A chemical, microbiological and (eco)toxicological scheme to understand the efficiency of UV-C/H_2O_2 oxidation on antibiotic-related microcontaminants in treated urban wastewater

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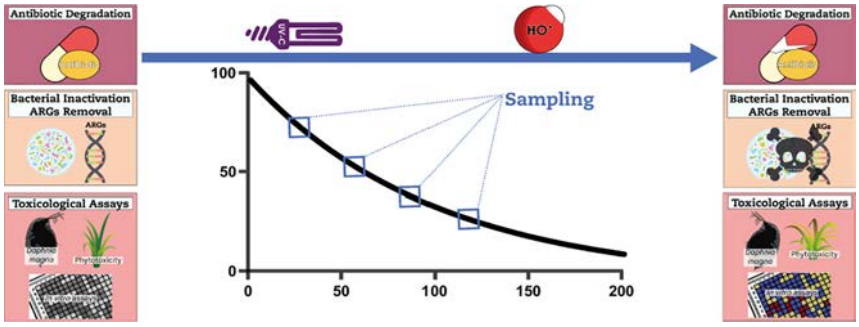
HIGHLIGHTS

- High UV fluence is required for the deg radation of antibiotics in wastewater.
- UV C/H_2O_2 at high UV fluence can po tentially lead to permanent bacterial damage.
- High UV fluence may decrease the phy totoxicity of the untreated wastewater.
- Antibiotics and their TPs exhibited toxic effects against *Daphnia magna*.
- UV C/H_2O_2 reduced oxidative stress and genotoxicity.

GRAPHICAL ABSTRACT



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ABSTRACT

An assessment comprising chemical, microbiological and (eco)toxicological parameters of antibiotic related microcontaminants, during the application of UV C/H_2O_2 oxidation in secondary treated urban wastewater, is presented. The process was investigated at bench scale under different oxidant doses (0 50 mg L^{-1}) with regard to its capacity to degrade a mixture of antibiotics (i.e. ampicillin, clarithromycin, erythromycin, ofloxacin, sulfa methoxazole, tetracycline and trimethoprim) with an initial individual concentration of 100 μg L^{-1}. The process was optimized with respect to the oxidant dose. Under the optimum conditions, the inactivation of selected bac teria and antibiotic resistant bacteria (ARB) (i.e. faecal coliforms, *Enterococcus* spp., *Pseudomonas aeruginosa* and total heterotrophs), and the reduction of the abundance of selected antibiotic resistance genes (ARGs) (e.g. bla_{OXA}, *qnrS*, *sul*1, *tetM*) were investigated. Also, phytotoxicity against three plant species, ecotoxicity against *Daphnia magna*, genotoxicity, oxidative stress and cytotoxicity were assessed.
Apart from chemical actinometry, computational fluid dynamics (CFD) modelling was applied to estimate the fluence rate. For the given wastewater quality and photoreactor type used, 40 mg L^{-1} H_2O_2 were required for the complete degradation of the studied antibiotics after 18.9 J cm^{-2}. Total bacteria and ARB inactivation was ob served at UV doses <1.5 J cm^{-2} with no bacterial regrowth being observed after 24 h. The abundance of most ARGs was reduced at 16 J cm^{-2}. The process produced a final effluent with lower phytotoxicity compared to the untreated wastewater. The toxicity against *Daphnia magna* was shown to increase during the chemical oxi dation. Although genotoxicity and oxidative stress fluctuated during the treatment, the latter led to the removal

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of these effects. Overall, it was made apparent from the high UV fluence required, that the particular reactor al though extensively used in similar studies, it does not utilize efficiently the incident radiation and thus, seems not to be suitable for this kind of studies.

1. Introduction

Antimicrobial resistance is an increasingly serious threat to global public health that requires immediate actions by governmental sectors and the society. However, strategies to mitigate the spread of antimicro bial resistance should take into consideration broader sources and routes of its dissemination associated with both natural and engineered environments (Berendonk et al., 2015). The environmental dimension of antimicrobial resistance has been identified as one of the six emerg ing issues of environmental concern according to the UN Environment Program (UN Environment, 2017). In June 2017, the European Commis sion adopted the EU One Health Action Plan against Antimicrobial Resis tance (EU, 2017). By recognizing that human and animal health are interconnected, the One Health approach encompasses the environ ment as another potential source of new resistant microorganisms.

In fact, urban wastewater treatment plants (UWTPs) are a unique in terface between human society and the environment as sewage con tains antibiotics and bacteria of human origin which can potentially provide a selective pressure for antibiotic resistant bacteria and antibi otic resistance genes (ARB&ARGs), prior to their release into the envi ronment and can act as reservoirs of antimicrobial resistance. A great concern for the dissemination of antimicrobial resistance lies in the fact that the ARGs tend to be shared among bacterial communities through horizontal gene transfer mechanisms. Sharing of genes is pro moted through mobile genetic elements, such as plasmids and integrons and can be driven by conjugation (i.e. bacterial mating), trans duction (i.e. viral mediated), and transformation (uptake of extracellu lar DNA from dead cells) (Pruden, 2014). Therefore, efforts to slow down the spread of antimicrobial resistance by inactivating the ARB may not be effective, unless they also remove the ARGs (Berendonk et al., 2015; Karkman et al., 2018; Pruden, 2014).

For this purpose, various combinations of Advanced Oxidation Pro cesses (AOPs) have been investigated for the most efficient removal of antibiotic residues (Michael et al., 2013), inactivation of ARB and re moval of ARGs from wastewater effluents in bench (Ferro et al., 2017; Sousa et al., 2017; Karaolia et al., 2018; Rizzo et al., 2018), pilot (Alexander et al., 2016; Michael et al., 2019) and full scale studies (Zhuang et al., 2015; Rodríguez Chueca et al., 2019). The role of operat ing parameters and oxidative damage mechanisms of AOPs in the com bat against ARB&ARGs present in urban wastewater has been recently reviewed by Michael Kordatou et al. (2018), indicating that the lack and/or heterogeneity of the available scientific data, as well as the differ ent methodological approaches applied in the various studies, make the accurate evaluation of the efficiency of the applied processes difficult and further mechanistic studies are required to establish the optimum operating conditions under which oxidative mechanisms target internal cell components.

While investigating the optimum operating conditions of an AOP, another aspect that should not be overlooked is the efficiency of the pro cess for attenuating the bioactivity and the potential toxic effects of transformation products (TPs) generated from the oxidation of both the dissolved effluent organic matter (dE_fOM) and chemical microcontaminants present in wastewater by these oxidative technolo gies (Jia et al., 2015). AOPs are expected to be carefully operated and monitored, and toxicity tests have been used to evaluate whether efflu ent detoxification takes place considering also the final use of the treated wastewater (Rizzo, 2011). Combined exposure to multiple chemicals can lead to cumulative adverse effects on human health and/or the ecosystem, even if single substances in the mixtures are below their individual safety thresholds (JRC, 2018; Kortenkamp and Faust, 2018). In order to get a holistic view of the hazards posed by treated wastewater effluents and "real life" chemical mixtures, effect based tools available for water quality monitoring, including *in vitro* and *in vivo* bioassays, and in silico approaches such as quantitative structure activity relationship (QSAR) models are also required. *In vitro* effect based bioassays can be used as analytical tools and can give an account of the mixture toxicity effects. Furthermore, if distinct modes of toxic action are targeted, effect based bioassays can also pro vide information on the characteristics of the compounds present in water samples (Escher and Leusch, 2011) for which further testing is recommended if their levels exceed effect based trigger values (EBTs). Attenuation of bioactivity has been found to be dependent on the mech anism of the treatment process and the bioassay endpoint (Jia et al., 2015). Therefore, concrete conclusions regarding the biological effect of the dE_fOM oxidation products can be drawn when the bioassay is also chosen, apart from the final use of the treated effluent, according to the mechanism of the AOP.

Among AOPs, the combined UV C/H_2O_2 process is of particular inter est and builds on the principles of the UV C disinfection, plus the homo lytic dissociation of H_2O_2. UV radiation is directly absorbed by DNA and thus, has a high potential to impart ARG damage. The mechanism of UV C/H_2O_2 inactivation, is both external and internal. When H_2O_2 is added, the generated hydroxyl radicals ($HO^•$) improve the disinfection capacity leading to efficient inactivation of ARB and removal of ARGs (Ferro et al., 2016, 2017; Zhuang et al., 2015; Rizzo et al., 2018; Rodríguez Chueca et al., 2019), as well as the enhancement of the degradation of various chemical microcontaminants (He et al., 2014; Michael et al., 2013). The optimum H_2O_2 dose is application specific and depends on several factors, including among others water quality, lamp type and power, reactor design, contaminant reactivity towards $HO^•$, contaminant treatment level, and direct photolysis contribution to the overall treat ment. Several UV light based systems are currently installed at water utilities worldwide to treat microcontaminants in both drinking water facilities and at water recycling facilities treating the wastewater sec ondary effluents for indirect or direct potable reuse (Stefan, 2018).

The majority of studies dealing with antibiotic related microcontaminants and the application of AOPs, are mainly focused either on the evaluation of the degradation of the parent compounds and the assessment of the biological effects (in terms of whole effluent toxicity) of the treated effluents that may be in duced by the partial oxidation of the parent compounds and the dE_fOM present in wastewater, or on the investigation of the effect of operating conditions and the mechanisms governing the inacti vation of ARB and removal of ARGs. According to the authors' knowledge, there is no study available in the scientific literature ad dressing concurrently all the above aspects.

In the present study, a chemical, microbiological and (eco)toxicolog ical scheme is proposed to be applied by utilizing UV C/H_2O_2 oxidation as a case study. Within this context, UV C/H_2O_2 oxidation at bench scale was investigated with regard to its efficiency to degrade a mixture of se lected antibiotics, to inactivate ARB, to reduce the abundance of ARGs, and to assess phyto and eco toxicity and other biological effects at cel lular level in urban secondary treated wastewater effluents. The se lected antibiotics were ampicillin, clarithromycin, erythromycin, ofloxacin, sulfamethoxazole, trimethoprim and tetracycline which are highly consumed antibiotics worldwide, belonging to different chemical

classes. Clarithromycin and erythromycin are also compounds of additional interest as they have been selected for inclusion in the updated EU "watch list" of substances known to potentially pose environmental implications to the aquatic environment (EU 2018/840). Under the optimum experimental conditions, the efficiency of the process to inactivate selected bacteria and ARB (i.e. faecal coliforms, *Enterococcus* spp., *Pseudomonas aeruginosa* and total heterotrophs) and to reduce the abundance of selected ARGs (i.e. 16S rRNA, bla_{OXA}, bla_{TEM}, $bla_{CTX\text{-}M}$, *qnrS*, *sul*1, *tetM*) was investigated. The selection of bacteria and ARB is based on the fact that they are human commensal and/or opportunistic and hospital pathogens, recognized carriers of acquired ARGs and indicators of faecal contamination in aquatic environments. Among the selected ARB, those conferring resistance to erythromycin, ofloxacin and trimethoprim were investigated based on the wide use of these antibiotics and their frequent detection in wastewater and aquatic environments. The examined genes represent a spectrum of typical documented prevalence in the environment. These genes have been proposed as possible indicators to assess the antibiotic resistance level in environmental settings, focusing mainly on genes spread in the environment and commonly associated to mobile genetic elements and thus, more prone to horizontal gene transfer (Berendonk et al., 2015). Phytotoxicity against three plant species (*Lepidium sativum*, *Sinapis alba* and *Sorghum saccharatum*), ecotoxicity against the crustacean *Daphnia magna*, genotoxicity, oxidative stress and cytotoxicity were also assessed.

2. Materials and methods

2.1. Chemicals and reagents

All antibiotic reference standards (ampicillin [CAS number 69 53 4], erythromycin [CAS number 114 07 8], clarithromycin [CAS number 81103 11 9], ofloxacin [CAS number 82419 36 1], sulfamethoxazole [CAS number 723 46 6], trimethoprim [CAS number 738 70 5], and tetracycline [CAS number 60 54 8]) were of high purity grade (more than 90%), and were supplied by Sigma Aldrich (Steinheim, Germany). The structures, molecular formulas, pKa and $\log_{Kow}$ values of the target antibiotics are given in Table S1 of the Supplementary Material. Hydrogen peroxide (H_2O_2, 30% w/w) was purchased from Merck (Darmstadt, Germany). Anhydrous sodium sulfite (Na_2SO_3), manganese oxide (MnO_2) and catalase from bovine liver were all purchased from Sigma Aldrich. For the chromatographic analysis, MeOH of LC MS grade (Sigma Aldrich) and formic acid (99%, Fluka, Buchs, Switzerland) were used. Ultrapure water (Millipore, 18.2 MΩ cm at 25 °C) was used throughout the experimental procedure and chromatographic analyses.

The UV C/H_2O_2 oxidation experiments were performed in secondary treated wastewater samples taken from the secondary clarifier of an UWTP located in Cyprus. All wastewater samples were grab samples collected in amber glass bottles and vacuum filtered through 0.45 μm glass fiber filters after arrival at the laboratory. The physicochemical parameters that have been used to characterize the wastewater samples prior to their use were determined according to the Standard Methods (Clesceri et al., 1998). The main average qualitative characteristics of the wastewater samples used during the experimental period are presented in Table S2. The inherent concentration of the examined antibiotics in the wastewater effluents is given in Table S3.

2.2. Experimental set up

The experiments were run in a photochemical apparatus, batch type bench scale cylindrical reaction vessel with a total capacity of 600 mL purchased from Ace Glass (Vineland, NJ, USA). Irradiation was provided by a 9 W low pressure mercury lamp (Radium, Puritec, UVC LPS 9) which emits at $\lambda = 254$ nm. The irradiance of the lamp entering into the reaction system *via* the sleeve was quantified employing chemical actinometry according to the protocols described by Bolton et al. (2011) and by Willett and Hites (2000). The calculations are provided in Text S1. Due to the anisotropic irradiation field expected in the photoreactor, average UV fluences were estimated to correct for the non uniformly illuminated region expected to be present at the top and the bottom of the photoreactor. To this purpose, a computational fluid dynamics (CFD) modelling approach was utilized, whose results are summarized in Text S2. In all CFD simulations, the actinometry based irradiance values were used as boundary condition for the radiation model. It should be mentioned that the CFD estimated average fluence doses can still be affected by the non uniform mixing in the system, which could explain the relatively high fluence rate values required for complete treatment reported in this paper. The axial contour plots for incident radiation of 254 nm monochromatic light for: (a) 15.61 m^{-1} (corresponds to filtered wastewater which was used for the antibiotics' degradation, phyto , eco toxicity and other biological effects experiments) and (b) 23.56 m^{-1} (corresponds to filtered wastewater which was used for the microbiological experiments) of water absorption coefficient (base e) are presented in Fig. 1.

The UV C lamp was turned on for 30 min in order to warm up so as the irradiance reaches its steady state value. After 30 min, the antibiotics spiked wastewater solution of 100 μg L^{-1} of each compound was introduced into the reaction vessel, and the UV C lamp with its quartz glass sleeve was immersed inside the solution (time zero, t_0). Bearing in mind that the typical environmental concentrations of antibiotics in the wastewater effluents are in the ng mg L^{-1} range, this concentration of 100 μg L^{-1} can be considered as (i) adequately high to characterize the degradation using available analytical techniques, and (ii) low enough to resemble real environmental conditions. The exterior of the reactor was covered by a black cloth in order to prevent external light penetration during the experimental duration. A water jacket maintained the reaction temperature at 22 ± 2 °C by circulating cooling water. Then, the appropriate amount of H_2O_2 was added to achieve the desired oxidant concentration in the range of 0 50 mg L^{-1}. At specific time intervals, samples were withdrawn from the reactor, transferred into vials containing MeOH or Na_2SO_3 or catalase to quench reactions in each sample for the chromatographic analysis, the determination of the dissolved organic carbon (DOC), the chemical organic carbon (COD), the microbiological as well as the phyto , eco toxicity and other biological effects experiments, respectively and processed for further analysis. The specific volume which was withdrawn from the reactor for the chromatographic analysis and the *in vitro* bioassays experiments was 900 μL at each time interval while for the determination for the DOC and the COD was 8 mL and as a result the overall volume of the wastewater was not significantly changed. For the microbiological as well as the phyto and eco toxicity effects experiments, the volume which was withdrawn from the reactor is discussed in detail below. It is noted that the results are given as a function of UV fluence to be reproducible by other laboratories and to enable, where applicable, any correlation of the results of the present study with those obtained in real scale UV AOP applications. All the experiments were performed in triplicate.

2.3. Analytical methods

The analysis of the samples with respect to the target antibiotics was carried out using an ACQUITY Ultra Performance Liquid Chromatography (UPLC) system interfaced to an ACQUITY Triple Quadrupole Detector (TQD) mass spectrometer (Waters Corporation). Data acquisition and data treatment were performed with MassLynx 4.1 software. The exact conditions of the analytical method for the determination of the antibiotics are given in Text S3.

An Aurora 1030 W TOC analyzer was employed, in order to monitor the DOC in the treated samples and therefore, to assess the extent of mineralization. The residual H_2O_2 remaining in the treated samples was measured using the spectrophotometric method employing ammonium metavanadate as described in Nogueira et al. (2005). The

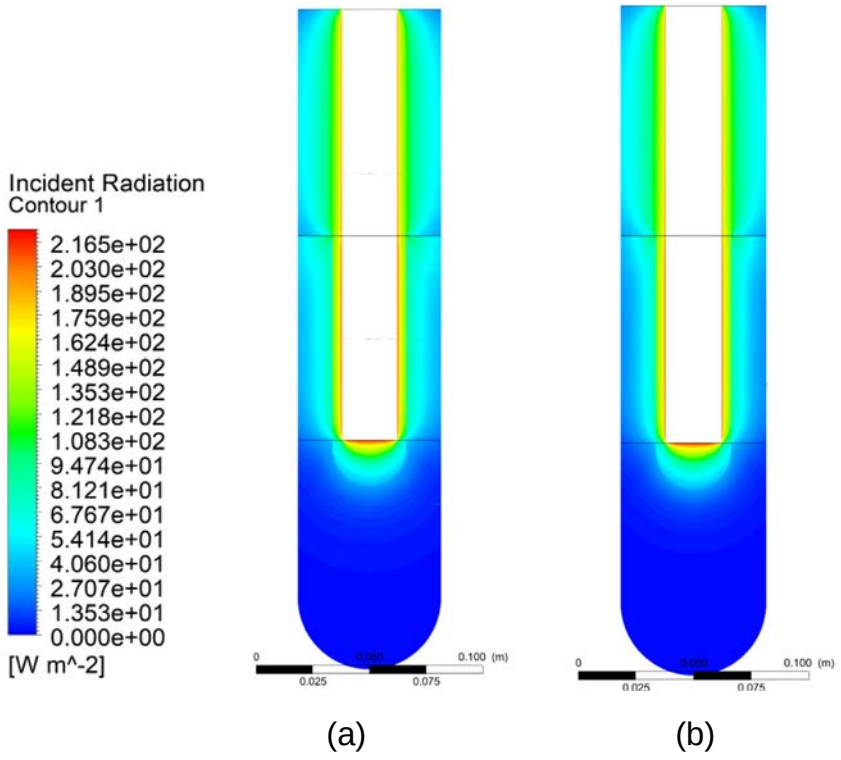


Fig. 1. Axial contour plots for incident radiation of 254 nm monochromatic light for: (a) 15.61 and (b) 23.56 m ^{1} of water absorption coefficient (base e).

absence of H_2O_2 in the samples was also checked using the Merckoquent® test sticks (Merck). COD determination was performed using the Merck Spectroquant® kits (Merck).

2.4. Enumeration of total and antibiotic resistant bacteria

The membrane filtration method was used for the enumeration of total bacteria and ARB in the samples taken during UV C/H_2O_2 oxidation, as described in (Novo and Manaia, 2010). Different selective media were prepared for each type of microorganism examined. m FC agar (Fluka) was prepared for faecal coliforms, Pseudomonas agar base (Fluka) was prepared for *Pseudomonas aeruginosa*, *Enterococcus* selective agar for *Enterococcus* spp. and Plate count agar (Fluka) for total heterotrophs enu meration, according to the manufacturers' instructions.

Where necessary, filtered volumes of 10, 50, 100 mL or sample serial dilutions were performed at 1:10, 1:100 and 1:1000 using a sterile sa line solution (NaCl, 0.85%) and samples were filtered using a sterile Millipore filtration apparatus, through cellulose acetate membrane fil ters of a pore size of 0.45 μm (Millipore). In order not to change signifi cantly the overall volume of the reactor, the experiment was reinitiated in the case of the withdrawal of 50 and 100 mL. The filters were then placed on the prepared solid agar medium and incubated for 24 h before enumeration. For the evaluation of the resistance of the examined bac teria, the prepared agar was spiked with the minimum inhibitory con centration (MIC) of each antibiotic (trimethoprim: 16 mg L^{-1}; ofloxacin: 8 mg L^{-1}; erythromycin: 8 mg L^{-1}) as determined in Clinical and Laboratory Standards Institute (CLSI) guidelines. The colonies which grew on each selective agar containing each antibiotic were con sidered resistant to the individual antibiotic present.

2.5. Determination of total genomic DNA and ARGs

After UV C/H_2O_2 oxidation, obtained samples were stored at 4 °C in the dark until molecular analysis, within 24 h of sampling. The DNA ex traction took place using the PowerWater® DNA isolation Kit (MoBio) following filtration of 600 mL of each sample with 47 mm diameter, 0.22 μm polycarbonate filter membranes (Millipore). The experiment was reinitiated after the withdrawal of the total volume of the reactor for each sample. The amount of the DNA of the extracts was measured using the Qubit (PEQLab BioTechnology, Munich, Germany). The proto col applied for the determination of total genomic DNA and ARGs is given in Text S4.

2.6. Phyto and eco toxicity assessment and classification

2.6.1. Phyto and eco toxicity assessment

The phytotoxicity of the treated samples was conducted with the Phytotestkit microbiotest kit (MicroBioTests Inc.) towards three plant species i.e. *Lepidium sativum*, *Sinapis alba* and *Sorghum saccharatum*. The procedure is described extensively elsewhere (Michael et al., 2012). Moreover, ecotoxicity tests were conducted according to the standard operating protocols for *Daphnia magna* (ISO 6341:2012). The procedure is described extensively elsewhere (Vasquez and Fatta Kassinos, 2013). The residual H_2O_2 was removed from the treated sam ples with catalase prior to analysis. 20 mL were withdrawn from the re actor and in order not to change significantly the overall volume, the experiment was reinitiated for each sample. The procedure followed for both the phytotoxicity and ecotoxicity assays is given in Text S5 and S6, respectively.

2.6.2. Phyto and eco toxicity classification

The system of toxicity classification developed by Persoone et al. (2003) was used to estimate phyto and eco toxicity of the treated sam ples: PE (Percent toxic effect) < 20% no significant toxic effect, class I, score 0, no acute hazard; 20% ≤ PE < 50% significant toxic effect, low toxic sample, class II, score 1, low acute hazard; 50% ≤ PE < 100% signif icant toxic effect, toxic sample, class III, score 2, acute hazard; PE 100% (single test), class IV, score 3, high acute hazard; PE 100% (all tests), class V, score 4, very high acute hazard. This system is based on two values: position in a five grade risk scale, and significance of the result

for each class. The data on toxicity of treated samples were then expressed as percentage effects (PEs) of inhibition in the seed germination (GI), shoot growth (SI), root growth (RI) and immobilization depending on the effect criterion of the respective test procedure scoring system. After determining the percent effect for each bioassay, the sample was classified as one of five classes according to the respective toxicity indicated by each one test.

2.7. In vitro bioassays assessment

The samples which were withdrawn at specific time intervals were evaporated to dryness under a gentle stream of nitrogen and redissolved in the same amount of dimethylsulfoxide (DMSO). Serial dilution series (1 , 10 , 30 and 100 times) were prepared in DMSO after which they were stored at −20 °C prior to analysis. The Nrf2, p53, p53 + S9 and Cytotox CALUX® bioassays (Chemically Activated Luciferase eXpression (CALUX®); BioDetection Systems BV, Amsterdam, the Netherlands) were then performed as described in Van der Linden et al. (2014).

Briefly, the cells were seeded into 96 well microtiterplates with DF medium without phenol red that was supplemented with 5% dextran coated charcoal stripped FCS (DCC medium). After 24 h of pre incubation for the Nrf2 and Cytotox CALUX® and 48 h of pre incubation for the p53 and p53 + S9 CALUX® (37 °C, 5% CO_2), the medium was replaced by medium containing serial dilutions of the water extracts (1% v/v DMSO) for agonistic activity testing (in triplicate). In case of the p53 + S9 CALUX®, a mixture containing S9 (MP Biomedicals; final concentration of 0.33 mg mL^{-1} medium), 200 μM NADPH (Applchem), 3 mM glucose 6 phosphate, 0.3 U mL^{-1} glucose 6 phosphate dehydrogenase, and 5 mM magnesium chloride was added. Three hours after addition of the S9 mixture, the medium containing the S9 mixture and serial dilutions of the water extract was removed and replaced by 200 μL of fresh DCC medium only and the cells were incubated for another 16 h. All incubations were performed in triplicate. After 24 h of total incubation, the medium was removed and the cells were lysed in 30 μL of Triton lysis buffer. The amount of luciferase activity was quantified using a 96 well plate luminometer reader (Mithras, Berthold Technologies Gmbh, Germany). On all plates, a dose response curve of the reference compounds was included for quantification of the response, which where curcumine, tributyltin acetate, actinomycin D and cyclophosphamide for the Nrf2, Cytotox, p53 and p53 + S9 CALUX®, respectively. For both the reference compound and sample extracts, a relative induction factor (IF) (relative to the DMSO solvent control) was calculated. Sample extracts were considered to be positive in the bioassays applied when a 1.5 fold relative induction was observed. Using the software package GraphPad Prism, the sample bioactivity was quantified following determination of the concentration of sample extract showing an IF of 1.5 and intrapolation in the reference calibration curve. Final results were expressed as curcumine, tributyltin acetate, actinomycin D and cyclophosphamide equivalences per L of samples for the Nrf2, Cytotox, p53 and p53 + S9 CALUX® bioassay respectively. Only dilutions that did not show any signs of cytotoxicity (relative induction in the Cytotox CALUX® bioassay >80%) were used for final evaluation of CALUX® analysis results.

3. Results and discussion

3.1. Effect of H_2O_2 concentration on the degradation of the target antibiotics and the removal of organic content of the wastewater

The optimization of the UV C/H_2O_2 process was performed with respect to the oxidant concentration for the degradation of the studied antibiotics in mixture. As a first step, preliminary photolytic experiments were carried out at zero concentration of H_2O_2 to examine the degradation of the mixture of the studied antibiotics in wastewater at a concentration of each antibiotic equal to 100 μg L^{-1}. Sulfamethoxazole and tetracycline exhibited substantial direct photolysis with both compounds being completely degraded at 2.6 J cm^{-2} and 4.7 J cm^{-2}, respectively (Fig. 2a). However, in the case of the rest of the studied compounds (i.e. ampicillin, clarithromycin, erythromycin, ofloxacin and trimethoprim) partial direct photolysis was observed, with their degradation being 87.5%, 79.9%, 71.6%, 95.1% and 50.3%, respectively, at the end of the treatment reaching 18.9 J cm^{-2} (Fig. 2b).

Several concentration levels of H_2O_2 (5, 10, 15, 30, 40 and 50 mg L^{-1}) were investigated in order to obtain the optimum oxidant dose for the complete degradation of the mixture of the selected antibiotics. While sulfamethoxazole may have been amenable to oxidation by HO• (Lekkerkerker Teunissen et al., 2012), it was completely already degraded by photolysis at 2.6 J cm^{-2}. For tetracycline, the gradual increase of the oxidant affected the degradation of the compound (100% degradation at 4.7 J cm^{-2} for 5 mg L^{-1} of H_2O_2 and at 1.6 J cm^{-2} for 40 mg L^{-1} of H_2O_2). At mild alkaline conditions, the tertiary amine group of the molecule becomes deprotonated causing an increase in the decomposition of the compound and the generated HO• are catalyzed by the hydroxyl ions in the wastewater as well as the electron rich functional groups on tetracycline (Khan et al., 2010). Ampicillin, whose pKa value falls within the inherent pH of the examined wastewater exists predominantly in its deprotonated form at pH 8.0 meaning that HO• attack is directed towards both the sulphur atom and non protonated amine (Jung et al., 2012). For ampicillin, the gradual increase of the oxidant slightly affected the complete degradation of the compound (100% degradation at 9.5 J cm^{-2} for all the tested concentrations of H_2O_2).

On the other hand, clarithromycin, erythromycin, ofloxacin and trimethoprim were shown to be more persistent to the UV C/H_2O_2 oxidation. For ofloxacin, this behavior can be attributed to its quinolone ring stability. However due to the photolability of fluoroquinolones, it is subjected to direct and/or indirect phototransformation (Boreen et al., 2003). The deprotonated form of trimethoprim which exists in the inherent pH of the wastewater is more nucleophilic and hence, more reactive towards HO• (Dodd and Huang, 2007). The persistent behavior of clarithromycin and erythromycin may be attributed to their rather complex chemical structure. The molecules of clarithromycin and erythromycin contain various deactivating electron withdrawing groups but no unsaturated C—C bonds, which HO• would prefer to attack *via* electrophilic mechanisms (Michael Kordatou et al., 2015). However, the increase of the oxidant enhanced their complete degradation, suggesting that oxidation by HO• was responsible for much of the degradation observed.

A compromise had to be made in order to obtain the optimum oxidant dose for the mixture of the tested compounds. The oxidant concentration of 40 mg L^{-1} was chosen as the optimum dose and used in further experiments, when using this concentration complete degradation of all the target antibiotics was achieved at 18.9 J cm^{-2}. It is noted that the optimum concentration falls within the range of the H_2O_2 concentrations applied in other relevant studies (He et al., 2014; Wols et al., 2013) and full scale applications (Rizzo et al., 2019b). Several studies have reported that beyond a certain level, an increase in the initial H_2O_2 dose cannot cause an increase in the substrate degradation because H_2O_2 itself can act as a scavenger of the produced HO• (Liao et al., 2016). In this study, the inhibition effect of H_2O_2 was observed at the highest concentration of the oxidant (50 mg L^{-1}), which resulted in lower degradation of the clarithromycin, erythromycin, ofloxacin and trimethoprim (*data not shown*).

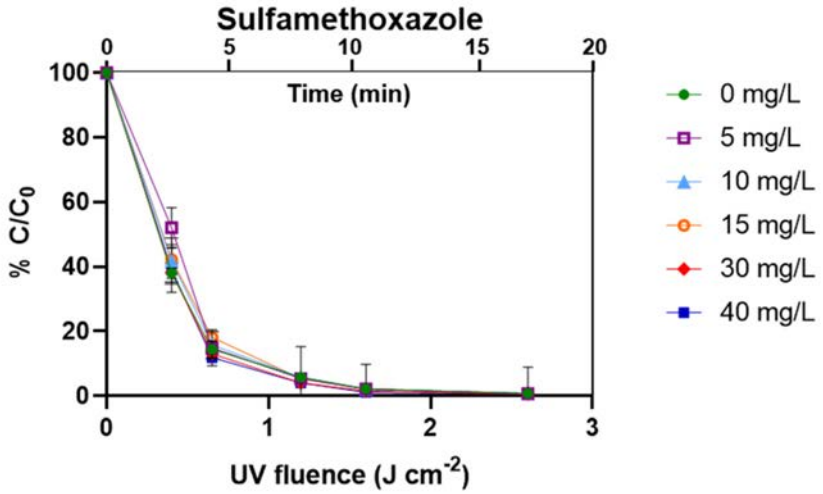
Based on the experimental set up of this study, high UV fluence is required for the degradation of chemical microcontaminants present in mixture in wastewater. As indicated in the method section, this could be due to a combination of effects. On the one hand, the wastewater matrix may contain scavengers at elevated concentrations, that would slow down the degradation of those compounds which are removed both by photolysis and hydroxyl radical attack. On the other hand, as illustrated in our CFD analysis (see Supplementary material), the laboratory scale photoreactor configuration is affected by a highly anisotropic fluence

rate distribution, which could have led to mixing limited degradation performance and lower degradation rates (experimentally shown as high fluence based rate constants). The synergistic effect of H_2O_2 and UV C irradiation for the degradation of the mixture of the selected anti biotics was demonstrated clearly with UV fluence reaching values of 18.9 J cm^{-2}. Previous research employing an experimental set up (i.e. a low pressure lamp of 15 W immersed in vertical position and isolated by a cylindrical quartz tube of 1 L) similar to this study has shown that the degradation of various pharmaceutical compounds including carba mazepine and trimethoprim in wastewater requires high UV fluence up to 120 J cm^{-2} (Paredes et al., 2018). The findings of this study confirm that when dealing with a mixture of chemical microcontaminants (as in the case of this study) containing recalcitrant compounds such as macrolides (clarithromycin, erythromycin), the process requires much higher UV fluence values than that commonly applied at UWTPs for dis infection in order to achieve sufficient degradation.

In general, the concentration of microcontaminants during oxidation obeys a pseudo first order kinetic law ($C = C_0 e^{-kt}$; where C and C_0 are the time dependent concentration and the initial concentration of the microcontaminant, respectively, and k is the pseudo first order rate constant, which is given in units of $time^{-1}$). However, it may be difficult to reproduce these time based rate constants in other laboratory set ups, since these parameters and results refer to the use of the particular reactor geometry and characteristics and also to the particular waste water matrix. For this purpose, in this study, fluence based rate con stants were derived for each antibiotic and linked directly to the fluences delivered in the UV C reactor. The fluence based rate constants were calculated using Eq. (1) (Bolton and Stefan, 2002):

$$\ln \frac{[C]_0}{[C]_{H'}} \qquad \ln \frac{[C]_0}{[C]_{t'}} \tag{1}$$

(a)



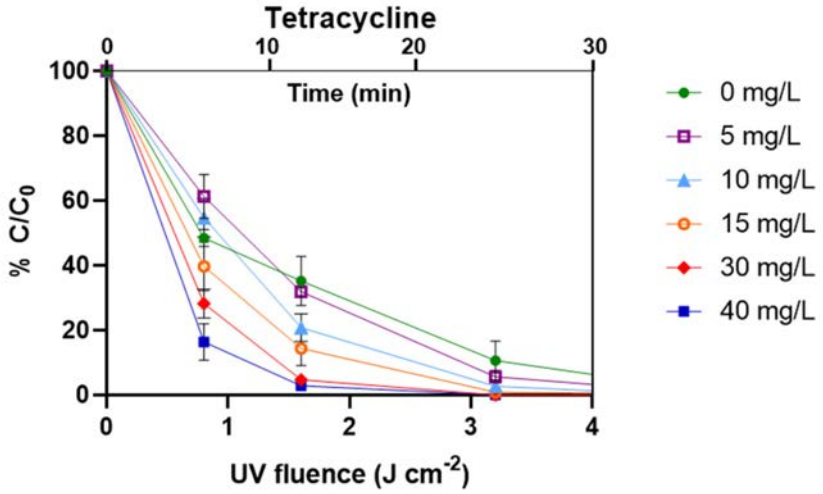


Fig. 2. Effect of H_2O_2 concentration on the degradation of antibiotics-spiked wastewater solutions under UV-C irradiation. Experimental conditions: wastewater effluent after CAS; $[A]_0 =$ 100 μg L^{-1}; pH = 7.92 ± 0.02; T = 22 ± 2 °C. (a) sulfamethoxazole, tetracycline (b) ampicillin, ofloxacin, clarithromycin, erythromycin, trimethoprim.

(b)

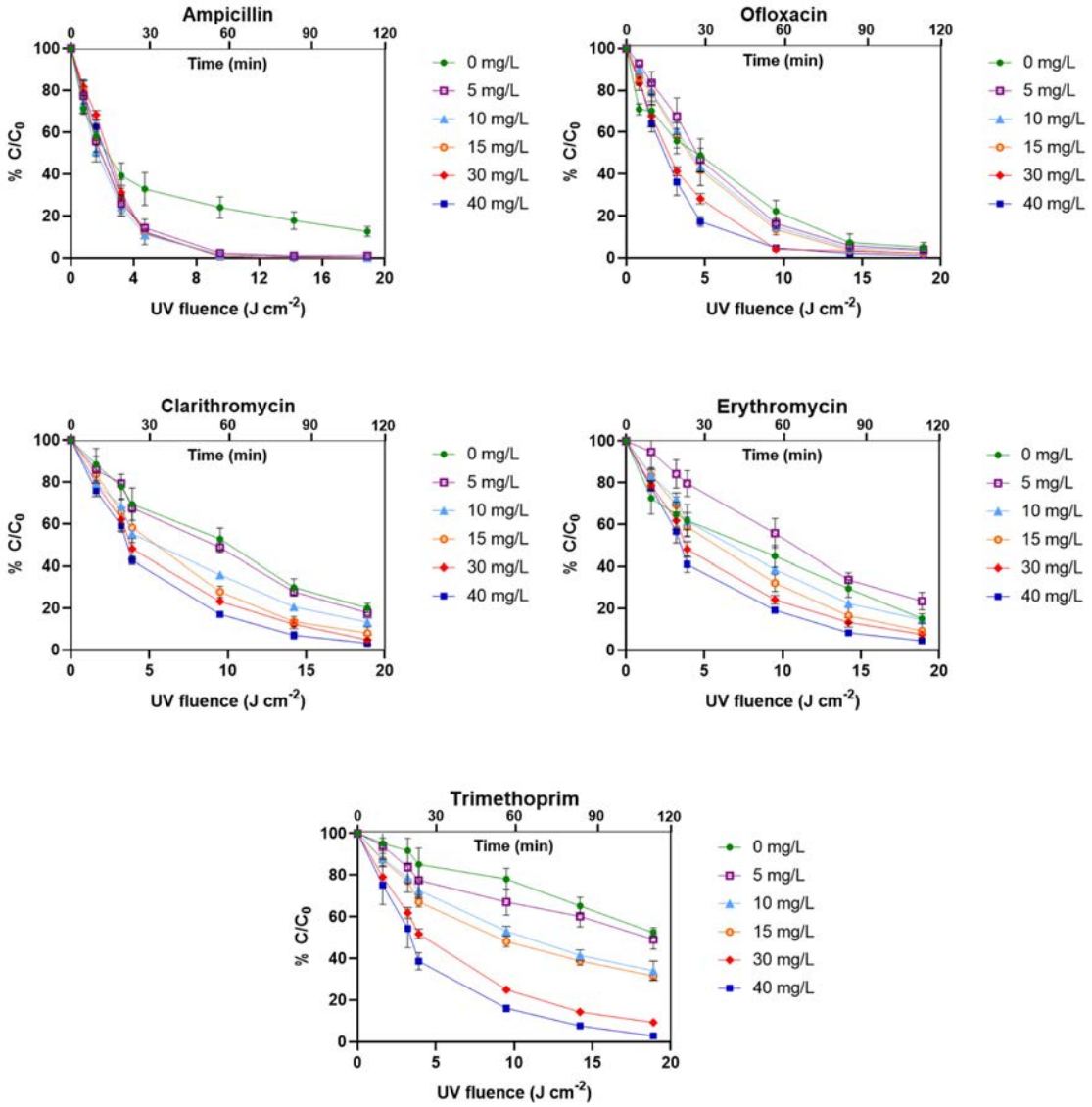


Fig. 2 (*continued*).

where t′ is the exposure time (min) corresponding to the fluence H′ (J m^{-2}) delivered to the UV C reactor to degrade the microcontaminant from C_0 to C.

$$k_1 t' \quad k_1' H' \quad k_1' E_{avg}' t' \tag{2}$$

$$k_1 \quad k_1' E_{avg}' \tag{3}$$

where k_1 is the time based rate constant (s^{-1}) of the microcontaminant, k_1' the fluence based rate constant (m^2 J^{-1}) and E_{avg}' is the average fluence rate (W m^{-2}) in the UV C reactor. Thus, the time based rate constants and the fluence based rate constants were calculated and given in Table 1.

Moreover, DOC measurements showed significant mineralization of the treated solution (DOC removal 38%) at 18.9 J cm^{-2}, while COD was reduced by 24% after the end of the treatment (*data not shown*). It should be noted that the DOC of the reaction solution corresponds to the inherent DOC of the wastewater effluents taking into consideration the low concentration of the spiked antibiotics.

3.2. Assessment of the efficiency of UV C/H_2O_2 oxidation to inactivate total and ARB

UV C/H_2O_2 oxidation was investigated with regard to its efficiency to inactivate total bacteria and ARB and thus, reduce the cultivable microbial populations. Fig. 3 shows the total bacteria and ARB resistant

Table 1
Time-based rate constants (s^{-1}) and fluence-based rate constants (m^2 J^{-1}) of the target antibiotics during UV-C/H_2O_2 oxidation.

Antibiotic	Time-based rate constants (s^{-1})	Fluence-based rate constants (m^2 J^{-1})
Ampicillin	8.55×10^{-4}	3.25×10^{-5}
Clarithromycin	3.48×10^{-4}	1.32×10^{-5}
Erythromycin	3.53×10^{-4}	1.34×10^{-5}
Ofloxacin	6.15×10^{-4}	2.34×10^{-5}
Sulfamethoxazole	66.18×10^{-4}	2.52×10^{-4}
Tetracycline	68.66×10^{-4}	2.61×10^{-4}
Trimethoprim	3.88×10^{-4}	1.47×10^{-5}

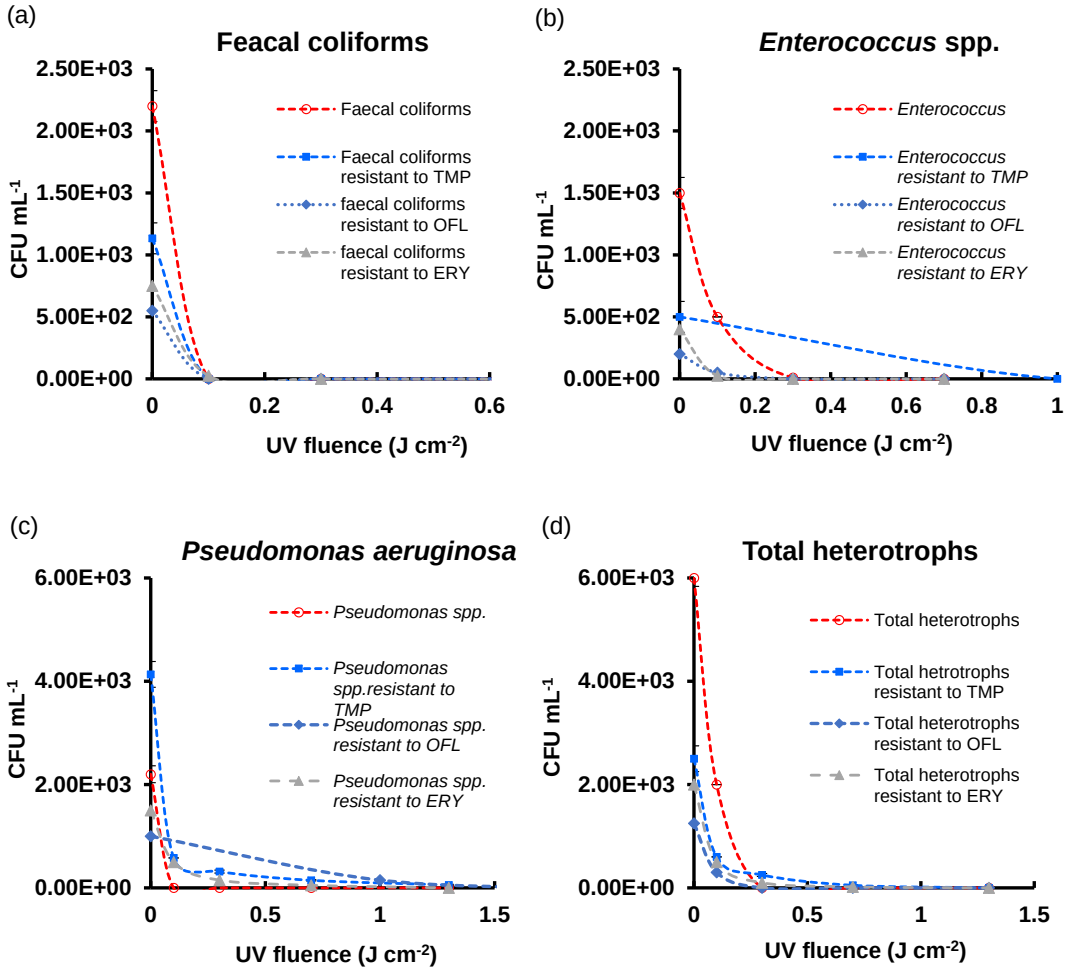


Fig. 3. Number of colonies of the selected bacteria and ARB resistant to trimethoprim, ofloxacin and erythromycin (a) faecal coliforms (b) *Enterococcus* spp. (c) *Pseudomonas aeruginosa* and (d) total heterotrophs (bacterial density expressed as CFU mL ^{1}) present in the treated samples upon UV-C/H_2O_2 oxidation. Experimental conditions: wastewater effluent after CAS; $[A]_0 = 100$ μg L ^{1}; $[H_2O_2] = 40$ mg L ^{1}; pH = 8.02 ± 0.02; T = 22 ± 2 °C.

to trimethoprim, ofloxacin and erythromycin expressed as CFU mL^{-1} as a function of UV fluence.

Comparing the populations of total heterotrophs and faecal coliforms, the former was shown to be more abundant and exhibited lower reduction rates than the latter. More specifically, the total heterotrophs were inactivated at 1.5 J cm^{-2}, compared to faecal coliforms which were rapidly inactivated at 0.1 J cm^{-2}. Guo et al. (2013) reported the high efficiency of UV treatment in removing erythromycin resistant heterotrophic bacteria at a UV fluence of 5 mJ cm^{-2}, with the corresponding log reduction being 1.4 ± 0.1. When the UV fluence increased to 20 and 50 mJ cm^{-2}, the counts of total resistant bacteria were below 1 CFU mL^{-1}.

Moreover, total enterococci were found to be less abundant than the previous two populations of total heterotrophs and faecal coliforms, a finding that is in agreement with the study by Novo and Manaia (2010). It was observed that total enterococci were inactivated at 0.3 J cm^{-2}. A study by Chen et al. (2016) showed over 3 log inactivation of total enterococci at 20 mJ cm^{-2} of disinfection with a low pressure mercury vapor lamp. *Pseudomonas* spp. and ARB to *Pseudomonas* spp. were shown to be more persistent than faecal coliforms and total enterococci and, requiring a higher UV dose (1.5 J cm^{-2}) for their complete inactivation.

Total bacteria and ARB can be effectively inactivated at lower UV fluence compared to that required for the degradation of a mixture of the chemical microcontaminants. The general lack of studies concurrently addressing both chemical microcontaminants and bacteria (total and ARB) during the application of UV C/H_2O_2 oxidation, certainly prevents scientists from reaching comprehensive conclusions in relation to the distinct effect of the UV fluence on microcontaminants of chemical and biological origin. The results presented herein suggest that the ability to estimate such an effect would allow design engineers to predict the overall effect of UV fluence, allowing thus to properly optimize the process at full scale application.

In addition, the findings of this study, have shown that under the experimental set up and conditions employed, both total bacteria and ARB were inactivated at higher UV fluence (0.1 1.5 J cm^{-2}) than that commonly applied at UWTPs for urban wastewater disinfection (40 100 mJ cm^{-2}) (Yoon et al., 2018). For example, Paredes et al. (2018) observed that the complete inactivation of total coliforms and *Escherichia coli* was not achieved with the typical UV fluence employed for disinfection at UWTPs. Higher UV fluence was required to achieve the complete inactivation of these pathogens, as observed in the lab scale experiments of the former study. Moreover, the inactivation of *E. coli* in the study of Taghipour (2004) required UV fluence up to 400 mJ cm^{-2}. In general, there is limited knowledge on the efficiency of UV C/H_2O_2 oxidation, in terms of UV fluence, to inactivate ARB, since the majority of the studies performed so far only covered the effect of treatment time on ARB without reporting the UV fluence required. Also, the different experimental conditions, scale (bench and pilot studies) and reactor configurations (e.g. collimated beam, cylindrical reactor with immersed UV C lamp) applied in each study, as well as the different matrices used, do not allow for an easy, uniform and concrete comparison among the various studies performed.

It is important to note herein that some studies performed at pilot scale, reported the bacterial inactivation during UV C driven oxidation processes as a function of both the experimental time (t) and the cumu lative energy per unit of volume (Q_{UV}) received in the photoreactor. Thus, an effort was made in this study to calculate the Q_{UV} values. The findings of (Rizzo et al., 2019a) have shown that the inactivation of antibiotic resistant *E. coli* up to the LOD in both wastewater and ground water under UV C irradiation in the presence of peracetic acid ([PAA] = 0.2 mg L^{-1}) was achieved for a cumulative energy (Q_{UV}) of 0.3 kJ L^{-1}. This value is in accordance with the Q_{UV} calculated in this study (0.1 0.8 kJ L^{-1}) under which complete bacterial inactivation occurred. One interesting observation in relation to the degradation of a mixture of contaminants of emerging concern (CECs) is that higher Q_{UV} values compared to bacterial inactivation seem to be required for their effec tive degradation. This was also observed in Cerreta et al. (2020), who examined the degradation of a mixture of four CECs (carbamazepine, diclofenac, sulfamethoxazole and imidacloprid) in wastewater under UV C oxidation process in the presence of free chlorine. The results demonstrated that 87% of degradation of total CECs was achieved at Q_{UV} 1.33 kJ L^{-1}, which is in the same order of magnitude in this study for complete degradation of seven CECs (Q_{UV} = 9.74 kJ L^{-1}).

The regrowth potential of all the examined types of bacteria was in vestigated after 24 h of storage of the treated effluents at 25 °C. No re growth was observed, indicating the permanent oxidative damage may have induced during the oxidation process. According to the scien tific literature, when UV C irradiation is applied, the damage is mainly at the genome level, due to the high absorption by the thymine and cyto sine bases. This stress induces responses of chaperones to repair the DNA damages, but soon this response is surpassed (Sinha and Häder, 2002). Similarly to UV C disinfection, the regrowth is influenced by the fluence of UV C received by the microorganisms. The superiority of UV C/H_2O_2 oxidation lies in the fact that UV C baseline damage is en hanced by the oxidative action of HO• of H_2O_2 (Giannakis et al., 2016).

UV C/H_2O_2 can potentially lead to permanent bacterial damage when operated at high UV fluence. The findings of this study clearly demonstrated that both total and ARB in spite of their stress status, they do not maintain viability and do not regrow when the oxidative stress is relieved. The rapid and efficient inactivation of total bacteria and ARB in this study may be also attributed to the fact that a higher ox idant dose (compared to other studies) of 40 mg L^{-1} has been employed, making thus both the inactivation more efficient and more rapid, with no regrowth taking place.

3.3. Assessment of the efficiency of UV C/H_2O_2 oxidation to remove total DNA and selected ARGs

The total DNA content throughout the UV C/H_2O_2 oxidation de creased down to an average yield of 0.04 ng μL^{-1} at 24 J cm^{-2} achieving an average reduction of 99% of total DNA concentration (*data not shown*). Moreover, the abundance of selected ARGs throughout the UV C/H_2O_2 oxidation was examined. The estimated average values expressed as copies mL^{-1} as a function of UV fluence are given in Fig. 4.

In the present study, the applied UV fluence of 16 J cm^{-2} was able to lead to the reduction of the abundance of *bla*$_{OXA}$, *bla*$_{TEM}$, *bla*$_{CTX-M}$, *qnrS*, *sul*1 and *tetM*. The reduction in the abundance of tetracycline gene *tetM* was higher than that of the sulfonamide gene *sul*1, indicating that the former was less recalcitrant than the latter. Apart from 16S rRNA and *sul*1, all the other ARGs were removed at 16 J cm^{-2}.

The experimental set up used in this study revealed that high UV fluence accompanied with high oxidant dose seems to be required for the efficient reduction of the abundance of ARGs. It was observed that despite the decrease of the selected ARGs during the treatment course, the UV C/H_2O_2 process was not effective in achieving complete elimina tion of some of the studied ARGs even after 24 J cm^{-2}. On the basis of the obtained findings, it can be inferred that the effective removal of ARGs requires higher UV fluence. Also, one important observation is that the UV fluence as reported in this study was higher than the UV dose commonly applied in UWTPs for wastewater disinfection, which is often less than 100 mJ cm^{-2}, indicating the potential persistence of these genes under the actually applied disinfection conditions. It is noted that such high UV fluence values (3500 5000 mJ cm^{-2}) were re ported in studies dealing with the removal of disinfection by products during drinking water treatment (Toor and Mohseni, 2007; Wang et al., 2015). Concentration of H_2O_2 is also an important parameter for the formation of HO• and the effectiveness of UV/H_2O_2 process. The in ability to generate HO• at low H_2O_2 concentrations is due to the small coefficient of H_2O_2 (19.6 M^{-1} cm^{-1} at 254 nm). The results presented herein suggest that the initial concentration of 40 mg L^{-1} H_2O_2 was in adequate to reduce the abundance of the selected ARGs and high UV fluences were required. The initial concentration of 40 mg L^{-1} H_2O_2 was chosen as the optimum one for the degradation of antibiotics be cause at the initial tested concentration of 50 mg L^{-1} H_2O_2, the well known self scavenging effect of H_2O_2 was observed.

In the study of Munir et al. (2011), no significant reduction ($p > 0.05$) was observed for ARGs removal, who reported that UV disinfection did not contribute significantly to the removal of *sul*1. It was considered that low effective fluence (as is the one applied at the UWTPs) might weaken the effect of UV treatment. This is in agreement with Gao et al. (2012) who demonstrated that low UV fluence (up to 8 mJ cm^{-2}) did not affect the frequency of conjugative transfer because UV disinfection only de creased the bacterial number, while it did not damage bacterial proper ties (e.g., the cell membrane), but it directly damaged the plasmid containing ARGs causing the death of donor (or recipient). The full scale application of UV C/H_2O_2 for the removal of ARGs is reported for the first time in the study of Rodríguez Chueca et al. (2019). Different UV C dosage (42 170 J L^{-1}), contact time (4 18 s) and 0.05 0.5 mM

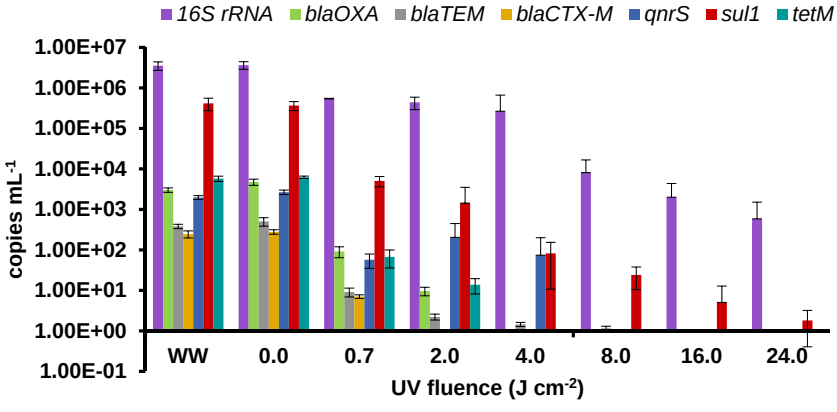


Fig. 4. The abundance of the examined ARGs in the UV-C/H_2O_2 treated samples as a function of UV fluence (J cm ^{2}). Mean values are presented in copies mL ^{1}.

of H_2O_2 were applied and the abundance of the studied ARGs (i.e. 16S rRNA, bla_{OXA}, *sul*1, *sul*2, *qnrS*, *int*1, bla_{TEM}) was decreased in ca. 0.60 log (7 s) and 0.21 log (4 s).

3.4. Phyto and eco toxicity assessment and classification

3.4.1. Phytotoxicity assessment

The phytotoxicity of the untreated (WW) and treated samples (under the optimum experimental conditions) towards *Lepidium sativum*, *Sinapis alba* and *Sorghum saccharatum* was expressed as the percentage of inhibition of GI, SI and RI. The phytotoxicity test applied in this study was considered suitable to evaluate the toxicity of the treated wastewater before its use for agricultural irrigation (Rizzo, 2011).

A phytotoxic effect against root growth was observed in all plant species as shown in Fig. 5. RI acquired its maximum value at 9.5 J cm^{-2} (*L. sativum* [53%], *S. alba* [65%], *S. saccharatum* [76%]), and from that time onwards, RI was significantly decreased to 10%, 10% and 12% for L. *sativum*, *S. alba* and *S. saccharatum*, respectively. The highest SI was also detected at 9.5 J cm^{-2} (*L. sativum* [41%], *S. alba* [48%], *S. saccharatum* [36%]), implying the formation of oxidation products with individual, additive, or/and synergistic phytotoxic effects. Finally, samples at 18.9 J cm^{-2} and beyond, displayed a decrease in SI down to approximately 7% level, indicating the removal of the toxic oxidation products throughout the process (*data not shown*). The treated samples did not induce inhibition on the germination of *L. sativum* and *S. alba* (*data not shown*), while a slight inhibition effect on seeds' germination was observed for *S. saccharatum*.

High UV fluence may decrease the phytotoxicity of the untreated wastewater. The results described above demonstrated the capacity of the applied UV C process to reduce the phytotoxicity to the examined plant species at UV fluence of 37.8 J cm^{-2} which resulted in the production of a treated effluent with lower phytotoxicity than that of the initial effluent (SI: *L. sativum* [7.5%], *S. alba* [7.5%], *S. saccharatum* [6.5%] and RI: *L. sativum* [10.5%], *S. alba* [10.5%], *S. saccharatum* [12.1%]). The

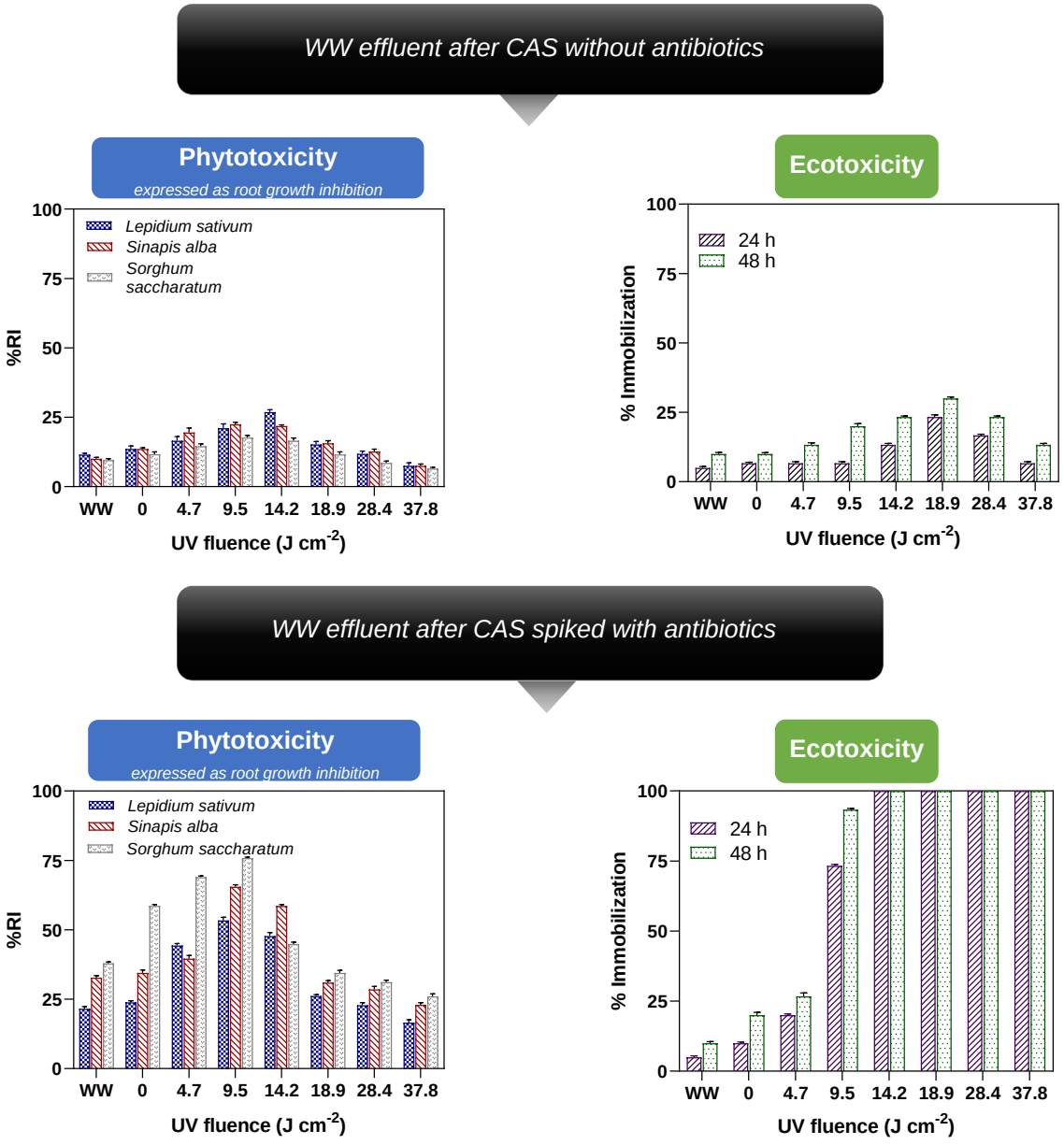


Fig. 5. Phytotoxicity [root growth inhibition (RI)] and ecotoxicity (evolution of toxicity to *Daphnia magna*). Experimental conditions: WW effluent after CAS; $[H_2O_2] = 40$ mg L^{-1}; pH = 8.01 ± 0.02; T = 22 ± 2 °C, wastewater (WW) effluent after CAS spiked with antibiotics; $[A]_0 = 100$ μg L^{-1}; $[H_2O_2] = 40$ mg L^{-1}; pH = 8.01 ± 0.02; T = 22 ± 2 °C.

phytotoxicity may potentially be attributed to the antibiotics and their TPs formed during the UV C/H_2O_2 process.

These results are in accordance with the study of Pan and Chu (2016) in which antibiotics exhibited a lower phytotoxic effect on GI rather than on SI and RI. RI values were always higher compared to those of SI, indicating the susceptibility of roots compared to the shoots to be affected more and confirming that root elongation was a more sensitive endpoint than shoot elongation and seed germination (An et al., 2009).

3.4.2. Ecotoxicity assessment

The evolution of toxicity to *Daphnia magna* during UV C/H_2O_2 process is illustrated in Fig. 5. It should be highlighted that the experiments were conducted in wastewater effluents under the optimum experimental conditions both in the presence and in the absence of antibiotics in order to examine the effect of the dE_fOM and its associated oxidation products towards the toxicity to *Daphnia magna*.

UV C/H_2O_2 was not able to remove the toxicity towards *Daphnia magna* even at high UV fluence. The toxicity of the treated samples at 9.5 J cm^{-2} of the UV C/H_2O_2 oxidation was increased to 73% and 93% after 24 h and 48 h of exposure, respectively, compared to the untreated wastewater (WW). From that point onwards, the immobilization of *Daphnia magna* was kept constant at 100% a fact that may be attributed to the oxidation of both the antibiotics and the dE_fOM leading to the generation of TPs with toxic effects. Since the toxicity of the treated samples at 9.5 J cm^{-2} was higher than that of the untreated wastewater, additional experiments were carried out to investigate whether the toxicity could be further reduced with higher UV fluences up to 37.8 J cm^{-2}. However, higher UV fluences did not reduce the toxicity.

Antibiotics and their TPs exhibited toxic effects against *Daphnia magna*. When the experiments were conducted under the same experimental conditions, but in the absence of the examined mixture of antibiotics, the highest immobilization was recorded at 18.9 J cm^{-2} of the treatment process (43.3 ± 0.5% and 53.3 ± 0.6% after 24 and 48 h of exposure, respectively). At 18.9 J cm^{-2}, a continuous decrease was observed. Finally, at 28.4 J cm^{-2} of the treatment process, the immobilization of daphnids after 24 h of exposure was 3.3%, while after 48 h of exposure the respective value was 6.7%.

These results indicate possible interactions among the components of the mixture. Whether these are additive, synergistic, antagonistic, etc. remains however unclear and further work is required to understand the specific mechanisms of action. Most studies that address the ecological hazard of antibiotics only account for the toxicity of single antibiotic exposure, usually under acute conditions, and do not take into account chronic additive or synergistic affects that can occur in mixtures. This is of concern given the fact that low level combinations of antibiotics are continuously released into the aquatic environment with aquatic species being exposed over the course of their life cycles.

3.4.3. Phyto and eco toxicity classification

A deeper analysis of the results of phyto and eco toxicity experiments was conducted and the system of toxicity classification developed by Persoone et al. (2003) was used to estimate the phyto and eco toxicity classification of the treated samples during UV C/H_2O_2 oxidation (Table S7).

Based on this classification, the toxicity presented a similar profile in the UV C/H_2O_2 experiments in wastewater, both in the presence and the absence of the examined antibiotics. The % class score weight was increasing 9.5 J cm^{-2} and then started to decrease until 37.8 J cm^{-2}. The maximum hazard was observed at 9.5 J cm^{-2} and was ranked as class III (PE value >50% was established or exceeded by at least one test, but the effect level was lower than 100%, samples are toxic, acute hazard) in the first case. However, most of the examined samples were classified as class II (a statistically significant percent effect of PE was found in at least one test; however, the value of the effect was below 50%; low toxic samples, low acute hazard). Moreover, no significant correlations were found between the daphnids immobilization and the phytotoxicity assays, so these analyses did not show a similar sensitivity to the treated samples. While assessing the sensitivity of the performed bioassays, the highest number of toxic responses was recorded in the toxicity tests towards *Daphnia magna*.

3.5. In vitro bioassays assessment

Four effect based reporter gene CALUX® bioassays based on the human U2OS cell line were applied to assess the potential genotoxicity (+/− S9), oxidative stress and cytotoxicity of the UV C/H_2O_2 treated samples. Cytotox CALUX® serves as a control for non specific activation or inhibition of luciferase expression. The capacity of the UV C/H_2O_2 process to reduce oxidative stress and genotoxicity (+S9) was demonstrated. Among the tested bioassays, p53 (+hepatic S9 as exogenous metabolic system) and Nrf2 CALUX® presented quantifiable responses (IF>1.5) to the treated samples of 9.5 J cm^{-2} in the wastewater samples, while no cytotoxicity was observed (Fig. 6). At 9.5 J cm^{-2}, these effects were reduced below the limit of detection, while clarithromycin, erythromycin, ofloxacin and trimethoprim were still present in the samples [degradation (%): 21.4%, 19.2%, 8.1% and 16.1%, respectively]. On the other hand, when the experiments were conducted under the same experimental conditions, but in the absence of the studied antibiotics, none of these biological effects were detected indicating that genotoxicity and oxidative stress activity could be attributed to the antibiotics' residues and their associated TPs (that are present even in very low concentrations) and not due to the dE_fOM components. To the

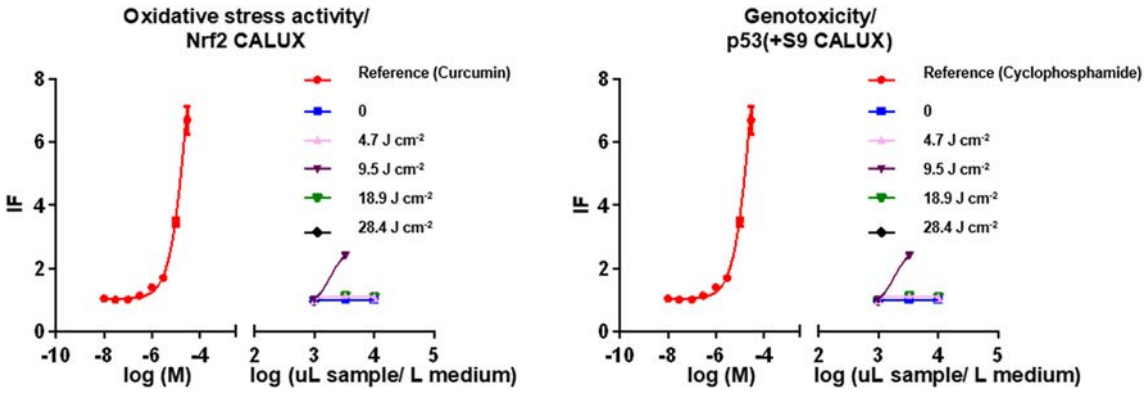


Fig. 6. Oxidative stress activity and genotoxicity during UV-C/H_2O_2 oxidation process. Experimental conditions: wastewater (WW) effluent after CAS spiked with antibiotics; $[A]_0$ = 100 μg L^{-1}; $[H_2O_2]$ = 40 mg L^{-1}; pH = 8.01 ± 0.02; T = 22 ± 2 °C.

authors' knowledge, this is the first time that the CALUX® assays are used to assess the genotoxicity and oxidative stress activity in UV C/H_2O_2 treated samples.

4. Conclusions

On the basis of the obtained findings of this study using the specific bench scale experimental set up, it can be confirmed that high UV fluence is required for the effective degradation of the mixture of the studied antibiotics in wastewater. Total bacteria and ARB can be effectively inactivated at lower UV fluence compared to that required for the mixture of the chemical microcontaminants. The effective inactivation of total bacteria and ARB, as well as the reduction of the abundance of ARGs require even higher UV fluence. This could be partly explained by the use of total bacteria as a disinfection efficiency indicator (which would lead to treatment performance controlled by the "strongest" bacterial strains in the total bacterial population), as well as by the non uniform irradiation and non optimal mixing occurring in the photoreactor. The latter led to achieving removals of the studied contaminants at high UV fluence, much higher than that applied at actual treatment plants during disinfection (i.e. 40 100 mJ cm^{-2}). As already mentioned, there is still limited knowledge on the efficiency of UV C/H_2O_2 oxidation, in terms of UV fluence, to inactivate ARB, as most of the studies report the removals based on the treatment time without reporting the UV fluence. The different experimental conditions moreover, the scale and reactor configurations of each study, and the different matrices used, do not allow for systematic comparisons. We would also like to report that the particular reactor used in this study, which has been used by various other groups in the past of the degradation of various chemical contaminants of emerging concern, seems to be unideal for studies like the present one. The specific configuration of the cylindrical photoreactor used, the discrepancies in UV fluence due to non uniform hydrodynamics and light distribution observed, support our conclusion.

Also, it is worth noting, that it is obvious both from this study and also other studies recently published, that *E. coli* disinfection is most usually achieved more easily and quickly than for other bacteria and hence, the disinfection fluence required at the actual treatment plants and reported for the *E. coli*, which serves as the most commonly used indicator, probably is not capable to achieve disinfection for other more persistent ones and also genes.

Higher doses of the oxidant could also potentially be used in order to inactivate ARB and reduce the abundance of ARGs. However, in the present study, a higher concentration of H_2O_2 caused a scavenging effect in the degradation of the antibiotics, a phenomenon that is frequently observed during the implementation of such processes. This fact implies that the optimization of the UV C/H_2O_2 process should consider both chemical and biological contaminants. It is therefore apparent, that the optimization of the UV C/H_2O_2 process should be a trade off with regard to the oxidant dose and the UV fluence, so that it can be effective in eliminating also the potential bacterial regrowth and avoiding subsequent recovery of the studied bacterial species.

Despite the benefits of the proposed assessment concept, there are still several limitations that have to be addressed in the future. When applying AOPs (in this case UV C/H_2O_2), the generation of oxidation TPs able to induce phyto and eco toxic and other biological activities, derived from either the antibiotics or the dE_fOM itself, necessitates the application of toxicological studies to evaluate the distinct effect of these unknown TPs due to their potential additive, antagonistic, and synergistic effects in combination with their parent compounds and other mixtures of chemicals.

The different aspects related to the environmental dimension of antimicrobial resistance need to be dealt with interdisciplinarity, as this is the only way through which an adequate response to tackle antibiotic related microcontaminants can be developed. An assessment scheme is needed to complement every advanced chemical oxidation process optimization, in order to obtain concrete results about the removal efficiency of selected chemical compounds (e.g. a mixture of antibiotics in this case) and their biological effects, the inactivation of ARB and the reduction of the abundance of ARGs, and thus, a comprehensive picture of the profile of the antibiotic related microcontaminants.

CRediT authorship contribution statement

Vasiliki G. Beretsou: Conceptualization, Investigation, Formal analysis, Visualization, Writing original draft, Writing review & editing. **Irene Michael-Kordatou:** Conceptualization, Writing review & editing. **Costas Michael:** Investigation, Writing review & editing. **Domenico Santoro:** Methodology, Validation, Writing review & editing. **Mahmoud El-Halwagy:** Methodology, Writing review & editing. **Thomas Jäger:** Investigation, Writing review & editing. **Harrie Besselink:** Writing review & editing, Resources. **Thomas Schwartz:** Writing review & editing, Resources. **Despo Fatta-Kassinos:** Conceptualization, Methodology, Supervision, Writing review & editing, Resources, Funding acquisition.

Declaration of competing interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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References

Alexander, J., Knopp, G., Dötsch, A., Wieland, A., Schwartz, T., 2016. Ozone treatment of conditioned wastewater selects antibiotic resistance genes, opportunistic bacteria, and induce strong population shifts. Sci. Total Environ. 559, 103–112. https://doi.org/10.1016/j.scitotenv.2016.03.154.

An, J., Zhou, Q., Sun, Y., Xu, Z., 2009. Ecotoxicological effects of typical personal care products on seed germination and seedling development of wheat (*Triticum aestivum* L.). Chemosphere 76, 1428–1434. https://doi.org/10.1016/j.chemosphere.2009.06.004.

Berendonk, T.U., Manaia, C.M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Bürgmann, H., Sørum, H., Norström, M., Pons, M.N., Kreuzinger, N., Huovinen, P., Stefani, S., Schwartz, T., Kisand, V., Baquero, F., Martinez, J.L., 2015. Tackling antibiotic resistance: the environmental framework. Nat. Rev. Microbiol. 13, 310–317. https://doi.org/10.1038/nrmicro3439.

Bolton, J.R., Stefan, M.I., 2002. Fundamental photochemical approach to the concepts of fluence (UV dose) and electrical energy efficiency in photochemical degradation reactions. Res. Chem. Intermed. 28, 857–870. https://doi.org/10.1163/15685670260469474.

Bolton, J.R., Stefan, M.I., Shaw, P.S., Lykke, K.R., 2011. Determination of the quantum yields of the potassium ferrioxalate and potassium iodide-iodate actinometers and a method for the calibration of radiometer detectors. J. Photochem. Photobiol. A Chem. 222, 166–169. https://doi.org/10.1016/j.jphotochem.2011.05.017.

Boreen, A.L., Arnold, W.A., McNeill, K., 2003. Photodegradation of pharmaceuticals in the aquatic environment: a review. Aquat. Sci. 65, 320–341. https://doi.org/10.1007/s00027-003-0672-7.

Cerreta, G., Roccamante, M.A., Plaza-Bolaños, P., Oller, I., Aguera, A., Malato, S., Rizzo, L., 2020. Advanced treatment of urban wastewater by UV-C/free chlorine process: micro-pollutants removal and effect of UV-C radiation on trihalomethanes formation. Water Res. 169. https://doi.org/10.1016/j.watres.2019.115220.

Chen, P.Y., Chu, X.N., Liu, L., Hu, J.Y., 2016. Effects of salinity and temperature on inactivation and repair potential of Enterococcus faecalis following medium- and low-pressure ultraviolet irradiation. J. Appl. Microbiol. 120, 816–825. https://doi.org/10.1111/jam.13026.

Clesceri, L.S., Greenberg, A.E., Eaton, A., 1998. Standard Methods for the Examination of Water and Wastewater. 20th ed. Public Health. American Public Health Association, Washington, DC.

Dodd, M.C., Huang, C.H., 2007. Aqueous chlorination of the antibacterial agent trimethoprim: reaction kinetics and pathways. Water Res. 41, 647–655. https://doi.org/10.1016/j.watres.2006.10.029.

Escher, B., Leusch, F., 2011. Bioanalytical Tools in Water Quality Assessment. IWA Publishing https://doi.org/10.2166/9781843393689.

EU, 2017. A European one health action plan against antimicrobial resistance (AMR). Eur. Comm. Last visit, 24 https://doi.org/10.1139/H07-078.

EU 2018/840, 2018. COMMISSION IMPLEMENTING DECISION (EU) 2018/840 of 5 June 2018. Off. J. Eur. Union 141, 9–12.

Ferro, G., Guarino, F., Castiglione, S., Rizzo, L., 2016. Antibiotic resistance spread potential in urban wastewater effluents disinfected by UV/H_2O_2 process. Sci. Total Environ. 560–561, 29–35. https://doi.org/10.1016/j.scitotenv.2016.04.047.

Ferro, G., Guarino, F., Cicatelli, A., Rizzo, L., 2017. β-Lactams resistance gene quantification in an antibiotic resistant *Escherichia coli* water suspension treated by advanced oxidation with UV/H2O2. J. Hazard. Mater. 323, 426–433. https://doi.org/10.1016/j.jhazmat.2016.03.014.

Gao, P., Munir, M., Xagoraraki, I., 2012. Correlation of tetracycline and sulfonamide antibiotics with corresponding resistance genes and resistant bacteria in a conventional municipal wastewater treatment plant. Sci. Total Environ. 421–422, 173–183. https://doi.org/10.1016/j.scitotenv.2012.01.061.

Giannakis, S., Voumard, M., Grandjean, D., Magnet, A., De Alencastro, L.F., Pulgarin, C., 2016. Micropollutant degradation, bacterial inactivation and regrowth risk in wastewater effluents: influence of the secondary (pre)treatment on the efficiency of advanced oxidation processes. Water Res. 102, 505–515. https://doi.org/10.1016/j.watres.2016.06.066.

Guo, M.-T., Yuan, Q.-B., Yang, J., 2013. Microbial selectivity of UV treatment on antibiotic-resistant heterotrophic bacteria in secondary effluents of a municipal wastewater treatment plant. Wat. Res. 47, 6388–6394. https://doi.org/10.1016/j.watres.2013.08.012.

He, X., Mezyk, S.P., Michael, I., Fatta-Kassinos, D., Dionysiou, D.D., 2014. Degradation kinetics and mechanism of β-lactam antibiotics by the activation of H_2O_2 and $Na_2S_2O_8$ under UV-254nm irradiation. J. Hazard. Mater. 279, 375–383. https://doi.org/10.1016/j.jhazmat.2014.07.008.

Jia, A., Escher, B.I., Leusch, F.D.L., Tang, J.Y.M., Prochazka, E., Dong, B., Snyder, E.M., Snyder, S.A., 2015. *In vitro* bioassays to evaluate complex chemical mixtures in recycled water. Water Res. 80, 1–11. https://doi.org/10.1016/j.watres.2015.05.020.

JRC, 2018. Something from Nothing? Ensuring the Safety of Chemical Mixtures. https://doi.org/10.2760/618648.

Jung, Y.J., Kim, W.G., Yoon, Y., Hwang, T.M., Kang, J.W., 2012. pH effect on ozonation of ampicillin: kinetic study and toxicity assessment. Ozone Sci. Eng. 34, 156–162. https://doi.org/10.1080/01919512.2012.662890.

Karaolia, P., Michael-Kordatou, I., Hapeshi, E., Drosou, C., Bertakis, Y., Christofilos, D., Armatas, G.S., Sygellou, L., Schwartz, T., Xekoukoulotakis, N.P., Fatta-Kassinos, D., 2018. Removal of antibiotics, antibiotic-resistant bacteria and their associated genes by graphene-based TiO_2 composite photocatalysts under solar radiation in urban wastewaters. Appl. Catal. B Environ. 224, 810–824. https://doi.org/10.1016/j.apcatb.2017.11.020.

Karkman, A., Do, T.T., Walsh, F., Virta, M.P.J., 2018. Antibiotic-resistance genes in waste water. Trends Microbiol. 26, 220–228. https://doi.org/10.1016/j.tim.2017.09.005.

Khan, M.H., Bae, H., Jung, J.Y., 2010. Tetracycline degradation by ozonation in the aqueous phase: proposed degradation intermediates and pathway. J. Hazard. Mater. 181, 659–665. https://doi.org/10.1016/j.jhazmat.2010.05.063.

Kortenkamp, A., Faust, M., 2018. Regulate to reduce chemical mixture risk. Science (80-.) 361, 224–226. https://doi.org/10.1126/science.aat9219.

Lekkerkerker-Teunissen, K., Benotti, M.J., Snyder, S.A., Van Dijk, H.C., 2012. Transformation of atrazine, carbamazepine, diclofenac and sulfamethoxazole by low and medium pressure UV and UV/H_2O_2 treatment. Sep. Purif. Technol. 96, 33–43. https://doi.org/10.1016/j.seppur.2012.04.018.

Liao, Q.N., Ji, F., Li, J.C., Zhan, X., Hu, Z.H., 2016. Decomposition and mineralization of sulfaquinoxaline sodium during UV/H_2O_2 oxidation processes. Chem. Eng. J. 284, 494–502. https://doi.org/10.1016/j.cej.2015.08.150.

Michael, I., Hapeshi, E., Michael, C., Varela, A.R., Kyriakou, S., Manaia, C.M., Fatta-Kassinos, D., 2012. Solar photo-Fenton process on the abatement of antibiotics at a pilot scale: degradation kinetics, ecotoxicity and phytotoxicity assessment and removal of antibiotic resistant enterococci. Water Res. 46, 5621–5634. https://doi.org/10.1016/j.watres.2012.07.049.

Michael, I., Rizzo, L., McArdell, C.S., Manaia, C.M., Merlin, C., Schwartz, T., Dagot, C., Fatta-Kassinos, D., 2013. Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: a review. Water Res. 47, 957–995. https://doi.org/10.1016/j.watres.2012.11.027.

Michael, S.G., Michael-Kordatou, I., Beretsou, V.G., Jäger, T., Michael, C., Schwartz, T., Fatta-Kassinos, D., 2019. Solar photo-Fenton oxidation followed by adsorption on activated carbon for the minimisation of antibiotic resistance determinants and toxicity present in urban wastewater. Appl. Catal. B Environ. 244, 871–880. https://doi.org/10.1016/j.apcatb.2018.12.030.

Michael-Kordatou, I., Iacovou, M., Frontistis, Z., Hapeshi, E., Dionysiou, D.D., Fatta-Kassinos, D., 2015. Erythromycin oxidation and ERY-resistant *Escherichia coli* inactivation in urban wastewater by sulfate radical-based oxidation process under UV-C irradiation. Water Res. 85, 346–358. https://doi.org/10.1016/j.watres.2015.08.050.

Michael-Kordatou, I., Karaolia, P., Fatta-Kassinos, D., 2018. The role of operating parameters and oxidative damage mechanisms of advanced chemical oxidation processes in the combat against antibiotic-resistant bacteria and resistance genes present in urban wastewater. Water Res. 129, 208–230. https://doi.org/10.1016/j.watres.2017.10.007.

Munir, M., Wong, K., Xagoraraki, I., 2011. Release of antibiotic resistant bacteria and genes in the effluent and biosolids of five wastewater utilities in Michigan. Water Res. 45, 681–693. https://doi.org/10.1016/j.watres.2010.08.033.

Nogueira, R.F.P., Oliveira, M.C., Paterlini, W.C., 2005. Simple and fast spectrophotometric determination of H_2O_2 in photo-Fenton reactions using metavanadate. Talanta 66, 86–91. https://doi.org/10.1016/j.talanta.2004.10.001.

Novo, A., Manaia, C.M., 2010. Factors influencing antibiotic resistance burden in municipal wastewater treatment plants. Appl. Microbiol. Biotechnol. 87, 1157–1166. https://doi.org/10.1007/s00253-010-2583-6.

Pan, M., Chu, L.M., 2016. Adsorption and degradation of five selected antibiotics in agricultural soil. Sci. Total Environ. 545–546, 48–56. https://doi.org/10.1016/j.scitotenv.2015.12.040.

Paredes, L., Omil, F., Lema, J.M., Carballa, M., 2018. What happens with organic micropollutants during UV disinfection in WWTPs? A global perspective from laboratory to full-scale. J. Hazard. Mater. 342, 670–678. https://doi.org/10.1016/j.jhazmat.2017.08.075.

Persoone, G., Marsalek, B., Blinova, I., Törökne, A., Zarina, D., Manusadzianas, L., Nalecz-Jawecki, G., Tofan, L., Stepanova, N., Tothova, L., Kolar, B., 2003. A practical and user-friendly toxicity classification system with microbiotests for natural waters and wastewaters. Environ. Toxicol. 18, 395–402. https://doi.org/10.1002/tox.10141.

Pruden, A., 2014. Balancing water sustainability and public health goals in the face of growing concerns about antibiotic resistance. Environ. Sci. Technol. 48, 5–14. https://doi.org/10.1021/es403883p.

Rizzo, L., 2011. Bioassays as a tool for evaluating advanced oxidation processes in water and wastewater treatment. Water Res. 45, 4311–4340. https://doi.org/10.1016/j.watres.2011.05.035.

Rizzo, L., Lofrano, G., Gago, C., Bredneva, T., Iannece, P., Pazos, M., Krasnogorskaya, N., Carotenuto, M., 2018. Antibiotic contaminated water treated by photo driven advanced oxidation processes: ultraviolet/H_2O_2 vs ultraviolet/peracetic acid. J. Clean. Prod. 205, 67–75. https://doi.org/10.1016/j.jclepro.2018.09.101.

Rizzo, L., Agovino, T., Nahim-Granados, S., Castro-Alférez, M., Fernández-Ibáñez, P., Polo-López, M.I., 2019a. Tertiary treatment of urban wastewater by solar and UV-C driven advanced oxidation with peracetic acid: effect on contaminants of emerging concern and antibiotic resistance. Water Res. 149, 272–281. https://doi.org/10.1016/j.watres.2018.11.031.

Rizzo, L., Malato, S., Antakyali, D., Beretsou, V.G., Đolić, M.B., Gernjak, W., Heath, E., Ivancev-Tumbas, I., Karaolia, P., Lado Ribeiro, A.R., Mascolo, G., McArdell, C.S., Schaar, H., Silva, A.M.T., Fatta-Kassinos, D., 2019b. Consolidated vs new advanced treatment methods for the removal of contaminants of emerging concern from urban wastewater. Sci. Total Environ. 655, 986–1008. https://doi.org/10.1016/j.scitotenv.2018.11.265.

Rodríguez-Chueca, J., Varella della Giustina, S., Rocha, J., Fernandes, T., Pablos, C., Encinas, Á., Barceló, D., Rodríguez-Mozaz, S., Manaia, C.M., Marugán, J., 2019. Assessment of full-scale tertiary wastewater treatment by UV-C based-AOPs: removal or persistence of antibiotics and antibiotic resistance genes? Sci. Total Environ. 652, 1051–1061. https://doi.org/10.1016/j.scitotenv.2018.10.223.

Sinha, R.P., Häder, D.P., 2002. UV-induced DNA damage and repair: a review. Photochem. Photobiol. Sci. 1, 225–236. https://doi.org/10.1039/b201230h.

Sousa, J.M., Macedo, G., Pedrosa, M., Becerra-Castro, C., Castro-Silva, S., Pereira, M.F.R., Silva, A.M.T., Nunes, O.C., Manaia, C.M., 2017. Ozonation and UV254nm radiation for the removal of microorganisms and antibiotic resistance genes from urban wastewater. J. Hazard. Mater. 323, 434–441. https://doi.org/10.1016/j.jhazmat.2016.03.096.

Stefan, M.I., 2018. Chapter 2, UV/hydrogen peroxide process. Advanced Oxidation Processes for Water Treatment. IWA Publishing.

Taghipour, F., 2004. Ultraviolet and ionizing radiation for microorganism inactivation. Water Res. 38, 3940–3948. https://doi.org/10.1016/j.watres.2004.06.016.

Toor, R., Mohseni, M., 2007. UV-H_2O_2 based AOP and its integration with biological activated carbon treatment for DBP reduction in drinking water. Chemosphere 66, 2087–2095. https://doi.org/10.1016/j.chemosphere.2006.09.043.

UN Environment, 2017. Frontiers 2017 - Emerging Issues of Environmental Concern, Frontiers 2017: Emerging Issues of Environmental Concern. https://doi.org/10.1016/S0140-6736(15)00298-6.

Van der Linden, S.C., von Bergh, A.R.M., van Vught-Lussenburg, B.M.A., Jonker, L.R.A., Teunis, M., Krul, C.A.M., van der Burg, B., 2014. Development of a panel of high-throughput reporter-gene assays to detect genotoxicity and oxidative stress. Mutat. Res. - Genet. Toxicol. Environ. Mutagen. 760, 23–32. https://doi.org/10.1016/j.mrgentox.2013.09.009.

Vasquez, M.I., Fatta-Kassinos, D., 2013. Is the evaluation of "traditional" physicochemical parameters sufficient to explain the potential toxicity of the treated wastewater at sewage treatment plants? Environ. Sci. Pollut. Res. 20, 3516–3528. https://doi.org/10.1007/s11356-013-1637-6.

Wang, D., Bolton, J.R., Andrews, S.A., Hofmann, R., 2015. Formation of disinfection by-products in the ultraviolet/chlorine advanced oxidation process. Sci. Total Environ. 518–519, 49–57. https://doi.org/10.1016/j.scitotenv.2015.02.094.

Willett, K.L., Hites, R.A., 2000. Chemical actinometry: using o-nitrobenzaldehyde to measure light intensity in photochemical experiments. J. Chem. Educ. 77, 900–902. https://doi.org/10.1021/ed077p900.

Wols, B.A., Hofman-Caris, C.H.M., Harmsen, D.J.H., Beerendonk, E.F., 2013. Degradation of 40 selected pharmaceuticals by UV/H_2O_2. Water Res. 47, 5876–5888. https://doi.org/10.1016/j.watres.2013.07.008.

Yoon, Y., Dodd, M.C., Lee, Y., 2018. Elimination of transforming activity and gene degradation during UV and UV/H2O2 treatment of plasmid-encoded antibiotic resistance genes. Environ. Sci. Water Res. Technol. 4, 1239–1251. https://doi.org/10.1039/c8ew00200b.

Zhuang, Y., Ren, H., Geng, J., Zhang, Yingying, Zhang, Yan, Ding, L., Xu, K., 2015. Inactivation of antibiotic resistance genes in municipal wastewater by chlorination, ultraviolet, and ozonation disinfection. Environ. Sci. Pollut. Res. 22, 7037–7044. https://doi.org/10.1007/s11356-014-3919-z.