of nitrite concentrations (see SI, Text S3), these results may be explained by the presence of an additional reactive species that is efficiently scavenged by isopropanol. A plausible hypothesis is that the wastewaters B and C might have contained industrially-derived photochemical sources of strong oxidizing species, such as aromatic quinones, whose excited triplet states have very high reduction potentials (Loeff et al., 1993) and thus act as strong oxidants (Bedini et al., 2012). The strong reduction in the rate constant for SMX observed upon addition of phenol (Figures 1b and 2b) concurs with an excited triplet-induced transformation pathway. Further hints on the origin of the enhanced phototransformation of SMX in wastewater
C were obtained by performing irradiation experiments using a dilution series of this wastewater (SI, Figure S2). The phototransformation rate constants of SMX increased linearly ($R^2=0.947$) with the concentration of wastewater. This trend is consistent with the assumption of a linear increase in concentration of photosensitizers (in this case all photoactive components of the wastewater). One should also consider a possible inhibiting effect by antioxidant components in the water, which would diminish upon dilution, but the net effect on the phototransformation rate constant could be in favor of an increase with concentration, as observed for the SD in PLFA solutions (see Section 3.2.). If hydroxyl radical was the main reactive species involved in the observed indirect phototransformation, one would expect no decrease in rate constant with decreasing concentration of wastewater, because a decrease in photosensitizer concentration would be counterbalanced by a proportional decrease in the concentration of scavengers, and consequently in first-order scavenging rate constant, thus leaving the steady–state concentration of hydroxyl radical unchanged. A similar experimental dilution series using river water B revealed no significant change in the rate constant of SMX phototransformation, consistent with the conclusions from Figure 1 that the dominant reaction pathway for SMX in river water is direct phototransformation.

### 3.2. Effect of concentration and type of DOM

To investigate the effect of DOM on the phototransformation rate constants of SD and SMX, buffered solutions with various concentrations of two well-characterized DOM extracts, SRFA and PLFA, were irradiated using the solar simulator. These fulvic acids were selected because they can be considered as representatives of two different types of aquatic DOM, the first of mainly allochthonous character and the second of pure autochthonous character, and therefore
they should be able to reflect to a great extent the variability of effects to be expected from
surface water DOM.
(Figure 3)

Figure 3. Solar simulator experiments: Dependence of the phototransformation rate constants of
SD, SMX and TMP (inset), all at 1 μM initial concentration, on the concentration of dissolved
SRFA and PLFA in buffered aqueous solution (pH 8.0). Error bars indicate the 95% confidence
intervals obtained from linear regression.

From Figure 3, two very distinct trends of SD phototransformation rate constants with respect to
DOM concentration are observed for the two fulvic acids. Upon SRFA addition (0.1–3.0 mgC L⁻¹)
the phototransformation rate constant of SD becomes on average slightly higher than the rate of
direct phototransformation (at 0 mgC L⁻¹ of SRFA), with a ≈30% increase at low DOM
concentration (0.1–0.2 mgC L⁻¹) followed by a slight decrease at higher concentrations. For
PLFA a marked, non-linear increase in the phototransformation rate constant is observed with
increasing DOM concentration. The enhancement of the rate with respect to direct
phototransformation reaches a factor of ≈3.6 at [DOM] = 1.9–2.5 mgC L⁻¹. The differential
trends in phototransformation rate constants of SD in the presence of autochthonous versus
allochthonous DOM are analogous to the results obtained for sulfadimethoxine in various waters
and DOM solutions (Guerard et al., 2009a, Guerard et al., 2009b). We suggest that the higher
inhibiting capacity of SRFA with respect to triplet-induced oxidation (Wenk et al., 2011, Wenk
and Canonica, 2012), which correlates with its higher electron donating capacity (Aeschbacher et
al., 2012), is responsible for the efficient suppression of the indirect phototransformation of SD in the presence of SRFA. In the investigated concentration range the role of PLFA as a photosensitizer prevails over its role as an inhibitor of SD phototransformation. In contrast to SD and in line with previous studies (see Introduction), the depletion rate constant of SMX is almost independent of DOM concentration for both fulvic acids (Figure 3). The main transformation pathway of SMX is attributed to direct phototransformation (0.048 h$^{-1}$) and indirect phototransformation seems to play a minor role. Indeed, the average increase of the phototransformation rate constant in the presence of PLFA (0.055 h$^{-1}$) and SRFA (0.057 h$^{-1}$) is less than 20% relative to the direct phototransformation rate constant. TMP was used as a reference compound to assess the photosensitizing capacity of DOM (mainly by excited triplet sates of DOM), since TMP is mainly degraded by DOM-induced phototransformation (Canonica et al., 1995, Faust and Hoigné, 1987), and its triplet-induced phototransformation is not inhibited by the presence of DOM (Canonica and Laubscher, 2008). For TMP (inset of Figure 3) the phototransformation rate constant is, as expected, directly proportional to DOM concentration for both fulvic acids, PLFA being a better photosensitizer than SRFA (when normalized to the carbon concentration). The behavior of TMP is in sharp contrast with that of SD and SMX, and stresses the importance of DOM-induced inhibition in the case of the phototransformation of SD.

3.3. Effect of river water composition on the phototransformation of sulfadiazine (SD) and 2,4,6-trimethylphenol (TMP)

Phototransformation rate constants of SD and TMP (reference compound) were measured in water samples that were collected during a one-day sampling campaign along the course of the Thur River, from its source to 80 km downstream. Eight different sampling points were chosen,
to consider in particular the possible impact of effluents from municipal wastewater treatment
plants (see Experimental Section).

(Figure 4)

Figure 4. Solar simulator experiments ($\lambda > 290$ nm): Phototransformation rate constants of SD
and TMP (both at 1$\mu$M initial concentration) in water samples taken along the course of the Thur
River. The DOM concentration is given in brackets as mgC L$^{-1}$, while pH varies in the range of
7.9–8.7 for the river water samples (see SI, Table S2) and is 8.0 for the ultrapure water solution.
Error bars indicate the 95% confidence intervals obtained from linear regression.

Figure 4 shows that the concentration of DOM in the Thur River increases from the source
downwards, and so does the rate constant for TMP phototransformation (hatched bars). The pH
of the river water samples also increases with distance from the river source (see SI, Table S2),
however this change is believed to be of minor importance for the phototransformation kinetics
of TMP and SD, since their speciation remains basically unchanged in the pH range under
consideration (7.9–8.7). The increase in TMP phototransformation rate constant correlates well
with the DOM concentration ($R^2 = 0.87$) (see SI, Figure S3). In contrast, the phototransformation
of SD does not seem to strongly depend on the DOM concentration in river water samples, i.e. in
a concentration range of 0.4–2.5 mgC L$^{-1}$. However, the phototransformation rate constants of
SD are on average significantly higher (by a factor of $\approx 2.7$) than in pure water, clearly indicating
that indirect phototransformation takes place. These results for SD show an intermediate behavior
with respect to the phototransformation rate constants in the presence of PLFA and SRFA,
respectively (Figure 3). For the lowest DOM concentration (river source: 0.4 mgC L\(^{-1}\)) the enhancement in the phototransformation rate constant is significant and approaches the effect of PLFA, however, at higher DOM concentrations the rate constants level off. A possible explanation is that, with increasing distance from the source, the fraction of antioxidant moieties in DOM increases. Probable sources of antioxidant moieties are runoff of agricultural areas, rich in allochthonous material, whose composition is expected to be more similar to that of SRFA, and input from wastewater treatment plants, whose EfOM apparently has a higher antioxidant capacity than the DOM of river water (see discussion of the results for wastewaters A1 and A2, Figure 1a).

4. Environmental Implications

As shown in previous studies and in the present paper, the photochemical transformation of sulfonamide antibiotics in surface waters and wastewaters is controlled by several processes, which are illustrated in Scheme 1.

(Scheme 1)

**Scheme 1.** Photochemical pathways for the transformation of a sulfonamide antibiotic (SA) in surface waters and wastewaters. Abbreviations: DOM\(_{\text{ox}}\)/EfOM\(_{\text{ox}}\) are oxidized forms of DOM/EfOM; PS are photoactive precursors and RS are their corresponding reactive species; SA\(^{\prime}\)\(_2\), SA\(^{\prime}\)\(_3\) are transformation intermediates of SA; SA\(_{\text{1,phot}}\), SA\(_{\text{2,ox}}\) and SA\(_{\text{3,ox}}\) are final reaction products of SA. Dashed arrows indicate reaction pathways that may be operative, depending on the specific RS.
A given sulfonamide antibiotic (SA) can undergo simultaneously direct phototransformation (process #1) and indirect (oxidative) phototransformation through excited triplet states of DOM/EfOM (process #2) or other reactive species (RS, process #3). RS is a collective term to describe any reactive species other than $^3$DOM*/$^3$EfOM*, such as hydroxyl radical, singlet oxygen, or oxidizing radicals derived from DOM/EfOM, and PS is the corresponding collective term to designate their precursors. Each of these three processes leads to a group of final products termed SA$_{1,\text{phot}}$, SA$_{2,\text{ox}}$ and SA$_{3,\text{ox}}$, respectively. For processes #2 and #3, DOM/EfOM can interfere in the route of product formation by reacting with transformation intermediates, SA$'_{2}$ and SA$'_{3}$ (process #4), leading to re-formation of the parent sulfonamide, SA (in principle, interference with process #1 is also possible, but it has been neglected in the scheme to keep it simple). In process #4, DOM/EfOM is assumed to function as an antioxidant (or electron donor) and thus reacts to give DOM$_{\text{ox}}$/EfOM$_{\text{ox}}$. For some RS, e.g. hydroxyl radical, DOM/EfOM can be effective scavengers, which is represented by process #5.

Despite their markedly different photochemical behavior, the phototransformation of SD and SMX can be explained in terms of Scheme 1. While the sunlight-induced transformation of SMX is generally dominated by direct phototransformation (process #1), SD undergoes both direct (process #1) and indirect phototransformation (processes #2 and #3), the latter being favored in waters containing DOM with a low antioxidant capacity (weak back-reaction through process #4). The photochemical fate of SD and SMX in surface waters is probably typical for the two classes of sulfonamide antibiotics identified by Boreen and coworkers (Boreen et al., 2004, 2005), namely the one containing a five-membered and six-membered heterocyclic ring substituent, respectively. However, SMX was also shown to be potentially affected by DOM/EfOM-induced photosensitized transformation, a process which may become important at increasing depth.
below the surface of a water body, and this effect is expected to concern the analogous compounds of the same sulfonamide class. A quantitative assessment of such an effect is planned. For both sulfonamides half-lives for conditions corresponding to the surface of a water body, mid-latitude, clear-sky midday conditions and season intermediate between summer and fall were in the range of 5–18 h. Considering the diurnal variation of sunlight intensity (including night time, factor of \( \approx 4 \)), partial cloud cover (factor of \( \approx 1.4 \)) and reduction of sunlight intensity by the screening effect in a water depth of 1 m (factor of \( \approx 3 \) for averaged half-life in a well-mixed water layer), one comes up with estimated half-lives in the order of 3–13 d. In rivers and creeks such half-lives may be further increased by the shadow caused by the presence of trees near the stream and water plants in the stream (Kari and Giger, 1995).

5. Conclusions

Sulfamethoxazole (SMX) and sulfadiazine (SD), as representatives of sulfonamide antibiotics, were shown to undergo both direct and indirect phototransformation in river waters, solutions of DOM extracts, and secondary effluents of wastewater treatment plants. The relative importance of direct and indirect phototransformation depends on the target compound, the type and concentration of DOM, and the spectrum of light used to induce the photochemical reaction. Under solar-simulated irradiation, direct phototransformation of SMX was largely predominant, and significant indirect phototransformation was only detected in a sample of a wastewater effluent. The reactive species responsible for this indirect pathway was possibly the hydroxyl radical, but its photochemical sources could not be identified with certainty. However, SMX was shown to also undergo DOM/EfOM-photosensitized transformation when UVA light from a
medium-pressure mercury arc lamp was used for irradiations. This process might become important with increasing depth in a water body.

In river waters, wastewater effluents and solutions of PLFA (an authochtonous aquatic DOM), SD phototransformation occurred mainly by DOM/EfOM photosensitization at DOM concentrations >0.4 mgC L\(^{-1}\). However, in solutions of SRFA, an allochtonous DOM with strong antioxidant capacity, indirect phototransformation of SD appeared to be much less important than direct phototransformation. SD constitutes an example of water contaminant whose photochemical fate is affected by both photosensitizing and antioxidant properties of DOM/EfOM (Wenk et al., 2011, Wenk and Canonica, 2012). Moreover, 2,4,6-trimethylphenol (TMP) was employed as a useful reference compound to assess the photosensitizing strength of DOM/EfOM, since its transformation is not affected by the presence of antioxidants (Canonica and Laubscher, 2008).

Phenol was successfully employed as a diagnostic compound to selectively inhibit the transformation of the sulfonamides photosensitized by DOM/EfOM (oxidation reaction attributed to their excited triplet states). Since phenol also scavenges hydroxyl radical, the combined use of a second hydroxyl radical scavenger, such as isopropanol, is recommended if the presence of hydroxyl radical in the irradiated solution cannot be \textit{a priori} excluded.

\textbf{Acknowledgements}

This work was supported by the Swiss National Science Foundation in the frame of the \textit{National research programme “Sustainable Water Management” (NRP 61)}. The authors thank Hans-Ulrich Laubscher for technical support and Iris Schilling for experimental assistance.

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Appendix A. Supplementary Data

Supplementary data related to this article can be found at

References


Loeff, I., Rabani, J., Treinin, A., Linschitz, H., 1993. Charge-transfer and reactivity of $n\pi^*$ and $\pi\pi^*$ organic triplets, including anthraquinonesulfonates, in interactions with inorganic anions: A comparative study based on classical Marcus theory. Journal of the American Chemical Society 115(20), 8933-8942.


## Table 1: Sampled river waters and wastewaters with selected water quality parameters.

<table>
<thead>
<tr>
<th>Water samples</th>
<th>DOC (mgC L(^{-1}))</th>
<th>Nitrate (mgN L(^{-1}))</th>
<th>Nitrite (µgN L(^{-1}))</th>
<th>Ammonium (µgN L(^{-1}))</th>
<th>Alkalinity (mM)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring water</td>
<td>0.4</td>
<td>0.3</td>
<td>&lt; 0.25</td>
<td>7.3</td>
<td>1.65</td>
<td>7.9</td>
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<tr>
<td>River water A</td>
<td>3.8</td>
<td>1.1</td>
<td>&lt; 0.25</td>
<td>-</td>
<td>4.40</td>
<td>8.1</td>
</tr>
<tr>
<td>River water B</td>
<td>2.5</td>
<td>2.8</td>
<td>11.2</td>
<td>12.7</td>
<td>4.99</td>
<td>8.5</td>
</tr>
<tr>
<td>Wastewater A1</td>
<td>6.6</td>
<td>13.9</td>
<td>106</td>
<td>79.6</td>
<td>6.21</td>
<td>8.0</td>
</tr>
<tr>
<td>Wastewater A2 (^a)</td>
<td>5.8</td>
<td>&lt; 0.25</td>
<td>1.7</td>
<td>17.7</td>
<td>1.18</td>
<td>7.5</td>
</tr>
<tr>
<td>Wastewater B</td>
<td>5.5</td>
<td>6.2</td>
<td>509</td>
<td>133</td>
<td>6.89</td>
<td>8.3</td>
</tr>
<tr>
<td>Wastewater C</td>
<td>5.6</td>
<td>2.7</td>
<td>25</td>
<td>191</td>
<td>8.37</td>
<td>8.0</td>
</tr>
</tbody>
</table>

Note: \(^a\) Wastewater A2 was derived from wastewater A1 by electrodialysis treatment.
Figure 1

(a) Sulfadiazine

- no addition
- + isopropanol
- + phenol

(b) Sulfamethoxazole

- no addition
- + isopropanol
- + phenol

Water samples include:
- ultrapure water
- SRFA
- PLFA
- spring water
- river water A
- wastewater A1
- wastewater A2
- wastewater B

Values in brackets indicate reaction rates.
Figure 4

The figure shows the rate constant $k_p$ (hour$^{-1}$) as a function of distance from the river source (kilometers). The data is represented for different distances: 0, 8, 19, 30, 32, 36, 60, and 80 km. The graph includes two categories: SD and TMP. SD data is represented by solid bars, while TMP data is represented by striped bars.

The y-axis represents the rate constant $k_p$ with values ranging from 0.00 to 0.30. The x-axis represents the distance from the river source in kilometers.

At each distance, the rate constant values are indicated within the bars. For example, at 0 km, the rate constant for SD is approximately 0.4, and for TMP, it is around 0.6. At 8 km, the rate constant for SD is about 0.8, and for TMP, it is around 1.2. This pattern continues for other distances with varying rate constant values.

The right side of the figure indicates the ultrapure water rate constant, which is less than 0.03.
Scheme 1