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Photocatalytic treatment of moxifloxacin in water: ecotoxicity assessment

Host University

Israel Deneke Haylamicheal

Promotors

Prof. dr. ir. Kristof Demeestere

Prof. dr. Colin Janssen

Tutor

ir. Xander Van Doorslaer

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Prof. dr. ir. Kristof Demeestere	Promoter	_____
Prof. dr. Colin Janssen	Promoter	_____
ir. Xander Van Doorslaer	Tutor	_____
Israel Deneke Haylamicheal	Author	_____

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The Author: Israel Deneke Haylamicheal _____

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Summary

Pharmaceuticals are regarded as emerging contaminants of concern due to their continuous release into the environment and persistence even at very low concentrations with potentials to cause adverse human health and environmental effects. The main concerns related to pharmaceuticals are associated with the emergence of antibiotic resistant bacteria that may complicate infection control efforts and ecotoxic effects on biota due to long-term exposures.

Moxifloxacin is a broad-spectrum, 4th generation fluoroquinolone, antibiotic whose consumption in Europe is on the rise. The growth in the consumption of moxifloxacin may increase its input into the environment with a potential for adverse effects. The use of advanced oxidation processes (AOPs) such as UV-A/TiO_2 proved to be efficient in removing moxifloxacin and other pharmaceuticals from water and wastewater matrices. However, evaluation of the applicability of a UV-A/TiO_2 treatment requires an integrated assessment that includes not only removal efficiency but also the ecotoxic effects of the treated solutions. Therefore, this study was conducted with the objective of assessing the effectiveness of a UV-A/TiO_2 treatment in removing the toxicity of a moxifloxacin solution using the freshwater algae *Pseudokirchneriella subcapitata*. Besides, assessments were done to qualitatively estimate the toxic effects of the photocatalytically generated degradation products, and to quantitatively predict the environmental (aquatic) risk of moxifloxacin in Belgium.

Photocatalytic treatments were conducted using a lab–scale batch reactor (200 mL) equipped with a UV-A pen ray (485 μW/cm^2). The experiments were done in two phases (first and second) starting with initial moxifloxacin concentrations (C_o) of 15 and 50 mg/L, respectively. The photocatalytic experiments were done in replicates (n=3) to reduce experimental errors. The removal of moxifloxacin from the reaction solution was monitored using HPLC coupled with a photodiode array detector. Degradation products of moxifloxacin were identified using HPLC–ESI–LRMS. The toxic effects of moxifloxacin and the photocatalytically treated solutions were assessed using the 72 h freshwater algae–*P. subcapitata*–growth inhibition test.

The result showed that the UV-A/TiO_2 treatment can eliminate moxifloxacin (C_o=15 mg/L) from a water matrix, and complete removal was achieved in 15 min of irradiation. Moreover, the degradation kinetics was described by a pseudo first-order model having a disappearance rate constant of k=0.274 min^{-1}. Even though complete removal of moxifloxacin was achieved, no significant mineralization (TOC removal) (p=0.90) was observed after 30 min of photocatalytic treatment.

Moxifloxacin is found to be one of the most toxic fluoroquinolone to *P. subcapitata* and its EC-50 (0.78 mg/L) was seven times lower than that of ciprofloxacin (5.57 mg/L). The UV-A/TiO_2 treatment assured not only the complete removal of residual moxifloxacin (C_o=15 mg/L) from the treated moxifloxacin solutions but also the toxicity. Generally, a decline in the toxic effects of the treated

solutions was observed with increasing degradation times. The decrease in toxicity was observed to be mainly associated with the photocatalytic removal of residual moxifloxacin from the treated solutions. This was supported by the fact that the residual moxifloxacin concentration (C_o=50 mg/L) in the treated solutions contributed to 94% and 64% of the growth rate inhibitions observed on *P. subcapitata* after 30 and 60 min of UV-A irradiation, respectively.

The result also demonstrated that one or more of the degradation products of moxifloxacin (C_o=50 mg/L) possess toxic potency and the 100% solutions induced an algal growth rate inhibition of 30 ± 17% and 13 ± 6% after 90 and 150 min of UV-A/TiO_2 treatment, respectively. The toxicity of the degradation products may have resulted from the conservation of the quinolone moiety along with most important functional groups (i.e., carboxyl, carbonyl, and fluoro). However, the structure–activity relationship revealed that most of the degradation products are generally less toxic to *P. subcapitata* than the mother compound due to reduced lipophilicity caused by the loss or transformation of the diazobicyclo group at position C7. The change in diazobicyclo group also disturbs the acid–base speciation of the individual degradates and the fraction of uncharged species that is able to penetrate the cell membrane and induce toxicity.

The environmental (aquatic) risk assessment revealed that under the current condition, moxifloxacin is unlikely to present a risk to aquatic organisms (RCR <1). However, the absence of environmental risk does not address the emergence of antibiotic resistant bacteria and sub-lethal effects (e.g., genotoxicity) that may prevail after long-term exposures at very low concentrations.

To understand ecotoxic effects at environmentally relevant concentrations, future studies should focus on the chronic effects of moxifloxacin and its photocatalytically treated solutions using higher test organisms such as daphnia and fish. Moreover, attention should be given to the study of the genotoxic effects of moxifloxacin and its photocatalytically treated solutions on aquatic organisms. This is because the use of standard test organisms and test concentrations much higher than relevant in the environment may underestimate toxicities that can have adverse effects on ecosystems in the long-term.

Table of contents

Acronyms and abbreviations

ANOVA	Analysis of variance
AOP	Advanced oxidation process
ARB	Antibiotic resistant bacteria
ARG	Antibiotic resistant gene
BOD	Biochemical oxygen demand
CBI (Dutch)	Centre for the promotion of imports from developing countries
C_o	Initial concentration
COD	Chemical oxygen demand
CV	Coefficient of variation
DDD	Defined daily dose
DID	Defined daily dose/1000 inhabitants/day
DNA	Deoxyribonucleic acid
DOC	Dissolved organic carbon
DT	Degradation time
E_o	Oxidation-reduction potential
EC	Effect concentration
ECHA	European chemicals agency
EQC	EQulibrium criterion
ERA	Environmental risk assessment
ESAC	European surveillance of antimicrobials consumption
EU	European Union
H	Henry's law constant
HPLC–ESI–LRMS	High-pressure liquid chromatography-electrospray ionization-low resolution mass spectrometry
I	Growth rate inhibition
IEP	Isoelectric point
K_{aw}	Air-water partitioning coefficient
K_d	Solid-water partitioning coefficient
K_{ow}	Octanol-water partitioning coefficient
m/z	Mass-to-charge ratio
NED	Normal equivalent deviation
OECD	Organization for economic development and cooperation
PEC	Predicted environmental concentration
PNEC	Predicted no-effect concentration
redox	Oxidation-reduction reaction

RCR	Risk characterization ratio
rps	Revolution per second
SAR	Structure–activity relationship
STP	Sewage treatment plant
TOC	Total organic carbon
UV	Ultra-violet

List of tables

List of figures

1. Introduction

The continued release of pharmaceuticals into the environment has raised concerns about their potential adverse human health and environmental effects as many classes of pharmaceuticals have been found to resist biotic and abiotic degradation and cause toxic effects on organisms. There is also a strong link between the emergence of antibiotic resistant bacteria and the continued release of antibiotics into the environment. Pharmaceuticals are being introduced into the environment mainly through sewage treatment plants (STPs) due to the inefficiency of the treatment processes applied in these facilities. In recent years, quite a number of researches have focused on the use of advanced oxidation processes (AOPs) for the removal of pharmaceuticals from water and wastewater matrices. In this regard, results show that AOPs are efficient in removing pharmaceuticals, but not necessarily the toxicity of the treated solution. Therefore, evaluation of the overall applicability of AOPs demands assessment of the toxic effects of the AOP treated aqueous matrix.

This thesis research was conducted mainly with the purpose of evaluating the effectiveness of the UV-A/TiO_2 treatment in removing the toxicity of moxifloxacin solutions, using the fresh water algae *P. subcapitata* as model organism. The study further assessed the toxic potency of the photocatalytically generated degradation products and environmental (aquatic) risk of moxifloxacin in Belgium.

This thesis is structured into five main sections consisting of (i) literature review, (ii) study justification and objectives, (iii) materials and methods, (iv) result and discussion, and (v) conclusions and recommendations. The literature review discusses the occurrence of pharmaceuticals in the environment, their fate in STPs, issues related to ecotoxicity and the development of antibiotic resistant bacteria due to the environmental exposure of bacteria to antibiotics. Moreover, the literature review contains sections that summarize the main AOPs studied for the removal of pharmaceuticals from water and wastewater matrices. In the end, the literature review attempts to address the general physical-chemical and environmental properties and ecotoxic effects of fluoroquinolones (antibiotic groups to which moxifloxacin belongs). Because of the limitations of relevant literature particularly focusing on moxifloxacin, the reader may find sections that talk about pharmaceuticals in general often times. However, discussion may become very specific to fluoroquinolones whenever relevant literature is found. The reader may also find words such as substrate or target compound/pollutant frequently, which means the compound under study.

The study justification and objectives part explains the scope of the study and contains the two main specific objectives of the research. The material and methods section deals with the methods used in the UV-A/TiO_2 photocatalysis, toxicity tests, environmental risk assessment, as well as the statistics and software used. The reader will also find that the photocatalytic treatment of moxifloxacin was done in two experimental phases. The first phase experiment was conducted to evaluate the ecotoxic effects of the photocatalytically treated moxifloxacin solutions. In the second phase, the experiment

further investigated the toxic effects of photocatalytically generated degradation products of moxifloxacin. Different initial concentrations of moxifloxacin were used in the first (C_o=15 mg/L) and second (C_o=50 mg/L) experimental phases. Initial concentrations of moxifloxacin much higher than expected in the environment were chosen for easy analytical detection of residual moxifloxacin and identification of degradation products, and for the quantification of toxic responses. The results and discussion part first addresses the photocatalytic removal of moxifloxacin followed by discussions on the results of the toxicity experiments. Moreover, a section is devoted to qualitatively explain the toxic effects of the photocatalytic degradation products of moxifloxacin on *P. subcapitata* using the structure–activity relationship. Finally, conclusions and recommendations are made based on the findings of the research.

Footnotes are sometimes used to define or explain technical terms.

2. Literature review

2.1. Pharmaceuticals in the environment

Pharmaceuticals constitute a large group of healthcare products intended for human and veterinary uses. Reports show that the production and consumption market of pharmaceuticals has been steadily increasing in recent years both globally and in the European Union (EU). In 2007, for instance, the production market of pharmaceuticals in the EU was approximately € 163 billion (at ex-factory prices) with an annual average growth of 3.5% since 2003. On the other hand, the consumption market in the region increased by annual average rate of 7.3% between 2003 and 2007 amounting, at ex-factory prices, an estimated € 141 billion in 2007 (CBI, 2010).

Despite the benefits of pharmaceuticals to mankind, a growing concern about their potential adverse impacts on biota (Kostich and Lazorchak, 2008) and human health has emerged as a result of their continued input into the environment (Baran et al., 2011). Since pharmaceuticals are present at very low levels (ng/L–µg/L) in wastewater (Daneshvar et al., 2010), conventional STPs fail to effectively and efficiently remove them (Bendz et al., 2005; Cooper et al., 2008). Besides, some pharmaceutical groups being recalcitrant escape through STPs and end up in surface water (Sim et al., 2010), groundwater (Fram and Belitz, 2011), and soil (Martín et al., 2012). For instance, studies indicate the wide presence of pharmaceuticals in surface waters (ng/L–µg/L) in countries like Belgium (Van De Steene et al., 2010), Germany (Scheurer et al., 2012), Portugal (Madureira et al., 2010), Spain (Ortiz de García et al., 2013), and USA (Gibs et al., 2013).

The main sources of pharmaceuticals to STPs include sewage from residential areas (i.e., private residences, dormitories, hotels, public and private institutions, etc.), animal farming, and effluent from healthcare facilities and pharmaceutical industries (Fig. 1) (Kümmerer, 2009b). Pharmaceuticals are released to sewer systems from residential areas, healthcare facilities, and animal farms as a result of the excretion of unmetabolized residues via feces and urine (Brown et al., 2006; Heberer, 2002), mainly because the metabolism of pharmaceuticals in biological systems is highly inefficient (Carballa et al., 2004; Hapeshi et al., 2010). Even more, the biotransformed metabolites of pharmaceuticals may retain the basic structure of their mother compounds (Robson, 1992; Stass and Kubitza, 1999). Therefore, they can display some form of biological activity and may contribute to the overall environmental risk. This suggests that in the assessment of environmental risk, the contribution of pharmaceutical metabolites to the overall risk should be considered since they can be present in significant proportions relative to the mother compound (Table 1).

Besides, direct release of unused and expired pharmaceuticals to sewers from one or more of these sources cannot be ignored (Heberer, 2002). Incomplete treatment of industrial influent containing pharmaceutical residues also contribute to the overall load of pharmaceuticals to STPs (Kümmerer, 2009a).

Table 1–Degree of excretion of unchanged pharmaceuticals and their metabolites from human body (urine and feces) after single dose treatment

Pharmaceutical	Excreted (%)		Reference
	Unchanged	Metabolites	
Moxifloxacin	41–55	45–59	Stass and Kubitza (1999)
Pefloxacin	<10*	60–85	Robson (1992)
Difloxacin	10*	22	Granneman et al. (1986)
Gatifloxacin	80–100	–	Grasela (2000)
Amoxicillin	80–90	10–20	Hirsch et al. (1999)
Ampicillin	30–60	20–30	Hirsch et al. (1999)
Penicillin G	50–70	30–50	Hirsch et al. (1999)

* Only urine excretion

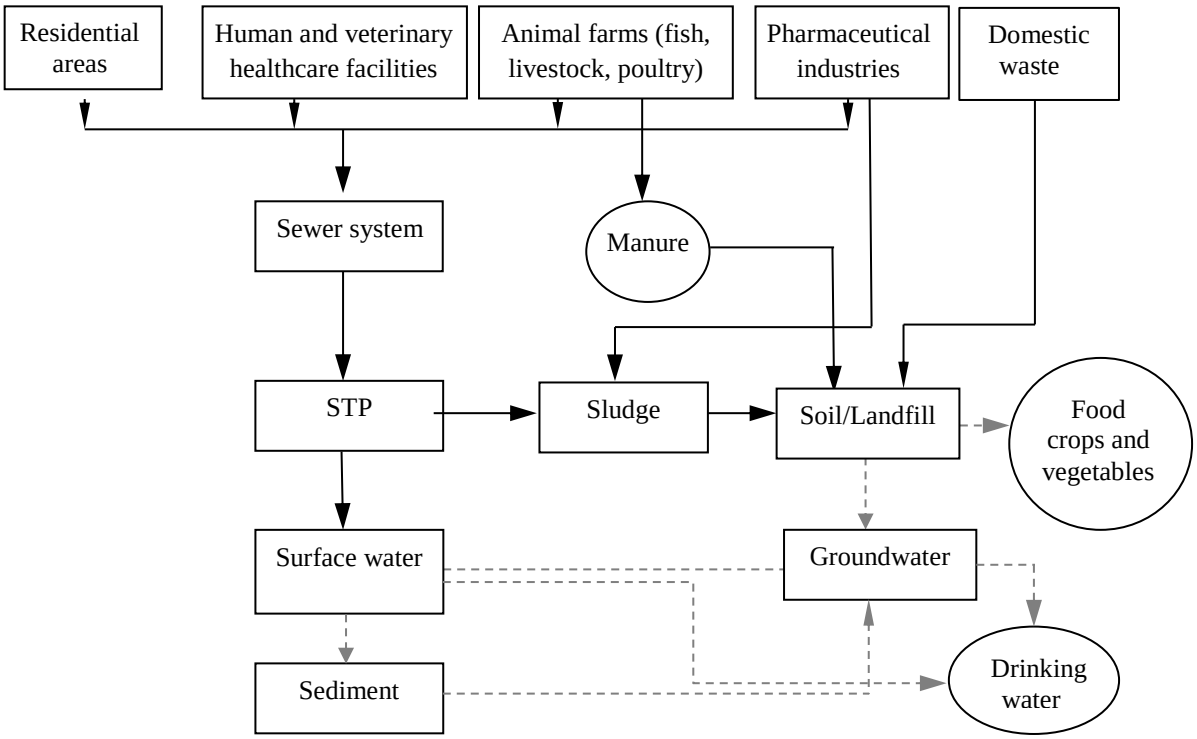


Fig. 1. Sources and release pathways of pharmaceuticals into the environment. (Adapted from Santos et al. (2010)). Solid lines–pathway of direct release; broken lines–pathway of indirect release.

The major pathways of release into the environment are via STP effluent discharge to surface water and sludge disposal to land (Santos et al., 2010). The effluent discharge leads to contamination of surface water and sediments (Gibs et al., 2013), and exposure of aquatic organisms, whereas sludge disposal can lead to groundwater contamination from direct application of sludge as biosolid (Homem and Santos, 2011). Additionally, contamination of groundwater by pharmaceuticals from landfill leachate and surface water percolation constitute indirect pathways.

2.2. Fate of pharmaceuticals in STPs

One of the challenges in wastewater treatment is the failure of STPs to degrade pharmaceutical residues and render them harmless. This inefficiency results in the release of these residues into the environment structurally unchanged. Once an influent loaded with pharmaceutical residues reaches a STP, properties such as partitioning coefficients (e.g., octanol-water (K_{ow}), solid-water (K_d), and air-water (K_{aw})), and chemical structure (including type and number of substituents present) determine the extent of their biodegradation, adsorption to sludge, photolysis, hydrolysis, and volatilization (Byrns, 2001; Jia et al., 2012; van Leeuwen and Vermeire, 2007).

Many pharmaceuticals are largely removed in STPs by adsorption to sludge via electrostatic or hydrophobic interactions, and/or cation exchange depending on the type of pharmaceutical and the prevailing pH condition in the wastewater (Jia et al., 2012; Yamamoto et al., 2009). Sorption to sludge has been reported as a major removal pathway for antibiotics, especially fluoroquinolones from wastewater (Conkle et al., 2010; Jia et al., 2012). Despite the substantial removal of fluoroquinolones (40–100%) in STPs (mainly by adsorption to sludge), concerns still remain because of their occurrence in surface water and soil as a result of effluent discharge and land application of sludge as a biosolid (Giger et al., 2003; Heberer, 2002; Hu et al., 2010).

The high sorptive removal of fluoroquinolones in STPs may be due to the fact that fluoroquinolones possess high log K_d values despite their low log K_{ow} and high water solubility (Table 2) (Girardi et al., 2011; Golet et al., 2003; Picó and Andreu, 2007; Vieno et al., 2007). The primary sorption mechanism of fluoroquinolones to solids in wastewater is via electrostatic interaction. Apart from that, hydrophobic intermolecular forces also play a role in the adsorption process (Conkle et al., 2010; Golet et al., 2003). When electrostatic interaction is the main mechanism of adsorption to sludge, K_{ow} may not be a good estimator of K_d because the relationship between K_{ow} and K_d is based on the assumption that there is a hydrophobic interaction between the molecule and solid matter (van Leeuwen and Vermeire, 2007). The electrostatic interaction is the result of fluoroquinolones ability to exhibit anionic, cationic, or zwitterionic properties depending on pH that would help them interact with opposite charges on the adsorbing solid surface (Dorival-García et al., 2013a).

Biodegradation in STPs is affected by the antibacterial activity and structural stability of pharmaceuticals. Therefore, the role of STPs in removing many pharmaceuticals is limited (Heidler and Halden, 2007; Jia et al., 2012; Lajeunesse et al., 2012); exceptions are pharmaceuticals including but not limited to enalapril, ketoprofen, and naproxen that degrade biologically with high degree of efficiency (>80%) (Jelic et al., 2011).

Table 2–STP removal efficiencies and relevant physical and environmental properties of fluoroquinolones

Fluoroquinolone	STP removal efficiency (%)	log K_d	log K_{ow}	Water solubility (g/L)
Ciprofloxacin	83 (Golet et al., 2003)	3.69 (soil) (Conkle et al., 2010)	0.28 (Vieno et al., 2007)	30 (Vieno et al., 2007)
	90 (Vieno et al., 2007)	n.d.a	n.d.a	n.d.a
Norfloxacin	88 (Golet et al., 2003)	3.76 (soil) (Conkle et al., 2010)	-1.03 (Vieno et al., 2007)	178 (Vieno et al., 2007)
	100 (Vieno et al., 2007)	n.d.a	n.d.a	n.d.a
	66 (Xu et al., 2007)	n.d.a	n.d.a	n.d.a
Ofloxacin	77 (Brown et al., 2006)	3.64 (soil) (Conkle et al., 2010)	-0.39 (Vieno et al., 2007)	28.3 (Vieno et al., 2007)
	86 (Vieno et al., 2007)	n.d.a	n.d.a	n.d.a
	57 (Xu et al., 2007)	n.d.a	n.d.a	n.d.a
Enrofloxacin	75 (Jia et al., 2012)	2.7–3.7 (soil) (Golet et al., 2003)	n.d.a	n.d.a
Sarafloxacin	n.d.a	4.64 (soil) (Picó and Andreu, 2007)	-0.09 (Völgyi et al., 2012)	n.d.a
Moxifloxacin	60 (Dorival-García et al., 2013b)	2.86 (sludge) (Dorival-García et al., 2013b)	-0.28 (Langlois et al., 2005)	19.6 (Varanda et al., 2006)
	40 (Jia et al., 2012)	n.d.a	n.d.a	n.d.a
Levofloxacin	n.d.a	n.d.a	-1.35 (Michot et al., 2005)	n.d.a
Gatifloxacin	50 (Xiao et al., 2008)	n.d.a	-0.71 (Völgyi et al., 2012)	n.d.a
	43 (Jia et al., 2012)	n.d.a	n.d.a	n.d.a

n.d.a: no data available

The role of biodegradation in the removal of fluoroquinolones from STPs is not yet clear because there are only few studies in this regard and the results are contradictory. For instance, closed bottle tests on the biodegradability of ciprofloxacin showed that the compound is not biodegradable up to 40 days of incubation (Al-Ahmad et al., 1999; Kümmerer et al., 2000). This result is consistent with the antibacterial nature of fluoroquinolones. On the other hand, Halling-Sørensen et al. (2000) stated that half of the initially spiked ciprofloxacin (250 µg/L) was biodegraded in 2.5 days in an activated sludge reactor. In addition, a recent study by Dorival-García et al. (2013b) observed that fluoroquinolones such as moxifloxacin, ofloxacin, ciprofloxacin, and norfloxacin biodegraded in an activated sludge membrane bioreactor with half-life time ranging from 4–10 days. The observed biodegradability of fluoroquinolones in activated sludge might be because of the presence of antibiotic resistant bacteria that are able to metabolize the compounds (Dorival-García et al., 2013b).

Many pharmaceuticals are high molecular weight compounds with very low Henry's law constants (i.e., $<10^{-15}$ atm$\cdot$m^3/mol) (Dorival-García et al., 2013b), thus they are barely volatile. Due to the presence of high load of suspended solids in a wastewater, in addition to the structural stability of many pharmaceuticals, abiotic degradations such as hydrolysis and photolysis barely take place in STPs. Therefore, the role of hydrolysis, volatilization, and photolysis in removing many pharmaceuticals from wastewater in STPs is negligible (Kosjek and Heath, 2011; Kümmerer, 2009a).

Wastewater characteristics and operational conditions of treatment processes in STPs also affect the concentration of pharmaceuticals in effluent water and sewage sludge. A case in point, increasing the concentration of total organic carbon (TOC) and solid residence time in STPs have shown to increase the concentration of pharmaceuticals in the sludge due to increased sorption (Li et al., 2013). Nevertheless, high removal efficiencies of STPs do not assure complete degradation of the pharmaceuticals as many of them strongly adsorb to sludge. Clearly, such treatments merely transfer pharmaceuticals from one environmental matrix (e.g., wastewater) to the other (e.g., soil) without eliminating or significantly reducing the risk. Once pharmaceuticals are in the soil, they can stay longer by strongly adsorbing to soil particles and may become inaccessible for degradation.

2.3. Concerns related to the release of pharmaceuticals into the environment

One of the factors that contributed to the growing concern regarding the release of pharmaceuticals into the environment is their persistence in different environmental compartments. For instance, Walters et al. (2010) found in their mesocosm study that among the initially detected 15 pharmaceuticals, five of them namely azithromycin, carbamazepine, ciprofloxacin, and norfloxacin had half-life time between 1–3 years in the soil-sewage sludge mixture indicating high resistance to biotic and abiotic degradation. In addition, studies have shown the inefficiency of solar photolysis in degrading psychiatric pharmaceuticals such as oxazepam, diazepam, and alprazolam in the aquatic environment (Calisto et al., 2011). Similarly, Yamamoto et al. (2009) reported that pharmaceuticals

including ibuprofen, atenolol, and carbamazepine showed resistance to solar photodegradation when irradiated for 50–70 h. The biodegradability of pharmaceuticals in coastal surface water samples was also investigated by Benotti and Brownawell (2009). In this study, they observed that pharmaceuticals such as antipyrine, carbamazepine, and trimethoprim were found to be non-biodegradable with half-life times between 35 to >100 days. Pharmaceuticals such as carbamazepine (140 ng/L) and sulfathiazole (10 ng/L) were also detected in finished drinking water (n=12) after conventional drinking water treatment (i.e., flocculation-sedimentation-activated carbon filtration) indicating that some pharmaceuticals can escape such treatments (Stackelberg et al., 2004; Stackelberg et al., 2007).

When pharmaceuticals, especially antibiotics, end up in the environment structurally unchanged, there is a possibility that virulent bacteria may develop resistance due to long term exposure and further complicate infection control efforts (Gao et al., 2012; Wellington et al., 2013). In this regard, there is an increasing evidence linking the high prevalence of antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs) in STP effluent and sludge to the co-presence of antibiotics (Rizzo et al., 2013; Wellington et al., 2013). For example, Gao et al. (2012) found a significant correlation (n=8; R^2=0.75–0.83) between the number of ARB and antibiotic (tetracycline and sulfonamide) concentrations (0.26–1.54 μg/L) in raw wastewater and treated effluent. In this case, the effect of the antibiotics at very low concentrations on susceptible bacteria causes a selective pressure resulting into the proliferation of ARB (Schwartz et al., 2003) through horizontal gene transfer (Wellington et al., 2013).

The release of pharmaceuticals into surface water also affects aquatic organisms such as fish. For example, Galus et al. (2013) observed that exposure of zebra-fish (*Danio rerio*) to a diluted wastewater effluent containing a spiked mixture of pharmaceuticals (acetaminophen, carbamazepine, gemfibrozil and venlafaxine) at a concentration of 0.5 μg/L and 10 μg/L induced a significant reduction in embryo production after 6 weeks of exposure. A similar study observed that the pharmaceutical dutasteride caused a significant decline in fecundity of fish (fathead minnow) at a spiked concentration of 10, 32, and 100 μg/L and affected many of the endocrine functions of both fish sexes in a 21 day toxicity test (Margiotta-Casaluci et al., 2013).

Likewise, when soil is exposed to pharmaceutical residues there is a potential for adverse effects on soil dwelling organisms from the toxic effects of pharmaceuticals, and on humans through plant uptake (food chain). For instance, Thiele-Bruhn and Beck (2005) reported that the presence of pharmaceutical residues such as sulfapyridine and oxytetracycline in soil caused 10% inhibition of microbial activity at an effective dose (ED-10) ranging from 0.003–7.35 μg/g soil (48 h incubation). Moreover, Boleas et al. (2005) observed that oxytetracycline (spiked dose of 100 μg/g soil) caused significant effects on soil microbes enzymatic activities in a 21 day test period.

Edible plants such as cabbage, carrot, lettuce, green onion, and corn were also seen accumulating pharmaceuticals from soil indicating caution on the direct application of manure and sludge to agricultural soil (Boxall et al., 2006; Kumar et al., 2005).

The occurrence of persistent pharmaceuticals in the environment at low levels may bring adverse effects on biota and human health with long-term exposures. Especially, the continued release of persistent antibiotics into the environment makes infection control efforts difficult as it accelerates the emergence of antibiotic resistant bacteria, which also makes the discovery of new antibiotics a costly process. Although many studies conducted on the ecological impacts of pharmaceuticals use initial concentrations far greater than the relevant environmental concentrations, it is likely that pharmaceuticals are able to disturb ecosystems and their functions if they are continuously released into the environment. Besides, the evidence that pharmaceuticals can accumulate in edible plants should be alarming since it would be difficult to predict their long-term effects on human health. Therefore, adopting the precautionary principle should guide the necessity for the removal of pharmaceuticals from wastewater.

The failure of STPs in degrading many classes of pharmaceuticals provided the opportunity for many researches to focus on the use of advanced oxidation processes (AOPs) for the removal/degradation of pharmaceuticals from water and wastewater matrices to prevent the adverse effects that may result from the release of pharmaceuticals into the environment.

2.4. Advanced oxidation processes for the removal of pharmaceuticals

AOPs, such as ozonation, sonolysis, Fenton-oxidation, photolysis, and photocatalysis are processes that are mainly based on the generation and use of highly reactive species such as hydroxyl radicals ($HO^{\bullet}$) to oxidize target compounds. This is because hydroxyl radicals are highly unstable and reactive (E_o= +2.59 V; pH <12). Therefore, their reactions with target compounds are extremely rapid and non-specific (Petri et al., 2011).

2.4.1 Fenton based processes

The classical Fenton process is based on a redox reaction that generates $HO^{\bullet}$ when ferrous ion (Fe^{2+}) and hydrogen peroxide (H_2O_2) react in an acidic medium as follows (Brillas et al., 2009):

$$Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + H_2O + HO^{\bullet} \quad (1)$$

Recently, advanced Fenton-based processes such as photo-Fenton, electro-Fenton, and microwave assisted Fenton processes have shown interesting results in enhancing the transformation and/or mineralization efficiency of pharmaceuticals in water and wastewater matrices. Coupling Fenton based processes with other AOPs increases the processes of $HO^{\bullet}$ generation, regenerating Fe^{2+}, and/or the continuous production of H_2O_2 by electrolysis, which lead to the increase in the concentration of $HO^{\bullet}$ in the treatment system (Brillas et al., 2009; Sirés et al., 2010).

Fenton-based processes proved to be highly efficient in removing target compounds from water and wastewater matrices with substantial mineralization measured as lost TOC or dissolved organic carbon (DOC) (Table 3). However, these processes still generate degradation products that possess toxicity. Treatment efficiency comparison of the various Fenton-based processes is not possible due differences in the operational parameters used. Obviously, the efficiencies obtained with Fenton-based processes are affected by operational parameters such as pH and the concentration of Fe^{2+} and H_2O_2 (Brillas et al., 2009).

Overall, Fenton-based processes provide advantages in terms of the ease of chemical handling and use of low cost chemicals (Brillas et al., 2009; Pignatello et al., 2006). However, they are limited to processes occurring in acidic medium (pH 2–4) (Klavarioti et al., 2009). The accumulation of excess iron sludge at the end of the treatment process is also a challenge (Neyens and Baeyens, 2003).

2.4.2 Ozonation

Ozonation is a well-established AOPs, which utilizes the strong oxidizing power of ozone (E_o= +2.07 V) for the removal of recalcitrant and trace organic compounds from drinking water and wastewater (Camel and Bermond, 1998; Fiehn et al., 1998; Kishimoto et al., 2005). Ozone is very selective and reacts directly with a limited number of compounds. For instance, compounds with C=C bond or aromatic compounds having electron donor groups (e.g., phenol, alkyl, or methoxy) are highly susceptible to ozone attack, whereas organics with amide and carboxylic groups are resistant (Nakada et al., 2007).

Ozonation proceeds via two possible mechanisms in degrading organic compounds depending the pH of the prevailing condition. These include direct electrophilic attack of target organic compounds (pH <7) and indirect oxidation (Eq. 2–5) through generated secondary reactive oxidants (pH >7) such as $HO^{\bullet}$, $HO_2^{\bullet}$, and $O_2^{\bullet-}$ (Wang and Xu, 2012).

$$O_3 + OH^- \rightarrow HO_2^- + O_2 \quad (2)$$

$$HO_2^- + O_3 \rightarrow O_3^{\bullet-} + HO_2^{\bullet} \quad (3)$$

$$HO_2^{\bullet} \leftrightharpoons H^+ + O_2^{\bullet-} \quad (4)$$

$$O_2^{\bullet-} + O_3 \rightarrow O_3^{\bullet-} + O_2 \quad (5)$$

$$O_3^{\bullet-} + H_2O \rightarrow HO^{\bullet} + O_2 + OH^- \quad (6)$$

The use of ozonation for the removal of pharmaceuticals from water and wastewater matrices found to be efficient. The complete removal of target compounds using ozonation can be possible by selecting optimum operational parameters. Despite the differences in the use of treatment operational parameters, the use of ozonation for pharmaceuticals removal from water and wastewater matrices resulted in the mineralization of less than a quarter of the original substrate concentration (Table 4).

This indicates that the generated degradation products are resistant to ozonation. This is also shown by the poor enhancement in biodegradability of the degradation products.

Coupling ozone with H_2O_2, UV irradiation, catalyst, UV/catalyst, or sonolysis enhances its treatment efficiency by generating non-selective and reactive oxidant species such as $HO^{\bullet}$, perhydroxyl radical ($HO_2^{\bullet}$) and superoxide ion ($O_2^{\bullet-}$) that can strongly react with target compounds (Abouzlam et al., 2013; Kishimoto et al., 2005; Petri et al., 2011). A recent study reported that by coupling ozonation with sonolysis/Fe^{2+}, a substantial enhancement in the mineralization (46% DOC removal) of diclofenac was obtained (Ziylan and Ince, 2013). Similarly, photocatalytic (UV-A/TiO_2) ozonation resulted in higher than 60% mineralization (TOC removal) from a mixture of pharmaceuticals containing atenolol, hydrochlorothiazide, ofloxacin, and trimethoprim (Rodríguez et al., 2013).

The generation of toxic degradation products (De Witte et al., 2010) and high operational cost for oxygen supply and ozone generation (Lucas et al., 2010) are some of the main drawbacks in the application of this AOP.

Table 3–Summary of Fenton-based AOPs used to treat pharmaceuticals in water and wastewater matrices

AOP	Pharmaceutical(s) studied	Experimental details	Main findings	Reference
Solar photo-Fenton	Ofloxacin, trimethoprim	Wastewater matrix; treated volume 85.4 L; initial spiked substrate concentration 0.1 mg/L; pH 2.8–2.9; treatment duration 180 min; $[Fe^{2+}]_o$=5 mg/L; $[H_2O_2]_o$=75 mg/L.	Complete removal of the pharmaceuticals; 21% DOC removal; 50% COD removal; final solution after 180 min treatment induced 13% (24 h) and 33% (48 h) immobilization of *D. magna*.	Michael et al. (2012a)
Fenton	Amoxicillin, ampicillin, cloxacillin	Artificial wastewater matrix; treated volume 0.5 L; initial substrate concentration 103–105 mg/L; $[Fe^{2+}]_o$=17–60 mg/L; $[H_2O_2]_o$= 510–1836 mg/L; pH 3; maximum treatment duration 60 min.	Complete substrate removal in 2 min; improvement of biodegradability (BOD_5/COD ratio) from 0 to 0.37 in 10 min; DOC and COD removals of 54% and 81%, respectively in 60 min.	Elmolla and Chaudhuri (2009)
Electro-Fenton	Cefalexin	Deionized water matrix; initial substrate concentration 200 mg/L; DC power; I= 6.66 mA/cm^2; $[Fe^{2+}]_o$= 56 mg/L; pH 3; treatment duration 480 min; working electrodes: RuO_2/Ti anode and activated carbon cathode.	Complete removal of cephalexin after 270 min of treatment; 49% TOC removal; 72% COD removal; biodegradability enhanced by 0.26 (BOD_5/COD).	Ledezma Estrada et al. (2012)
Microwave-assisted Fenton	Amoxicillin	Deionized water matrix; initial substrate concentration 0.45 mg/L; treated volume 50 mL; effective power 162 W; frequency 2450 MHz; $[Fe^{2+}]_o$= 0.095 mg/L; $[H_2O_2]_o$= 2.35 mg/L; pH 3.5; treatment duration 5 min.	Complete removal of amoxicillin in 5 min.	Homem et al. (2013)
Fenton	Flumequine	Deionized water matrix; initial substrate concentration 0.5 mg/L; treated volume 1 L; pH 2.8; $[Fe^{2+}]_o$= 28 mg/L; $[H_2O_2]_o$= 68 mg/L; treatment duration 60 min.	40% flumequine removal achieved in 15 min; reduction in anti-bacterial activity (*E. coli*) observed.	Rodrigues-Silva et al. (2013)
Photo-Fenton	Flumequine	Deionized water matrix; initial substrate concentration 0.5 mg/L; treated volume 1 L; pH 2.8; $[Fe^{2+}]_o$= 14 mg/L; $[H_2O_2]_o$= 340 mg/L; Hg–lamp (P=15 W, λ=254 nm); treatment duration 60 min.	94% flumequine removal achieved in 60 min; anti-bacterial activity (*E.coli*) virtually eliminated.	Rodrigues-Silva et al. (2013)

2.4.3 Sonolysis

Sonolysis (sonochemical reaction) makes use of sonic or ultrasonic waves (frequency >16 KHz) to create an oxidative environment through the formation, growth, and sudden collapse of micro-bubbles in liquids (Fig. 2) called cavitation or "*cold boiling*" (Adewuyi, 2001). The collapse of these bubbles result in extremely high localized temperature and pressure greater than or equal to 5000 K and 1000 atm, respectively (Adewuyi, 2001; Flint and Suslick, 1991). Though these conditions are momentary, they are able to generate highly reactive species such as $HO^{\bullet}$, hydrogen ($H^{\bullet}$), and $HO_2^{\bullet-}$ radicals that are responsible for rapid chain reactions (De Bel et al., 2011; Wang and Xu, 2012).

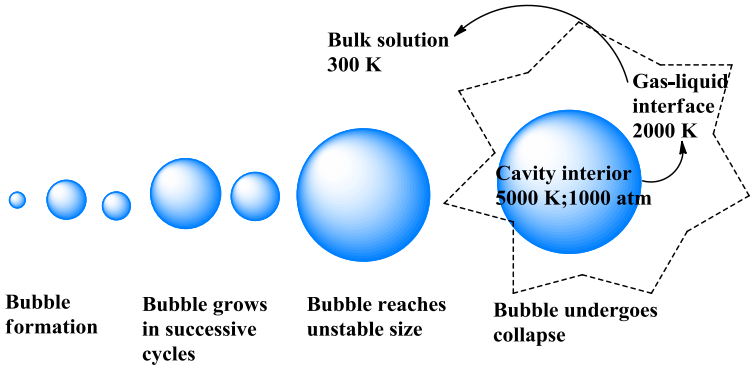


Fig. 2. Steps in cavitation bubble formation and collapse (Wang and Xu, 2012).

The "hot spot" theory is the most widely accepted one used to explain environmental sonochemsity. It states that sonochemical reactions are highly heterogeneous reactions that generate free radicals and heat from the bubble of cavitation (Eq. 7–10). The free radicals then react with target compounds within the collapsing bubbles, at the interface of the bubbles, and in the surrounding liquid. Inside the center of the bubble, bond cleavage of water, water vapor and other gases produce free radical species because of the harsh conditions generated on bubble collapse (Adewuyi, 2001).

$$H_2O \rightarrow H^{\bullet} + HO^{\bullet} \quad (7)$$

$$H^{\bullet} + O_2 \rightarrow HO_2^{\bullet} \quad (8)$$

$$HO_2^{\bullet} + HO_2^{\bullet} \rightarrow H_2O_2 + O_2 \quad (9)$$

$$HO^{\bullet} + HO^{\bullet} \rightarrow H_2O_2 \quad (10)$$

Although the use of sonolysis in water and wastewater treatment is a relatively new technique (Michael et al., 2012b), recent studies show that its efficiency of pharmaceuticals removal is quite encouraging. For example, sonolysis (35 KHz) was able to remove 70% the antibiotic Penicillin G (initial concentration 200 mg/L) from a synthetic wastewater matrix (pH 3) after 70 min (Saghafinia et al., 2011). Similarly, 57% ciprofloxacin (initial concentration 15 mg/L) disappeared when sonicated (520 KHz) in deionized water (pH 7) for 120 min (De Bel et al., 2009). High removal rates were also reported for pharmaceuticals such as levodopa (91%) and paracetamol (95%) when sonicated (574 KHz) for 240 min in pure water at an initial concentration of 25 mg/L (Isariebel et al., 2009).

Table 4–Summary of ozonation treatment used in the removal of pharmaceuticals from water matrices

Pharmaceutical (s) studied	Experimental details	Main findings	Reference
Diclofenac	Wastewater matrix; initial spiked substrate concentration 200 mg/L; O_3 dose 220 mg/L; pH 7.06; treatment duration 30 min.	> 99% removal of diclofenac in 30 min; 24% TOC removal in 1 h; BOD_5/COD ratio <0.1 after 30 min; degradation products are poorly biodegradable; toxicity on *V. fisheri* slightly decreased.	Coelho et al. (2009)
Oxytetracycline	Wastewater matrix actually containing oxytetracycline; O_3 dose 657 mg/L; treatment duration 120 min; T=20^{o}C; O_3 flow rate 300 mL/min; pH 7.	> 96% substrate elimination; 29% COD removal.	Zheng et al. (2010)
Ciprofloxacin	Deionized water matrix; initial substrate concentration 0.2 mg/L; O_3 flow rate 7.5 mg/min; pH 9; treatment duration 30 min.	> 90% substrate removal; < 20% COD reduction.	Vasconcelos et al. (2009b)
Ciprofloxacin	Hospital wastewater matrix; initial spiked substrate concentration 15 mg/L; O_3 flow rate 120 mL/min; pH 7; maximum treatment duration 90 min.	95% substrate removal in 79 min; degradation products exhibit anti-bacterial activity (*E.coli*).	De Witte et al. (2010)
Bezafibrate	Deionized water matrix; initial substrate concentration 181 mg/L; pH 6; O_3 dose 35 mg/L; maximum treatment duration 105 min.	Complete substrate removal in 10 min; 20% TOC removal; BOD_5/COD ratio after 10 min of treatment equals 0.15 indicating poor biodegradability of degradation products; biodegradability enhanced by increasing treatment duration; one or more of the degradation products generated after 10 min treatment more toxic to *V. fisheri* than the mother compound.	Dantas et al. (2007)
Sulfamethoxazole	Deionized water matrix; initial substrate concentration 200 mg/L; O_3 dose 400 mg/L; without pH adjustment; maximum treatment duration 60 min.	Complete substrate removal in 15 min; 18% TOC removal after 60 min of treatment; BOD_5/COD ratio enhancement from 0 to 0.28 was observed after 60 min treatment; degradation products generated in the first 30 min were more toxic to *V. fisheri* than the mother compound.	Dantas et al. (2008)

In fact, higher removal efficiencies could also be achieved by controlling operational parameters such as initial substrate concentration, pH, irradiation time, power, frequency, and air sparging (Naddeo et al., 2009b), or by coupling sonolysis with UV, catalyst, or UV/catalyst. In the latter case, Hapeshi et al. (2012) observed that the removal of ofloxacin (initial spiked concentration 10 mg/L) from a wastewater effluent was the (~100%) for sonolysis (20 KHz)/UV-A/TiO_2 followed by sonolysis/TiO_2 (62%), and the lowest (15%) for sonolysis after 120 min of treatment. However, a sonolysis/UV-A combination resulted in 90% removal of ofloxacin after 30 min of treatment. The enhancement in removal efficiency was attributed to the increase in the generation of reactive free radicals and the creation of additional cavitation activity. Even more, on a large-scale, the economic feasibility of a hybrid sonolysis such as sonolysis/UV/O_3 is reported to be more cost-effective than sonolysis alone (Mahamuni and Adewuyi, 2010).

A biodegradability, mineralization, and ecotoxicity test on a mixture of pharmaceuticals including diclofenac (2.5 mg/L), amoxicillin (10 mg/L), and carbamazepine (5 mg/L) spiked into a real wastewater matrix indicated that the treatment increased the BOD_5/COD ratio by 30% after 60 min sonication (20 kHz, pH 7.5). However, only 11% TOC removal was possible; and, a 30% decrease in growth rate inhibition on *P. subcapitata* was observed (Naddeo et al., 2009b). In another study, a TOC removal of 36% was attained when diclofenac (40 mg/L) in pure water was sonicated (20 KHz) for 40 min (Naddeo et al., 2009a).

Coupling sonolysis with other AOPs increases the generation of reactive free radicals resulting in better removal efficiency. However, like other AOPs, the mineralization efficiency of sonolysis is limited.

2.4.4 Photolysis and photocatalysis

Photolysis involves the direct use of shorter wavelength radiation (e.g., UV) to cleave the bond of a target compound and initiate a reaction, or proceeds with the generation of highly reactive oxidants such as $HO^{\bullet}$ free radical that will react with the target compounds (Fatta-Kassinos et al., 2011). On the other hand, photocatalysis makes use of a semiconductor catalyst, and light of a specific energy to activate the catalyst start a redox reaction with a target compound. It is sub-divided into homogenous photocatalysis, where the catalyst is in the same phase as the target compound, and heterogeneous photocatalysis, where the catalyst is in a different phase from the target compound (Parmon et al., 2002). Transition metal oxides such as TiO_2, ZnO, ZrO_2, CeO_2, etc., are the ones that can be used as heterogeneous photocatalysts, among which the most studied is TiO_2.

Generally, photocatalytic processes that use TiO_2 are faster and more efficient than solar or UV assisted photolysis (Paul et al., 2010). Moreover, the use of TiO_2 for the removal of trace organic pollutants from water and wastewater provides a number of advantages including the ability to operate

using solar light (UV-A), low cost, chemical stability over a wide pH range, and biological and chemical inertness (Herrmann, 1999; Hoffmann et al., 1995).

2.4.4.1 UV/TiO_2 photocatalysis

The use of TiO_2 as a catalyst coupled with UV irradiation has gained wide attention in this decade for the removal of pharmaceuticals from water and wastewater with encouraging results (Calza et al., 2006; Méndez-Arriaga et al., 2008; Nasuhoglu et al., 2012; Sousa et al., 2012). The TiO_2 acts as an initiator for light induced redox reactions because of its electronic structure characterized by an electron filled valence band and empty conduction band (Linsebigler et al., 1995). When the TiO_2 is illuminated with a photon of energy higher than or equal to the band gap energy (E_b= +3.2 eV; $\lambda \leq 388$ nm) of the TiO_2, an electron will be excited from the valence band into the conduction band forming a photo-hole (h^+) in the valence band (Herrmann, 2005; Hoffmann et al., 1995). The formed hole in the valence band and the excited electron in the conduction band are capable of undergoing redox reactions with adsorbed reactants (Linsebigler et al., 1995) according to the following general reaction equations (Eq. 11–17, Fig. 3):

$$(TiO_2) + h\nu \rightarrow h^+ + e^* \quad (11)$$

$$M + e^* \rightarrow M^{\bullet -} \quad (12)$$

$$H_2O + h^+ \rightarrow H^+ + HO^\bullet \quad (13)$$

$$P + h^+ \rightarrow P^{\bullet +} \quad (14)$$

$$M^{\bullet -} + P^{\bullet +} \rightarrow D \quad (15)$$

$$HO^\bullet + P \rightarrow D \quad (16)$$

$$h^+ + e^* \rightarrow N + \text{Energy} \quad (17)$$

Annotations: $\mathbf{e}^*$: excited state conduction band electron; **M**: electron acceptor; **P**: electron donor; **D**: degradation intermediate; **N**: the neutral center; *hv*: light.

Conduction band electron and valence band hole can recombine and dissipate the input energy (Klavarioti et al., 2009) if no electron acceptor is available, or directly react with electron donors (e.g., target compound) and acceptors (e.g., O_2) adsorbed on the TiO_2 surface (Hoffmann et al., 1995). Besides, indirect redox reactions occur through the formation of $HO^\bullet$ generated by the oxidation of water by the hole (Herrmann, 1999, 2005).

Sorption of electron donors and acceptors onto the catalyst surface is a critical step in UV/TiO_2 photocatalysis. In this process, one or more interaction mechanisms including van der Waals forces, hydrogen bonding, and complexation play the binding role (Hoffmann et al., 1995).

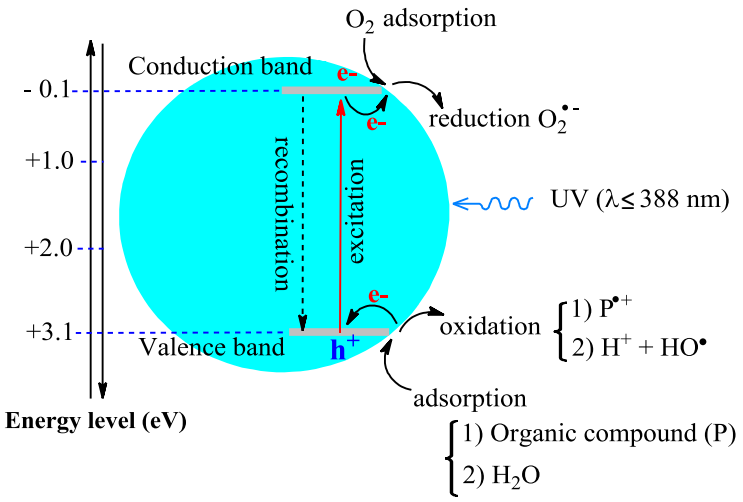


Fig. 3. Photocatalytic redox processes in spherical TiO_2 (Herrmann, 2005).

Studies show that the use of UV/TiO_2 in removing pharmaceuticals from water and wastewater matrices proved to be efficient with significant reduction in toxicity. Comprehensive reviews on the use of UV/TiO_2 for removal of pharmaceuticals from these matrices are published recently (Klavarioti et al., 2009; Tong et al., 2012). The reviews indicated that several factors influence the efficiency of substrate (i.e., target compound) removal including pH, presence of electron acceptors, catalyst composition and loading, substrate concentration, light source, matrices used, and treatment duration. High substrate concentrations lower reaction rates by saturating the catalyst active sites, while pH does the same by controlling the charge character of the catalyst surface and the substrate. Increasing catalyst loading does not increase reaction rate indefinitely, because with increasing catalyst loading, some catalyst particles may be shielded from the incident light by others, and their activity may be hindered.

Enhancements in removal efficiency can also be obtained by optimizing operational parameters such as pH, catalyst particle size and concentration, initial concentration of substrate and electron acceptor (e.g., oxygen), incident light intensity, and temperature (Ahmed et al., 2011; Hoffmann et al., 1995; Van Doorslaer et al., 2012). For instance, when a solution containing a mixture of pharmaceuticals such as metronidazole, atenolol, and chlorpromazine was treated using UV-C/TiO_2 (PC-500; immobilized on ceramic plate), it was observed that increasing the initial substrate concentration at constant irradiation time (90 min) resulted in reduced substrate removal efficiency. In addition, optimal removal efficiency (>85%) was achieved at 10 mg/L individual substrate concentration and 150 min irradiation time. In the same study, high mineralization (90% TOC removal) was realized after 16 h of treatment (Khataee et al., 2013). Even if it was possible to achieve a substantial level of mineralization, the treatment duration is very long and this may increase the cost of energy.

A study by Van Doorslaer et al. (2012), using UV-A/TiO_2 (Degussa, P-25), indicated optimal degradation for the antibiotic moxifloxacin at 5 g/L TiO_2, 25^oC and an air flow rate of 60 mL/min. In a separate study, the highest removal rates for ciprofloxacin and moxifloxacin after UV-A/TiO_2 (Degussa, P-25) treatment were found at neutral pH (TiO_2 0.5 g/L) (Van Doorslaer et al., 2011). On the other hand, efficient photocatalytic degradation (100%) of the antibiotic oxolinic acid was favored at lower pH and 1 g/L catalyst loading (UV-A/TiO_2, Degussa P-25) (Giraldo et al., 2010).

Despite the potential high removal efficiency the UV/TiO_2 treatments, in many cases it is limited by the generation of degradation products with poor biodegradability, toxicity (Rizzo et al., 2009b; Vasconcelos et al., 2009a), and low rate of mineralization (Chong and Jin, 2012; Giraldo et al., 2010).

2.4.5 End-points of AOP treatment efficiency and effectiveness

AOPs are efficient in removing target compounds from water and wastewater matrices. However, they are characterized by limited mineralization and the generation of degradation products that may possess toxicity. Therefore, in the evaluation of the overall treatment efficiency and effectiveness of AOPs in removing target compounds from water and wastewater matrices, and rendering the final product suitable for discharge into the environment or reuse, a careful selection of measurable end-points is essential. Table 5 presents the important end-points and measurement variables that are used to assess the efficiency and effectiveness of AOPs treatment.

Table 5–End-points of AOPs performance evaluation

End-point	Measurement variable	Reference
Removal efficiency	Degradation kinetics	Van Doorslaer et al. (2011)
Mineralization rate	TOC removal rate	An et al. (2010)
	DOC removal rate	Hapeshi et al. (2010)
Biodegradability enhancement	Change in BOD_5/COD ratio	Naddeo et al. (2009b)
	Ready biodegradability	Vasconcelos et al. (2009a)
Antibacterial activity	Growth inhibition zone diameter	Van Doorslaer et al. (2013)
	Minimum inhibitory concentration	Sturini et al. (2012)
Ecotoxicity measured as EC-50	Growth rate (percent inhibition)	Rizzo et al. (2009a)
	Percent bioluminescence inhibition	Coelho et al. (2009)
	Growth and reproduction effects	Martins et al. (2012)
	Percent immobility	Hapeshi et al. (2010)
	Survival (mortality) rate	Martins et al. (2012)
	Root biomass	Rizzo (2011)
	Germinated plant seed number and root length	Rizzo et al. (2009a)

2.5 Fluoroquinolones

2.5.1 Structure and activity

Fluoroquinolones are compounds that contain a fluorine atom on the quinolone moiety (Fig. 4). They are a group of synthetic broad-spectrum anti-bacterial drugs that are widely used to treat gram-negative, gram-positive, and anaerobic bacterial infections (Kowalski et al., 2003; Oliphant and Green, 2002). They inhibit DNA synthesis by interacting with DNA gyrase and type IV topoisomerase enzymes resulting in rapid bacterial death (Oliphant and Green, 2002).

So far, four generations of quinolones have been developed by changing substituents on the quinolone ring to enhance cell penetration ability and broaden their spectrum of anti-bacterial action. The first generation of quinolone was non-fluorinated (e.g., nalidixic and oxolinic acids) with limited spectrum of anti-bacterial action and lower cell penetration ability. The other generations such as ciprofloxacin (2^{nd} generation), levofloxacin (3^{rd} generation), and moxifloxacin (4^{th} generation) possess a fluorine atom on the quinolone ring (Kowalski et al., 2003; Mather et al., 2002). The addition of fluorine atom enhances the lipophilicity and metabolic stability of the compounds (Khetan and Collins, 2007). The enhancement in lipophilicity increases the cell penetration ability of fluoroquinolones, thus influences their biological activity.

Fluoroquinolones are the third largest group of antibiotics accounting for 17% of the global market share with a sell of US$ 7.1 billion in 2009. Besides, their production had grown by 5% between 2005 and 2009 (Hamad, 2010). Evidently, this was also reflected by a significant growth in fluoroquinolones consumption between 1997 and 2009 in Europe (Adriaenssens et al., 2011).

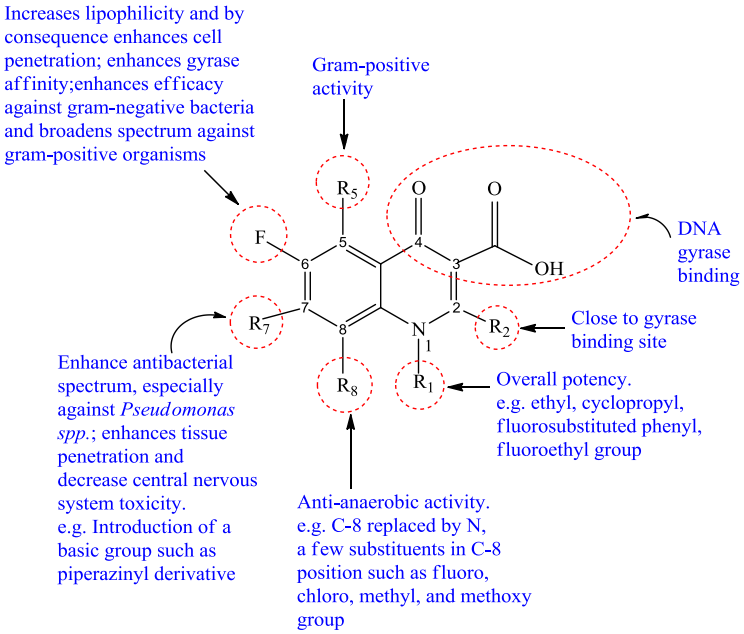


Fig. 4. A general fluoroquinolone structure with specific sites of anti-bacterial activity (Picó and Andreu, 2007; Sukul and Spiteller, 2007).

The main structural similarity among fluoroquinolones is that the carboxyl, carbonyl and fluoro groups are always attached to position C3, C4, and C6 of the quinolone ring, respectively (Fig. 5). However, the difference lies on the type of substituent groups that are attached to N1, C5, C7, C8 positions of the quinolone ring. Moreover, the difference can be because of stereoisomerism as in the case of levofloxacin and ofloxacin.

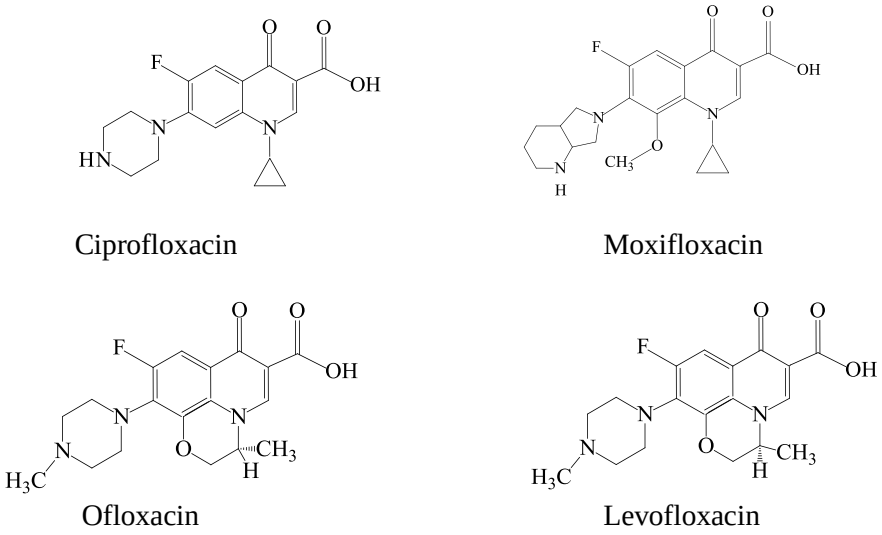


Fig. 5. Chemical structure of some fluoroquinolones.

Fluoroquinolones are one of the five groups of antibiotics usually detected in the environment (Jia et al., 2012). This is mainly because they resist biodegradation, thus can have longer half-life times and accumulate in the environment to reach detectable levels (Khetan and Collins, 2007; Walters et al., 2010). In this regard, reports show that fluoroquinolones have been detected in surface water, soils and sediments in different places (Table 6).

The concerns with regard to the occurrence of fluoroquinolones in the environment are associated with the development of antibiotic resistance by pathogenic bacteria, and ecotoxic effects on aquatic and soil organisms. Therefore, complete removal of this group of antibiotics from wastewater matrix using advanced oxidation processes is necessary. AOPs have demonstrated to be efficient in removing fluoroquinolones from aqueous media. Nevertheless, the effectiveness of any AOP should, eventually, be evaluated by its ability to eliminate the environmental and human health risk posed by the release of fluoroquinolones into the environment. This is done by undertaking ecotoxicity evaluation of the target fluoroquinolone and its treated solution with properly selected sensitive test organisms and measurement of end-points or response variables that enable the estimation of effect concentrations.

Table 6–Concentrations of fluoroquinolones in different environmental compartments

Fluoroquinolone	Concentration in surface water (µg/L)	Concentration in soil** and sediment* (µg/kg)	Country	Reference
Ciprofloxacin	0.077	2.5–10*	USA	Gibs et al. (2013)
		450**	Switzerland	Golet et al. (2003)
	0.019		Switzerland	Golet et al. (2002)
	0.03		USA	Kolpin et al. (2002)
	0.013		Germany	Christian et al. (2003)
Ofloxacin	0.9	7.7–21*	USA	Gibs et al. (2013)
	0.028		Austria	Ferdig et al. (2005)
Enrofloxacin	0.067–0.103		Portugal	Pena et al. (2007)
Norfloxacin		0.35**	Switzerland	Golet et al. (2003)
	0.12		USA	Kolpin et al. (2002)
	0.0023–0.008		Hong Kong	Gulkowska et al. (2007)
	0.025		Austria	Ferdig et al. (2005)
Moxifloxacin	0.017		Austria	Ferdig et al. (2005)
	0.006		China	Xiao et al. (2008)
Gatifloxacin	0.0029		China	Xiao et al. (2008)

2.5.2 Ecotoxicity

Understanding the ecotoxicity of fluoroquinolones and their degradation products after AOP treatment is essential to assess the effectiveness of the treatment processes. Because, in the end, the goal of any treatment process should be to eliminate or significantly reduce target pollutants so that the potential risks on human health and the environment are adequately controlled when the treated product (effluent) is discharged into the environment.

For any given fluoroquinolone, toxicity decreases with increasing biological complexity of the test organisms. For instance, bacteria/cyanobacteria are more sensitive to fluoroquinolones than eukaryotic green algae, while green algae are more sensitive than daphnia. For example, *M. aeruginosa* (cyanobacteria) is the most sensitive to ciprofloxacin with an EC-50 (50% effect concentration)[1] ranging from 0.005–0.017 mg/L. On the other hand, *D. magna* was found to be the least sensitive with EC-50 of 65.3 mg/L (Table 7). Besides, comparison of the EC-50s among the different generations of fluoroquinolones on *P. subcapitata* revealed that toxicity increased with increasing chronology. For instance, clinafloxacin (4th generation) is the most toxic to *P. subcapitata*, followed by enrofloxacin and levofloxacin (3rd generations), while ciprofloxacin, lomefloxacin and ofloxacin (2nd generations)

[1]EC-50–The concentration, which affects 50% of a test population after a specified exposure time.

showed the least toxicity. The increase in toxicity is attributed to structural improvements that enhanced lipophilicity, metabolic stability and binding affinity of the fluoroquinolones.

Ofloxacin was found to be non-toxic to *D. magna* after an acute toxicity test (48 h) at an environmentally relevant concentration (ng/L–μg/L), whereas partial immobilization was observed only at 10 mg/L. Another study showed that *D. magna* are insensitive (10% immobility) to acute toxicity (48 h) effects of fluoroquinolones such as clinafloxacin, enrofloxacin, levofloxacin, ofloxacin, ciprofloxacin, and lomefloxacin up to 10 mg/L (Robinson et al., 2005). On the other hand, initial ofloxacin concentrations of 0.4 mg/L and 0.015 mg/L caused almost complete growth inhibition (> 95%) of the bacteria *P. putida* and *V. fischeri*, respectively (Vasquez et al., 2013). Yet, acute bioluminescence inhibition test on *V. fischeri* by ciprofloxacin showed no toxic effect up to 0.3 mg/L (Vasconcelos et al., 2009a), while enrofloxacin caused 27% growth inhibition on *V. fischeri* only at higher initial concentration (10 mg/L) (Li et al., 2011). A study by Ebert et al. (2011) indicated that enrofloxacin and ciprofloxacin induced different levels of acute toxicity to different test organisms. Among them, *A. flosaquae* was the most sensitive and *D. subspicatus* the least sensitive to these fluoroquinolones.

Paul et al. (2010) found that the inhibitory effect of ciprofloxacin (C_o=33 mg/L) on the growth of *E. coli* progressively declined with increasing UV-A/TiO_2 treatment (max. 145 min) concluding that the degradation products generated possess lower anti-bacterial toxicity than ciprofloxacin. On the other hand, degradation products of enrofloxacin generated after 60 min of solar irradiation induced 2.5*x* higher growth inhibition on *V. fischeri* than the mother compound (Li et al., 2011). Strikingly, the photolytically treated (max. 420 min) solution of moxifloxacin (C_o=0.05 mg/L) induced the highest growth inhibition on *E. coli* compared with the photodegradation products of ciprofloxacin, danofloxacin, and levofloxacin (Sturini et al., 2012). There are also reports that the degradation products of fluoroquinolones after AOP treatment can induce genotoxic effects. For instance, Vasquez et al. (2013) reported that degradation products of ofloxacin generated after photolytic $(UV)^2$ and photocatalytic (UV/TiO_2) treatments induced genotoxic effects on the human liver cells–hepatoma cell lines (HepG2). In addition, a similar study by Garcia-Käufer et al. (2012) identified that photolytic $(UV)^2$ degradation products of ciprofloxacin caused genotoxic effects to hepatoma cell lines (HepG2).

This shows that the degradation products of fluoroquinolones can induce subtle effects that may go unnoticed when using standard test organisms in ecotoxicity studies. This is an indication that no generalization can be made about the effectiveness of a specific treatment technique based on single ecotoxicity test as the toxic effects of treated solutions on test organisms are influenced by the type of treatment method, treatment parameters selected, and toxicity test used.

[2] The UV light source was a mercury lamp that emits polychromatic radiation in the wavelength range of 200–436 nm.

Table 7–EC-50s of selected fluoroquinolones on test organisms

Fluoroquinolone	Generation	Test organism	Test type and duration	EC-50 (mg/L) [95% CI]	Reference
Ciprofloxacin	2nd	*M. aeruginosa*	Growth and reproduction test, 5 days	0.017 [0.014, 0.020]	Robinson et al. (2005)
			Growth and reproduction test, 5 days	0.005 [0.004, 0.006]	Halling-Sørensen et al. (2000)
		A. flos-aquae	Growth inhibition test, 72 h	0.0363 [0.0226, 0.0597]	Ebert et al. (2011)
		L. minor	Growth inhibition test, 7 days	3.75[n.d.a]	Martins et al. (2012)
			Growth inhibition test, 7 days	0.203 [0.041, 0.364]	Robinson et al. (2005)
		P. subcapitata	Growth inhibition test, 72 h	18.7 [16.2, 21.2]	Robinson et al. (2005)
			Growth inhibition test, 72 h	2.97 [2.41, 3.66]	Halling-Sørensen et al. (2000)
		P. subcapitata	Growth inhibition test, 96 h	4.83 [3.44, 7.32]	Martins et al. (2012)
		D. magna	Acute immobilization test, 48 h	65.3 [54.9, 79.1]	Martins et al. (2012)
Enrofloxacin	2nd	*M. aeruginosa*	Growth and reproduction test, 5 days	0.049 [0.041, 0.056]	Robinson et al. (2005)
		A. flos-aquae	Growth inhibition test, 72 h	0.465 [0.387, 0.562]	Ebert et al. (2011)
		L. minor	Growth inhibition test, 7 days	0.322 [0.028, 4.24]	Ebert et al. (2011)
		P. subcapitata	Growth inhibition test, 72 h	3.1 [2.6, 3.6]	Robinson et al. (2005)
		D. subcapitatus	Growth inhibition test, 72 h	28.4 [23.9, 35.4]	Ebert et al. (2011)

Lomefloxacin	2nd	*M. aeruginosa*	Growth and reproduction test, 5 days	0.186 [0.172, 0.20]	Robinson et al. (2005)
		L. minor	Growth inhibition test, 7 days	0.106 [0.045, 0.167]	Robinson et al. (2005)
		P. subcapitata	Growth inhibition test, 72 h	22.7 [19.9, 25.5]	Robinson et al. (2005)
Ofloxacin	2nd	*M. aeruginosa*	Growth and reproduction test, 5 days	0.021 [0.018, 0.024]	Robinson et al. (2005)
		L. minor	Growth inhibition test, 7 days	0.126 [0.052, 0.201]	Robinson et al. (2005)
		P. subcapitata	Growth inhibition test, 72 h	12.1 [10.4, 13.7]	Robinson et al. (2005)
Sarafloxacin	2nd	*P. subcapitata*	Growth inhibition test, 72 h	16 [9.8, 25]	Lützhøft et al. (1999)
Levofloxacin	3rd	*M. aeruginosa*	Growth and reproduction test, 5 days	0.0079 [0.0064, 0.0094]	Robinson et al. (2005)
		L. minor	Growth inhibition test, 7 days	0.051 [0.0086, 0.094]	Robinson et al. (2005)
		P. subcapitata	Growth inhibition test, 72 h	7.4 [6.4, 8.4]	Robinson et al. (2005)
Clinafloxacin	4th	*M. aeruginosa*	Growth and reproduction test, 5 days	0.103 [0.086, 0.120]	Robinson et al. (2005)
		L. minor	Growth inhibition test, 7 days	0.062 [0.021, 0.103]	Robinson et al. (2005)
		P. subcapitata	Growth inhibition test, 72 h	1.1 [0.93, 1.3]	Robinson et al. (2005)

Full name of the test organisms: *Anabaena flos-aquae*, *Desmodesmus subcapitatus*, *Lemna minor*, *Microcystis aeruginosa*, *Pseudokirchneriella subcapitata*, *Vibrio fischeri.*
n.d.a: no data available

3. Study justification and objectives

Moxifloxacin is a 4th generation fluoroquinolone antibiotic whose consumption in Europe is on the rise (Adriaenssens et al., 2011). In Belgium, it is one of the largely prescribed fluoroquinolones in recent years (Fig. 6). Due to the incomplete metabolism of moxifloxacin in the human body, almost half of the consumed moxifloxacin is eliminated structurally unchanged (Stass and Kubitza, 1999), leading their way to STPs and subsequently into the environment.

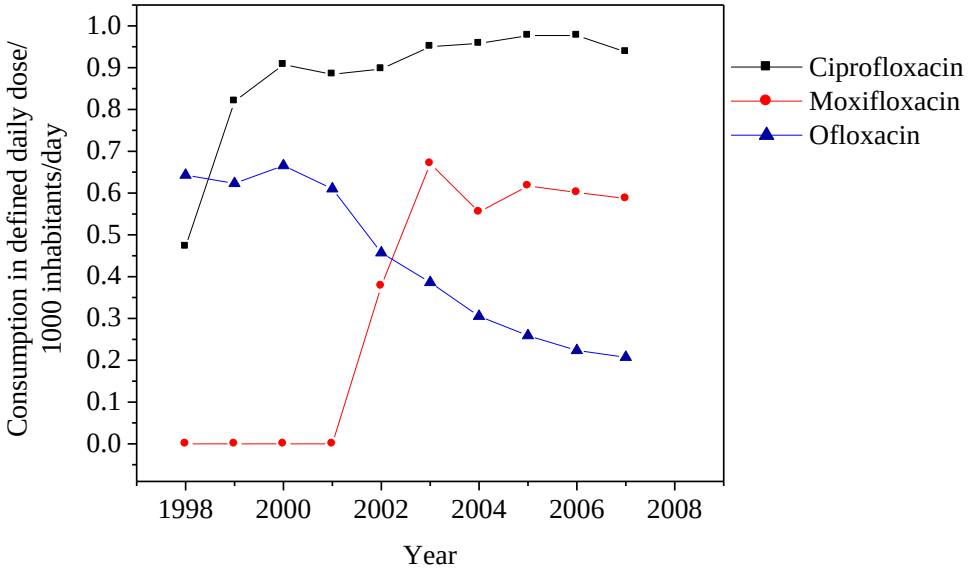


Fig. 6. Consumption of some fluoroquinolone antibiotics in Belgium (1998–2007).
Drug consumption is given in defined daily dose (DDD)/1000 inhabitants/day. The DDD is a standardized (and statistical) measure of drug consumption. Data source: European surveillance of antimicrobials consumption (ESAC); data were obtained upon request.

Ecotoxicity data for the fourth generation fluoroquinolone members are scarce. However, an ecotoxicity study made by Robinson et al. (2005) (see also Table 4) showed that the EC-50 of the clinafloxacin (a 4th generation fluoroquinolone) on *P. subcapitata* is greatly lower than the EC-50s of many of the preceding generations of fluoroquinolones. This may be the result of improvements in biological activity of the fourth generation fluoroquinolones. Moxifloxacin being a 4th generation fluoroquinolone, it was found worth investigating to what extent it would be toxic to the fresh water algae *P. subcapitata* (primary producer) so as to control the risk due to the release of moxifloxacin into the environment. Moreover, environmental risks may be controlled by applying AOP treatments to remove moxifloxacin from water and wastewater matrices. In this regard, Van Doorslaer et al. (2011) showed that the UV-A/TiO_2 photocatalytic treatment can completely remove moxifloxacin from a water matrix. Nevertheless, evaluation of the overall effectiveness of a UV-A/TiO_2 treatment requires an integrated assessment scheme that includes not only removal efficiency but also the ecotoxic effects of the treated solution that contain residual moxifloxacin and degradation products. To the author's best knowledge, there are no studies that investigated the ecotoxic effects of moxifloxacin

and its photocatalytically treated solution using the freshwater algae *P. subcapitata*. Therefore, this study was mainly conducted to:

- Evaluate the ecotoxic effects of moxifloxacin and its photocatalytically treated solutions on the freshwater algae *P. subcapitata*, and investigate if the photocatalytically generated degradation products of moxifloxacin possess toxicity; and
- Assess the environmental (aquatic) risk of moxifloxacin because of its release into the environment in Belgium.

4. Materials and methods

To address the first research objective, laboratory experiments were conducted in two phases. In the first phase, an aqueous solution of moxifloxacin having an initial concentration (C_o) of 15 mg/L was used to evaluate the ecotoxic effects of photocatalytically treated reaction solutions. In the second phase, a higher initial moxifloxacin concentration (C_o=50 mg/L) was used to investigate if the degradation products induce toxic effects on *P. subcapitata*. Initial concentrations of moxifloxacin–much higher than environmental concentrations–were used to obtain analytically detectable levels of residual moxifloxacin and degradation products without the need for pre-concentration. Besides, the use of such higher initial concentrations enabled the quantification of toxic effects on *P. subcapitata* induced by the photocatalytically treated solutions.

4.1. UV-A/TiO_2 photocatalytic treatment

4.1.1. Reactor set-up and reaction solution

A lab-scale reactor vessel (Pyrex, 200 mL) fitted with a circular stainless steel cover and a quartz tube as a light source inlet was used for the treatment of a moxifloxacin solution. The reactor vessel was 10 cm high and 7 cm long in diameter. A pen ray lamp (UVP, UK) (485 μW/cm^2 at 3 cm distance, λ=300–440 nm) was also used as a UV-A light source (Fig. 7).

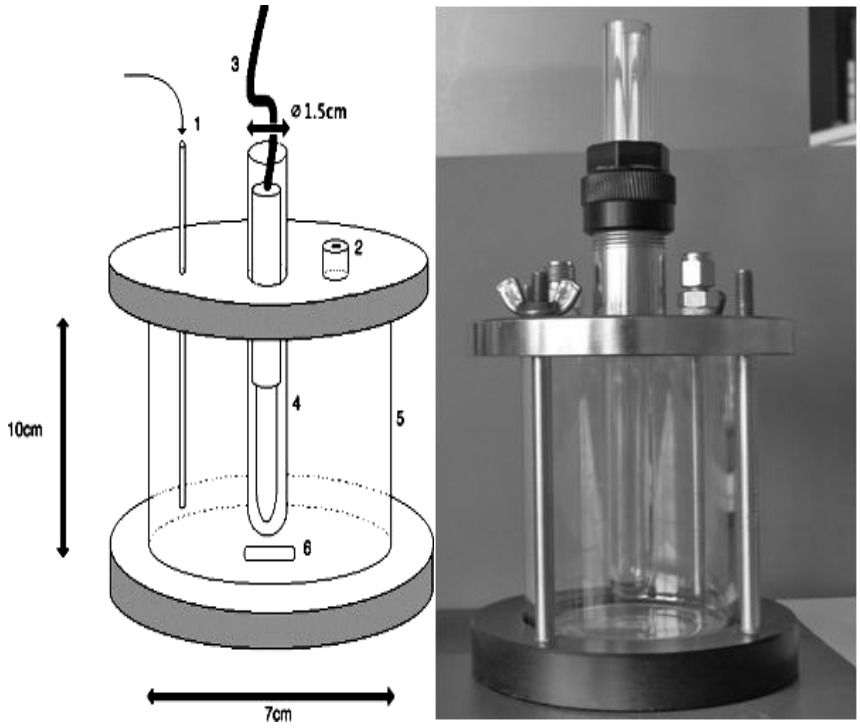


Fig. 7. Reactor set-up: air inlet (1), sampling port (2), UV-A pen ray lamp (3), quartz tube (4), reactor vessel (5), and stirrer bar (6) (Van Doorslaer et al., 2011).

Reaction solutions were prepared from a stock solution of moxifloxacin (1000 mg/L) that was previously made from reagent-grade moxifloxacin.HCl (Table 8) (BAY12-80369, Bayer Co., Berlin). The stock solution was prepared by weighing 0.0545 g of moxifloxacin.HCl on a microbalance and dissolving it in 50 mL of deionized water in a volumetric flask. In the first phase of the experiment, a reaction solution containing 15 mg/L initial moxifloxacin concentration was made by adding 3 mL of moxifloxacin from the stock solution and 4 mL of phosphate buffer into the reactor. Then, the reactor was filled with deionized water to make up 200 mL reactor solution. Similarly, in the second phase of the experiment, a reaction solution containing 50 mg/L initial moxifloxacin concentration was prepared by transferring 10 mL of moxifloxacin and 4 mL of phosphate buffer into the reactor. Finally, the reactor was filled with deionized water to make up 200 mL reactor solution. Phosphate buffer was made by dissolving 2.10 g of KH_2PO_4 (CAS: 7778-77-0, 99%$^+$, ACROS organics, Belgium) and 1.66 g of K_2HPO_4 (CAS: 7758-11-4, 98%$^+$, ACROS organics, Belgium) in 50 mL deionized water. Before photocatalytic treatment, pH of the reaction solutions was adjusted to seven by titrating with NaOH (CAS: 1310-73-2, ACROS organics, Belgium) using a calibrated pH meter (JENWAY 3310).

Table 8–Physical-chemical and environmental properties of moxifloxacin

Parameters	**Value**
log K_d (L/kg) (sludge) 28^{o}C	2.86[a]
log K_{ow}	-0.28 (pH=7.4)[b]
pK_{a1}	6.3[a]
pK_{a2}	9.3[a]
Molecular weight (g/mol)	401.43
Molecular formula	$C_{21}H_{24}FN_3O_4$
CAS number [c]	186826-86-8
IUPAC name [c]	3-Quinolinecarboxylic acid, l-cyclopropyl-6-fluoro-l,4-dihydro-8-methoxy-7-(octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-4-oxo

a: Dorival-García et al. (2013b)

b: Langlois et al. (2005)

c: Bayer (2009)

4.1.2. Photocatalytic treatment

In the first phase of photocatalytic treatment, degradation times (i.e., treatment duration) of 0, 10, 20, and 30 min were selected. The selection was based on a report by Van Doorslaer et al. (2011) who studied the UV-A/TiO_2 treatment of moxifloxacin (C_o=15 mg/L). On the other hand, in the second phase of the photocatalytic experiment, degradation times of 0, 30, 60, 90, and 150 min were chosen

based on an earlier finding of the degradation products of moxifloxacin (C_o=50 mg/L) after UV-A/TiO_2 treatment (Van Doorslaer et al., 2013). To reduce experimental errors in both phases of the photocatalytic treatment, three replicates of reaction solutions were treated for each degradation time.

In all photocatalytic experiments, the concentration of TiO_2 (Degussa-P25, anatase-rutile ratio 80:20, particle size 21 nm, BET specific surface area 50 ± 15 m^2/g) was maintained at 1 g/L by adding 0.2 g of TiO_2 into the reactor vessel. Once the catalyst was added, the moxifloxacin–TiO_2 mixture was placed in the dark to reach an adsorption–desorption equilibrium in 30 min at a stirring speed of 13 rps before UV-A irradiation. This condition was considered as the 0 min degradation time. Ten minutes earlier to UV-A irradiation, dry air (O_2/N_2 ratio 20:80, Praxair, Belgium) started to be sparged (60 mL/min) into the moxifloxacin–TiO_2 mixture and continued until the end of the treatment. At the same time, the UV-A lamp was switched on for 10 min inside a box to stabilize irradiation intensity and heat release. After dark adsorption–desorption equilibrium, the UV-A lamp was inserted into the reactor and was allowed to irradiate the mixture for the intended duration.

During UV-A irradiation, the reactor was completely covered with aluminum foil to prevent the effect of external light sources. Moreover, the reactor temperature was kept at 25^{o}C by immersing the reactor vessel into a thermostatic water bath (Fig. 8). Temperature reading was being monitored by a digital thermostat and an analogue thermometer immersed in the water bath. Once the UV-A irradiation was completed, the reaction mixture was filtered using a suction pump in stepwise: first by VWR filter paper (CAT: 516-0816, particle retention 5–13 μm, VWR international) for quick removal of aggregated catalyst from the mixture, and then by a mixed cellulose ester membrane filter (REF: GSTF 04700, pore size 0.22 μm, Merck millipore, Germany).

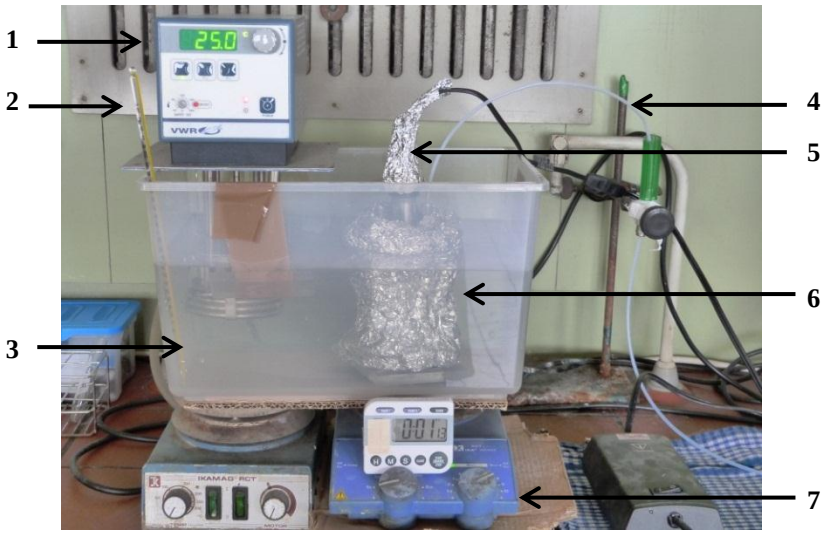


Fig. 8. The complete photocatalytic treatment setup: digital thermostat (1), analog thermometer (2), water bath (3), air flow tube (4), UV-A pen ray lamp (5), reactor vessel covered with aluminum foil (6), and magnetic stirrer (7).

4.1.3. Analytical determinations

Residual moxifloxacin concentration in the reaction solutions was analyzed using high performance liquid chromatography (HPLC) (Finnigan, Germany) coupled with a photodiode array detector (Surveyor Thermo Scientific, USA). The stationary phase was a Luna C18 (2) column (150 mm×3.0 mm, 3 μm; Phenomenex, USA), while the mobile phase consisted of a mixture of water and acetonitrile adjusted to gradient condition (Table 9). The detection of moxifloxacin was performed at a wavelength of 296.0 ± 4.5 nm (flow rate 0.4 mL/min; injection volume 10μL; column temperature 35^{o}C) by collecting 1mL aliquot using a spinal needle syringe. The aliquots were then filtered by a syringe driven 0.2 μm Spartan mini disk filter (CAT: 10463042, Whatman GmbH, Germany), and transferred into 1.5 mL HPLC vials.

Table 9–HPLC mobile phase gradient condition

Time (min)	%	
	Acetonitrile	(H_2O + 0.1% formic acid)
0	0	100
8	30	70
14	100	30
17	0	100
19	0	100

The identification of degradation products (C_o=50 mg/L) was done using high performance liquid chromatography-electrospray ionization-low resolution mass spectrometry (HPLC–ESI–LRMS) by taking samples at 0, 30, 60, 90, and 150 min of degradation time (n=3). In this photocatalytic experiment, the same sample volume and filtration procedure was followed as for the determination of residual moxifloxacin explained previously. The HPLC coupled to the mass spectrophotometer had a Luna C18 (2) column (150 mm × 2.0 mm, 3 μm, Phenomenex, USA) stationary phase kept at 35^{o}C, and a binary mobile phase containing formic acid in water (0.1% *v/v*) and in methanol (0.1% *v/v*). The mobile phase was flowing at a rate of 170 μL/min and started with an isocratic 10% organic phase for one minute, which then rose to 60% in 20 min and to 100% in the following five minutes. The organic phase was maintained steady for 10 min before returning to the starting condition in 1 min. It was then equilibrated for 20 min prior to the next run. The MS detection was performed using a Thermo Finnigan double focusing magnetic sector MAT95XP mass spectrometer (Finnigan, Germany) fitted with an electrospray ionization source in positive-ion mode. The spray voltage was 3 kV with nitrogen as a sheath gas at 4 bar and a capillary temperature of 250^{o}C.

Total organic carbon (TOC) (n=3) was analyzed, for reaction solutions in the first phase of the experiment only, using TOC analyzer (TOC-$V_{CPH/CPV}$, Shimadzu) equipped with a non-dispersive infrared detector. TOC was analyzed using the combustion catalytic oxidation method (airflow 150 mL/min; *T*=680^{o}C).

4.2. Ecotoxicity assessment

4.2.1. Algal toxicity test procedure and test organism

The toxicity test was based on the OECD guidelines for the testing of chemicals using the 72 h freshwater algae, *Pseudokirchneriella subcapitata*, growth inhibition test (OECD, 2011) and using the standard operational procedures of the ALGALTOXKIT F^{TM} freshwater toxicity test with microalgae (MicroBioTests, 2004).

The test organism was selected for this study after a preliminary sensitivity test that revealed *P. subcapitata* as the most sensitive to moxifloxacin compared with *D. magna* and the rotifer *Brachionus calyciflorus* (data not presented). The toxicity test relied on the observation of growth inhibition by test solutions on an exponentially growing algae in a batch culture over a period of 72 h (OECD, 2011). The system's response was measured as the reduction of algal growth rates in a series of algal cultures exposed to different concentrations (i.e., *percent solution*[3] in this case) of the test solution.

The test organism, *P. subcapitata*, was obtained originally from the Culture Collection of Algae and Protozoa (CCAP 278/4, 121 Oban, Scotland) and has been cultured at the Laboratory of Environmental Toxicology, Ghent University, in ES-medium at 1/2 strength (Provasoli, 1968). The medium was prepared by filtering (0.45 µm carbon filter) and sterilizing tap water followed by supplementing it with 1.4 mg/L $FeSO_4 \cdot 7H_2O$, 15 mg/L $NaH_2PO_4 \cdot 2H_2O$, 150 mg/L $NaNO_3$, and 2.35 mg/L $MnCl_2 \cdot 4H_2O$, which was then maintained at pH 8.3 under continuous aeration. Four days prior to the start of the 72 h algal growth inhibition test, new algal pre-culture was prepared and allowed to grow exponentially (T=25^{o}C) in a 100 mL Erlenmeyer flask. The pre-culture was placed on a shaking table and was continuously illuminated sideways using white cool fluorescent light (5180 lux). The pre-culture was subsequently used to inoculate replicates of the test concentrations with algae.

4.2.2. Test concentrations, controls and color corrections

Range finding[4] tests were performed for all degradation time test solutions to find appropriate test concentrations for definitive tests. The definitive tests[5] were setup with different test concentrations (n=5) (Table 10), replicates per test concentration (n=9), and controls (n=3 per control). The test concentrations were prepared by adding appropriate volume of the test solution into 100 mL volumetric flasks.

[3] Since we are dealing with photocatalytically treated solutions that contain mixtures of compounds, the concentrations of the toxicity test solutions can only be expressed in volume percent relative to the volume of the final solution. For instance, a 20% concentration means that 20 mL of the photocatalytically treated solution is mixed with 80 mL of diluent to make up a 100 mL test solution.

[4] **Range finding test**–A toxicity test conducted to estimate the test concentrations of photocatalytically treated solution to be used in a definitive test.

[5] **Definitive test**–The tests and procedures necessary to definitively establish to a high level of certainty the presence or absence of a particular toxic effect.

Table 10–Test concentrations used in definitive tests

Experimental phase	Degradation time (min)	Test concentrations (percent solution)
First	0	0.05, 0.5, 1, 10, 13
		1,5, 10, 15, 100
	10	7, 17, 33, 50, 100
	20, 30	1, 5, 10, 50, 100
Second	0	1, 5, 10, 20, 100
	30, 60, 90,150	5, 10, 20, 50, 100

Along with the test concentrations, two types of controls[6] namely catalyst-phosphate and algal culturing medium were prepared. The catalyst-phosphate control solution was prepared using the same procedure as the reaction solutions without moxifloxacin, but kept for 30 min in the dark. Moreover, the same dilution factors (n=5) as the test concentrations were used to prepare the catalyst-phosphate control dilution series. On the other hand, the algal medium was used as a standard control (i.e., solution that only contained the algae medium) in all toxicity tests. The medium was prepared by adding 10 mL of nutrient stock A and 1 mL of nutrient stock B, C, and D (Table 11) in deionized water to make up a liter of final solution. The solution was well shaken to allow mixing and aerated for 30 min before use. The catalyst-phosphate controls were used to normalize the effects of the catalyst (TiO_2) and the phosphate buffer on the growth of *P. subcapitata* in the test solutions, while the standard control was used to monitor the growth performance of algae.

Different test concentrations (% solutions) were prepared by adding appropriate volumes of the photocatalytically treated solutions into 100 mL volumetric flasks and diluting them with a diluent solution aerated for 30 min. The diluent was prepared by adding 40 mL phosphate buffer into a 2 L volumetric flask and filling it with deionized water to make up 2 L of final solution. Afterwards, 1 mL of nutrient A and 0.1 mL each of nutrients B, C, and D were added into the test solutions in the volumetric flasks. Then, 25 mL solution from each volumetric flask was transferred into long cell vials as color corrections for the test concentration's absorbance measurement in the subsequent 72 h. Exactly the same procedures were followed to prepare dilutions for the catalyst-phosphate controls that correspond to the dilution factors of the test concentrations. On the other hand, the standard control was prepared using algal medium aerated for 30 min. The aerated algal medium was used to fill a 100 mL volumetric flask. Then, the same volume of nutrients were added into the volumetric

[6] **Control**–A treatment that duplicates all the conditions of the test concentrations but contains no test material. It is used to determine the absence of toxicity under basic test conditions.

flask as mentioned above. Finally, 25 mL solution was transferred into long cell vials as color corrections.

Similar procedures as mentioned in the preceding paragraph, were followed while performing algal toxicity tests for pure moxifloxacin (n=5), ciprofloxacin (n=2) and potassium dichromate (n=3) solutions. Toxicity study of moxifloxacin was conducted for two reasons. First, to determine the EC-50 of moxifloxacin and compare it with an experimentally determined EC-50 of the most commonly studied fluoroquinolone–ciprofloxacin. Second, to use the EC-50 of moxifloxacin in the assessment of environmental (aquatic) risk posed by the release of the compound in surface waters in Belgium. On the other hand, potassium dichromate was used for internal quality control. In these toxicity tests, algal medium was used both as a diluent and as a standard control.

Table 11–Composition of nutrients in algal culturing medium

Nutrients	Concentration in stock solution
Stock solution A: macro nutrients	
NH_4Cl	1.5 g/L
$MgCl_2.6H_2O$	1.2 g/L
$CaCl_2.2H_2O$	1.8 g/L
$MgSO_4.7H_2O$	1.5 g/L
KH_2PO_4	0.16 g/L
Stock solution B: iron	
$FeCl_3.6H_2O$	64 mg/L
$Na_2EDTA.2H_2O$	100 mg/L
Stock solution C: trace elements	
H_3BO_3	185 mg/L
$MnCl_2.4H_2O$	415 mg/L
$ZnCl_2$	3 mg/L
$CoCl_2.6H_2O$	1.5 mg/L
$CuCl_2.2H_2O$	0.01 mg/L
$Na_2MoO_4.2H_2O$	7 mg/L
Stock solution D: bicarbonate	
$NaHCO_3$	50 g/L
$Na_2SiO_3.9H_2O$	

(OECD, 2011)

4.2.3. Algal density measurement

A spectrophotometer (JENWAY, 6300) fitted with a 670 nm filter and a holder for 10 cm long test vial (Fig. 9) was used to measure the absorbance of algal cell density. The algal density measurements were done once every 24 h for 72 h. Test vials were used as vessels for culturing the inoculated algae in the test solutions as well as for direct measurement of absorbance.

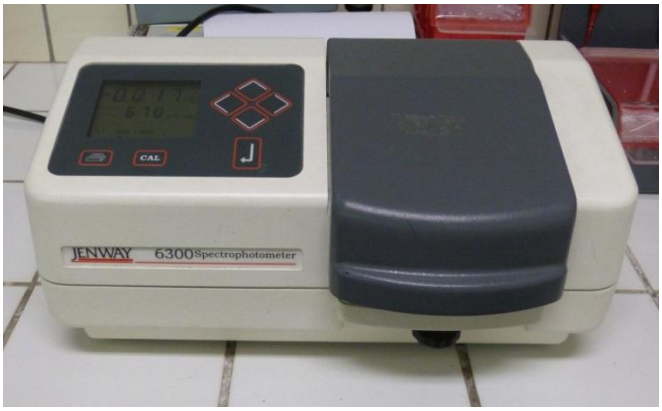


Fig. 9. Spectrophotometer used for algal absorbance measurement.

The pH of the controls and the highest and lowest test concentration solutions were measured using a pH meter (HANNA, HI98140 GLP) before the addition of algal inoculum and after the 72 h test.

All the test concentrations and controls were inoculated with exponentially growing pre-cultured algae to make a final concentration of 10, 000 cells/mL in the test vials. To determine the volume of pre-cultured algae that would result in 10, 000 cells/mL in 75 mL, test solutions three dilutions (i.e., 20×, 50×, and 100×) of the pre-cultured algae were prepared in 50 mL volumetric flasks. The absorbance of the diluted algal pre-cultures were measured using the long test vials after zero calibration of the spectrophotometer using a blank (i.e., algal medium). Based on the measurement of absorbance and the regression equation that relates absorbance to algal cell density: $\boldsymbol{N = 752,\ 425 \times A - 14,\ 394}$ (**N**=cell number/mL; **A**=absorbance) the average algal cell density in the algal pre-culture was determined. Finally, the volume of pre-cultured algae added to the test solutions and controls to bring 10, 000 cells/mL algal concentration was calculated using the dilution equation.

Once the algal pre-culture was added to all test concentrations and controls, the inoculated culture were divided into three replicates of 25 mL each and were transferred into labelled long vials. Then, they were randomly placed on transpartent plastic trays along with color correction vials and kept on a glass shelf in an incubation room (25^{o}C). Light was being illuminated from the bottom (3000–4000 lux) supplied by cool white fluorescent lamps for 72 h. Furthermore, the lids of the long vials were slightly opened, while plastic strips were slid between the lid and vial to allow for gas exchange during the 72 h period (Fig. 10). The same procedures were followed for culturing algae in pure moxiflxacin, ciprofloxacin, and potassium dichromate solutions.

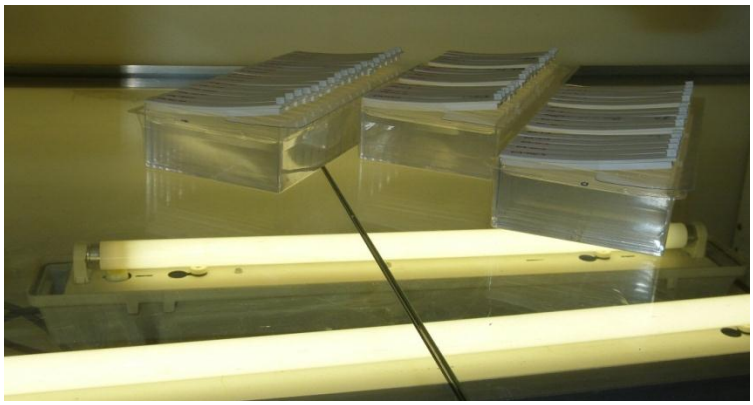


Fig. 10. Test solutions inoculated with algae on the incubation shelf.

The absorbance of the growing algae in the test vials was measured once every 24 h for 72 h. Zero calibration of the spectrophotometer using a blank always preceded the direct measurement of absorbance. Besides, to ensure homogenous distribution of the algae while measuring absorbance, the test vials were gently shaken for 10 seconds. Recording of absorbance was done within 10 seconds after shaking the test vials before the algae start to settle (Fig. 11).

Fig. 11. Algal absorbance measurement. Gentle shaking of the algal culture by turning the test vial upside down to distribute the algae evenly (1&2) and measurement of light absorbance at 670 nm wavelength (3&4).

4.2.4. Test validity criteria and internal quality assurance

All toxicity tests were checked for validity using the OECD (2011) guidelines. The following criteria were evaluated to verify the validity of all tests:

1. An exponential increase in algal biomass in all control cultures by at least a factor of 16 within 72 h;
2. The average percent coefficient of variation (CV%) for the day-by-day specific growth rates (days: 0–1, 1–2, and 2–3) in all control cultures not exceeding 35% (Table 12);

Table 12–A description on how to calculate CV% for day-by-day specific growth rates

Sectional growth rates (μ) (d^{-1})			
μ(0-1)	μ(1-2)	μ(2-3)	Sectional CV (%)
x_1	y_1	z_1	$CV_1\%=SD\ (x_1,y_1,z_1)\ /\ mean\ (x_1,y_1,z_1) \times 100$
x_2	y_2	z_2	$CV_2\%=SD\ (x_2,y_2,z_2)\ /\ mean\ (x_2,y_2,z_2) \times 100$
x_3	y_3	z_3	$CV_3\%=SD\ (x_3,y_3,z_3)\ /\ mean\ (x_3,y_3,z_3) \times 100$
Average day-by-day CV% = mean ($CV_1\%$, $CV_2\%$, $CV_3\%$)			

SD: standard deviation

3. The CV% of average specific growth rates (μ) during the whole test period in all replicate control cultures not exceeding 7% (Table 13);

Table 13–A description on how to calculate CV% of μ for the whole test period

	ln (cell density)				growth rates (μ) day 0 - day 3(d^{-1})
Day	0	1	2	3	
Row1	n_o	x_1	y_1	z_1	$Slope_1$ (row1 /day 0 - day 3)
Row1	n_o	x_2	y_2	z_2	$Slope_2$ (row2 /day 0 - day 3)
Row1	n_o	x_3	y_3	z_3	$Slope_3$ (row3 /day 0 - day 3)
CV% of average μ during the whole test period					= SD (slopes) / mean (slopes) ×100

SD: standard deviation

n_o: initial algal cell density (i.e., 10, 000 cells/mL)

4. The change in pH in all control cultures before and after the test period should not exceed 1.5 units.

It is worth mentioning that the actual controls tested for validity of the toxicity tests were all the dilutions of the catalyst-phosphate controls. Moreover, internal quality assurance of the test results and performance of the test organism were done twice before and once in the middle of the toxicity tests using the reference toxicant potassium dichromate.

4.3. Environmental (aquatic) risk assessment

Assessment of the presence or absence of environmental (aquatic) risk due to the release of moxifloxacin into surface water in Belgium, was done based on the European Chemicals Agency's guidance documents on environmental exposure estimation (ECHA, 2010), characterization of concentration-response for the environment (ECHA, 2008), and risk characterization (ECHA, 2012). The environmental (aquatic) risk was derived by calculating the risk characterization ratio (RCR). The

RCR is the ratio of the predicted environmental concentration (PEC) and predicted no-effect concentration (PNEC) (Eq. 18), which is either greater or less than one. If RCR <1, it means that the environmental risk associated with the release of moxifloxacin into surface water is adequately controlled and the compound is unlikely to pose risk to aquatic ecosystems. If RCR >1, the environmental risk is not adequately controlled and the release of moxifloxacin will pose a potential risk to aquatic ecosystem (ECHA, 2012).

$$\mathbf{RCR} = \frac{\mathbf{PEC}}{\mathbf{PNEC}} \tag{18}$$

To estimate the PEC, worst case scenario (TIER I) assumptions were made when data were not available, while default values were used from the guidance documents. The PEC was computed using the level–III EQC–2.02 (EQuilibrium Criterion) steady state multimedia fate modeling software (Trent University, 2003). On the other hand, the PNEC was determined by dividing acute toxicity data with an appropriate assessment factor (ECHA, 2008).

4.4. Statistical analysis

Ecotoxic effect of the test solutions were estimated by determining the EC-50[7] value when toxic effects were strong enough to cause 50% growth rate inhibition or higher. Otherwise, statistical tests such as one-way ANOVA and student t-tests were conducted to determine the absence/presence of toxic effects in the test concentration solutions.

Average specific growth rate ($\mu_{0\text{-}3}$) was used as a response variable in the toxicity test. Whereas, growth rate inhibition (I%) was considered as an end point. The average specific growth rate (μ) (Eq. 19) and growth rate inhibition (I%) (Eq. 20) are calculated as follows:

$$\mu_{0\text{-}3} = \frac{\ln(B_{3d}) - \ln(B_{0d})}{t_3 - t_0} \tag{19}$$

Where:

$\mu_{0\text{-}3}$	average specific growth rate from day 0 to day 3 (day^{-1})
B_{0d}	algal biomass concentration at day 0 (cells/mL)
B_{3d}	algal biomass concentration at day 3 (cells/mL)
t_0 and t_3	day 0 and day 3 respectively (day)

[7] EC-50 values are expressed in percent solutions (*v/v*) that causes 50% growth rate inhibition on *P. subcapitata.*

$$I\% = \frac{\mu_C - \mu_T}{\mu_C} \times 100 \quad (20)$$

Where:

I% percent inhibition of average specific growth rate

μ_C average specific growth rate in the control (day^{-1})

μ_T average specific growth rate in the test concentration solution (day^{-1})

A log-logistic regression model (Eq. 21) was used to determine the EC-50 values of the test solutions and their associated 95% confidence intervals. They are estimated by plotting the average specific growth rates against their test concentrations using the statistical software STATISTICA 7 (StatSoft, Inc., 2004).

$$y = \frac{k}{1 + \left(\frac{x}{\exp(a)}\right)^s} \quad (21)$$

Where:

k: average specific growth rate of the catalyst-phosphate controls (day^{-1})

x: concentration on linear scale (percent solution)

a: ln (*x*–50)

s: slope parameter

y: average specific growth rate (day^{-1})

In addition, the probit transformation (Eq. 22) was used to determine EC-10 values of the test solutions. The probit transformation is based on the NED (normal equivalent deviation), which is the fraction of the inhibited algal growth rate (P), expressed as units of standard deviation from the mean of a standard normal distribution. The NED was calculated using MS Excel with the function NORMINV (P, 0, 1) (i.e., mean = 0; standard deviation = 1).

$$\text{Probit}(P) = \text{NED}(P) + 5 \quad (22)$$

Normality of data was checked using the Shapiro–Wilk test before applying parametric statistical tests. Similarly, variance homogeneity tests were conducted before using one-way ANOVA. Furthermore, in all statistical data analyses, the significance level was set at 0.05. Student t-tests and one-way ANOVA were used for testing statistically significant differences in the average specific growth rates between/among catalyst-phosphate control dilutions, and mean TOC among the different degradation time solutions. Student t-tests were also performed to determine if there was statistically significant difference in the average specific growth rates between 100% solutions and their corresponding catalyst-phosphate controls. All data treatments and graphical presentations were done using STATISTICA 7 (StatSoft, Inc., 2004), Origin 6 (Microcal software, Inc., 1999) software and MS Excel (2007).

5. Results and discussion

5.1. UV-A/TiO_2 treatment of moxifloxacin in water

The photocatalytic degradation profile of a moxifloxacin solution (C_o=15 mg/L) is illustrated in Figure 12. The result shows that the UV-A/TiO_2 treatment can eliminate the compound from water, and complete removal was achieved in 15 min of photocatalytic treatment. Moreover, the degradation kinetics can be described by a pseudo first-order model having a disappearance rate constant (k=0.274 min^{-1}) consistent with the value (k=0.227 min^{-1}) reported previously for moxifloxacin (C_o=15 mg/L) at neutral pH (Van Doorslaer et al., 2011).

Even though complete removal of moxifloxacin was achieved, no significant mineralization (TOC removal) (p=0.90) was observed (Fig.12). The absence of significant mineralization indicates that moxifloxacin is transformed into other degradation products. Comparison of the mineralization rate of moxifloxacin with other fluoroquinolones proved that the degradation products of moxifloxacin after UV-A/TiO_2 treatment are more resistant to mineralization. For instance, 35–65% mineralization (DOC ≈ TOC removal) of ofloxacin (C_o=5–20 mg/L) was observed after 30 min of UV-A/TiO_2 treatment (TiO_2=0.25–1 g/L) (Hapeshi et al., 2010; Vasquez et al., 2013). Additionally, ~10% mineralization (TOC removal) was reported for a mixture containing ofloxacin, norfloxacin, ciprofloxacin, and enrofloxacin after 30 min of photocatalytic treatment (solar/TiO_2) (Li et al., 2012).

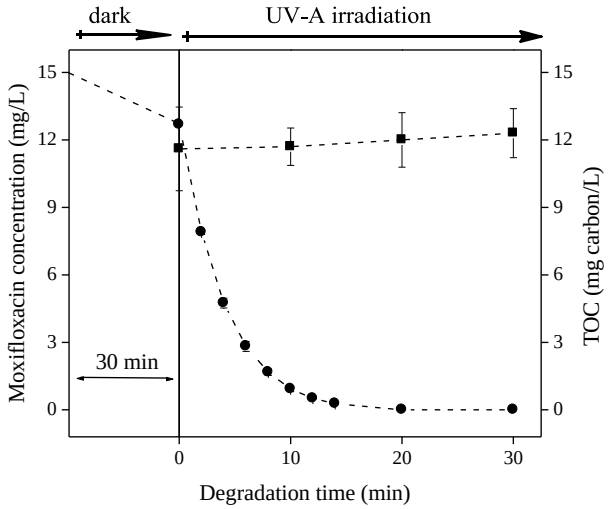


Fig. 12. Residual moxifloxacin concentration in solution (●) and TOC of the treated solutions (■) at 25°C, pH 7, stirring speed 13 rps, air flow 60 mL/min, and catalyst and initial moxifloxacin concentration of 1 g/L and 15 mg/L, respectively (n=3). During dark-adsorption (30 min), 15% of the initially added moxifloxacin was adsorbed on the catalyst surface.

The elimination of moxifloxacin from the photocatalytically treated solutions does not necessarily guarantee removal of toxicity because of the absence of mineralization and the generation of degradation products with unknown toxic effects. Therefore, assessing the toxic effects of moxifloxacin and the photocatalytically treated solutions was found to be necessary as a way of evaluating the effectiveness of the treatment.

5.2. Ecotoxicity assessment

5.2.1. Test validity and internal quality control

All data obtained from toxicity tests were first checked for validity using the OECD algal toxicity test validity criteria (OECD, 2011) before estimating effect concentrations (i.e., EC-50 /10). Data from eight non-consecutive weeks of definitive ecotoxicity tests show that the change in pH in all dilutions of the catalyst-phosphate controls, and the standard (algae media) control were within the criteria (≤ 1.5 units). In addition, in all dilutions of the catalyst-phosphate controls and the standard control, algal biomass grew >16×, and the CV% of average specific growth rates in the replicates was less than 7%. Nevertheless, the CV% of the day-by-day average specific growth rates in all dilutions of catalyst-phosphate control replicates was above the requirement (i.e., ≥35%), except for the standard control. Even though one of the criteria was not met, all data were considered acceptable for the purpose defined in this research since the main criteria (e.g., biomass growth factor and pH changes) were within acceptable range.

The performance of the test organism, *P. subcapitata*, and the reproducibility of test results were assessed by determining the EC-50 of a reference toxicant potassium dichromate with 95% confidence interval (Fig. 13).

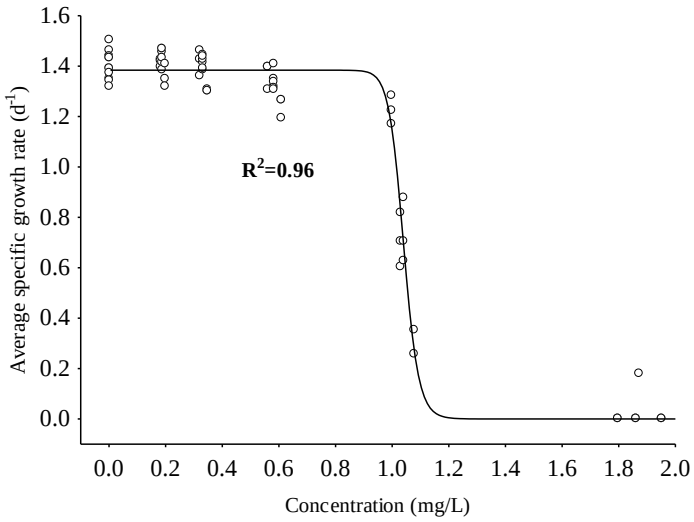


Fig. 13. A concentration-response curve of *P. subcapitata* exposed to $K_2Cr_2O_7$. The solid line is plotted using the log-logistic model for EC-50 determination (n=3).

The experimentally determined EC-50 value of potassium dichromate: 1.04 mg/L [1.036 mg/L, 1.045 mg/L] is in the same order of magnitude as those reported by Halling-Sørensen et al. (2000) (EC-50=0.59 mg/L [0.46 mg/L, 0.75 mg/L]) and Paixao et al. (2008) (EC-50=0.98 mg/L [0.85 mg/L, 1.12 mg/L]) emphasizing a good algal performance and assurance of test reproducibility. Besides, there was no statistically significant difference in the average specific growth rates of the standard controls (p=0.195) and the undiluted catalyst-phosphate controls (p=0.149) across the definitive toxicity test

weeks. This affirms that the performance of *P. subcapitata* was consistent throughout the test period (Fig. 14). Generally, the average specific growth rates of the standard control was significantly higher than the undiluted catalyst-phosphate controls ($p<0.05$). This may be due to the toxic effects of the catalyst and/or the phosphate buffer present in the solutions.

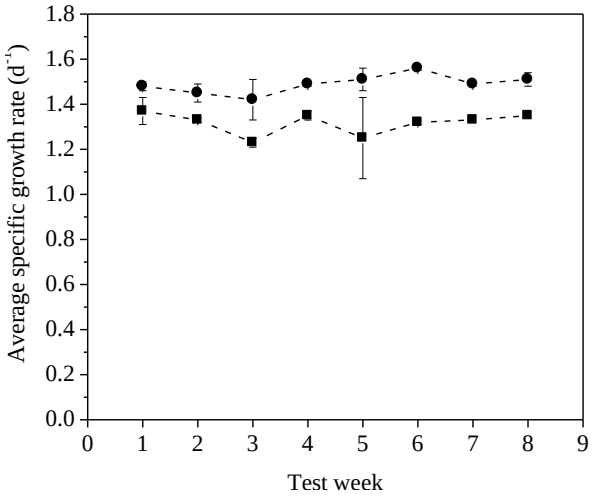
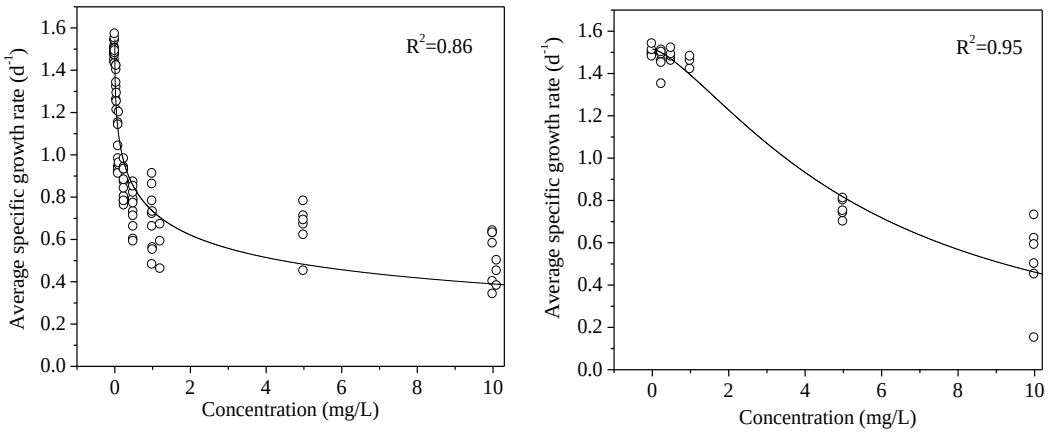


Fig. 14. Average specific growth rates of *P. subcapitata* in the standard (●) and undiluted catalyst-phosphate (■) controls during the definitive toxicity test weeks.

5.2.2. Moxifloxacin and its photocatalytically treated solutions

The toxicity of moxifloxacin on *P. subcapitata* was investigated by determining its EC-50 (Fig.15a). Moreover, its toxicity was compared with the EC-50 of ciprofloxacin (experimentally determined) (Fig. 15b).



a **b**

Fig. 15. A concentration-response curve for *P. subcapitata* exposed to moxifloxacin (a) (n=5) and ciprofloxacin (b) (n=2). The solid lines are plotted using the log-logistic model for EC-50 determination.

The result shows that the EC-50 of moxifloxacin for *P. subcapitata* is seven times lower than ciprofloxacin (Table 14). Besides, moxifloxacin is found to be the most toxic to *P. subcapitata*

compared with a number of other fluoroquinolones whose EC-50 s are reported in literature (see Table 7). For example, the toxic effect of moxifloxacin on *P. subcapitata* is higher than that of enrofloxacin, levofloxacin, lomefloxacin, sarafloxacin, and ofloxacin, while it has comparable toxicity with clinafloxacin. This is because moxifloxacin and clinafloxacin are fourth generation fluoroquinolones with enhanced cell penetration ability and extended inhibitory effects.

Table 14–EC-50 of moxifloxacin and ciprofloxacin with 95% confidence intervals (CI)

Fluoroquinolone	EC-50 (mg/L) [95% CI]
Moxifloxacin	0.78 [0.56, 1.09]
Ciprofloxacin	5.57 [4.86, 6.38]

When moxifloxacin (C_o=15 mg/L) was treated with UV-A/TiO_2, toxic effects on *P. subcapitata* were induced by the 0 and 10 min degradation time test solutions. But, no statistically significant differences (α=0.05) in the average specific growth rates of the algae were observed between the highest tested concentration (i.e., 100% solution) and the corresponding catalyst-phosphate control for the 20 min (p=0.31) and 30 min (p=0.17) degradation time test solutions indicating the absence of algal toxicity (Table 15).

Table 15–EC-50 values of photocatalytically treated moxifloxacin solutions

Degradation time (min)	EC-50 * (% solution) [95% CI]	
	A	**B**
0	7.8 [5.6, 11]	4.5 [2.6, 7.8]
10	71.1 [62.4, 80.9]	n.d
20	No toxicity	n.d
30	No toxicity	15.0 [9.5, 23.9]
60	n.d	38.2 [30.6, 47.8]
90	n.d	Toxicity < EC-50
150	n.d	Toxicity < EC-50

Note: A: C_o = 15 mg/L moxifloxacin
B: C_o = 50 mg/L moxifloxacin
n.d: not determined
* Estimated using log-logistic model

Generally, an increasing trend in the average specific growth rates of the algae was observed when the photocatalytic treatment duration of a moxifloxacin solution was increased. Fig. 16 shows that the average specific growth rate in the 100% solution increases with increasing degradation time. This illustrates the decline in the toxic effects of the treated solutions with increasing degradation time. It also coincides with the photocatalytic removal of residual moxifloxacin concentration from the treated solutions. For example, the concentration of moxifloxacin after dark-adsorption (0 min) was 12.7 mg/L and it decreased by a factor of 14 after 10 min of UV-A irradiation. At the same time, the EC-50

increased by a factor of 9. Besides, no algal toxicity was observed when the residual moxifloxacin concentration in the treated solutions fell below the limit of detection after 20 min of irradiation.

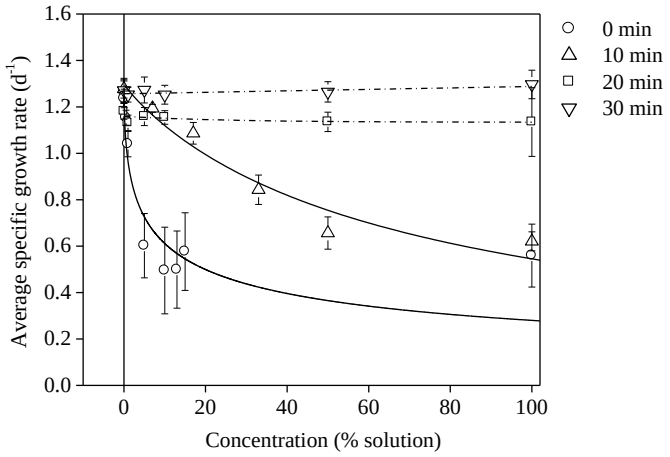


Fig. 16. An overlay of concentration-response plots for 0, 10, 20 and 30 min degradation time solutions at 15 mg/L initial moxifloxacin concentration. The solid lines are fitted with the log-logistic regression model; the broken lines are drawn to guide the eye. (*See appendix I for individual concentration-response graphs*).

Higher initial moxifloxacin concentration (C_o=50 mg/L) and longer treatment duration (max. 150 min) were used in this study to investigate the toxic potency of the degradation products on *P. subcapitata*, and to estimate the contribution of moxifloxacin and its degradates to the overall toxicity of the mixture. Generally, an increase in average specific growth rates of the test organism with increasing degradation time was observed in the treated solutions (Fig.17). This was confirmed by the increase in the EC-50 values of the test solutions until 60 min of photocatalytic treatment. Moreover, after 90 and 150 min of UV-A irradiation the solutions caused less than 50% growth rate inhibition on *P. subcapitata* (Table 15).

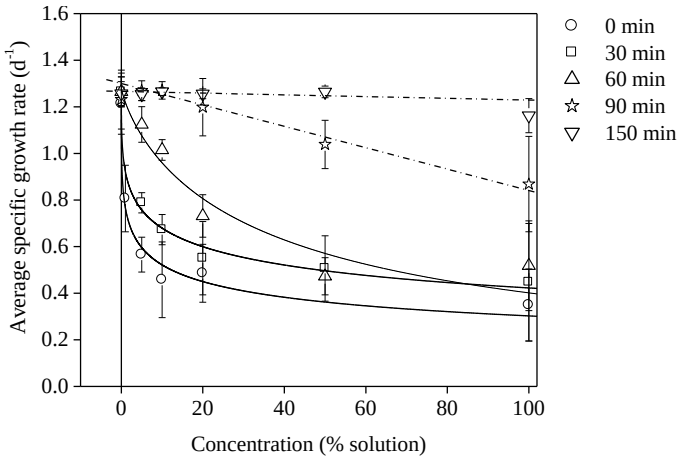


Fig. 17. An overlay of concentration-response plots for 0, 30, 60, 90 and 150 min degradation time solutions at 50 mg/L initial moxifloxacin concentration. The solid lines are fitted with the log-logistic model; the broken lines are drawn to guide the eye. (*See appendix II for individual concentration-response graphs*).

In this experiment (C_o=50 mg/L), the residual moxifloxacin concentration after dark-adsorption was 47.7 mg/L and it dropped by a factor of 6 and 85 after 30 and 60 min of UV-A irradiation, respectively. Besides, no residual moxifloxacin concentration was detected after 90 and 150 min of treatment. Correspondingly, the EC-50 increased by a factor of 3 and 9 after 30 and 60 min of irradiation, respectively. Moreover, after 90 and 150 min of UV-A irradiation, the treated solutions were able to cause 30 ± 17% and 13 ± 6% growth rate inhibition on *P. subcapitata*, respectively (Fig. 18) even if no residual moxifloxacin concentration was detected in the solutions. This suggests that one or more of the formed degradation products induce toxic effect on *P. subcapitata*. Other studies have also established the fact that photo-(catalytic) degradation products of fluoroquinolones exhibit biological activity (Paul et al., 2010; Sturini et al., 2012; Van Doorslaer et al., 2013).

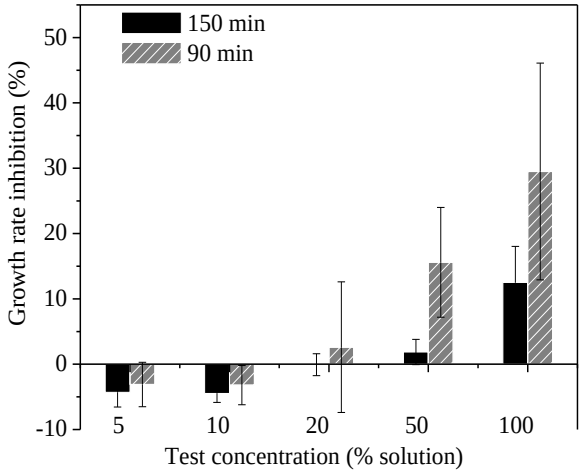


Fig. 18. Growth rate inhibition (%) of 90 and 150 min degradation time solutions.

Toxicity on *P. subcapitata* was not observed when the residual moxifloxacin concentration (C_o=15 mg/L) was below the limit of analytical detection. This may be attributed to the absence of sufficient quantity of degradation products to induce toxicity. On the other hand, at 50 mg/L initial moxifloxacin concentration, considerable toxic effect on the test organism was noticeable even after the complete removal of moxifloxacin. Because at such initial moxifloxacin concentration, it is possible to produce sufficient quantity of degradation products that can induce toxicity.

When the initial moxifloxacin concentration was increased from 15 to 50 mg/L, which is more than tripling, the EC-50 of 0 min degradation time of the latter concentration should have decreased considerably compared with the former (Table 15). The expectation was that there would be a substantial decline in EC-50 of the 0 min solution by increasing the initial concentration of moxifloxacin. Surprisingly, this did not happen and we are unable to fully explain it.

To further investigate the contribution of residual moxifloxacin concentration (C_o=50 mg/L) to the total toxicity of the treated solutions, a comparison was performed between the experimentally

determined growth rate inhibitions (I%) of 100% solutions (C_o=50 mg/L) and calculated I% for solutions having the same residual moxifloxacin concentration as the 100% solutions (Fig. 19). For the latter, I% was calculated using the log-logistic relationship between residual moxifloxacin concentration (mg/L) and average specific growth rates for the 0 min degradation time solution. The result showed that the residual moxifloxacin concentration (C_o=50 mg/L) in the treated solutions contributed to 94% and 64% of the growth rate inhibitions observed on *P. subcapitata* after 30 and 60 min of UV-A irradiation, respectively. This demonstrates that as long as residual moxifloxacin concentration is present in the treated solutions it is the main contributor to the overall algal toxicity.

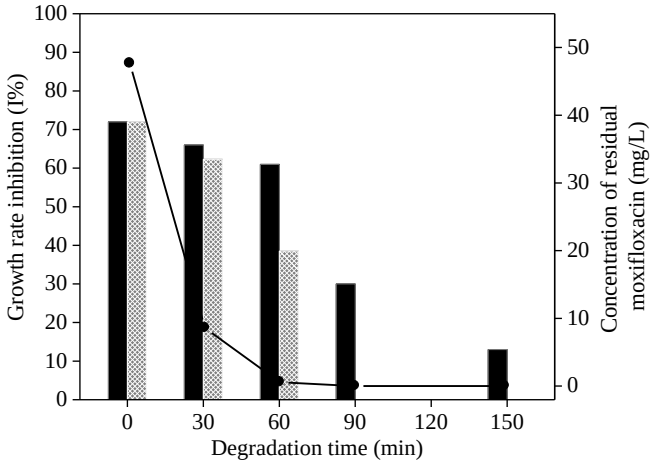


Fig. 19. Growth rate inhibition of 100% solutions and residual moxifloxacin concentration as a function of degradation time. I% measured in 100% solutions (black bar); I% calculated from residual moxifloxacin concentration (gray bar); and residual moxifloxacin concentration (solid line).

To examine the relationship between the photocatalytic degradation time and the evolution of toxicity (C_o=50 mg/L), EC-10 values were determined using the Probit transformation. The result demonstrated that toxicity removal as a function of degradation time best fits to a logistic curve (Fig. 20). It is obvious to see from Fig. 20 that until 60 min of degradation time, the drop in toxicity is exponential. Beyond 90 min, the toxicity reduction slows down and forms a plateau. This may be because moxifloxacin was completely degraded and fewer degradation products were present after 90 and 150 min of photocatalytic treatments. The pattern explains the existence of an optimum treatment duration beyond which any increase in degradation time will not bring significant changes in the measured effect concentration.

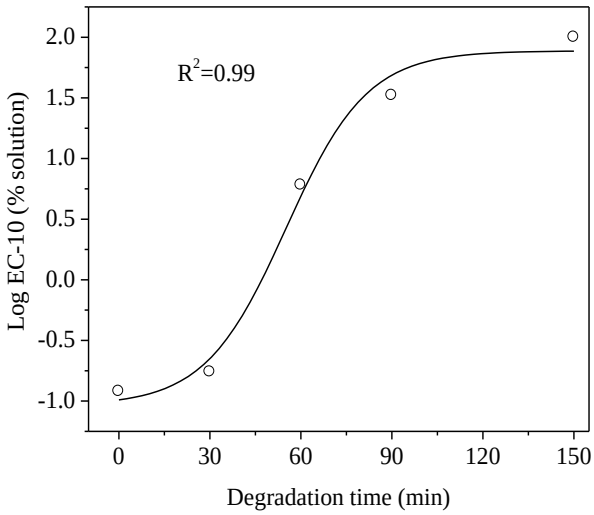


Fig. 20. Change in toxicity of photocatalytically treated moxifloxacin solution as a function of degradation time (C_o=50 mg/L).

5.2.3. Structure–activity relationship of the degradation products

Photocatalytic treatment of an aqueous moxifloxacin solution (C_o=50 mg/L) and identification of its degradation products were performed previously by Van Doorslaer et al. (2013) under the same conditions as this study. The authors proposed the degradation pathways and the chemical structures for the degradation products of moxifloxacin. In their study, the authors mainly used high–resolution HPLC–ESI–MS to determine the molecular composition of the identified degradates and to propose their chemical structures. However, in this study, identification of the degradation products of moxifloxacin (C_o=50 mg/L) was carried out using HPLC–ESI–LRMS. The analytical work was done only to confirm the similarity of the generated degradation products with those reported by Van Doorslaer et al. (2013).

Nominal masses (*m/z*) of the degradation products were identified as $[M+H]^+$ and used to consult their proposed structure from Van Doorslaer et al. (2013). The analysis identified the presence of 17 degradation products out of which seven have lower molecular mass than moxifloxacin (i.e., moxifloxacin *m/z*=402) (Table 16 & 17).

Table 16–Determination of photocatalytically generated degradation products of moxifloxacin (n=3; 25°C, pH 7, stirring speed 13 rps, air-flow 60 mL/min, and catalyst and moxifloxacin concentration of 1 g/L and 50 mg/L, respectively)

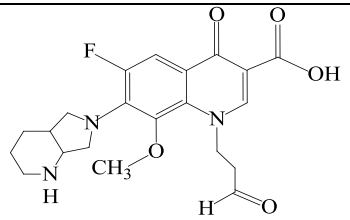
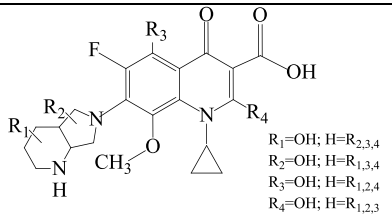
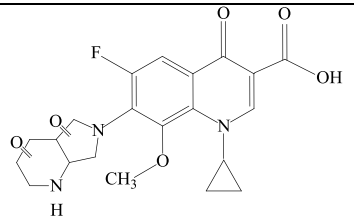
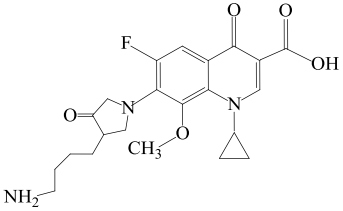
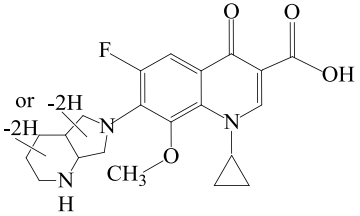
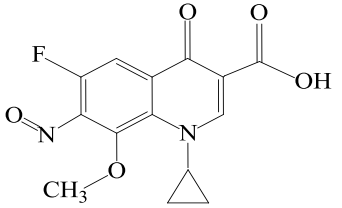
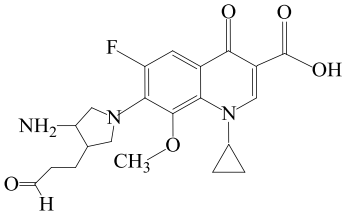
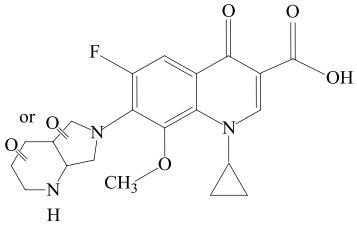
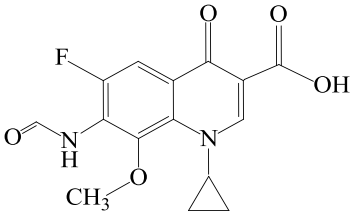
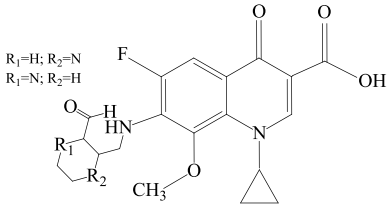
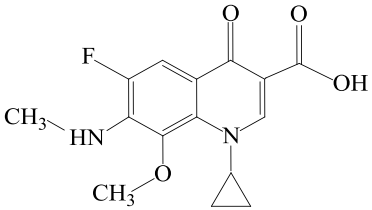
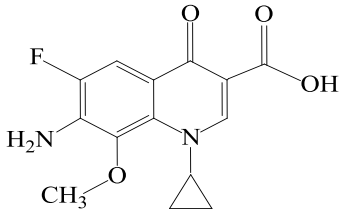
Nominal mass (*m/z*) as ($[M+H]^+$)	Chromatographic retention time (min)	Molecular formula [n]	Structure no. (see Table 17)
293	24.54	$C_{14}H_{13}O_4N_2F$	12
307[a]	23.16	$C_{14}H_{11}O_5N_2F$	10
307[b]	24.51	$C_{15}H_{15}O_4N_2F$	8
321	21.86	$C_{15}H_{13}O_5N_2F$	11
400[a]	16.82	$C_{21}H_{22}O_4N_3F$	6
400[b]	18.15		6
400[c]	21.62		6
416[a]	11.16	$C_{21}H_{22}O_5N_3F$	7
416[b]	15.10		7
416[c]	17.50		7
416[d]	18.31		7
416[e]	22.77		7
418[a]	15.23	$C_{21}H_{24}O_5N_3F$	1–5
418[b]	16.82		1–5
418[c]	14.54		1–5
430[a]	12.61	$C_{21}H_{20}O_6N_3F$	9
430[b]	18.22		9

Note: *m/z* value represents the molecular mass of a degradation product measured as molecular ion $[M+H]^+$.

Numbering of the structures is directly taken from Van Doorslaer et al. (2013) to avoid confusion.

The alphabetical superscripts are used to differentiate the different degradates having the same molecular mass.

Table 17–Chemical structures of the photocatalytically generated degradation products of moxifloxacin [n]

No.		No.		No.	
1		5		9	
2		6		10	
3		7		11	
4		8		12	

[n]: Proposed structures taken from Van Doorslaer et al. (2013).

Profiles of the different degradation products as well as the residual moxifloxacin concentration present in the treated solutions are given as a function of degradation time in Fig. 21. The occurrence of the identified degradation products is expressed as peak areas. The peak areas do not indicate actual concentration, but the response of the instrument. Therefore, no comparison of concentrations among the degradation products is possible. This is because no standards are available to calibrate the instrument for the degradation products. Generally, more degradation products were observed for the treated solutions of 30 min (n=16) and 60 min (n=13) degradation times than 90 min (n=8) and 150 min (n=6). This may be due to the increased treatment duration that might have led to the mineralization of some of the degradation products and/or further transformation of the previously generated degradation products to fewer forms or to undetectable compounds.

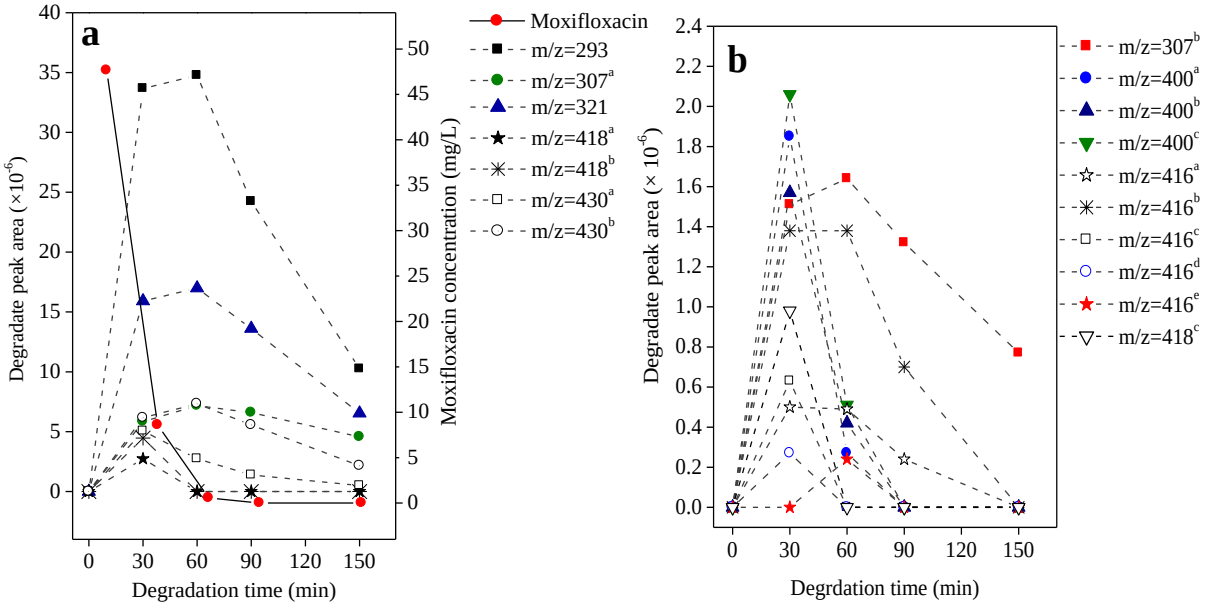


Fig. 21. Integrated peak areas of the degradation products generated and residual moxifloxacin concentration present after photocatalytic treatment at 25°C, pH 7, stirring speed 13 rps, air flow 60 mL/min, and catalyst and moxifloxacin concentration of 1 g/L and 50 mg/L, respectively. During dark-absorption (30 min), 5% of the initially added moxifloxacin was adsorbed on the catalyst surface.

To explain the toxicity of the degradation products on *P. subcapitata* as well as to compare them with the toxicity of the mother compound–moxifloxacin–on the same test organism, a qualitative structure–activity relationship (SAR) study was done. It was assumed that the mode of action of moxifloxacin on *P. subcapitata* is similar to its mode of action on bacteria, although moxifloxacin is designed mainly to inhibit the activities of topoisomerases such as DNA gyrase and topoisomerase IV that are largely found in bacteria (Oliphant and Green, 2002). The assumption is made based on the fact that *P. subcapitata* possesses topoisomerase I and II enzymes (Champoux, 2001), and can provide a number of evolutionary conserved target sites as a result of bacterial ancestry of plastid organelles and conservation of certain metabolic pathways. Even if plastids have undergone several adjustments through evolution in eukaryotes, the main parts of the chloroplast have remained fundamentally

bacterial in nature (Brain et al., 2008). There are also reports that show DNA gyrase involvement in DNA replication and control of DNA topological state in the chloroplasts of plants (Cho et al., 2004; Wall et al., 2004). Evidently, fluoroquinolones have been reported inhibiting chloroplast DNA replication in plants (Brain et al., 2008). In addition, a recent study reported that the fluoroquinolone ciprofloxacin significantly reduced photosynthetic rate and chlorophyll content of *P. subcapitata* (Liu et al., 2011).

A closer look at the structures of the degradation products shows that all of them retained the quinolone moiety with the cyclopropyl, carboxyl, carbonyl, fluoro, and methoxy groups present. Similarly, others also reported that fluoroquinolones can retain the quinolone core structure after photo-(catalytic) degradation (Paul et al., 2010; Sturini et al., 2012). In such a case, it is reasonable to consider that the degradation products exhibit the same mode of action as their mother compound because of structural similarity. However, they may express different levels of toxicity due to differences in substituents mainly at position C7 (see Fig. 4). Besides, comparison of the mother compound with the degradation products was based on the observation of changes in substituents that subsequently affect the lipophilic/hydrophilic properties of the degradation products.

Moxifloxacin (see Fig.5), as a fourth generation fluoroquinolone, has enhanced and extended anti-bacterial activity compared with the previous generations of fluoroquinolones. It is believed that the quinolone core as well as the carboxyl (C3) and carbonyl (C4) groups are responsible for DNA gyrase binding, while the methoxy group (C8) is responsible for an anti-anaerobic bacterial activity. The cyclopropyl (N1), fluorine (C6), and diazobicyclo (C7) groups also improve lipophilicity and pharmacokinetics, hence cell penetration and the toxic potency of moxifloxacin (Paul et al., 2010; Picó and Andreu, 2007).

All the degradation products except degradation product 1 and 6, possess more polar substituent groups at position C7 than the diazobicyclo group in moxifloxacin. This decreases the lipophilicity of the degradation products their by reducing their ability to penetrate cell membrane and induce toxic effects. Moreover, the pH of a test solution is a significant factor in controlling the speciation of the mother compound and its degradation products, and consequently affects their toxicity. Generally, the pH of the test solutions in this study stayed stable and close to the isoelectric point (IEP)[8] of moxifloxacin in the 72 h period (min–max: 6.83–7.50). In this regard, Langlois et al. (2005) reported that in the pH range of 7.0–8.0, moxifloxacin exists mostly as uncharged (~10%) and zwitterionic (~90%) (net-neutral) species in dynamic equilibrium with each other. The zwitterion species are formed due to the deprotonation of carboxyl group (pKa_1=6.25) and protonation of the amine group (pKa_2=9.29) on the diazobicyclo ring. Since the neutral/uncharged form of moxifloxacin exhibits more lipophilicity than the zwitterionic form, it can penetrate cell membrane more easily and induce toxic

[8] Isoelectric point (IEP) is the pH at which a particular molecule or surface carries no net electrical charge.

effects. Moreover, maximum cell penetration by a fluoroquinolone occurs when the neutral species is present in a proportionally considerable concentration relative to the zwitterionic form (Takács-Novák et al., 1992). Therefore, changing the diazobicyclo group (C7) of moxifloxacin by other groups changes the pKa_2 of the compound and disturbs is acid-base speciation (Langlois et al., 2005) and may decrease the proportion of uncharged species in the solutions.

Degradation product 1 has its difference in functional group with moxifloxacin at position N1. This degradation product contains a more hydrophilic aldehyde group than the hydrophobic cyclopropyl group in moxifloxacin. Degradation product 6 will probably have the same toxicity as moxifloxacin since the only difference between them is the presence of a double bond in the diazobicyclo group of the degradation product. On the other hand, degradation products 2–5 and 7–12 are formed by either the complete loss (i.e., fragmentation) or transformation (i.e., attachment of substituents) of the diazobicyclo group. The new substituent groups attached at C7 position on these degradation products contain carbonyl, hydroxyl and amine groups, which are more hydrophilic than the diazobicyclo group. Since oxygen and nitrogen are capable of forming intermolecular hydrogen bonding with water, the presence of hydroxyl, carbonyl and/or amine groups on the substituents increases the hydrophilicity of the degradation products.

Overall, the presence of more polar substituent groups on the degradation products enhance water solubility, but reduce the ability of degradation products to penetrate cell membrane compared with moxifloxacin. Besides, the loss or transformation of the diazobicyclo ring may decrease the binding affinity of the degradates to DNA topoisomerase (Paul et al., 2010). All of these factors may be responsible for the observed lower toxicity of the degradation products compared with moxifloxacin.

5.3. Environmental (aquatic) risk assessment

The predicted environmental concentration in surface water (PEC_{water}) was computed using the EQC–2.02 software. The software was given the physical–chemical parameters of moxifloxacin as well as the degradation half-life time in and total emission estimates to water, air, soil, and sediment (Table 19). A number of assumptions and default values were used to derive the PEC_{water}. The assumptions include the following:

- All the excreted moxifloxacin in the wastewater is in the dissolved phase and there is neither biotic/abiotic transformation nor adsorption of moxifloxacin to solid before reaching STPs;
- Although the log K_{ow} of moxifloxacin is negative (Table 19), it is assumed to have a log K_d value comparable with other fluoroquinolones in soils. The use of K_{ow} to estimate K_d for moxifloxacin in soils and sediments was not possible because the relationship between K_{ow} and K_d is dependent on the notion that there is a hydrophobic interaction between the molecule and solid matter. In fact, the interaction of moxifloxacin with solids is mainly via electrostatic attraction, which is stronger than hydrophobic forces. As a result, the K_d range for moxifloxacin was directly estimated by

looking at reported soil K_d values for other fluoroquinolones. The log K_d for moxifloxacin, therefore, is predicted to be > 3 (*see Table 18 for log K_d values of other fluoroquinolones*);

- Moxifloxacin is regarded as an inherently biodegradable compound. This is because there are contradictory reports regarding the biodegradability of fluoroquinolones in general. For instance, closed bottle biodegradability tests conducted on ciprofloxacin and ofloxacin showed no biodegradation of the compounds up to 40 days (Al-Ahmad et al., 1999; Kümmerer et al., 2000). On the other hand, biodegradability tests on ciprofloxacin in an activated sludge reactor indicated that 50% biodegradation was achieved in 2.5 days (Halling-Sørensen et al., 2000). Besides, Dorival-García et al. (2013a) reported that moxifloxacin, ofloxacin, ciprofloxacin and norfloxacin biodegraded in an aerobic activated sludge–membrane bioreactor with half-life time ranging from 4–10 days. Because of this ambiguity, moxifloxacin was considered inherently biodegradable in the environment. This permits us to directly use the default DT50 values for moxifloxacin in soil and sediments for inherently biodegradable compounds from ECHA (2010);
- In STPs, it is assumed that hydrolysis, volatilization, and biodegradation of moxifloxacin are negligible. In addition, the main removal mechanism of moxifloxacin in STPs is through adsorption to sludge;
- In the environment, it is assumed that hydrolysis and volatilization are insignificant processes. However, a very slow biodegradation process is considered due to the inherent biodegradability of the compound; and,
- Only 80% of the wastewater is treated in STPs. The rest (20%) is directly discharged into surface water (ECHA, 2010).

Total emissions to the different environmental compartments were calculated by estimating the daily total emission of moxifloxacin into wastewater stream ($E_{total\_wastewater}$). Afterwards, the $E_{total\_wastewater}$ was used to estimate direct emissions to surface water ($E_{direct\ to\ water}$) and STPs (E_{STP}), emission to water via effluent ($E_{effluent}$), and emission to soil (E_{soil}). The emission values were calculated as follows:

- $E_{total\_wastewater}$ (kg/h) = (defined daily dose (DDD)/1000 inhabitants/day) × (0.40 g moxifloxacin/DDD) × (total population of Belgium in 2013) × (proportion of moxifloxacin eliminated from human body unchanged) × (percent use of the prescribed moxifloxacin) × (kg/1000 g) × (day/24 h);
- $E_{direct\ to\ water}$ (kg/h) = (20%) × $E_{total\_wastewater}$ (kg/h)
- E_{STP} (kg/h) = (80%) × $E_{total\_wastewater}$ (kg/h)
- $E_{effluent}$ (kg/h) = release fraction to effluent × E_{STP} (kg/h)
- E_{soil} (kg/h) = release fraction to sludge × E_{STP} (kg/h)
- E_{water} (kg/h) = $E_{effluent}$ (kg/h) + $E_{direct\ to\ water}$ (kg/h)

Default release fractions to air, effluent, and sludge in STPs are obtained from lookup tables provided by the European Chemicals Agency (ECHA) guidance document on environmental exposure estimation (ECHA, 2010). To be able to use the lookup tables, the log K_{ow} and log H values of the compound should be in the range of 0–6 and -4–5, respectively. Since the log K_{ow} for moxifloxacin is negative, the lookup table could not be used for our purpose. Therefore, the release fractions of moxifloxacin into the sludge and effluent were estimated directly from mass balance reports (i.e., fate) of moxifloxacin in STPs. The release fraction of moxifloxacin to sludge is estimated by taking the average percent removal of moxifloxacin in STPs via adsorption to sludge from literature, while the release fraction to effluent is obtained by subtracting the value of the release fraction to sludge from one.

Table 18–Parameters and emission values used to estimate the PEC_{water} for moxifloxacin

Physical-chemical and environmental parameters	Unit	Value	Comment		Reference
Molar mass	g/mol	401.43			
Environmental temperature	^{o}C	25	Ambient temperature assigned.		
Water solubility	g/m^3	0.0196			Varanda et al. (2006)
Vapor pressure	Pa	1.0×10^{-11}	Worst-case (minimum value).		
Log K_{ow}		-0.36		-0.28	Langlois et al. (2005)
			Average	-0.26	Völgyi et al. (2012)
				-0.53	Michot et al. (2005)
Melting point	^{o}C	250			Bayer (2009); Dorofeev et al. (2004)
Degradation time/half-life					
$DT50_{air}$	h	**1×10^{11}**	Worst-case, max. default value by the software.		
$DT50_{water}$	h	**3600**			ECHA (2010)
$DT50_{soil}$	h	**720, 000**			ECHA (2010)
$DT50_{sediment}$	h	**720, 000**			ECHA (2010)
Total emission to wastewater					
DDD moxifloxacin*	g	0.40			
DID**	DDD/1000 inhabitants/day	0.582	DID is an average of data from 2002–2007. The average DID is assumed to be the same for 2013.		
Mid-year population of Belgium 2013***	Inhabitant	11,113, 965	Projected population.		
Total body excretion	%	45	Unchanged moxifloxacin.		Stass and Kubitza (1999)
Percent consumption of the prescribed drug	%	100	Worst-case (maximum value).		
$E_{total\_wastewater}$	kg/h	**0.0485**			
Removal of moxifloxacin in STPs	%	61			Michael et al. (2012b)
	%	40			Jia et al. (2012)
	%	27	Calculated		Xiao et al. (2008)
Adsorption of moxifloxacin to sludge	%	60	Calculated		Dorival-García et al. (2013b)

Release fractions				
Release fraction to water via effluent		0.53	Calculated by assuming that the only removal mechanism is adsorption to sludge. An average value of STP removal efficiency was taken.	
Release fraction to air		0.0	Worst case (maximum value).	
Release fraction to soil via sludge		0.47	Calculated by assuming the only removal mechanism is adsorption to sludge. An average value of STP removal efficiency was taken.	
$E_{direct\ to\ water}$, E_{STP}, $E_{effluent}$				
$E_{direct\ to\ water}$	kg/h	0.0097		
E_{STP}	kg/h	0.0388		
$E_{effluent}$	kg/h	0.0206		
log K_d (soil)				
Ciprofloxacin		3.69		Conkle et al. (2010)
		2.60		Golet et al. (2003)
		4.79		Picó and Andreu (2007)
Norfloxacin		3.76		Conkle et al. (2010)
Ofloxacin		3.64		Conkle et al. (2010)
		4.64		Picó and Andreu (2007)
Enrofloxacin		2.7–3.7		Golet et al. (2003)
Sarafloxacin		3.55		Picó and Andreu (2007)
Total emissions				
E_{water}	kg/h	**0.0303**	Includes direct emission to surface water and indirect emission via STP effluent.	
E_{air}	kg/h	**0.0**	Worst-case (maximum value).	
E_{soil}	kg/h	**0.0182**	All the sludge goes to agricultural land (worst-case).	
$E_{sediment}$	kg/h	**0.0**	Worst-case (maximum value).	

*http://www.whocc.no/atc_ddd_index/?code=J01MA14&showdescription=yes

**European surveillance of antimicrobials consumption (ESAC): http://app.esac.ua.ac.be/public/index.php/en_gb/home

***Statistics Belgium, Federal government of Belgium: http://statbel.fgov.be/nl/modules/publications/statistiques/bevolking/downloads/bevolking_op_1_januari_2013-2061.jsp

The result showed that the PEC_{water} under the worst-case scenario is 0.203 ng/L (Fig. 22), and this value is assumed to be spatially uniform across surface waters in Belgium. The PEC_{water} is in the same order of magnitude as concentration ranges frequently reported for pharmaceuticals in aquatic environment (Gibs et al., 2013; Brown et al., 2006).

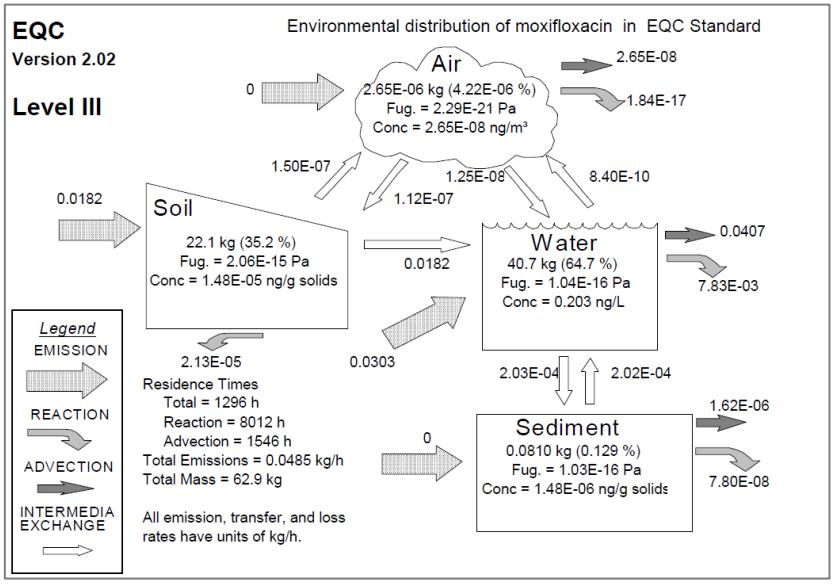


Fig. 22. A worst-case (TIER I) multimedia distribution model for moxifloxacin under steady state conditions.

To derive the $PNEC_{water}$, the EC-50 (acute) of moxifloxacin for algae[9], daphnia and fish representing three trophic levels are required, and the lowest EC-50 is divided by the highest assessment factor (i.e., 1000) (ECHA, 2008). It was assumed that daphnia and fish are less sensitive to the acute toxicity of moxifloxacin than algae, because in our preliminary sensitivity test, *D. magna* was found less sensitive than *P. subcapitata*. Moreover, it was expected that fish would be the least sensitive to the acute toxicity effects of moxifloxacin because of its organismal complexity. Therefore, the EC-50 of moxifloxacin for P. *subcapitata* is considered the lowest value. Since the EC-50 of moxifloxacin for *P. subcapitata* is 0.78 mg/L (780, 000 ng/L, see table 14), the $PNEC_{water}$ is calculated to be 780 ng/L. This means that the RCR is less than one.

$$RCR = \frac{PEC_{water}}{PNEC_{water}} = 0.00026 < 1$$

Therefore, under the current condition, the environmental (aquatic) risk due to the release of moxifloxacin into surface water is adequately controlled and the compound is unlikely to represent a risk to aquatic ecosystem. However, one must bear in mind that this conclusion does not addresses the concerns of antibiotic resistant bacteria development and sub-lethal effects such as genotoxicity that may develop over long-term exposure to very low concentrations (e.g., ng/L) of chemicals.

[9] The 72 h algal growth inhibition test is actually a chronic toxicity test. Nevertheless, in environmental risk assessment it is considered as an acute toxicity test.

6. Conclusions and recommendations

6.1. Conclusions

The use of UV-A/TiO_2 treatment completely removes moxifloxacin (C_o=15 mg/L) from a water matrix with pseudo first-order degradation kinetics (k=0.274 min^{-1}). But, the lack of significant mineralization ($p<0.05$) after 30 min of treatment shows the formation of persistent degradation products. The presence of higher number of degradation products of moxifloxacin (C_o=50 mg/L) after 30 and 60 min of degradation time than 90 and 150 min may indicate the mineralization of some of the degradation products and/or further transformation of the previously generated degradation products to fewer forms or to undetectable compounds.

The photocatalytic treatment is shown to be capable of eliminating the toxicity of a moxifloxacin solution, and toxicity removal as a function of degradation time is observed to follow a logistic curve. Residual moxifloxacin is observed to be the main toxicant in the treated solutions, but one or more of the degradation products are also toxic to *P. subcapitata*. This is because all the identified degradation products conserved the quinolone moiety as well as a number of important substituent groups that are essential for biological activity. The toxic effects of most of the degradation products are lower than the mother compound since they have a polar substituent group than the diazobicyclo group at position C7 on the quinolone ring, which reduces their ability to penetrate cell membrane and cause toxic effects.

The estimated EC-50 of moxifloxacin (0.78 mg/L) classifies the compound as one of the most toxic fluoroquinolones to *P. subcapitata*. Therefore, it is possible to assume that the potential ecological impact because of moxifloxacin release into the environment would be far greater than its predecessor fluoroquinolones. However, the risk assessment under the worst-case scenario shows that the release of moxifloxacin into surface water is unlikely to present a risk to aquatic ecosystem (RCR <1). On the other hand, the adequate control of environmental (aquatic) risk under the current condition does not address the concerns of antibiotic resistant bacteria development and sub-lethal effects (e.g., genotoxicity) that may prevail after long-term exposures at very low concentrations.

6.2. Recommendations

Future studies should focus on the chronic effects of moxifloxacin and its photocatalytically treated solutions using higher test organisms such as daphnia (e.g., *D. magna* reproduction test, 21 day) and fish (e.g., *F. minnow*, short-term screening for oestrogenic and androgenic activity, 21 day). Moreover, attention should be given to the study of the genotoxic effects of moxifloxacin and its degradation products using the DNA unwinding assay (e.g., fish RTG2 cell lines: rainbow trout gonad tissue). This is because the use of standard test organisms and test concentrations much higher than environmentally relevant may underestimate toxicity that can have profound effects on ecosystems in the long-term.

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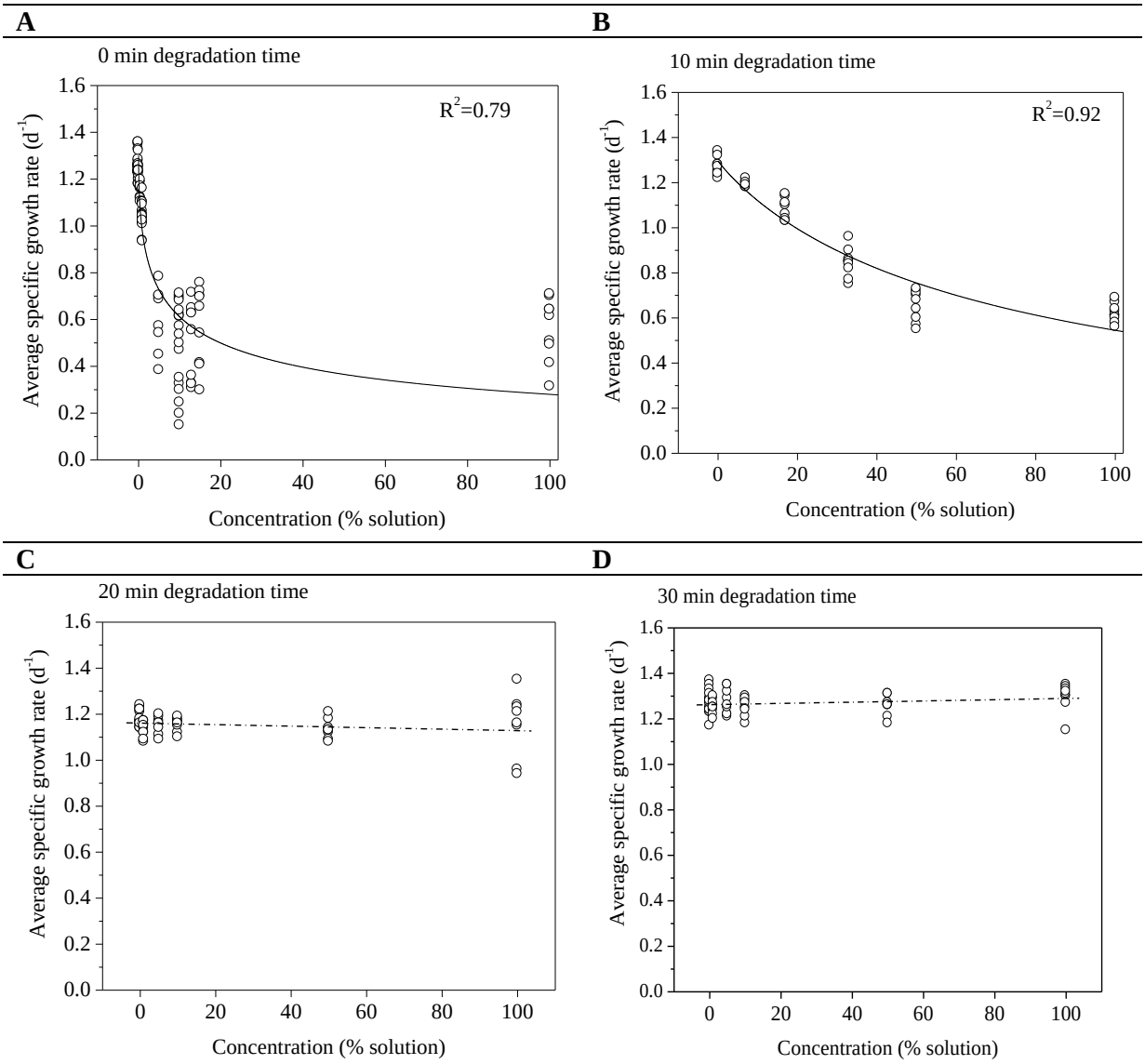
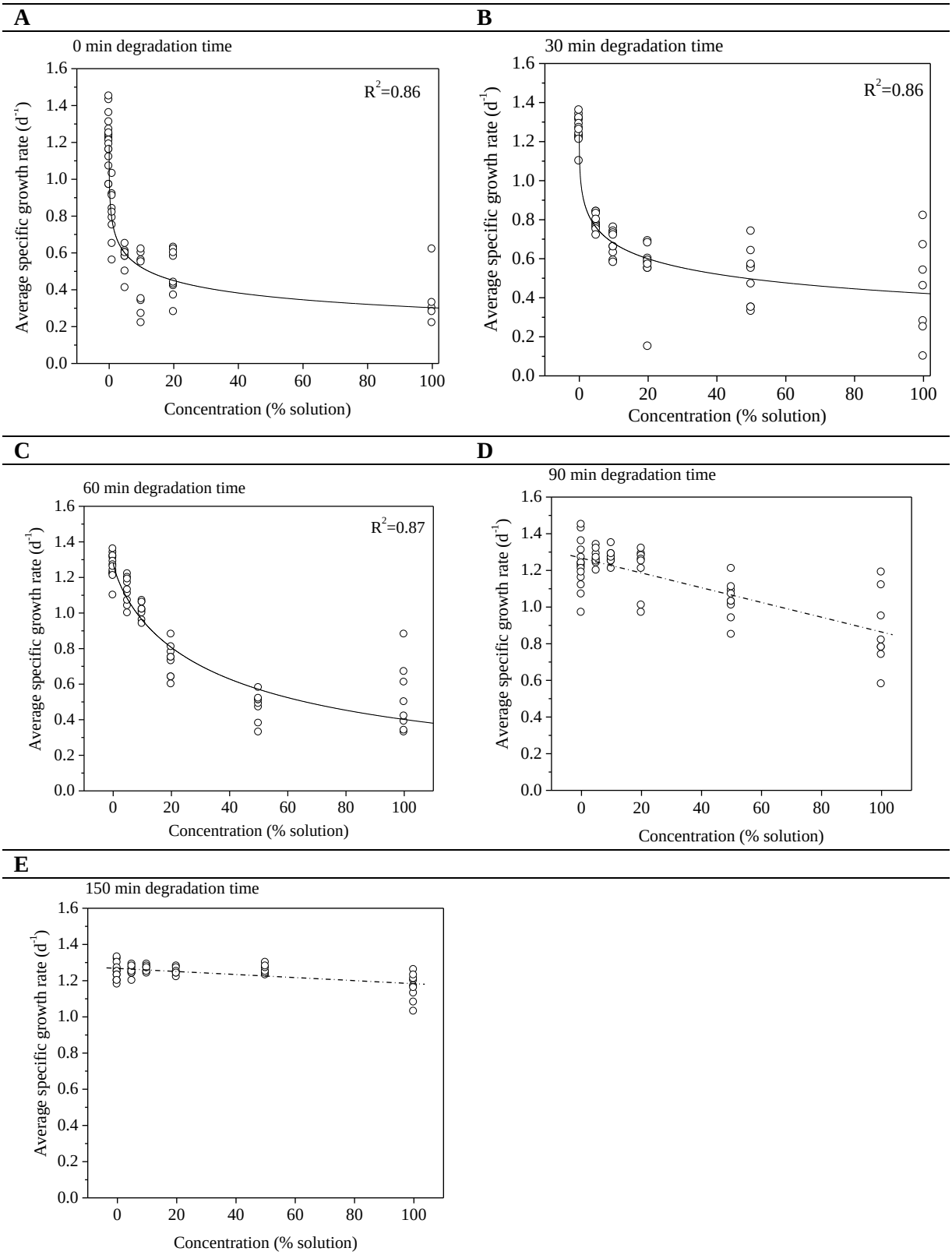
Appendices

Appendix I



Concentration-response plots for 0, 10, 20 and 30 min degradation time solutions at 15 mg/L initial moxifloxacin concentration. The solid lines are fitted with log-logistic regression model; broken lines are drawn to guide the eye.

Appendix II



Concentration-response plots for 0, 30, 60, 90 and 150 min degradation time solutions at 50 mg/L initial moxifloxacin concentration. The solid lines are fitted with log-logistic model; broken lines are drawn to guide the eye.