- The sunlight degradation of five Fluoroquinolones was studied in WWTPs effluent
- The photodegradation was studied at environmentally significant concentrations
- Photoproducts were identified and their distribution profiles were monitored
- The toxicity of the photoproducts was studied by long-term *V. fischeri* assay
- Photoproducts contribution to the overall biotoxic effect (μg L^{-1} level) was proved

Fluoroquinolones in wastewaters effluent: sunlight-induced degradation and photoproducts ecotoxicity

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Abstract: The photodegradation of Ciprofloxacin (CIP), Enrofloxacin (ENR), Danofloxacin (DAN), Marbofloxacin (MAR) and Levofloxacin (LEV), five widely used Fluoroquinolones (FQs), was studied in urban WWTP secondary effluent, under solar light. The degradation profiles and the kinetic constants were determined at the micrograms per litre levels (20-50 μg L^{-1}). The photo-generated products were identified by high-pressure liquid chromatography coupled to electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS). The toxicity of the photoproducts was assessed by *V. fischeri* light emission inhibition assay performed on irradiated and not-irradiated FQs solutions, at environmentally significant concentrations. Attention was focused on the evaluation of the photoproducts contribution to the overall biotoxic effect of these emerging pollutants. Data from chronic exposure experiments (24-48 h) were primarily considered. Results confirmed the major usefulness of chronic toxicity data with respect to the acute assay ones and proved the not negligible biotoxicity of the FQs photodegradation products.

Keywords

Bioluminescent assay; Ecotoxicity; Fluoroquinolones; Photoproducts; Wastewaters

1. Introduction

In the last decades increasing attention has been paid to the occurrence, behaviour, and fate of pharmaceutically active compounds introduced in the environment. Despite this, the still limited knowledge about the environmental fate and effect of pharmaceuticals requires intensive, further researches (Babić et al., 2013). Fluoroquinolones (FQs) represent an important class of emerging pollutants in water and soil environmental systems (Andreu et al., 2007; Speltini et al., 2010; Speltini et al., 2011). These drugs are the most frequently detected in water, followed by sulphonamides, tetracycline, and macrolides (Kusari et al., 2009). FQs are amphoteric molecules obtained by modification of the quinolone core structure by insertion of a fluorine atom in C-6 position and a piperazinyl – or piperazine derivative – group at C-7. They act through inhibition of bacterial DNA gyrase and topoisomerase IV enzymes. The fluorine atom at C-6 position of the ring provides a more than 10-fold increase in gyrase inhibition and up to 100-fold improvement in minimum inhibitory concentrations, while substituent groups at position C-7 play a key role in determining the antibacterial spectrum and bioavailability. These synthetic antibiotics are widely employed both in human and veterinary medicine due to their high potency, broad activity spectrum, good bioavailability, high serum levels, and a potentially low incidence of side-effects (Andersson and MacGowan, 2003). After administration, FQs are only partially metabolised, thus large part of the ingested dose (>50%) is excreted with no structural modification. A minor fraction of the ingested dose is excreted as Phase I (addition of reactive functional groups through oxidation, reduction or hydrolysis) or Phase II (covalent conjugation to polar molecules, e.g. glucoronic acid, sulphate, acetic acid or amino acids) metabolites (Van Doorslaer et al., 2011; Reemtsma and Jekel, 2006).

The poor metabolization poses serious issues. Variable amounts of these antibacterial agents are regularly released in their active form by the wastewater treatment plants (WWTPs), not capable of a quantitative abatement. Ciprofloxacin (CIP) has been determined at concentration up to 5.6 $\mu g\ L^{-1}$ in WWTPs effluents (Andreozzi et al., 2003) and FQs have been frequently detected in environmental waters at concentrations ranging from $ng\ L^{-1}$ to $\mu g\ L^{-1}$ (Speltini et al., 2010). In spite of the continuous release of FQs the accumulation to high concentrations is hampered by FQs photosensitivity. Indeed, photochemistry represents the main transformation path of these compounds resistant to hydrolysis, thermal decomposition, and biodegradation (Andreozzi et al., 2003; Speltini et al., 2010; Sturini et al., 2014). Nevertheless, complete mineralization is hard to achieve in water systems under the most common environmental conditions (Kusari et al., 2009), leading to the persistence of various photoproducts together with residual parent drugs (Babić et al., 2013; Prabhakaran et al., 2009; Sturini et al., 2010; Sturini et al., 2014). The degradation kinetics is influenced by the organic and inorganic matrix constituents and by adsorption on suspended particulate, both having large effects on the degradation rates (Andreozzi et al., 2003; Schmitt-Kopplin et al., 1999; Sturini et al., 2010; Sturini et al., 2014).

In view of the growing demand for decontaminated water supplies, various research groups focused on photocatalysis for the remediation of FQs-contaminated waters, in order to develop efficient water purification processes (Maraschi et al., 2014; Sturini et al., 2012a; Van Doorslaer et al., 2011; Vasquez et al., 2013). The formation of photo-generated products has to be considered to realistically value the overall environmental impact of FQs pollution. In this context, recent works showed that beside the parent drugs also their photoproducts exert antimicrobial activity, contributing to stimulate bacterial resistance (De Bel et al., 2009; Kusari et al., 2009; Sturini et al. 2012b; Sukul et al. 2009).

More recently, different studies have been focused to assess the ecotoxicity of photolyzed aqueous FQs solutions (Li et al., 2011; Sirtori et al., 2012; Vasconcelos et al., 2009; Vasquez et al., 2013). The results currently available indicate that despite the degradation of the parent drugs, after photolysis the solutions preserved significant toxicity, reasonably ascribed to the formation of bioactive products (Li et al., 2011; Sirtori et al., 2012; Vasconcelos et al., 2009). However, as underlined by Vasconcelos et al. (2009), the concentrations used in these studies (Li et al., 2011; Sirtori et al., 2012) were higher than those normally measured in the environment, and only data from short-time assays are available up to now (Li et al., 2011; Sirtori et al., 2012; Vasconcelos et al., 2009). With regard to this, it should be considered that in the case of the bioluminescence inhibition assay, long-term assays (e.g. 24 h) are required to obtain more realistic results, because short-term assays underestimate or even fail to detect the toxicity (Backhaus et al., 1997).

The photolytic degradation of FQs in untreated urban WWTP effluents under solar light, at the low micrograms *per* litre levels, has not been reported as yet. Indeed, Babić et al. (2013) investigated the photolytic degradation of CIP, Enrofloxacin (ENR) and Norfloxacin (NOR) in simulated pharmaceutical industry wastewater. Other studies focused on the photocatalytic abatement of Ofloxacin (OFL) in secondary treated effluents (Michael et al., 2010), or on UV-A radiation of 15-50 mg L^{-1} Moxifloxacin (MOX) solutions in deionised water (Van Doorslaer et al., 2013) or hospital effluent (Van Doorslaer et al., 2015), as well as on 2 mg L^{-1} CIP in wastewater effluent under UV (Keen and Linden, 2013).

To the authors' best knowledge, only one paper (Vasquez et al., 2013) reported the chronic effects (24 h) of photolyzed OFL solutions at the low μg L^{-1} concentration levels on *V. fischeri* light emission, proving that UV degradation processes create genotoxic byproducts.

On the basis of the current state of the art, we deemed interesting to study the photodegradation of five widely employed FQs - CIP, ENR, Danofloxacin (DAN), Marbofloxacin (MAR), Levofloxacin (LEV) - in urban WWTP secondary effluent, under solar light, at the micrograms per litre levels, and to assess the ecotoxicity of the photoproducts from each FQ by long-term *V. fischeri* assay. The degradation profiles have been determined for each drug, the kinetic constants calculated and compared with those previously found in raw river water (Sturini et al., 2012a), and the photoproducts identified by high-pressure liquid chromatography coupled to electrospray ionization

93 tandem mass spectrometry (HPLC-ESI-MS/MS). Differently from the current literature, biotoxicity experiments were
94 conducted to specifically discern the contribute of the photoproducts from that of residual FQs, focusing the work at
95 low FQs concentrations, in the range 1-90 μg L^{-1}.
96
97 **2. Experimental section**
98 *2.1. Reagents and materials*
99 All chemicals employed were reagent grade or higher in quality and were used without any further purification.
100 Analytical grade CIP, DAN, ENR, LEV and MAR were supplied by Fluka (Sigma-Aldrich). HPLC gradient grade
101 acetonitrile (ACN) and methanol (MeOH) were from VWR and H_3PO_4 (85%, w/w) from Carlo Erba Reagents;
102 HCOOH (98-100%, w/w) was obtained from Merck. Ultra-pure water (resistivity 18.2 MΩ cm^{-1} at 25°C) was produced
103 at laboratory by means of a Millipore (Milan, Italy) Milli-Q system. FQs stock solutions were prepared in pure water
104 and stored in the dark at 4°C until use. Working solutions were renewed daily.
105 The luminescent bacteria *V. fischeri* were employed as lyophilized aliquots, which were prepared from fresh cultures
106 maintained at the laboratory starting from an original batch supplied by the Pasteur Institute (Paris, France). The 96-
107 wells "Black Cliniplate" microplates were supplied by Thermo Scientific (Vantaa, Finland). Nutrient broth components
108 were obtained from Sigma-Aldrich.
109
110 *2.2. Analytical determinations*
111 The HPLC system consisted of a pump Series 200 (Perkin Elmer) equipped with vacuum degasser and a programmable
112 fluorescence detector (FD). The FD excitation/emission wavelengths selected were 297/507 nm for MAR, 280/500 for
113 LEV and 280/450 nm for CIP, DAN and ENR. After an equilibration period of 10 min, 50 μL of each sample were
114 injected into a 250 × 4.6 mm, 5 μm Ascentis RP-Amide (Supelco) coupled with a similar guard-column. The mobile
115 phase was 25 mM H_3PO_4-ACN (85:15) for 30 min at a flow rate of 1 mL min^{-1}.
116 The HPLC-UV system consisted of a Shimadzu (Milan, Italy) LC-20AT solvent delivery module equipped with a
117 DGU-20A3 degasser and interfaced with a SPD-20A UV detector. The injection volume was 20 μL. The analysis
118 wavelength selected was 275 nm. 20 μL of each sample were injected into a 250 × 4.6 mm, 5 μm Analytical Ascentis
119 C18 (Supelco) coupled with a similar guard-column. The mobile phase was 25 mM H_3PO_4-ACN (85:15), at a flow rate
120 of 1 mL min^{-1}.

121 The HPLC-ESI-MS/MS analyses were performed by using an Agilent 1100 HPLC with a Luna C18 (150 × 4.6 mm, 5
122 μm) column, maintained at 30°C. The mobile phase was HCOOH 0.5% (v/v) in ultrapure water-ACN (90:10), at a flow
123 rate of 1.2 mL min^{-1}, and the injection volume was 5 μL.
124 The WWTPs sample was analyzed on a Poroshell column (2.1 × 50 mm, 2.7 μm), with MeOH/water-0.1% HCOOH
125 (22:78) as the mobile phase (flow rate 0.5 mL min^{-1}), allowing a better sensitivity. The MS/MS-system consisted of a
126 linear trap Thermo LXQ. ESI experiments were carried out in positive-ion mode under the following constant
127 instrumental conditions: source voltage of 4.5 kV, capillary voltage of 20 V, capillary temperature of 275°C and
128 normalized-collision energy 35.
129
130 *2.3. Wastewater samples*
131 Wastewater samples were collected over five consecutive days, excluding Saturday and Sunday. 24-h composite, flow-
132 proportioned samples of wastewater were collected from the secondary effluent of a WWTP located in Northern Italy.
133 Before performing kinetic experiments the samples were pooled in order to have a representative sample of the effluent.
134 The main physical-chemical parameters of the wastewater matrix are reported in Table 1.
135 The sample was analyzed for its native FQs content by a validated method (Sturini et al., 2009) and confirmed by
136 HPLC-ESI-MS/MS.
137
138 *2.4. Irradiation experiments*
139 Kinetics experiments were performed by using unfiltered WWTP samples (500 mL, pH 6.9) enriched with 50 μg L^{-1} of
140 each drug (20 μg L^{-1} for DAN). Samples were photolyzed in an open glass container (20 mm depth, exposed surface
141 280 × 200 mm) by using a solar simulator (Solar Box 1500e, CO.FO.ME.GRA) set at a power factor 250 W m^{-2},
142 equipped with a UV outdoor filter of soda lime glass, IR. At the planned times, aliquots (1 mL) of each sample were
143 withdrawn and immediately injected in the HPLC-FD system, after 0.45 mm filtration. All experiments were performed
144 in triplicate.
145 In order to identify the photoproducts, 10 mL of each FQ 10^{-3} M in WWTP effluent were irradiated in 10 mL quartz
146 tube by means of 10×15 W phosphorus-covered low pressure mercury arcs, emission maximum centred at 310 nm (UV
147 flux measured by a 310 nm sensitive probe, 12 W m^{-2}), and immediately injected in the HPLC-UV system prior to
148 HPLC-ESI-MS/MS analysis.
149 Samples for ecotoxicity tests were prepared starting from 20 mL 10^{-4} M (25 mg L^{-1}) aqueous solutions in tap water
150 (named A) of each FQ. Samples were irradiated (310 nm, 150 W) for different times (2 min for DAN, 5 min for CIP, 7

151 min for ENR, 15 min for LEV, 20 min for MAR) in order to collect the highest amount of photoproducts, verified by
152 HPLC-UV. After photolysis, each solution (named B) was properly diluted to prepare a suitable concentrations interval
153 for the *V. fischeri* assay.
154
155 *2.5. Toxicity assays*
156 The biotoxicity was evaluated by the application of the ISO standard test based on *V. fischeri* luminescence inhibition
157 and currently employed as reference assay in water quality controls (ISO 11348-3 2009). Being *V. fischeri* a marine
158 organism the samples were added with NaCl to the 3% w/v. The lyophilized bacteria, reconstituted by 1 mL of distilled
159 water, were diluted by addition of a volume (from 10 to 30 mL) of the specific nutrient broth (NaCl 15 g, Peptone 2.5 g,
160 NaCl 15 g, Peptone 2.5 g, Yeast extract 1.5 g, Glycerol 1.5 mL, HEPES 1.19 g, in 500 mL, pH 7). To each well of the
161 microplate were added 200 µL of bacteria suspension and 100 µL of sample or blank (3% NaCl in tap water). Emitted
162 light was recorded by a "Victor light" microplate luminometer (Perkin-Elmer, USA) at various intervals till 24-48 h to
163 evaluate the chronic exposure effects. The light emission value calculated for each sample was the average of 8
164 replicates.
165 Bioluminescence inhibition was determined for each drug, in parallel, for the irradiated B and not irradiated C solutions.
166 Solution B contained the maximum amount of photoproducts and a residue of the parent compound (7% for DAN, 7%
167 for ENR, 6% for CIP, 5% for LEV and MAR), while solution C contained the same amount of parent compound as in B
168 and was prepared by dilution of solution A. The percent distribution of the different photoproducts in solution B is
169 reported in Table 2, and their molecular structures can be found in Supplementary data.
170
171 **3. Results and discussion**
172 *3.1. Photodegradation of FQs in urban WWTP secondary effluent*
173 One of the aims of this research was to investigate the sunlight-induced degradation of FQs in WWTP final effluents,
174 which are considered the major contributors to the spread of human antibiotics in the environment (Zuccato et al.,
175 2010). Before the irradiation experiments, FQs background concentration was determined by a previously reported
176 method (Sturini et al., 2009) and confirmed by HPLC-ESI-MS/MS. CIP and LEV, the two most administered FQs for
177 human use (Lillenberg et al., 2010), were found at concentrations of 30 and 55 ng L^{-1}, respectively (RSD<9%, n=3).
178 The HPLC-ESI-MS chromatogram is reported in Fig. 1. These results confirmed that WWTPs are unable to completely
179 remove such chemically stable molecules, which can therefore reach environment in their pharmacologically active
180 form and stimulate bacterial resistance. Moreover, their photodegradation generates ecotoxic photoproducts, also at low
181 concentration levels (Li et al., 2011; Sturini et al., 2012b).

182 The WWTP secondary effluent sample was spiked with 20-50 μg L^{-1} of each FQ before photolysis since these
183 concentrations were representative of the FQs occurrence in this kind of matrix (Batt et al., 2006; Hartmann et al., 1998;
184 Speltini et al., 2010) and high enough to accurately determine the degradation profiles of FQs avoiding any pre-
185 concentration step. Samples were not filtered and no pH adjustment was done because filtration could modify sample
186 characteristics and because photolysis is pH dependent (Sturini et al., 2010).
187 The photodecomposition profiles are reported in Fig. 2. DAN was decomposed in 15 min, CIP and ENR in about 35
188 min, MAR required 50 min, while LEV resulted the most persistent among the considered FQs (5 h). Good
189 reproducibility (RSD<5%, *n*=3) was observed for all photodegradation experiments.
190 Experimental data were fitted by a first-order mono-exponential law by means of the Fig.P application (Fig.P Software
191 Corporation), according to eq. 1:

192 $$y = A \times e^{-kt} \qquad (1)$$

193 The kinetic constants (*k*), expressed in min^{-1}, are reported in Table 3.
194 Comparing these results with those previously found in river water under the same irradiation conditions (Sturini et al.,
195 2012a) it was observed that the degradation rates were different (see Table 3). Except for MAR, slower decays occurred
196 in WWTP secondary effluent with respect to raw river water. It is evident that the matrix substantially influenced the
197 photodegradation of such compounds. As it can be seen in Table 1, the WWTP effluent was characterized by higher
198 DOC values (6 mg L^{-1}) with respect to the river water (1 mg L^{-1}) and this can contribute to slow down the degradation
199 kinetics in WWTP effluent (Sturini et al., 2012a, Li et al., 2011). Moreover, chloride and sulphate concentrations were
200 higher in WWTP effluent, while nitrate concentration was similar in the two kinds of water (see Table 1).
201 The unaffected MAR photodegradation rate was ascribed to the primary photoreaction mechanism of MAR, which
202 involves a unimolecular process (Pretali et al., 2010). Indeed, N-N bond fragmentation readily initiates its
203 photodegradation, after triplet excited state population, leading to direct photolysis products (see Supplementary Data).
204 All other FQs mainly photodegrade via bimolecular reactions. After triplet excite state population, two degradation
205 pathways are available, that is type 2 nucleophilic substitution on the carbon 6 initiated by the addition of a water
206 molecule, or hydrogen abstraction (mainly from electron rich piperazine ring), the latter being the preferred route.
207 Bimolecular reactions are considerably slower than the unimolecular reaction, as evident from the quantum yields,
208 Φ=0.043 for MAR (Pretali et al., 2010), Φ =0.022 for ENR (Wammera et al., 2013) and Φ =0.0012 for LEV (Pretali et
209 al., 2010). Indeed, bimolecular photoreactions are more influenced by matrix scavenging processes that can deactivate
210 the reactive triplet state before it can react. LEV, the less reactive among the FQs investigated, always showed the
211 highest persistence, as expected.

212 During degradation various photoproducts were generated. These were identified by HPLC-ESI-MS/MS (the molecular
213 structures are reported in Supplementary Data) and resulted not different from those obtained in tap and raw river
214 waters (Sturini et al., 2012a) under the same experimental conditions. Their decomposition time ranged from 30 min for
215 DAN to 3 h for LEV. Summing up, sunlight was able to photolyse quantitatively the antibiotics also in WWTP
216 effluents.
217
218 *3.2. Ecotoxicity of FQs photoproducts*
219 Irradiated (B) and not irradiated (C) FQ solutions were tested to estimate the contribution of the photoproducts to the
220 environmental toxicity of each FQ. The *V. fischeri* test was selected to evaluate the FQs biotoxicity because of its
221 widespread application in monitoring and quality control activities on surface and sea water bodies (Girotti et al., 2008).
222 The bioluminescence inhibition was observed for FQ solutions in the concentration range 5-30 μg L^{-1}; higher
223 concentrations (up to 100 μg L^{-1}) always reduced to zero the light emission, while very low concentrations (< 5 μg L^{-1})
224 never induced a significant reduction in the bioluminescence intensity of samples with respect to the controls.
225 The additive, toxic effect of the photoproducts was evaluated by comparing the intensity of the light emitted by the
226 wells containing the B samples and that emitted by the C samples containing wells (C samples contained the same FQs
227 residue concentrations as in B but *not* the photoproducts). The trend obtained for all the tested solutions was always
228 similar to that reported in Fig. 3. The 1:20 dilution of the samples (concentration range 21-29 μg L^{-1}) produced a
229 significant reduction of the emitted light; in case of CIP, DAN and ENR both the irradiated and not irradiated solutions
230 strongly inhibited the light emission, while only the irradiated LEV and MAR solutions produced a similar effect (Fig.
231 3a). In the range 14-19 μg L^{-1} (dilution 1:30 of B and C solutions) a strong inhibition of the emitted light by both kinds
232 of samples was still observed only for the CIP solutions. For the other FQs, the not irradiated solutions produced no or
233 not so significant inhibition. On the contrary, the solutions containing the photoproducts resulted definitely more toxic,
234 with the exception of MAR samples (Fig. 3b).
235 It was immediately clear that these data confirmed the toxicity peculiar to the photoproducts. Their noxious effects on
236 the bacterial metabolism were in addition to those of the parent compound, in some cases doubling or multiplying the
237 inhibitory effects of the samples containing the equivalent amount of not irradiated parent FQ.
238 Since some samples produced a strong inhibition even at the lower concentrations range, we performed additional tests
239 at a fixed, lower concentration (9 μg L^{-1}). As shown in Fig. 4, the chronic toxicity data indicated CIP as the most toxic
240 FQ among the tested ones, still reducing consistently the light emission at such low concentration. A slight recovery of
241 light intensity was observed at 48 h after contact (Fig. 4b) probably because of the particular property of bacteria to
242 metabolize also toxic compounds. At this low concentration, the solutions of all other compounds did not show the

243 additive effect of the photoproducts presence (Fig. 4a). The differences in the B and C samples light emission ratio at 48
244 h are surely influenced by the bacterial growth oscillations in the very limited environment represented by the
245 microplate wells.
246 The above reported data represented just the effects on this strain of marine bacteria and did not mean that the usually
247 low concentrations of FQs detected in the urban WWTP effluent must be of no concern, as on different organisms
248 and/or because bioaccumulation phenomena, different responses or information can be obtained by different ecosystem
249 components.
250

251 **4. Conclusions**

252 In this research the sunlight degradation of five widely used FQ antibiotics has been studied in WWTP secondary
253 effluent. It has been proved that also in this kind of matrix photochemistry (under solar light) is an important removal
254 pathway for these otherwise persistent anthropogenic contaminants. FQs residuals in the few tens of ng L^{-1}
255 concentration range were actually determined in our WWTP effluent.
256 However, during the first steps of the photolytic process various photoproducts were formed and clear evidences were
257 obtained to ascribe a toxic effect specifically to the FQs photodegradation products. These outcomes posed the need for
258 further investigation in order to assess the real environmental impact of the specific contaminants after
259 photodegradation at different degrees. The simple biotoxicity assays here employed confirmed the prevalent importance
260 of the information obtained from the long-term (24-48 h) contact tests (chronic toxicity), an experimental condition
261 close to the real occurrence in the environment for persistent pollutants. Nevertheless, the carrying out of further
262 toxicity tests on organisms different from *V. fischeri* is compulsory to clarify these controversial effects of the chemical
263 contaminants photodegradation and to design the more effective treatments able to remove these pollutants.
264
265
266

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271
272
273

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Table captions

Table 1 Main physical-chemical parameters of wastewater and river water samples.

Table 2 Relative percentage distribution of FQ photoproducts obtained by irradiation of antibiotic standard solutions (25 mg L^{-1}).

Table 3 Kinetic constants (*k*) determined under natural sunlight in urban WWTP secondary effluent and in river water for comparison, individually fortified with 20-50 μg L^{-1} of each FQ.

Figure captions

Fig. 1. HPLC-ESI-MS chromatogram obtained for the SPE extract of the WWTP effluent.

Fig. 2. Degradation profiles obtained under solar light in urban WWTP secondary effluent for CIP (◊), DAN (×), ENR (+), LEV (Δ), MAR (□).

Fig. 3. Light emission recorded for solution B (striped bars) and solution C (white bars) of the tested FQs in the concentration range 21-29 μg L^{-1} (a) and 14-19 μg L^{-1} (b) at 24 h after contact. Error bars represent the standard deviation (*n*=8).

Fig. 4. Light emission recorded for solution B (striped bars) and solution C (white bars) of the 9 μg L^{-1} FQs solutions at 24 h (a) and 48 h (b). Error bars represent the standard deviation (*n*=8).

Table 1
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Parameters	WWTP secondary effluent (mg L^{-1})	River water[a] (mg L^{-1})
COD	25	4
DOC	6	1
BOD_5	< 10	< 10
Cl^-	162	9
SO_4^{2-}	121	29.3
NO_3^-	5.3	6.5
P TOT	0.9	0.03

[a] (Sturini et al., 2012a)

Table 2
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FQ	Photoproduct	Relative percentage distribution (%)
CIP	C1	24
	C2	31
	C3	1
	C4	45
DAN	D1	16
	D2	7
	D3	20
	D4	18
	D5	11
	D6	28
ENR	E	1
	E1	0.5
	E2	4
	D	7
	C	21
	A	25
	B	40
LEV	L1	12
	L2	1
	L3	1
	L4	1
	L7	85
MAR	G	48
	F	52

Table 3
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FQ	WWTP secondary effluent k (min^{-1})	River water k (min^{-1}) [a]
CIP	0.110(6)	0.22(2)
DAN	0.31(5)	0.66(3)
ENR	0.077(6)	0.24(3)
LEV	0.010(4)	0.19(2)
MAR	0.061(3)	0.061(2)

[a](Sturini et al., 2012a)

Figure 1

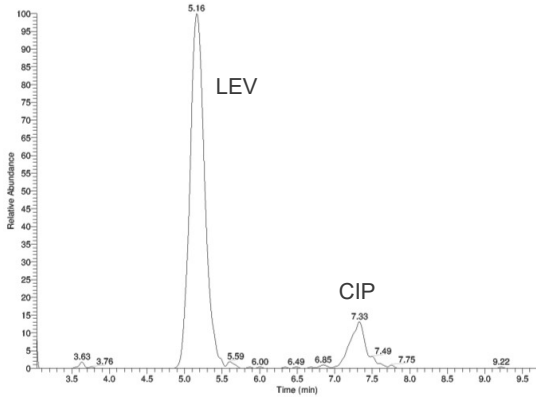

5.16
LEV
CIP
7.33
3.63
3.76
5.59
6.00
6.49
6.85
7.49
7.75
9.22
Relative Abundance
Time (min)

Figure 2

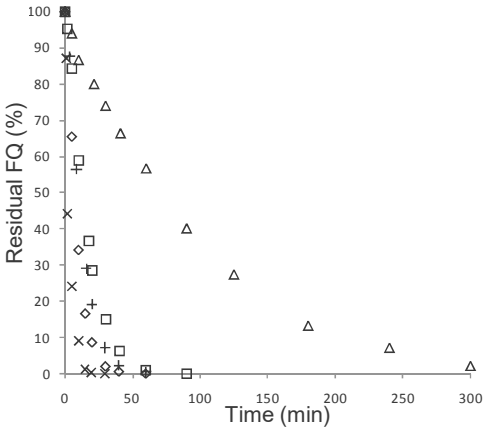

Residual FQ (%)
100
90
80
70
60
50
40
30
20
10
0
0
50
100
150
200
250
300
Time (min)

Figure 3

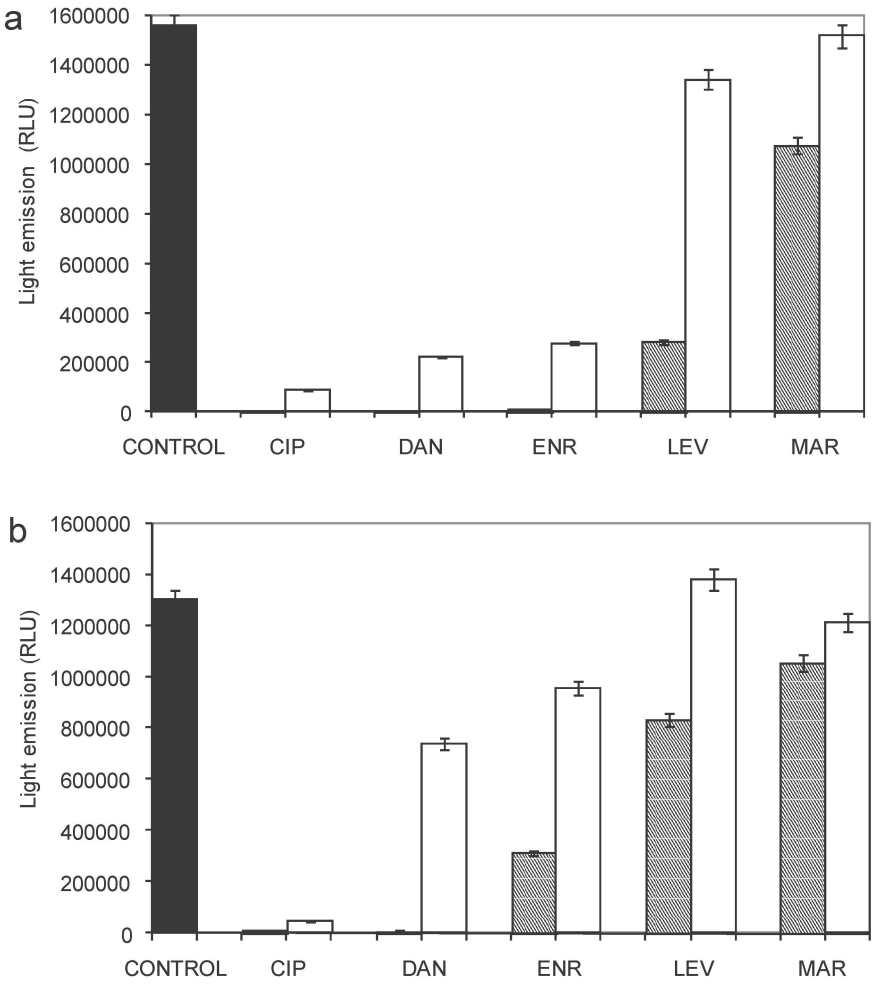
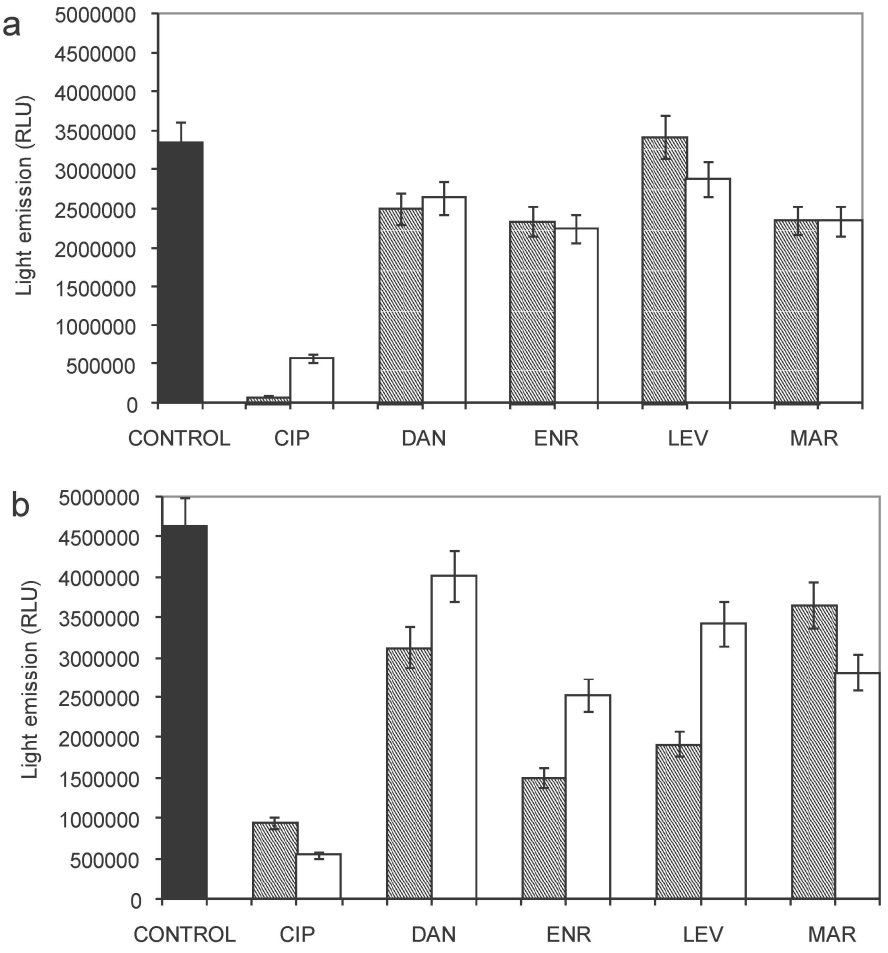

a
Light emission (RLU)
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
CONTROL
CIP
DAN
ENR
LEV
MAR
b
Light emission (RLU)
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
CONTROL
CIP
DAN
ENR
LEV
MAR

Figure 4


a
Light emission (RLU)
5000000
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
CONTROL
CIP
DAN
ENR
LEV
MAR
b
Light emission (RLU)
5000000
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
CONTROL
CIP
DAN
ENR
LEV
MAR

Supplementary Material

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